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*12,573 volunteer abstracts, 18 symposia abstracts, 24 history of neuroscience abstracts, and 58 teaching of neuroscience abstracts.

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

Policy on the Use of Animals in Neuroscience Research

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

Introduction

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

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

General Policy

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

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

Local Committee Review

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

Other Laws, Regulations, and Policies

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

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

Recommended References

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Policy on the Use of Human Subjects in Neuroscience Research

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

Recommended References

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

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

POLICY ON ETHICS

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

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

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

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

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

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

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

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

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

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

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

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580.	Long-term potentiation: physiology III	Slide				Wed AM	
596.	Long-term potentiation: physiology IV	Poster				Wed AM	
597.	Long-term potentiation: physiology V	Poster				Wed AM	
598.	Long-term potentiation: physiology VI	Poster				Wed AM	

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

THEME D: NEUROTRANSMITTERS, MODULATORS, TRANSPORTERS, AND RECEPTORS

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

OTHER

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

3

SYMPOSIUM. TYROSINE PHOSPHORYLATION PATHWAYS IN NEURONAL SIGNALLING. J. M. Baraban, Johns Hopkins Univ. School of Medicine (Chairperson); D. Kaplan, Montreal Neurolog. Inst.; J. Schlessinger, New York Univ. School of Medicine; J. Settleman, Mass. Gen. Hosp. Cancer Ctr.; M.E. Greenberg, Harvard Medical School, and Children's Hosp.

Recent studies of tyrosine phosphorylation in the nervous system indicate that intracellular signalling pathways utilizing this uncommon form of protein phosphorylation exert pervasive, powerful influences in both developing and mature neurons. The presentations will highlight advances in understanding the organization and function of these signalling pathways in the nervous system. J. Baraban will present an overview of tyrosine phosphorylation pathways to orient neuroscientists unfamiliar with this area of research. D. Kaplan will discuss how multiple divergent signalling pathways are triggered by neurotrophin receptor activation. J. Schlessinger will report on recent developments in this rapidly advancing field. J. Settleman will present studies linking novel tyrosine phosphorylation pathways to neuronal development *in vivo*. M. Greenberg will review recent studies implicating a network of cytoplasmic tyrosine phosphorylation pathways as key regulators of neuronal survival and death.

4

SENSORIMOTOR INTEGRATION IN SUPERIOR COLLICULUS: WHAT DOES THE COLLICULUS CONTROL? R. H. Wurtz, NIH-NEI (Chairperson); D. P. Munoz, Queen's Univ., Kingston; E. L. Keller, Smith-Kettlewell Inst.; D. Guitton, Montreal Neurol. Inst.; D. L. Sparks, Univ. Pennsylvania.

The sensorimotor activity of the superior colliculus (SC) controls movement, but exactly what movement does the descending output of the colliculus control? The speakers will address two issues.

The first issue is whether the colliculus lies in a feedback loop and controls the dynamics of rapid eye movements. Munoz will describe the distribution of neural activity among the different populations of cells within the SC, show evidence for a shift in the spatial distribution of activity during a saccade, and consider a novel hypothesis to describe how the SC controls saccade dynamics. Keller will consider the difficulty in verifying this hypothesis because one needs to know the neural population activity over the entire area of the colliculus at each instant during a saccade, since the neural data exist as recordings from individual cells during many saccades.

The second issue is whether the SC controls gaze (eye and head) rather than just eye movement alone. Guitton will consider evidence from the cat which cannot deviate its eye in the orbit more than about 25 deg and so relies extensively on combined eye-head movements. Evidence from the discharge of SC output cells, microstimulation of the SC map, and activity of neural elements downstream of the SC indicates that the cat sends gaze related signals to brainstem eye and head motor circuits. In the primate, Sparks will show that the SC is involved in the coordination of eye and head movements during large gaze shifts. The most parsimonious explanation is that the SC generates a single functional signal of desired gaze displacement that is transmitted to separate eye and head controllers.

Work of the speakers was supported by the National Eye Institute, Medical Research Council of Canada, and the McKnight Endowment for Neuroscience.

BRAIN METABOLISM AND BLOOD FLOW I

7.1

DOSE-DEPENDENT EFFECTS OF FLUOXETINE ON REGIONAL CEREBRAL GLUCOSE METABOLISM (rCMRglc) IN AWAKE RATS.

U. Fre*, A. Merico, C. Ori[1], P. Pietrini[2], M. Dam G. Pizzolato and L. Battistin
Neurology and Anesthesiology[1] Departments, 35100 Padova, ITALY and Psychiatry Department[2], 56100 Pisa, ITALY.

Fluoxetine has a wide therapeutic spectrum (e.g. antidepressant, antiobsessional, antipanic and anorectic effects) which may not enterily depend on its serotonin (5-HT) reuptake blocking properties. The aim of this study was to assess the effects of graded doses of fluoxetine on rCMRglc, a putative index of neural function. Experiments were performed in male 3-mo-old Fischer-344 rats (Charles River Italia, Como, Italy) according to the [^{14}C]2-Deoxy-D-glucose ([^{14}C]DG) (Amersham International, Arlington Heights, IL, U.S.A.) autoradiographic technique. rCMRglc was determined in 56 brain regions 30 min after i.p. injection of saline or of 0.4, 4 or 40 mg/kg fluoxetine hydrochloride. rCMRglc values of each brain region were analyzed for statistical significance by Bonferroni multiple comparison test by comparing the mean of fluoxetine-treated groups to the saline-treated group. At a 0.4 mg/kg dose fluoxetine significantly ($P < 0.05$) reduced rCMRglc in the hypothalamus and caudate nucleus only (3 brain regions, 5%); at 4 mg/kg dose fluoxetine reduced rCMRglc in the hypothalamus, caudate nucleus, prefrontal and frontal cortices, locus coeruleus and dorsal raphe (6 regions, 11%). At 40 mg/kg dose, fluoxetine produced widespread rCMRglc reductions (25 brain regions, 45%). Regional CMRglc effects of fluoxetine may be related to fluoxetine anorectic (e.g. hypothalamus), antiobsessional (e.g. caudate) and antidepressant (e.g. frontal cortex) actions. The findings further suggest that fluoxetine exherts its pharmacological actions by modulating both 5-HT and adrenergic efferent outputs from the raphe and coeruleus nuclei to basal ganglia and cortical areas.

7.2

PHARMACOKINETIC AND PHARMACODYNAMIC EFFECTS OF PHYSOSTIGMINE: IMPLICATIONS FOR BRAIN IMAGING STUDIES.

M.L. Furey*, P. Pietrini, G.E. Alexander, M.J. Mentis, A. Dani, U. Shetty, S.I. Rapoport, M.B. Schapiro and U. Freo. Lab. Neurosci., National Institute on Aging (NIA), NIH, Bethesda MD 20892, USA

To identify the time during infusion at which the pharmacokinetic and pharmacodynamic effects of physostigmine (physo) become stable, we measured plasma drug levels, performance indices on a working memory (WM) task and regional cerebral blood flow (rCBF) using positron emission tomography and $H_2{}^{15}O$ during infusion to healthy subjects of saline (6F/3M; mean age=40yr) or physostigmine (3 F/9M; 45yr). Eight scans alternated between rest and a WM delayed (6 sec) match-to-sample face recognition task. After 2 control scans, the drug group received a 10 min loading dose of physo (1.93 mg/hr), followed by a maintenance drip (0.816 mg/hr) to study completion. After stereotaxic normalization of images, absolute rCBF was divided by the subject's global CBF and scaled to the group's mean baseline global CBF. Physo plasma levels increased slightly over time ($p<.05$), and became stable by 30 min. Physo decreased reaction time (RT) ($p<.05$), and this effect became generally stable by 20-40 min of infusion. During physo, CBF increased slightly in right inferior temporal cortex at rest, while CBF in right prefrontal cortex progressively decreased during task ($p<0.01$). These effects reached a plateau by 30-40 min. CBF and RT effects did not correlate with plasma drug levels, indicating that plasma levels do not reflect the central nervous system pharmacodynamic response. These results also show that pharmacokinetic and pharmacodynamic physo effects vary early during infusion, so that functional studies using physo require a 30-40 minute infusion period to obtain stable effects prior to initiating the study. Thus, in studying brain functions, identification of the time point at which the effects of infused drugs become stable is essential to separate the effects of experimental variables from the concomitant changes in drug effects over time. (Supported by NIA).

7.3

7-NITROINDAZOLE ATTENUATES CORTICAL HYPEREMIA DURING RABBIT CORTICAL SPREADING DEPRESSION (CSD). D.M. Colonna*, D.D.Deal and D.W. Busija. Depts. of Anesthesia* and Physiology & Pharmacology, Bowman Gray School of Medicine of Wake Forest University, Winston-Salem, NC 27157-1009.

CSD is a wave-like, temporary depolarization of excitable brain cells. Associated with the depolarization is a dilation of pial arterioles and an increase in cerebral blood flow (CBF). NO's role in promoting hyperemia during CSD remains controversial. Recent reports implicate constitutive brain NO synthase (bNOS) in promoting hypercapneic cerebrovasodilation[(1)]. We hypothesized that bNOS activity promotes CSD induced cortical hyperemia. New Zealand White rabbits were anesthetized with thiopental and urethane. They were instrumented for CBF determinations *via* colored microspheres. CSD was initiated by KCl microinjection. CBF (ml•100g^{-1}•min) was measured at 4 timepoints: baseline (BL), during the first CSD (CSD1), 45min after 15mg/kg, 7-nitroindazole, i.p.(7-NI, a selective inhibitor of constitutive bNOS), (in corn oil), and after a second CSD (CSD2). 7-NI did not affect resting mean arterial blood pressure (MABP, mmHg), demonstrating its lack of endothelial NOS (eNOS) inhibition, nor reduce resting CBF. *7-NI reduced CSD hyperemia (CSD1 *vs* CSD2, RM-ANOVA, $p<0.05$). 7-NI did not reduce resting cortical CBF (BL *vs* 7-NI), though at higher doses and in other species 7-NI decreased CBF up to 20-60%. The fact that CSD induced cerebral hyperemia was so robust after 7-NI suggests many possibilities: that bNOS was inadequately inhibited at this dose of 7-NI, that eNOS plays a significant role in this response, or that other substances (*e.g.*, CGRP) promote cortical hyperemia during CSD. Reference: 1.) *J.Cereb. Blood Flow Metab.* 15:774, 1995

	BL	CSD 1	7-NI	CSD 2
CBF	42±8	124±19	38±4	90±8*

Data presented as mean ± s.e.m.

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7.4

ANOXIC STRESS REDUCES OXYTOCIN-INDUCED PIAL ARTERIOLAR DILATION IN PIGLETS

R. Errico*[1], F. Bari[1,2], T. Louis[3], and D. Busija[1] [1]Bowman Gray Sch. of Med., Winston-Salem, NC 27157, [2] Albert Szent-Györgyi Med. Univ., H-6720 Szeged, Hungary, and [3]East Carolina Univ. Sch. of Med., Greenville, NC 27858.

Current evidence indicates that oxytocin (OT) plays a role in regulating cerebral vascular tone in newborns. We compared the effects of hypoxia, global cerebral ischemia and asphyxia on pial arteriolar response to OT in anesthetized, newborn piglets. Pial arteriolar responses to 10^{-8} and 10^{-6} M of OT were evaluated before and 1 hr following either 10 min of hypoxia, 10 min of global cerebral ischemia or 5 min of asphyxia. Hypoxia was produced by inhalation of 7.5% O_2 - 92.5% N_2. Global cerebral ischemia was achieved by increasing intracranial pressure and confirmed visually by cessation of cortical blood flow. Asphyxia was achieved by turning off the ventilator. In control conditions, OT dilated pial arterioles by 9±1% at 10^{-8} and by 16±1% at 10^{-6} M ($n=36$, $P<0.05$). For the time control animals, arteriolar dilation to OT did not change with repeated applications (9±1%, 15±1% vs. 10±1% and 13±2%, $n=10$). One hour after hypoxia, dilatory effect of OT was diminished at either dose (2±2%, at 10^{-8} and -3±2 % at 10^{-6}, $n=8$). One hour after asphyxia, OT had no effect at 10^{-8} M and conversely had a mild vasoconstrictor effect at 10^{-6} M (-4±3 %, $n=9$). After ischemia, OT did not dilate arterioles at 10^{-8} M and had vasoconstrictor activity at the 10^{-6} M concentration (-6±4%, $n=6$). Topical superoxide dismutase applied before during and after asphyxia failed to preserve vasodilation to OT ($n=5$). We conclude that the absence of pial arteriolar dilation to OT after ischemia may be involved in the pathomechanism of perinatal brain injury. Supported by NIH grants: HL-30260, Hl-46558 and HL-50587.

7.5

FACTORS MODULATING N-METHYL-D-ASPARTATE (NMDA)-INDUCED DILATATION OF PIAL ARTERIOLES IN PIGLETS.

F. Bari*[1,2], R. Errico[1], T. Louis[3], and D. W. Busija[1]. [1]Bowman Gray Sch. of Med., Winston-Salem, NC 27157, [2]Albert Szent-Györgyi Med. Univ., H-6720 Szeged, Hungary, and [3]East Carolina Univ. Sch. of Med., Greenville, NC 27858.

Anoxic/hypoxic stress transiently reduces NMDA-induced cerebral arteriolar dilation, but mechanisms are unclear. The purpose of this study was to examine the roles of oxygen radicals and extracellular adenosine as mediators of reduced vasodilation to NMDA. In anesthetized newborn piglets, we examined pial arteriolar responses using intravital microscopy. Topically applied NMDA resulted in dose-dependent dilatation of pial arterioles (9±1% and 28±5% above baseline to 10^{-5} and 10^{-4} M, respectively). Thirty minutes after 15 min hypoxia (inhalation of 7.5% O_2-92.5% N_2), arteriolar responses to NMDA were reduced to 3±3% at 10^{-5} M and to 12±5% at 10^{-4} M ($P<0.05$, n=6). In a separate group (n=6), topical application of superoxide dismutase (100 U/ml) 30 min before, during and after hypoxia preserved the arteriolar responses to NMDA. In other animals (n=6), we found that coadministration of a non-vasoactive level of adenosine (10^{-6} M) with NMDA (10^{-4} M) reduced pial arteriolar dilatation (31±6% to 20±4% with adenosine, $P<0.05$). We conclude that reduced responsiveness of cerebral blood vessels to NMDA caused by hypoxia appears to be due to oxygen radicals and elevation of extracellular adenosine concentration.

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7.6

EFFECTS OF NMDA RECEPTOR ACTIVATION ON COLLATERAL CEREBRAL BLOOD FLOW.

S.C. Robertson*, C.A. Mazzochi, C.M. Loftus. Div. of Neurosurgery, Univ. of Iowa College of Medicine, Iowa City,IA 52242

Recent evidence suggests that interruptions in the glutamate receptor excitotoxic pathway may confer neuronal protection in part by local alterations of rCBF by neuronal as well as endothelial forms of NO synthase. To our knowledge there is no work linking collateral cerebral flow to neuronal glutamate receptor agonism and antagonism. We studied the effects of NMDA on rCBF and specifically collateral blood flow following middle cerebral artery occlusion.

In seven dogs under 1% halothane anesthesia, a left sided craniectomy was performed. A branch of the MCA was cannulated and collateral-dependent tissue was identified by using the "shadow flow" technique. Regional cerebral blood flow (rCBF) was measured using radiolabeled microspheres. Cerebral vessel pressures in branches of the middle cerebral artery (MCA) were measured using a glass micropipette and servo-null system. After baseline and shadow flows and pressures were obtained, NMDA(300μM)in ACSF was perfused over the exposed brain and repeat flow and pressure measurements were obtained. After 30 min. the NMDA perfusion was stopped and recovery flow measurements was obtained at 30 and 60 min.

NMDA increased collateral and normal cerebral blood flow by 33.1% and 23.0% respectively, while causing a decrease in both small and large cerebral vascular resistance. Systemic and cerebral vascular pressures remained unchanged.

NMDA receptor activation, presumably through activation of endothelial and neuronal NO synthase, causes cerebral vasodilation and increased cerebral blood flow. This response is thought to increase blood flow to ischemic regions of the brain in an effort to minimize infarct. Proposed neuroprotective agents should be designed to maintain cerebral blood, while attenuating glutamate neurotoxicity.

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7.7

Ca^{++}-SENSITIVE K^+ CHANNELS (K_{Ca}) AND THE PERMISSIVE FUNCTION OF NITRIC OXIDE (NO) AND CYCLIC GMP IN HYPERCAPNIA-INDUCED PIAL ARTERIOLAR DILATION.

Q. Wang* and D.A. Pelligrino. Dept. of Anesthesiology, Univ. of Illinois-Chicago, IL 60612.

Classically, NO-mediated cerebrovasodilation (CVD) is elicited by increased NO synthesis. That NO, in turn, diffuses to the vascular smooth muscle (vsm) cell, *increasing* cyclic GMP (cGMP) production. Recent findings have suggested that, in hypercapnia (HC) and other vasodilating conditions, NO synthase (NOS) inhibitors may act to suppress CVD by reducing basal NO and, subsequently, vsm cGMP levels below a critical threshold. Thus, cGMP functions in a "permissive" manner in promoting CVD. We sought to examine whether the permissive function of cGMP in the CVD elicited by HC relates to the capacity for cGMP to support activity of the hyperpolarizing K_{Ca} channels in vsm. A closed cranial window technique was used to monitor pial arteriolar diameter changes in fentanyl/N_2O-anesthetized, male S-D rats. Topical suffusions of the K_{ATP} channel opener, levcromakalim (CR, 50 μM) and the K_{Ca} channel opener, NS-1619 (NS, 50 μM), were sequentially applied, followed by exposure to HC ($PaCO_2$ = 60-65 mmHg). The neuronal NOS (nNOS) inhibitor, 7-nitroindazole (7-NI), was then injected ip (40 mg/kg). 30 min later, the following sequences were used: HC → HC + 8-bromo-cGMP (8-Br, 30 μM) → HC + 8-Br + iberiotoxin (IBX, 0.1 μM, the K_{Ca} channel blocker) → IBX + NS + 8-Br → IBX + CR + 8-Br. Pial arteriolar CO_2 reactivity was attenuated by 7-NI, restored by 8-Br, and, upon addition of IBX, the restoration was reversed. The specificity of IBX was confirmed by its ability to reduce NS- but not CR-induced dilations. These results, not only confirmed that the NO-dependent portion of the cerebral arteriolar response to HC derives from nNOS, but also suggested that the NO acts in a permissive manner, via maintaining vascular smooth muscle cGMP levels. That apparent obligatory action of cGMP may be due to its capacity to support vsm K_{Ca} channels, and implies that NOS inhibitors may reduce cerebrovascular responses to HC by affecting vsm membrane polarization.

7.8

NITRIC OXIDE SYNTHASE ACTIVITY IN BRAIN AND ENDOTHELIUM: RELATION TO HYPERCAPNIC RISE OF CEREBRAL BLOOD FLOW IN RATS.

M. Lauritzen, M. Fabricius*, J.Rubin, M. Bundgaard. Inst. of Medical Physiology & Biochemistry, University of Copenhagen, Dept. of Clinical Neurophysiology Rigshospitalet & Glostrup Hospital, DK-2200 Copenhagen, Denmark.

We examined whether attenuation of the hypercapnic increase of cerebral blood flow (CBF) associated with nitric oxide synthase (NOS) inhibition was related to local neuronal or aortic endothelial NOS activity, or local endothelial/neuronal NOS-dependent vasodilation. Halothane anaesthetized rats were ventilated, and CBF was measured by laser-Doppler flowmetry over the parietal and cerebellar cortex. I.v. N^G-nitro-L-arginine (L-NNA) (30 mg/kg) inhibited brain and aortic NOS activity by 67-70%. Topical L-NNA (1mM) inhibited brain NOS activity by 91-94%, while aortic NOS activity remained constant. In contrast i.v. L-NNA attenuated the hypercapnic CBF rise much more efficiently than topical L-NNA. 7-nitroindazole, another NOS inhibitor, attenuated endothelial and neuronal NOS activity equally well, and inhibited the hypercapnic CBF increase as effectively as L-NNA.Topical L-NNA and 7-nitroindazole abolished local eNOS dependent vasodilation after 15 min, while hypercapnic CBF was only slightly reduced. L-NNA injected into the tissue abolished nNOS dependent vasodilation while hypercapnic CBF was unchanged. The findings suggest that local NOS activity, whether neuronal or endothelial, is unimportant for the hypercapnic rise of CBF.

7.9

PRESERVED METABOLIC ACTIVATION TO SOMATOSENSORY STIMULATION DESPITE ABOLISHED BLOOD FLOW RESPONSE FOLLOWING NEURONAL NOS INHIBITION.

N. Cholet, J. Seylaz, G. Bonvento*. Laboratoire de Recherches Cérébrovasculaires, CNRS UA 641, Université Paris 7, IFR 6, 75010 PARIS, France.

The mediators of the local cerebral blood flow (CBF) increases that occur during functional brain activation are not clearly defined. We have previously demonstrated that inhibition of neuronal NO synthase (nNOS) by 7-Nitroindazole (7-NI), abolished the vasodilatory response to whisker stimulation in the somatosensory cortex and ventral posteromedial nucleus of thalamus (VPM) but did not modify the vasodilation in the first order relay stations of the trigeminal pathway. In the present study, we sought to determine whether this differential cerebrovascular influence of nNOS could be ascribed to a specific action on cerebral metabolism. Rats were anesthetized (α-chloralose 40 mg/kg, sc) and ventilated without curarizing agent. Local cerebral glucose utilization (CGU) was measured using the quantitative [^{14}C]2-deoxyglucose autoradiographic method in control rats (n=8) and 25 min following 25 mg/kg ip 7-NI injection (n=9). CGU was determined bilaterally in six regions of the trigeminal pathway during mechanical stimulation (4 Hz) of the right C whiskers. In control rats, whiskers stimulation increased significantly CGU from 37-79% in the ipsilateral trigeminal nuclei and from 38-71% in the contralateral VPM and somatosensory cortex. Under 7-NI, CGU increases were not significantly different from control, ranging from 47-75 % and from 28-74% in the first and second order relay stations, respectively. These results show that nNOS does not affect the metabolic response to somatosensory stimulation and suggest that neurally-derived NO is an important mediator of the associated vasodilatory response.

7.10

fMRI REVEALS GLUCOSE MODULATION OF HUMAN BRAIN RESPONSES TO VISUAL STIMULI

R.B. Barlow*, E.A. Boudreau, D.C. Moore, S.C. Huckins, A.M. Lindstrom, and D.H.P. Streeten. SUNY Health Science Center and Syracuse University, Syracuse, NY 13210

Glucose, a major energy source for brain function, can modulate human visual sensitivity (Barlow et al, *Invest. Ophthal. Vis. Sci. 34* (4): 785, 1993.). We used fMRI to determine whether changes in blood glucose can also modulate cortical activity in response to visual stimuli (GE Signa, 1.5 Tesla, Advanced NMR Inc., gradient echo-echo planar sequence, TE 60ms,TR 3.5s). Subjects who had fasted overnight to induce mild hypoglycemia entered the imager and were instructed to view the center of a grid of alternating (5Hz) vertical and horizontal bars shown in 15sec on/off cycles during multislice scanning of the occipital pole. Remaining in the imager, the subjects ingested a 75gm glucose drink, waited 20-30min and were rescanned. We monitored blood glucose levels with a *One Touch* home kit and a standard laboratory assay throughout the experiment.

fMRI reveals increased activation at the posterior occipital pole and in some cases along the calcarine fissure after blood glucose levels increased an average of 61+13mg/dl (74+7 to 135+14mg/dl, n=6). This is equivalent to eating a substantial breakfast. We conclude that physiological changes in blood glucose levels can modulate cortical activity in response to visual stimuli. Whether blood glucose changes act pre- and/or post-synaptically in the cortex is not yet known.

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7.11

DECREASED FRONTAL LOBE GLUCOSE METABOLISM IN FEMALE COMPARED TO MALE ALCOHOLICS: A PET STUDY
D Hommer*, W. Williams, R. Momenan, P. Ragan, , U. Ruttimann, D. Rio, M. Eckardt National Institute on Alcohol Abuse and Alcoholism, DICBR, Laboratory of Clinical Studies, Bethesda, MD 20892-1256

Previously we reported that female alcoholics have a significantly smaller corpus callosum than either male alcoholics or age matched female controls (Arch. Neurol. 53:359, 1996). We now report that alcoholic women have significantly lower cerebral metabolic rate for glucose (CMRglc) in the frontal lobes than alcoholic men. The reduction in frontal lobe CMRglc occurs despite a significantly higher overall brain glucose uptake among alcoholic women compared with alcoholic men. Data were collected from 8 alcoholic women and 31 alcoholic men using ^{18}F-2-fluoro-2-deoxyglucose (FDG) positron emission tomography(PET). PET data were co-registered with MRI scans and tested for pixelwise group differences by a Gaussian random fields method. Analysis was performed using both absolute pixel CMRglc values as well as using means-adjusted pixel values obtained by subtracting the overall mean brain CMRglc from each pixel CMRglc. Both mean-adjusted and absolute CMRglc produced similar results. In a smaller sample of normal subjects (10 males & 5 females) no significant differences in frontal lobe glucose utilization were seen.

ISCHEMIA: MEDIATORS

8.1

ACIDOSIS ALONE AND ACIDIC OXYGEN-SUBSTRATE DEPRIVATION INJURY OF PRIMARY ASTROCYTE CULTURES INVOLVE THE SODIUM/BICARBONATE COTRANSPORTER.
M.C. Papadopoulos*, L.L. Haak, L. Hill, A.N. van den Pol, and R.G. Giffard, Depts. of Anesthesia and Biology, Stanford University, Stanford, CA 94305; Section of Neurosurgery, Yale University, Stamford, CT 06520.

Several studies show that animals subjected to cerebral ischemia with elevated blood sugars suffer more severe injury than control animals subjected to the same insult with normal blood sugars. This correlates with more severe lactic acidosis and glial cell death. We have previously shown that cortical astrocytes are more vulnerable to acidosis injury than are neurons in primary culture. We sought the mechanism of glial acid injury by exposing primary cultures of mouse astrocytes to acidosis under different ionic conditions. Cultures exposed to balanced salt solutions(BSS) at pH 7.4 were uninjured after 24 h; cultures kept at pH 6.8 in normal BSS containing sodium and bicarbonate suffered 70-95% death. When choline was used to replace the sodium or chloride replaced bicarbonate, injury at pH 6.8 was reduced more than 80%. We then asked whether ion substitution could prevent astrocyte injury by oxygen-substrate deprivation at pH 6.8. We found that removing sodium or bicarbonate was again able to reduce injury to near background levels. Intracellular calcium measured with fura-2, increased 350nM over baseline at pH 6.8 in conditions which led to injury, and was stable when sodium or bicarbonate was absent. This is the first data which implicates the sodium/bicarbonate cotransporter in glial cell injury.

8.2

LACTATE, NOT GLUCOSE, IS THE ENERGY SUBSTRATE THAT FUELS RECOVERY OF NEURONAL FUNCTION AFTER HYPOXIA IN VITRO.
A. Schurr*, R.S. Payne, J.J. Miller[a] and B.M. Rigor. Depts. of Anesthesiology and Pathology[a], University of Louisville, School of Medicine, Louisville, KY 40292.

Elevated glucose levels *in vitro* significantly increased the degree of recovery of neuronal function from hypoxia in hippocampal slices (*Brain Res.*, **421**:135, 1987). This function can be supported by aerobic metabolism of lactate alone (*Science*, **240**:1326, 1988). When given equicaloric amounts of either lactate or glucose, slices supplied with the former showed a higher degree of recovery of neuronal function from hypoxia than with the latter (*Soc. Neurosci. Abst.*, **21**:213, 1995). During ischemia/hypoxia the brain produces high levels of lactate. Thus, we studied here the role of endogenously-formed lactate in recovery of neuronal function after hypoxia.

Rat hippocampal slices were prepared and maintained as described elsewhere (*Brain Res.*, **614**:10, 1993). Slices were perfused with standard artificial cerebrospinal fluid (10 mM glucose) under an atmosphere of 95% O_2/5% CO_2. A CA1 population spike, evoked by stimulation of the Schaffer collaterals, was used as a measurement of neuronal function. Hypoxia was produced by replacing O_2 with N_2 in the gas atmosphere. Tissue glucose and lactate levels were determined using standard assays.

When supplied with 20 mM instead of 10 mM glucose to increase anaerobic lactate production, all slices recovered their neuronal function upon reoxygenation from 23-min hypoxia. Of slices that were perfused with 20 mM 2-deoxyglucose (2DG) instead of glucose to inhibit glycolysis during the first 13 min of hypoxia, none recovered their neuronal function, although glucose was abundant (20 mM) during the last 10 min of hypoxia and during reoxygenation. In contrast, of slices that produced lactate for the first 10 min of hypoxia before the addition of 2DG for the last 13 min of hypoxia, 80% recovered their neuronal function upon reoxygenation. A similar degree of recovery was observed when glucose was absent during reoxygenation, however, it was short-lived due to the fast diminishing intracellular lactate pools.

We concluded that, upon reoxygenation after a long hypoxic insult, anaerobically accumulated lactate is an obligatory aerobic energy substrate that fuels the recovery of neuronal function.

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8.3

Nitric Oxide Dynamics as Monitored by Citrulline Immunoreactivity in Cerebral Ischemia

M. J. L. Eliasson[1], Z. Huang[2], M. J. Schell[3], M. A. Moskowitz[2], and S. H. Snyder[1,3]*. Dept. of Neuroscience[3], and Pharmacology and Molecular Sciences[1], Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205. Stroke Research Laboratory[2], Mass General Hospital, and Neurosurgical Service, Harvard Med. Sch., Charlestown, MA 02129.

Nitric oxide (NO) or its reactant products have been implicated as major mediators of tissue injury following focal cerebral ischemia. To localize the proposed synthesis of NO following MCA occlusion, we utilized the observation that endogenous citrulline in the brain appears to be formed solely by the enzyme nitric oxide synthase (NOS). Using an antibody highly specific for citrulline conjugated to glutaraldehyde we studied mice with unilateral occlusion of the middle cerebral artery (MCA) for 30, 60, 120, and 180 minutes, respectively.

We have observed a dramatic increase in citrulline immunoreactivity (IR) unilateral to the occlusion which is augmented with longer occlusion times. The IR is localized to neurons and the endothelium in the territory of the occluded vessel. Our findings support the notion of NO overproduction in cerebral ischemia and provide a tool to investigate the effect of stroke-protective agents on NOS.

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8.4

ANOXIA-TOLERANT CA1 NEURONS FROM RAT HIPPOCAMPUS: ANOXIA-EVOKED CALCIUM CHANGES AND NMDA RECEPTOR INACTIVATION.
Philip Bickler* Anesthesia Dept. UCSF, San Francisco, CA 94143

Neurons from neonatal mammals survive hypoxia that kills mature neurons. I found that 5 min. anoxia/6 hr. recovery killed only 20% of CA1 neurons in post-natal day (PND) 3-7 hippocampal slices, compared to 66% in PND 18-22 slices (calcein-AM/ethidium stains). I tested the hypothesis that survival of hypoxia is associated with limited glutamate release, small intracellular calcium changes, and inactivation of NMDA receptor (NMDAR) function. Glutamate release was measured with the glutamate dehydrogenase assay, and anoxia and NMDAR-mediated calcium changes in CA1 were made with fura-2.

Hypoxia (PO_2 = 5 mm Hg, 10 min) increased calcium in PND 18-22 slice CA1 by 5 times basal; in PND 3-7 CA1 the changes were only twice basal ($p<0.001$). Despite the large difference in calcium changes, anoxia-evoked glutamate release only doubled between PND 3 and PND 22 ($n=40$, $p<0.01$), and 100 μM NMDA-evoked calcium changes decreased by 50% ($n=60$, $p<0.01$). This suggests NMDARs are hypoxia-inactivated in neonates to avoid large calcium changes. To test this, NMDAR-mediated calcium changes were measured after 5 min hypoxia (PO_2 10 mmHg, no ATP loss). In PND <12 slices, hypoxia decreased NMDAR activity in CA1 by 52% ($p<0.05$), and in PND >16 slices by only 23% (NS). NMDAR blockade worsened hypoxia survival in PND 3-7 slices (57 vs. 80% cell survival), but enhanced it in 18-22 slices. I suggest NMDAR inactivation allows a limited and protective increase in cytosolic calcium in CA1 in neonates. In older slices, the greater calcium changes are lethal.

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8.5

X-RAY MICROANALYSIS OF ANOXIC Ca^{2+} ENTRY MECHANISMS IN CNS AXONS. R.M.LoPachin[1]* and P.K.Stys[2]. [1]Montefiore Medical Center, 111E. 210th St., Bronx, NY 10467. [2]Loeb Institute, University of Ottawa, Ottawa, Canada, K1Y 4E9.

Stys et al. (1992) have proposed that, in anoxic axons, reverse operation of Na^+-Ca^{2+} exchanger mediates Ca^{2+} entry triggered by a presumed rise in Na_i. Here we used x-ray microanalysis and procedures that modify transaxolemmal Na^+ and Ca^{2+} movements to specifically examine ionic influx routes and elemental changes in anoxic axons. Perfusion of rat optic nerve with zero-Na^+/Li^+-substituted medium or Na^+ channel blockade by TTX (1μM) prevented axonal increases in Na^+ and Ca^{2+} normally associated with anoxia. Incubation with zero-Ca^{2+}/EGTA perfusate impeded mitochondrial and axoplasmic Ca^{2+} accumulation during anoxia but did not affect Na^+ increase or K^+ loss. Inhibition of Na^+-Ca^{2+} exchange with bepridil (30 μM) did not alter anoxia-induced changes in Na^+ or K^+ concentrations but did prevent increases in axonal Ca^{2+}. Neither nifedipine (5μM) nor nimodipine (5μM) influenced the effects of anoxia on intraaxonal Na^+, K^+ or Ca^{2+}. These findings suggest that during anoxia, Na^+ enters axons primarily via voltage-gated Na^+ channels and that subsequent increases in axoplasmic Na^+ are functionally coupled to Ca^{2+} import. Intracellular Na^+-dependent, Ca^{2+} entry is consistent with reverse operation of the axolemmal Na^+-Ca^{2+} exchanger. Additional studies with modified perfusates and conducted after 60 mins of reoxygenation indicated that ionic deregulation during anoxia is premonitory for severe ionic disruption associated with reoxygenation. We suggest that reverse Na^+-Ca^{2+} exchange is a general route of Ca^{2+} entry during axon injury. Supported by grants NIEHS RO1-ES03038 and MRC MT-11595.

8.6

INHIBITION OF THE Na,K-PUMP INDUCES A GIANT MEMBRANE DEPOLARIZATION IN MAMMALIAN CENTRAL NEURONS B. Hu*, D. M. Mooney, V. Senatorov and P.K. Stys. Loeb Research Institute, Ottawa Civic Hospital, Ottawa, ON, Canada, K1Y 4E9.

Inhibition of the electrogenic Na,K-pump is thought to induce a significant membrane potential depolarization due to a breakdown of ionic gradients, although this phenomenon has not been clearly demonstrated in neurons. We examined this issue in rat thalamic neurons maintained *in vitro* in explants. In sharp-electrode intracellular recording mode, short (20s) applications of Na,K-pump inhibitor strophanthidin (<100 μM) induced two different types of membrane depolarizations: a small (<13 mV) electrogenic response and a large, prolonged membrane depolarization that could exceed 40 mV. Glutamate receptors blockers CNQX/APV did not prevent the large membrane response. Computer modeling suggests that if Na^+ influx and K^+ efflux are allowed to continue uncorrected following pump inhibition, they may lead to a significant decrease in transmembrane ionic gradients, thereby causing a giant membrane depolarization. This mechanism is especially prominent in a small neuron having a large dendritic area. In support of this prediction, in whole-cell recording mode where cell ionic gradients were maintained by equilibration with the intra-pipette solution, strophanthidin failed to induce the large membrane depolarization. The small, electrogenic response remained, however, intact. The large membrane depolarization was also not observed in the medium containing Na^+ channel blocker TTX. These data indicate that small central neurons may undergo sustained membrane depolarization as a result of breakdown of ionic gradients, such as that occurs in anoxia. *Supported by grants from MRC and the Heart and Stroke Foundation of Ontario.*

8.7

INHIBITION OF Na,K-ATPase INDUCES CELL DEATH IN SINGLE, ISOLATED MAMMALIAN CENTRAL NEURONS. V.V.Senatorov*, P.K. Stys and B.Hu, Loeb Medical Research Institute, Ottawa Civic Hospital, Ottawa, Ontario, Canada K1Y 4E9.

Inhibition of Na^+,K^+-ATPase induces neuronal death, which may involve both direct cytotoxic effect due to Na^+/Ca^{2+} accumulation and indirect excitotoxicity as a result of abnormal release/uptake of glutamate. Using imaging techniques, we assessed the direct cytotoxic mechanism of pump inhibition by isolating central neurons from their afferent nerve terminals and glial contact and exposing them to ouabain, a Na^+/K^+-pump inhibitor.

Acutely dissociated rat thalamic neurons were plated and perfused with oxygenated Krebs solution at 32° C. Monensin, a Na^+ ionophore was used to induce a homogenous enhancement of membrane permeability to Na^+. Cell survival was assessed using a membrane destruction assay based on calcein AM and ethidium homodimer. $[Na^+]_i$ was monitored with the Na^+ fluorophore Sodium Green™. Combined application of ouabain (250 μM) and monensin (20 μg/ml) induced a sequence of neuronal responses, typically consisting of an initial rise of $[Na^+]_i$,cell swelling and membrane blebs, followed by membrane disintegration. Most cells (75%) died within 4 hrs after ouabain and monensin application while no cell death was observed within the same period in the control group. The percentage of cell death dropped significantly if monensin or ouabain was used alone. These data suggest that an enhanced Na^+ membrane permeability coupled with cessation of ion pumping is sufficient to induce rapid membrane destruction in central neurons devoid of glutamatergic contacts. *Supported by grants from MRC of Canada and Heart and Stroke Foundation of Ontario.*

8.8

ANOXIA INDUCED POTASSIUM CHANNEL SUPPRESSION IN CA3 NEURONS OF THE RAT HIPPOCAMPUS. N. Hershkowitz* and A.N.Katchman. Department of Neurology, Georgetown University School of Medicine, Washington, DC 20007.

There is little indication that a physiologically significant suppression of K^+ channels occur in tissue preparations during anoxia. In this report we demonstrate a reduction in a potassium channel conductance in the *in vitro* slice preparation following anoxia which leads to increase cellular excitability. Whole cell patch recordings were made in CA3. pyramidal neurons in current clamp mode from rat hippocampal slices. Input resistance was monitored by hyperpolarizing current pulses. Anoxia was induced by switching perfusion and ambient gas of an interface chamber from a 95%O_2/5%CO_2 to a 95%N_2/5%CO_2 mixture. After the onset of anoxia, CA3 cells initially exhibited a small depolarization or hyperpolarization associated with a decrease in input resistance (53 ± 3% from preanoxic control; n=17). This was followed by a transient depolarization (the depolarizing nub) which was associated with a recovery of input resistance (85 ± 3 % of preanoxic control). The nub evoked single as well as bursts of action potentials in the CA3 neurons. The possibility that the nub associated increase in resistance may result from a depolarization-induced turning off of an inwardly rectifying current was examined. Thus, during normoxia current injections which elicited depolarizations of a similar magnitude as the nub (+20 mV) resulted in only a 1.0 ± 3.0 % reduction in input resistance. This suggested that voltage induced alterations of an inward rectifier does not cause the observed alterations in conductance. Finally, no nub-like depolarization, or significant increases in resistance was observed in 7 cells recorded with an electrode containing the non specific potassium channel blocker, cesium. In conclusion, CA3 neurons recorded in whole cell patch current mode exhibited an excitatory depolarization which results from an anoxia-induced suppression of a potassium conductance. (Supported by NIH grant NS32426 and an Epilepsy Foundation of America Research Grant)

8.9

MECHANISMS OF DELAYED AMINO ACID EFFLUX DURING REPERFUSION AFTER FOCAL ISCHEMIA: A POSSIBLE ROLE FOR CARRIER DYSFUNCTION. K.Matsumoto*, E.H.Lo, A.R. Pierce, T.Kano, C.Evans, R.Newcomb. Ctr for Imaging & Pharm Res, Harvard Med Sch, Boston, MA 02129; Neurex, Menlo Park, CA 94325; Neuropsychiatric Institute, UCLA, Los Angeles, CA 90024.

We have demonstrated a large delayed component to increases in extracellular amino acids after transient focal ischemia in rabbit cortex (Matsumoto et al, J Cereb Blood Flow Metab 16:114, 1996). Since this may underlie a process of secondary excitotoxicity in stroke, we have begun to explore the mechanisms involved. New Zealand White rabbits (n=15) were subjected to 2 h MCA occlusion and 4 h reperfusion. A chiral amino acid analysis procedure was used to determine concentrations of L-Asp, L-Glu, D-Ser, L-Ser, L-Gln, PEA, Gly, Tau, L-Ala, and GABA in brain microdialysates. The response of normal brain to depolarization was assessed with 10 min perfusion of 100 mM K+ pre-ischemia. The transmitters L-Asp, L-Glu and GABA showed sharp transient efflux that rapidly renormalized. Gly showed a rapid but prolonged release. Tau and PEA showed delayed release and Gln showed a prolonged decrease. There were no changes in D-Ser, L-Ser or L-Ala. At 30 min post-reperfusion, K+ depolarization efflux of GABA was increased 5-fold. After 2h reperfusion, K+ efflux of L-Asp, L-Glu and GABA was elevated 3-10-fold. Delayed Ca-independent events (Gln, PEA, Tau) were either attenuated or abolished. Amplification of transmitter release and attenuation of delayed events suggest dysfunction of carrier systems in reperfusion injury after transient cerebral ischemia. Supported in part by NINDS NS32806 and Neurex.

8.10

PRESYNAPTIC CONTROL OF GABA RELEASE AT INHIBITORY TERMINALS IS BLOCKED BY ANOXIA IN THE RAT HIPPOCAMPUS. M. Avoli*, R. Köhling and M. Barbarosie. Montreal Neurological Institute and Dept. of Neurology & Neurosurgery, McGill University, Montreal, Quebec, H3A 2B4, Canada

Field-potential and $[K^+]_o$ recordings were made in rat hippocampal slices to establish whether anoxia modifies the control of GABA release from inhibitory interneurons. Spontaneous, negative field potentials (ampl.=1.4±0.6mV, mean±SD; interval=40.9±15.7s; n=10 slices) occurred in CA3 stratum radiatum in the presence of 4-aminopyridine (4AP, 50μM) and ionotropic excitatory amino acid antagonists.These events were associated with $[K^+]_o$ elevations (peak= 5.3±0.7mM; dur.=23.4±3.5s; n=3) and were blocked by the $GABA_A$ receptor antagonist bicuculline methiodide (10μM; n=5), the $GABA_B$ receptor agonist baclofen (100μM; n=6) or the μ-opioid receptor agonist (D-Ala2-N-Me-Phe,Gly-ol)enkephalin (DAGO, 10μM; n=4). Hence, they represented monosynaptic, synchronous, inhibitory field postsynaptic potentials (IPSPs). Brief (4-5 min) anoxic episodes induced a slow elevation of the $[K^+]_o$ baseline to 5.2±0.3 mM (n=3) and an increase in the rate of occurrence of the field IPSPs. The depressant effects of baclofen (n=6) or DAGO (n= 4) on these IPSPs disappeared during anoxia, and reappeared once oxygen supply was restituted. Our findings demonstrate that 4AP-induced monosynaptic IPSPs in the hippocampus are resistant to anoxia, thus supporting the view that $GABA_A$-mediated post-synaptic responses are not influenced by oxygen deprivation. These results also reveal that anoxia blocks the presynaptic control of GABA release exerted by $GABA_B$ and μ-opioid receptors at CA3 inhibitory interneuron terminals. Hence, G protein coupled mechanisms, modulating transmitter release in the hippocampus, may be impaired during oxygen arrest; such phenomenon may also occur at excitatory terminals thereby increasing excitotoxic damage. Supported by MRC of Canada and Quebec Heart and Stroke Foundation

8.11

Cerebral Injection of Antiinflammatory Cytokine IL-10 Increases the Size of Cerebral Infarcts in the Rat Qi-Hui Zhai, Nancy Futrell*, Fang-Jie Chen
Cerebrovascular Disease Laboratory, Medical College of Ohio

Inflammation following cerebral infarction may contribute to brain tissue damage. Interleukin-10 (IL-10) is an antiinflammatory cytokine. Systemically, it can inhibit the synthesis of proinflammatory cytokines, such as TNF-α, IL-1β, and IL-6, by deactivating macrophages. As mRNAs for IL-10, TNF-α and IL-1β are all present six hours after cerebral infarction, and as there is evidence that TNF-α and IL-1β worsen tissue damage, we studied the hypothesis that IL-10 would ameliorate tissue damage following cerebral infarction. Twelve male Fisher (8-9 weeks old) rats were studied. Six experimental rats received an intracerebral injection of 0.5μg recombinant murine IL-10 in 10μl of normal saline into the territory of the right middle cerebral artery (MCA), with a control group of six rats given a similar injection of 10μl of normal saline alone. Thirty minutes later, MCA occlusion was performed using a craniotomy and cauterization of the right MCA. Rats were sacrificed by transcardiac perfusion 24 hours later. Coronal sections were cut at 7μm thickness using a microtome and stained H&E. Infarct volume was measured by the MCID-M4 computerized image analysis system. The mean infarct volume was 102.83 ± 25.12 mm^3 in the IL-10 group and 54.16 ± 21.90 mm^3 in the control group ($0.005<P<0.01$, t-test). Larger infarcts following the injection of an anti-inflammatory cytokine contrast the results of smaller infarcts following administration of anti-ICAM antibodies, which also have an antiinflammatory effect. This suggests that the role of the inflammatory response in the propagation of ischemic cerebral injury may be complex, and the administration of anti-inflammatory therapeutic strategies may be not always beneficial. The precise roles of the multiple mediators and inflammatory cells need to be further studied before optimal modulation of this response following cerebral ischemia can be designed.

The study is supported by PHS-RO1-AG11759

8.12

THE ROLE OF STRESS-ACTIVATED PROTEIN KINASES (JNK/SAPK) IN ISCHEMIA AND REPERFUSION. D.A. Shackelford* and R.Y. Yeh
Dept. of Neurosciences, UCSD, La Jolla, CA 92093-0624

The MAP (mitogen-activated protein) kinase families, including ERK (extracellular signal regulated kinases), JNK/SAPK (cJUN amino terminal or stress-activated protein kinases), and p38, are instrumental in transducing extracellular signals to the nucleus. The effect of ischemia and reperfusion on JNK activity was investigated using the rabbit spinal cord ischemia model (RSCIM). Activity was determined in animals occluded for 15 min or 60 min and reperfused for 2-30 min to determine the correlation with neurological outcome. The former animals will generally recover motor function upon reperfusion, whereas the latter will be permanently paraplegic. JNK activity decreased 45 % and 52 % after 15 min and 60 min of occlusion, respectively ($p < 0.001$). Upon reperfusion for 15 min, the activity increased to 99 % of control levels in animals occluded for 15 min, but was still significantly reduced (57 % of control level) in animals occluded for 60 min. Immunoblot analyses did not reveal a significant loss of JNK protein, however, there was a transient increase in JNK associated with the particulate fraction during reperfusion. These results indicate that JNK activity decreases during ischemia and then during reperfusion, JNK activity increases to essentially normal levels following short durations of ischemia (15 min) associated with recovery, but not after long durations (60 min) that result in permanent paraplegia. JNK phosphorylates the transcription factors, cJUN and ATF2, thereby increasing transcription of target genes that may be crucial for a regenerative response to ischemic/reperfusion injury. *(Supported by grants from NIH and UCSD Academic Senate)*

CALCIUM CHANNELS: STRUCTURE, FUNCTION, AND EXPRESSION I

9.1

MOLECULAR CLONING OF CALCIUM CHANNEL SUBUNITS USING P-CHANNEL ANTIBODY. B.D. Cherksey*, V.S. Sapirstein, M. Saito, M. Sugimori and R. Llinás Depts. of Physiology & Neuroscience and Psychiatry, NYU Medical Center, 550 First Ave., NY, NY 10016.

A polyclonal antibody was prepared against affinity purified P-channel protein from bovine cerebellum. Previous reports showed this antibody reacts with Purkinje cell mostly at the dendritic level (but that no reactivity was observed in this region prior to dendrogenesis.) Western blot analysis shows the antibody reacts with three molecular species of 38Kd, 46 Kd and 130-15OKd. This spectrum of reactivity was brain specific (Hillman et al., Proc. Natl. Acad. Sci. USA, 88:7076-7089, 1991) to include human cerebellum. The high molecular weight protein was also present in heart. We used this antibody to probe a human cerebellar lambda gt-11 cDNA library. Initial screening yielded 8 clones which were purified by successive screenings. Analysis of these cDNA showed that they represented three independent clones of 1.7kb, 3.4 kb and 3.7 kb. Each of these was subcloned into plasmids (BlueScript) and analyzed by nucleotide sequencing. Based on the position of the open reading frames it appears that at least two if not all three clones represent partial sequences. Comparison of the nucleotide sequences in the open reading frame of the 3.7 kb clone showed 55% matching with a known α2B calcium channel subunit. Preliminary results indicate the 3.4kb clone is similar to β channel subunits. The 1.7kb clone shows homology with different α1 calcium channel subunits. Its amino acid sequence predicts 5 transmembrane domains with three exhibiting clear helical structure. From the above we conclude that the antibody reacts with an α1 α2 and β subunit indicating it recognizes a specific calcium channel. Support: NIA-AG09480, NS13742.

9.2

A NEUROBLASTOMA T-TYPE CALCIUM CHANNEL IN THE ABSENCE OF ALPHA-1E mRNA. W. GOTTSCHALK[+], D.S. KIM[#], D. GILBERT[%*], H. CHIN[#], E.F. STANLEY[+] SMS[+], LNC[#], ROSU[%], NINDS, NIH, Bethesda, MD 20892.

The T-type calcium channel plays an important role in the function of a large variety of cell types but relatively little is known about its molecular structure. It has been suggested that this channel is derived from the alpha-1E subunit of the calcium channel family (Soong *et al*, Science 260, 1993). We have tested this hypothesis for a T-type calcium channel observed in a neuroblastoma cell line.

The predominant calcium current in the 140-3 cell line, a hybrid derived from mouse neuroblastoma and rat glioma, is activated at about -40 mV and is inactivated at a holding potential of -50 mV. It has a unitary conductance of 7 pS in 110 mM Ba^{2+}. This channel is blocked by high concentrations of nifedipine (10 uM) and nickel (100 uM), but is insensitive to a variety of other putative T-channel blockers.

Southern blot hybridization analysis demonstrated the presence of the alpha-1E gene in these cells but several approaches, including Northern blot hybridization analysis, RPA and RT-PCR failed to detect any alpha-1E mRNA. A set of specific PCR primers detected some alpha-1C message in these cells.

Our results essentially dissociate this variant of the T-type channel from the alpha-1E subunit and suggest that some heretofore unidentified protein forms the pore of this channel. This work is supported by the NINDS, NIH, Intramural Research Program.

9.3

THE Ca CHANNEL β_3-SUBUNIT DOES NOT SIGNIFICANTLY AFFECT GATING OR MODULATION OF α_{1B} CURRENTS EXPRESSED IN OOCYTES. Diane Lipscombe* & Zhixin Lin. Department of Neuroscience, Brown University, Providence, RI 02912.

It has been suggested that co-expression of Ca channel β-subunits with α_{1B} is required for normal N-type Ca channel function; including expression, channel gating and modulation by protein kinase C. We report that co-expression of the Ca channel β_3 subunit with α_{1B} does *not* significantly affect N channel gating or PKC-mediated modulation, in *Xenopus* oocytes. Ca channel rscgα_{1B} clones, constructed from our rat superior cervical ganglia cDNA libraries, were inserted in to the *Xenopus* β-globin expression vector (pBSTA, A. Goldin). N-type Ca channel expression was achieved in frog oocytes within 2-3 days, following injection of up to 23ng of rscgα_{1B} cRNA. The main effect of co-expressing rscgα_{1B} with the Ca channel β_3 subunit (Campbell lab), was a significant enhancement in Ca channel current density. Ca channel current amplitudes were, on average, 0.5 ± 0.4 μA (n=24) for α_{1B} alone; and 2.92 ± 0.7 μA (n=25) when co-expressed in oocytes with β_3 (+10 mV; 5 mM Ba as charge carrier; oocytes were injected with 46 nl of 50 mM BAPTA prior to recording). Under these recording conditions, we observed little effect of β_3 on channel gating kinetics; the average time to reach peak current, following voltage steps to 0 mV, was 15 ± 2 ms (n=11) in the absence of β_3, and 22 ± 1 ms (n=7) in its presence; the rate of current inactivation, was 317 ± 30 ms (n=11) in the absence of β_3 and 363 ± 33 ms (n=7) in its presence (480 ms depolarization; 0 mV). Finally, up-modulation of α_{1B} currents by PMA (phorbol-12-myristate 13-acetate) was also independent of the presence of β_3. 100 nM PMA, but not 4-α-PMA, induced 2.3 ± 0.3 fold (n=8) and 1.9 ± 0.2 fold (n=14) increases in the peak of the N channel current in the absence and presence of β_3, respectively. *NIH NS29967.*

9.4

REGULATION OF THE L-TYPE CALCIUM CHANNEL BY THE $\alpha_2\delta$ SUBUNIT IN TRANSFECTED MAMMALIAN CELLS. Ricardo Felix*, Christina A. Gurnett and Kevin P. Campbell. Howard Hughes Medical Institute/Dept. of Physiology and Biophysics, University of Iowa College of Medicine, Iowa City, IA 52242.

The regulatory effects of the calcium channel $\alpha_2\delta$ ancillary subunit on the L-type calcium channel were studied using a mammalian expression system. Cloned pore-forming α_{1C} subunit was transiently expressed in tsA201 cells alone or in combination (molar ratio 1:1) with the rat brain $\alpha_2\delta$ subunit, and whole-cell barium currents were recorded using the patch-clamp technique. Transfection with α_{1C} subunit only resulted in the expression of voltage-gated currents that inactivated slightly during a 150-ms depolarizing pulse. Coexpression of the $\alpha_2\delta$ subunit caused a 3-fold increase in macroscopic current amplitude, shifted the activation curve to more hyperpolarized potentials and altered markedly both activation and inactivation kinetics of the current. In addition, equilibrium radioligand binding experiments showed that the coexpression of $\alpha_2\delta$ subunit with α_{1C} leads to an increase in the affinity for the dihydropyridine isradipine without affecting the total number of binding sites. These data suggest that the $\alpha_2\delta$ subunit regulation of the cardiac L-type calcium channel activity in mammalian cells involves a modification in both gating and binding properties of the α_{1C} subunit.

Howard Hughes Medical Institute

9.5

A DROSOPHILA MUTANT IDENTIFIES AN AMINO ACID AFFECTING CALCIUM CHANNEL ACTIVATION RATE AND PEAK CURRENT. L.M. Hall*, D. Ren, D.F. Eberl, H. Xu, and M. Chopra. Dept. of Biochemical Pharmacology, SUNY at Buffalo, Buffalo, NY 14260.

Null mutations in the gene *l(2)35Fa* cause recessive embryonic lethality. Deletion mapping, transformation rescue, and DNA sequencing of mutant alleles have shown that this gene encodes the calcium channel α_1 subunit Dmca1D. A missense mutation causing a C to Y change in a highly conserved region of domain IS1 causes reduced viability in the organism. Expression in Xenopus oocytes of channels bearing this mutant change shows that channel activation rate and peak current are reduced. Additional *in vitro* mutagenesis shows that it is size and polarity of the amino acid at this position, rather than ability to form S-S bonds, which is important. Electrophysiological recordings from larval muscle show reduced current levels in mutant relative to wild-type. This mutant change persists in the presence of the T-type calcium channel blocker amiloride showing that the Dmca1D α_1 subunit is the amiloride-insensitive channel in muscle. This Drosophila mutant has identified the molecular component responsible for one pharmacologically distinct calcium channel expressed in Drosophila muscle and has identified an amino acid important for channel function in the organism and in heterologous expression systems. Supported by NIH grant HL39369.

9.6

KINETIC DIFFERENCES IN THE INTERACTION OF ω-CONOPEPTIDES WITH B-CLASS AND N-TYPE Ca^{2+} CHANNELS. D. M. Rock*[1], S. J. Stoehr[1], J. Offord[2] and A. Palma[3]. [1]Neuroscience Therapeutics and [2]Dept. of Biotechnology, Parke-Davis Pharmaceutical Research Division of Warner-Lambert Co., 2800 Plymouth Rd., Ann Arbor, MI 48105 and [3]Neurex Corp., Menlo Park, CA 94025.

Several peptides isolated from naturally occurring toxins have been useful in identifying voltage-gated Ca^{2+} channel subtypes in neuronal preparations. Molecular biological experiments have shown that differences in the α_1 subunits (α_{1A}-α_{1E}) code for different subtypes of Ca^{2+} channels, and the α_1 subunit contains sites for the interactions with toxins. Based mainly on sensitivity to the specific N-type Ca^{2+} channel blockers ω-conopeptide GVIA and MVIIA, recombinant B-class Ca^{2+} (α_{1B}) channels have been classified as neuronal N-type Ca^{2+} channels.

Using whole-cell voltage clamp techniques, we compared the kinetics of ω-conopeptide interactions with human neuronal B-class Ca^{2+}(α_{1B}) channels expressed in HEK-293 cells and N-type Ca^{2+} channels in rat superior cervical ganglion (SCG) neurons. All ω-conpeptides tested (SNX-111 [MVIIA], SNX-230 [MVIIC] and SNX-238 [MVIID]) blocked Ca^{2+} channels in both B-class cell lines and SCG neurons, but there were differences in the kinetics and potencies of the block. On rates were slower in B-class compared to N-type channels for all conopeptides. SNX-230 produced a rapid, irreversible block of B-class channels but rapidly and reversibly blocked N-type channels. SNX-238 produced a rapid, incomplete and reversible block of both B-class and N-type Ca^{2+} channels, with a lower potency in SCG neurons. The kinetics of ω-conopeptide interactions suggest that recombinant B-class and N-type Ca^{2+} channels are not identical. Potential explanations for these differences will be discussed. [Supported by Warner-Lambert Co. and Neurex Corp.]

9.7

AUTORADIOGRAPHY OF L-TYPE AND N-TYPE CALCIUM CHANNELS IN AGED RAT BRAIN. K.M. Kelly*, A. Kume[+], R.L. Albin[+] and R.L. Macdonald[+#], Depts. of Neurology[+] and Physiology[#], U. of Michigan, Ann Arbor, MI 48104-1687.

Brains from male (F344xBN)F1 rats aged 6, 17 and 32 mo were studied to determine whether there was age-dependent change in the number of L-type and N-type calcium channels in different brain regions. The L-type calcium channel antagonist PN200-110 and the N-type channel antagonist ω-conotoxin GVIA were used to determine specific binding densities. Binding of the ligands was performed using methods described previously for the L-type channel (Cortes et al., J Neural Trans 1984:60;169) and the N-type channel (Filloux et al., Devel Brain Res 1994:78;131). The autoradiographic distribution of ligand binding from hippocampus (CA1, CA3, dentate gyrus), entorhinal cortex (laminae I-III, IV-VI) and neocortex (lamina I-III, IV-VI) was quantified with computer-assisted densitometry. Five to ten readings per area from each animal (n=4 at each age) were averaged. One-way ANOVA noted a significant variance in the mean value of binding density between different age groups only in neocortex laminae IV-VI for [^{3}H]PN200-110 binding ($p<0.05$). Post-hoc testing indicated that the mean value of the 17 mo old group was significantly less than those of the 6 and 32 mo old groups ($p<0.05$). No other significant differences were found for either ligand within the different age groups for any other brain region.

These results indicate no age-dependent change in the number of binding sites for [^{3}H]PN200-110 (except at 17 mo for neocortex laminae IV-VI) and [^{125}I]ω-conotoxin GVIA suggesting no significant overall change in the number of L-type and N-type calcium channels in rat brains 6-32 mo of age.

9.8

NONHOMOGENEOUS DISTRIBUTION OF DIFFERENT CALCIUM CHANNEL SUBTYPES ON PRESYNAPTIC TERMINALS IN THE HIPPOCAMPUS. C. A. Reid, J. D. Clements and J. M. Bekkers*, Division of Neuroscience, John Curtin School of Medical Research, Australian National University, Canberra, Australia.

The N-type (ω-Conotoxin GVIa-sensitive) and Q-type (ω-Agatoxin GIVa-sensitive) presynaptic calcium channels have been shown to support the release of the neurotransmitter glutamate at excitatory synapses in the hippocampus. Several recent studies have proposed that a mixed *population of calcium channels co-exist at a single release site* and contribute jointly to the local calcium transient that triggers release. However, debate exists as to whether one calcium channel subtype alone can support release from a synaptic terminal. Whole-cell recordings were made from single cultured rat hippocampal neurons that form autaptic synapses. Probability of release (Pr) was measured by analysing the progressive block of the NMDA receptor- mediated synaptic current by the irreversible open channel blocker MK-801 (2μM). The time course of the progressive block could be fitted by the sum of two exponentials, consistent with there being at least two types of presynaptic terminals, one with a high Pr, the other with a low Pr. Although ω-Conotoxin GVIa (1μM) blocked the EPSC by 41% ± 4.7 (Ave. ± se) there was no significant change in either the fast (τf control = 8.2 ± 1.1 vs τf ω-Ctx = 7.3 ± 1.5) or slow (τs control = 53.1 ± 4.7 vs τs ω-Ctx = 48.0 ± 5.1) time constants of the MK-801 progressive block. A similar reduction in the EPSC by cadmium (a non-specific calcium channel blocker) significantly increased both the fast (τf cadmium = 17.3 ± 3.2) and slow (τs cadmium = 81.0 ± 8.3) time constants ($p< 0.01$). These results suggest Pr is unchanged at a proportion of terminals following block of N-type calcium channels. Our conclusion is that functional N-type calcium channels are absent from some presynaptic terminals. (Supported by Australian Department of Health)

COGNITION: FUNCTIONAL NEUROIMAGING

10.1

RELATION OF TASK PERFORMANCE TO CEREBRAL BLOOD FLOW ACTIVATION PATTERNS DURING FACE PERCEPTION. G.E. Alexander*, M.J. Mentis, J.R. Moeller[1], M.L. Furey, P. Pietrini, B. Horwitz, C.L. Grady. Laboratory of Neurosciences, NIA, NIH, Bethesda, MD 20892; [1]Dept. of Biological Psychiatry, NYSPI, New York, NY 10032.

Face perception has been associated with cerebral activation of a ventral pathway including occipitotemporal and frontal brain regions. To investigate the interactions of regional cerebral blood flow (rCBF) during face perception in relation to task performance and age, we analyzed rCBF with high-resolution positron emission tomography and $H_2{}^{15}O$ in 9 healthy elderly (mean age = 67±6 yrs) and 9 young, right-handed, adult males (mean age = 28±4 yrs) using the Scaled Subprofile Model (SSM; Moeller, et al., 1987). SSM analysis of scan subtractions during face matching and sensorimotor control tasks in the young and old groups combined extracted a first principal component pattern whose expression was positively correlated with task accuracy ($r=0.57$, $p<0.02$) and did not differ between the two age groups. This pattern was predominantly characterized by higher activity in left lingual and fusiform gyri and bilateral inferior and middle frontal association regions with relatively lower activity in lateral inferior and middle occipitotemporal regions, bilaterally. Separate SSM analyses for the young and old groups each produced patterns which were correlated with the pattern from the combined groups analysis. These results suggest that individual differences in face matching performance are associated with the interaction of brain regions previously implicated in object perception and effortful information processing, and that this functional interaction occurs in both young and elderly subjects. This research was supported by the NIA Intramural Research Program.

10.2

INVOLVEMENT OF THE SUPERIOR PARIETAL CORTEX IN MENTAL TRANSFORMATIONS OF THE BODY INDUCED BY TOUCH. E. Bonda*, S. Frey and M. Petrides. Montreal Neurological Institute, McGill University, Montreal, Quebec, H3A 2B4, Canada.

The neural systems underlying body-space mental representation were studied by measuring changes in regional cerebral blood flow (CBF) with positron emission tomography (PET) in human subjects. The experimental paradigm involved identification of the left or the right hand of the experimenter that was presented in different orientations on the palm of the subject's right hand. The subjects were required to decide whether it was the left or the right hand that was presented. In order to perform this task, the subjects had to move mentally the position of their own arm to adopt that of the experimenter's arm. The control condition involved the same type of tactual stimulation without the requirement of mental transformations of the subject's body. The distribution of CBF was measured by means of the water bolus $H_2{}^{15}O$ methodology during the performance of these tasks. Comparison of the distribution of CBF between the experimental and the control tasks revealed significant increases in the caudal superior parietal cortex, including the intraparietal sulcus, and the adjacent medial parietal and cingulate cortex. The results indicate that there is a dorso-medial parietal system underlying the mental experience of the body in an interactive relation with space. These findings, taken together with earlier related findings obtained through the visual modality (Bonda et al., 1995, Proc. Natl. Acad. Sci.), establish that the involvement of this system in body-space interactions is amodal in nature.

Funded by the McDonnell-Pew Program in Cognitive Neuroscience and MRC (Canada).

10.3

BRAIN AREAS INVOLVED IN THE EXECUTIVE CONTROL OF TASK SWITCHING AS REVEALED BY PET

J.E. Evans, E.J. Lauber*, D.E. Meyer, J. Rubinstein, L. Gmeindl, L. Junck, R.A. Koeppe.
Department of Psychology and Nuclear Medicine, University of Michigan, and Department of Psychology, University of Georgia.

The executive mental processes needed to switch between two simple tasks was examined using both behavioral and PET (O15 water) methodologies. Normal adult subjects were required to make perceptual judgments of color or shape, or to alternate between them, and respond with finger presses on a 3-button mouse. Subtraction of the activation images obtained in the average of the single-task conditions from the average of the dual-task conditions revealed peak activation foci in the left dorsolateral prefrontal cortex, left posterior parietal cortex, left premotor cortex, right cerebellum, and left anterior cingulate gyrus. These results are consistent with findings in the literature that implicate these brain areas in context updating or task designation (left prefrontal cortex), accuracy monitoring and response inhibition (anterior cingulate cortex), and movement preparation (premotor cortex).

Funding provided by the General Clinical Research Center, University of Michigan Medical Center.

10.4

WITHIN SUBJECT NEUROACTIVATION MAPPING WITH WHOLE BRAIN ISOTROPIC EPI AND 3-D PET: A DIRECT COMPARISON.

D.R. Weinberger*, A. Santha, A. Mattay, J. Frank, B. Kirkby, J. van Horn, G. Esposito, K.F. Berman. Clinical Brain Disorders Branch, NIMH Neuroscience Center at St. Elizabeths, Washington, D.C. 20032 and LDRR, Bethesda, Md. 20892

"BOLD" contrast fMRI is "in vogue" as a technique for in vivo human brain mapping, though its merits relative to state-of-the-art 3-D PET mapping are debatable. We have compared directly whole brain, multi-slice, isotropic (3.75mm) EPI and 3-D O-15 water PET (6.5 mm isotropic) in seven normal volunteers performing repetitive sequential finger movements. The fMRI protocol involved 18 "on-off" repetitions; the PET protocol involved twelve scans (i.e. six movement-rest pairs). The fMRI datasets were smoothed to the PET resolution and voxel density and then coregistered to each other. A rigorous T statistical analysis was applied with Bonferroni correction for the calculated number of "resels" unique to each method. Significantly activated voxels in primary sensorimotor cortex (SM) and in supplementary motor cortex were seen in every subjects with both techniques. Ipsilateral cerebellar activation was seen in every subject with fMRI, and in five subjects with PET. Only one study (PET) showed any activation of the contralateral basal ganglia region. Foci of activation were generally overlapping in SM, but less so in other motor regions. At these critical T thresholds, the number of activated voxels in SM and in other motor regions, especially cerebellum, were usually greater with the fMRI technique. These data show that the neuroactivation maps of this fMRI method and of 3-D PET, at least during motor function, are comparable.

10.5

REGIONAL DIFFERENCES IN THE DEVELOPMENT OF WHITE MATTER DEMONSTRATED WITH MRI.

M.A. Eckert*[a], D.C. Wilson[b], O.F. Agee[c], T.H. Lucas[d] & C.M.Leonard[d]. Depts. of Psychology[a], Mathematics[b], Radiology[c], Neuroscience[d] Univ. of FL, Gainesville, FL, 32610.

The acquisition of myelin increases conduction speed and improves the temporal fidelity of neural processing. Postmortem studies show myelination to proceed in a regional order that correlates with the order of functional development. Sensory and motor areas reach mature levels of myelin within the first few years, while association areas continue to develop myelin into adulthood. Therefore, the state of myelination is assumed to represent the functional development of the brain. The cholesterol in myelin has been shown to produce high signal intensities on T1 weighted MR images. The percent of high signal intensity voxels can be quantified as an index of white matter development. The method used here to quantify voxels has replicated postmortem findings of regional differences in myelin development. Children ($\bar{x}$ age = 8 yrs) demonstrated a significantly lower percentage of high signal voxels in the association cortex of the temporal lobe than adults ($\bar{x}$ age = 34 yrs) in the right ($F_{(1,38)}$=83.14, p<.0001) and left hemispheres ($F_{(1,38)}$=26.44, p<.001). However, there was no difference in the visual cortex of the right ($F_{(1,57)}$ =0.29, p>.05) or left hemispheres ($F_{(1,57)}$ =1.13, p>.05). We plan to use this technique to investigate structural correlates for language development. Adult levels of myelination may reflect the attainment of mature levels of functioning and boundaries for sensitive periods of development. (Supported in part by MOD FY96-1021 and NIDCD2922.)

10.6

ADJUSTING FOR TEMPORAL AND SPATIAL CORRELATIONS IN ANALYSIS OF MEAN FMRI SIGNAL INTENSITY CHANGES.

J.J. Locascio*, P. J. Jennings, and S. Corkin. Dept. of Brain and Cognitive Sciences and the Clinical Research Center, Mass. Institute of Technology, Cambridge, MA 02139.

Although functional magnetic resonance imaging (fMRI) methods yield a richness of temporal and spatial data for even a single subject, universally accepted data analysis techniques have not been developed yet that fully use all the potential information from fMRI of the brain. Specifically, temporal correlations and confounds are a problem in assessing change within pixels. Spatial correlations across pixels are a problem in determining regions of activation, and in correcting for multiple significance tests. We propose methods of addressing these issues in the analysis of mean fMRI signal intensity changes for individual subjects. Our approach to temporally based confounds within pixels is to employ autoregressive-integrated-moving average (ARIMA or "Box-Jenkins") time series analysis. To adjust for performing multiple significance tests across pixels, taking into account between-pixel correlations, we propose adjustment of p-values with computer intensive "resampling methods." Our objective is to produce 2- or 3- dimensional brain maps that provide, at each pixel in the map, an estimated p value with absolute meaning. That is, each p value approximates the probability of having obtained by chance the observed signal effect at that pixel, given that the null hypothesis is true. Time series analysis and resampling methods have been employed with success in fMRI analysis, but to our knowledge no one has tested their use in combination as we are proposing. Simulated and real data examples are provided.

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10.7

SENSITIVITY OPTIMIZATION AND EXPERIMENTAL DESIGN IN FUNCTIONAL MAGNETIC RESONANCE IMAGING

TE Conturo, RC McKinstry, E Akbudak, AZ Snyder, T Yang, ME Raichle*
Depts of Radiology & Neurology, Washington U., St. Louis, MO 63110

Introduction: The fMRI signal responses are very small. The blood oxygenation level-dependent signal change (the BOLD signal) is ~2-4% for robust stimuli and even smaller for cognitive tasks. As sensitivity is very dependent on MRI acquisition parameters, these variables must be optimized.

Methods: Sensitivity to detect activation was modeled as the contrast-to-noise ratio (CNR) given by the time-averaged activated pixel intensity minus the time-averaged control relative to noise. Timing intervals TR and TE, tip angle α, in-plane resolution, slice thickness, RF coil, number of time frames per run (N_{frame}) and number of runs (N_{run}) were considered. Normal volunteers were studied with 8 Hz photic stimulation on a Siemens 1.5 T MR unit. Echo planar imaging was used with N_{frame} = 128, N_{run} = 1 and 3 on/off blocks.

Results: For practical maximum N_{frame}, TR > 1 s and $\alpha \approx 90°$ are preferred. Optimally, TE ≈ 90 ms but shorter TE can be chosen without severe penalty. N_{run} should be maximized depending on subject tolerance. The measured sensitivity of surface coils was up to 4x that of a head coil. For 1.9x1.9x5 mm voxels with the head coil, CNR was only 3:1 but increased by a factor of 12 to 37:1 using 3.1x3.1x7 mm voxels with a surface coil. For large voxels there was uniform grey matter (GM) BOLD signal. For small voxels signal was inhomogeneous with higher signal in the sulci (suggesting vessels) and lower signal in GM. The measured GM CNR was approximately proportional to voxel volume as predicted. The low-resolution CNR was much larger than the sulcal high-resolution CNR, indicating that the large-voxel BOLD signal is dominated by GM signals with only minor effects from blurred sulcal signals. The CNR predictions may aid tailoring of experiments to anticipated BOLD responses.

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10.8

RELIABILITY AND CORTICAL UNFOLDING OF WITHIN-SUBJECT fMRI DATA FOR WORD-STEM COMPLETION

R.L. Buckner*, A.M. Dale, R.B.H. Tootell, S.E. Petersen†, M.E. Raichle† and B.R. Rosen. MGH-NMR Center, Boston MA 02129. † Wash. Univ. Med. Sch., St. Louis, MO 63110.

Previous PET studies of word-stem completion have demonstrated a pathway of brain areas activated in averaged subject-groups (Buckner et al. *J. Neurosci.* 1995). The present study sought to determine if these areas (left inferior prefrontal, SMA, motor, and visual) could be reliably detected within-subject using fMRI. 8 subjects were imaged during 6 to 8 runs of word-stem completion (4 fixation and 3 word-stem completion blocks per run; each block 30 sec with 12 trials; whole brain fMRI using BOLD contrast, 1.5T GE scanner with ANMR EPI upgrade, 3x3x7mm voxels, 17 axial slices along the AC-PC plane). For each subject, data were divided into two sets. Reliability was assessed in two ways. (1) Regions were defined on activations in the first data sets and tested for replication in the second, independent, data sets using a KS test. (2) Correlations between KS maps in the first data sets (for a slice containing the left inferior prefrontal activation) and the second sets were determined on a voxel by voxel basis. Results showed high reliability within-subject: (1) 94% of the regions tested replicated (p<.05, 88% were p<.01) and (2) the KS maps from the two separate data sets were highly correlated within-subject (mean r = .74, median r = .80). For two of the subjects, the cortical surface was reconstructed from hi-resolution anatomic images and unfolded (Dale & Sereno *J. Cog. Neuro.* 1993). KS activation data were projected onto these unfolded cortical surfaces revealing similarly localized prefrontal activation for each subject. Moreover, significant and reliable left inferior prefrontal activation could be detected in all 8 subjects with a signal change magnitude of about 1%. Such high reliability within and between subjects serves as a foundation for detailed functional-anatomic exploration of cortical areas during cognitive tasks.

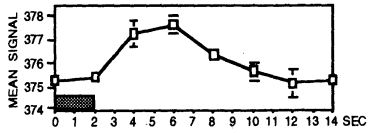
(Support: NIH Grant NS32979, Charles A. Dana Foundation, McDonnell Center)

10.9

fMRI IMPULSE RESPONSE CHARACTERISTICS FOR PREFRONTAL ACTIVATION DURING WORD-STEM COMPLETION P.A. Bandettini*, R.L. Buckner, K.M. O'Craven, R.L. Savoy and B.R. Rosen. MGH-NMR Center, Boston, MA 02129

Data presented in the companion abstract demonstrate within-subject reliability of prefrontal activation for a word-stem completion task. In the present study, we demonstrate that significant fMRI signal changes can be elicited in prefrontal areas using single trials for the same task. Use of single trial responses may be optimal for tasks that would benefit from mixed, rather than blocked, design.

Using a region defined on data from a block-designed word-stem completion task (see companion abstract for methods), fMRI time series data from 4 subjects were analyzed for separate runs containing spaced single word-stem completion trials (15 trials per run, 1 trial every 14 sec, 4 runs per subject). Shown below are signal averages for the left inferior prefrontal region for the first subject. (Shaded area represents approximate word-stem on time. Data are shown with standard error bars.) This ~1% signal change was reproducible across subjects.



These studies demonstrate that single trials can be used to map cognitive function. This cognitive "impulse response" approach may be optimal for cognitive tasks requiring stimulus or task randomization, or in cases where individual trials differ based on performance measures obtained from the subject.

(Support: Grant P1DA09467, Charles A. Dana Foundation, The Rowland Institute)

10.10

DETECTION OF CORTICAL AREAS WITH TRANSIENT BRAIN ACTIVITY USING ECHO-PLANAR MAGNETIC RESONANCE IMAGING. S. Konishi, R. Yoneyama, H. Itagaki, I. Uchida, H. Kato, K. Nakajima, K. Okajima, H. Koizumi and Y. Miyashita* Department of Physiology, the University of Tokyo School of Medicine, Tokyo 113, Japan. Central Research Laboratory, Hitachi, Ltd., Tokyo 185, Japan.

With 'transient' regions of interest (ROIs), we identified transiently activated visual cortical areas using functional magnetic resonance imaging (fMRI) at 1.5 T in five human subjects. The validity of this method was confirmed by comparing them with conventionally determined continuously activated visual cortical areas. Ventral extrastriate cortices in the occipital lobe were activated by transient (0.2 or 2 s) or continuous (20 s) visual stimulation. The transient activation was assessed by estimating the pixel-wise statistical significance of signal increase at each of time points using a paired *t*-test to delineate transient ROIs, and the spatial distribution of the transient ROIs was compared with that of the conventionally determined ROIs derived from continuous activation. Ninety-five and 89 percent of the total areas of transient ROIs derived from 0.2 and 2 s activation, respectively, appeared at 5, 7 or 9 s after the onset of stimulus presentation. Eighty-nine and 76 percent, respectively, of them overlapped with conventional ROIs derived from 20 s activation. These results indicate that transient ROIs can be delineated by setting a time window for them of around 7 s after the transient brain activation and that few of them are false positive areas, and also suggest that the delineation of transient ROIs might be a powerful strategy for the exploration of cognitive functions with fMRI.

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10.11

SUBORDINATE-LEVEL CATEGORIZATION IN HUMAN INFERIOR TEMPORAL CORTEX: CONVERGING EVIDENCE FROM NEUROPSYCHOLOGY AND BRAIN IMAGING. I. Gauthier[1], M. Behrmann[2], M. J. Tarr[3], A. W. Anderson[4], J. Gore[4], and J. L. McClelland[2]*, Departments of Psychology[1] and Diagnostic Radiology[4], Yale University, New Haven CT 06520; Department of Psychology[2], Carnegie Mellon University, Pittsburgh, PA 15213 and Dept. of Cogn. & Ling. Sciences[3], Brown University, Providence, RI 02912.

Several neuropsychological case studies of face-specific recognition deficits (prosopagnosia) and functional brain imaging studies appear to show that a portion of inferior temporal lobe -IT- (part of the fusiform and inferior temporal gyri) in humans is dedicated to face processing (Sergent et al., 1992; Haxby et al., 1994; Farah et al., 1995). However, face recognition can be considered a particular case of *subordinate-level recognition*, in which members of a visually homogeneous class must be distinguished. In several experiments using perceptual matching and match-to-sample tasks, we found that prosopagnosics are disproportionally sensitive to increasingly specific levels of categorization with both familiar and novel non-face objects. We also report an fMRI study in which the same region of IT associated with faces is engaged by the subordinate-level recognition of familiar non-face objects. Together, these results suggest that the level of visual categorization, not stimulus class membership *per se*, may be the mediating factor in dissociations generally found between face and object recognition in the human ventral pathway. At a minimum, such results provide more stringent controls for claims of face-specific mechanisms and neural substrates. (Support provided by ONR, Contract N000149303.)

10.12

CEREBELLAR CONTRIBUTIONS TO MOTOR REPRODUCTION OF TEMPORAL SEQUENCES VB Penhune*, RJ Zatorre and AC Evans Montréal Neurological Institute, McGill University, Montréal PQ H3A 2B4.

Motor reproduction of temporally complex auditory and visual sequences was investigated in a PET activation study (O^{15} water bolus) in normal volunteers (N=12). Reproduction of the sequences in both modalities was expected to activate regions of the brain, particularly the cerebellum, required for processing the temporal content of the sequences and for executing the motor response. In the baseline conditions, subjects reproduced either auditory or visual isochronous sequences by tapping on a computer keyboard to imitate both the durations and the number of elements. In the activation conditions, subjects reproduced a series of temporally complex novel sequences in the same way. Paired image subtractions confirmed that sensory input and motor output were similar in the baseline and activation conditions, since no statistically significant (t>3.5) rCBF increases were observed in either sensory or motor cortical regions. In both modalities, significant increases were observed in anterior and posterior cerebellar vermis, left lateral cerebellar cortex, cingulate, and right insula. In the auditory condition, additional increases were observed in right lateral cerebellar cortex and the left insula. In the visual condition, additional increases were observed in the right dentate gyrus, as well as right orbito-frontal and posterior dorso-lateral frontal regions. These data provide partial support for the hypothesized cerebellar control of temporal processing, by revealing neural systems engaged in timed motor output, regardless of the modality of sensory input. However, they also indicate that additional, modality-specific neural systems may be involved Supported by the NIH/NIMH and the Medical Research Council of Canada.

NEUROTOXICITY: EXCITOTOXIC INJURY

11.1

EFFECT OF MELATONIN ADMINISTRATION TO RATS ON KAINATE-INDUCED P53 IMMUNOREACTIVITY, DNA DAMAGE AND NEURONAL DEGENERATION IN THE BRAIN. **T. Uz*, A. Kharlamov, J.-Y. Joo, D. Franceschini[1], P. Giusti[1] and H. Manev. ASRI, Med. Coll. Pennsylvania and Hahnemann Univ., Pittsburgh, PA;[1] Dept. Pharmacol, Univ. Padova, Italy.**

The pineal hormone melatonin has recently been shown to be capable of reducing neurotoxic action of the glutamate receptor agonist kainate both in neuronal cultures and in the rat brain in vivo. The mechanism of melatonin's neuroprotection does not appear to involve a direct blockade of kainate-sensitive glutamate receptors, and it may include the antioxidative properties of this hormone. An i.p. injection of 10 mg/kg kainate to male rats resulted in neuronal damage in the hippocampus (CA1, CA3, and CA4), the entorhinal cortex, and the amygdaloid complex that was accompanied by signs of both necrosis and apoptosis. We characterized in melatonin-treated rats (4 x 2.5 mg/kg, i.p.) and in corresponding controls the temporal pattern and the localization of kainate-triggered markers of apoptotic cell injury (i.e., by p53 immunoreactivity and the in situ TUNEL assay of DNA damage), and we assayed neuronal damage using Nissl and GFAP-specific (glial fibrillary acidic protein) stainings. In addition, we investigated the correlation between the behavioral response to kainate and kainate-induced brain injury. The TUNEL and Nissl assays revealed the occurrence of DNA damage and neuronal degeneration (Nissl loss) as early as 3 hr after kainate; both markers increased further by 24 hr and then remained stable for the next 2 days. The p53 immunostaining was positive 24 hr after kainate. At that time we also observed an increased number of GFAP-positive cells in the stratum radiatum of the CA1. Melatonin reduced the appearance of all the above-noted markers of injury. In addition, melatonin reduced the intensity of behavioral responses to kainate. However, we observed the neuroprotective action of melatonin in animals in which the behavioral responses to kainate were comparable. Thus, it appears that pharmacological doses of melatonin are capable of reducing excitotoxicity via a mechanism that does not directly involve glutamate receptors. Currently we are investigating whether similar effects are mediated by endogenous melatonin. (T. Uz: Fogarty Award F05 TW/NS5271-01).

11.2

DECREASED LEVELS OF ENDOGENOUS MELATONIN POTENTIATE EXCITOTOXIC AND STROKE-INDUCED BRAIN DAMAGE. **H. Manev*, T. Uz, A. Kharlamov and J.-Y. Joo. ASRI, Medical College of Pennsylvania and Hahnemann University, Allegheny Campus, Pittsburgh, PA, 15212.**

Pharmacological administration of the pineal hormone melatonin to rats reduces kainate-triggered excitotoxic brain damage (Uz et al., this meeting). It is not clear yet whether endogenous melatonin would provide the same neuroprotective action and whether the pineal gland can be considered a part of endogenous neuroprotective systems which, if impaired, could put the brain at risk for neurodegenerative pathologies. We investigated the effect of endogenous melatonin on the response of the brain to excitotoxic (i.p. administration of 10 mg/kg kainate) and stroke (photothrombotic model with rose bengal and light)-triggered injury using two models of changed endogenous melatonin levels: a) diurnal rhythm, and b) pinealectomy. In addition, since it is believed that aging impairs pineal functioning, we characterized the effect of kainate in adult (2 months) and old (24 months) male rats. The extent of kainate-induced damage was assessed in brain slices using computer-assisted quantitative analysis of Nissl loss and in situ TUNEL labeling of DNA damage. The volume of stroke-triggered unilateral focal cortical lesions was also measured. An RIA assay (Alpco Ltd.) was used to measure the concentration of melatonin in the plasma. The extent of kainate-triggered DNA damage and the loss of Nissl staining were lower when kainate was injected at night (1:00 A.M.), i.e., in the presence of high endogenous levels of melatonin (5 times greater than levels at 12:30 P.M.). Fifteen days after pinealectomy, the daytime plasma level of melatonin could not be detected. Kainate injections into these animals resulted in greater damage than in the sham-pinealectomized rats. The volume of stroke-triggered lesion was also bigger in pinealectomized animals. In addition, kainate-triggered injury was greater in the brains, e.g., the CA3 of the hippocampus, of the old rats. Our results support the proposition that endogenous melatonin can influence the outcome of brain injury, and that this effect is not limited to kainate excitotoxicity only. Further studies are needed to evaluate the contribution the antioxidative action of melatonin makes to neuroprotection.

11.3

LOSS OF P53 INDUCTION IS ASSOCIATED WITH INHIBITION OF APOPTOSIS. D. Uberti, M. Belloni, C. Rizzini, L. Piccioni, F. Sinelli, F. Borsini*, P.F. Spano and M. Memo Div. Pharmacol., Dep. Biomed. Sci. & Biotechnol., Brescia University, School of Medicine. 25123 Brescia, Italy

P53 is a tumor suppressor protein involved in controlling cell cycle checkpoint that is important for maintaining the integrity of the genoma. The demostration that p53 promotes apoptosis in abnormally proliferating cells may have important implications for the central nervus sistem (CNS), where cell death is observed normally during development, in response to injury and in neurodegenerative disorders. Recent findings suggest that p53 could be associated with induction of cell death consequent to some forms of cellular damage in the SNC. A possible role for p53-related modulation of neuronal viability is suggested by the finding that p53 expression is increased in damaged neurons in models of ischemia and epilepsy. Immunocytochemical studies using p53 antibody has performed during the cell develpoment "in vitro". We found a significant increase of p53 immunoreactivity during the cell maturation. When the culture were exposed to kainic acid (60 μM) for 24 h a significant increase of p53 immunoreactivity associated with a decrease of cell viability resulted. Time-course experiments revealed the increases of p53 expression to be maximal 2 h after the kainic acid treatment. P53 levels returned to basal values after 8 h. To investigate the relationship between p53 expression and Kainic acid induced neuronal damage neurons were preincubated with a specific p53 antisense oligonucleotide. This treatment resulted in an inhibition of the kainic acid induced increase of p53 immunoreactivity and prevented kainic acid induced apoptosis. However p53 antisense oligonucleotide treatment did not completely antagonize the kainic acid-induced cell death. suggesting that this excitatory amino acid triggers at least two separate cell death programms, only one of them strictly p53-dependent.

This work is supported by C.N.R.

11.4

NMDA-INDUCED c-fos and hsp70 mRNA EXPRESSION IN NEONATAL RAT CEREBROCORTICAL SLICES K. Hasegawa*, M.T. Espanol, L. Litt, F.R. Sharp, P.H. Chan. Depts. of Anesthesia, Neurology, Neurosurgery. The University of California, San Francisco, CA 94143

The temporal course of c-fos and hsp70 gene induction was studied during cell death induced by N-methyl-D-aspartate (NMDA), and during cell death blockade by MK801 (dizocilpine) pretreatment. In each of 3 experiments, forty 350μm thick cerebrocortical slices, obtained from ten 7-day old Sprague-Dawley rats, were studied during hyperoxic perfusion. A 30 min exposure to NMDA (100μM) or NMDA+MK801 (50μM) was followed by 8 hours of perfusion. In control experiments untreated slices were perfused for 11.5 hours. In situ hybridization of c-fos and hsp70 mRNA was done in 20μm adjacent sections of slices frozen just before treatment, and at 0, 1, 2, 4, 8 hrs after treatment, using ^{35}S-labeled oligonucleotide probes. Three hrs after decapitation, control slices showed moderate c-fos mRNA expression that plateaued for 2 additional hrs, and then decreased slowly. In control slices hsp70 mRNA was always barely detectable. NMDA treatment caused prolongation of c-fos mRNA expression, compared to control slices. Addition of MK801 attenuated the prolonged expression of c-fos mRNA. NMDA administration caused widspread edematous injury (Nissl stained sections) that was significantly reduced by MK801 administration. This suggests that c-fos involves stimulation of NMDA-type receptors and plays a role in neuronal cell death. MK801 did not reduce the intensity of hsp70 mRNA induction, which occurred weakly 2 hrs after NMDA treatment. This suggests that hsp70 induction was mediated by mechanisms unrelated to NMDA-type receptors. Supported by NIH GM34767, NS14543, NS25372.

11.5

USE OF RNA DIFFERENTIAL DISPLAY TO STUDY THE MECHANISM OF ACTION OF QUINOLINATE. K.Dhanireddy[1], R.Kori[1], I.Rodriguez[2] and M.A.A.Namboodiri[1]* [1] Dept. Biol., Georgetown U., Washington, DC 20057, [2] Lab. Ret. Cell Mol. Biol. NEI, NIH, Bethesda, MD 20892.

Recent studies have shown that quinolinate (QUIN), an endogenous neorotoxin, is increased in the blood and CSF during a number of inflammatory neurological disorders, including neuroAIDS, and that QUIN may play a role in the neurotoxicity underlying these disorders. Based on the blocking of exogenous QUIN neurotoxicity by the NMDA receptor antagonist, MK801, QUIN is proposed to cause neurotoxicity via overexcitation of the NMDA receptor system. However, because of the relatively weak agonist nature of QUIN, it is not clear if QUIN is acting directly or indirectly on the NMDA receptor system. Presently, we have initiated studies on the mechanism of action of QUIN using the RNA differential display technique, a powerful tool for the study of differential gene expression.

A GnRH secreting neuronal cell line (GN cell line) was cultured in a custom synthesized medium in the presence of 5% fetal calf serum. At approximately 50% confluence, the cultures were exposed to QUIN acutely (100um, 2h) or chronically (10um, 24h). Total RNA was extracted using RNAzol B, DNA contamination was removed by DNAse treatment and mRNA differential display was performed using the RNAimage kit (GenHunter Corp., TN). A number of differentially expressed cDNA bands were detectable in both acute and chronic QUIN treated sample, some of which were common. Further studies involving TA cloning of these cDNA bands, sequencing and Northern blot analysis are in progress.

11.6

THE GP120-INDUCED RISE IN $[Ca^{2+}]_i$ IN RAT HIPPOCAMPAL NEURONES IN CULTURE IS NOT MEDIATED BY NMDA RECEPTORS OR VOLTAGE-GATED CALCIUM CHANNELS. I. Medina, S. Ghose, B.A. Esplin* and Y. Ben-Ari. INSERM Unité 29, 123 bd Port-Royal, 75014 Paris; *Pharmacology Dept., McGill University, Montréal, PQ H3G 1Y6, Canada.

The HIV-1 envelope protein gp-120 generates a rise in intracellular calcium concentration ($[Ca^{2+}]_i$) in different types of rat brain cultures. The possible mechanisms of gp-120 action involve activation of the NMDA receptor-channels (Lipton et al., 1991), voltage-gated calcium channels (Dreyer et al., 1990) and change of glutamate uptake by astrocytes (Dreyer & Lipton, 1995). We have now studied the pathways involved in gp-120 induced rise of $[Ca^{2+}]_i$ in 14-20 days-old culture of P2 rat hippocampal neurones. We used these as gp-120 does not induce a rise in $[Ca^{2+}]_i$ in young cultures (<14 days) (see abstract Ghose et al.). $[Ca^{2+}]_i$ was measured using confocal scanning microscopy with Fluo-3AM.

We found that gp-120 (200 pM) increases $[Ca^{2+}]_i$ in 90% of the neurones (n=51) with rise time (10 to 90%) from 30 to 100 s. This effect was mediated neither by NMDA, nor by voltage-gated calcium channels: it was observed in presence of Mg^{2+} (2 mM), APV (50 μM), an antagonist of NMDA receptors and Cd^{2+} (100 μM) or D600 (50 μM), blockers of calcium channels. These results were confirmed by whole-cell patch-clamp recordings during which gp-120 induced no activation of NMDA or voltage-gated calcium channels or other change in membrane current (voltage clamp mode, -60 mV) or potential (current clamp mode) (n=24).

The rise of $[Ca^{2+}]_i$ in neurones was prevented effectively by thapsigargin (10 μM), (n=8 out of 9 studied neurones) suggesting an involvement of intracellular calcium stores in gp-120's action.

11.7

NEUROPROTECTIVE ACTION OF NMDA RECEPTOR ANTAGONISTS IN A RAT GENETIC MODEL OF GLUTAMATE TOXICITY. G. Müller, J. Bresink, H.H. Schneider*, and L. Turski. Research Laboratories of Schering AG, Müllerstr. 178, D-13342 Berlin, Germany.

The mutant strain of Wistar rats spa/spa is characterised by decreased mRNA expression of the GluR2(B) receptor subunit in the brain and progressive degeneration of cerebellar and hippocampal cells. The first abnormal signs in these animals can be observed at the age of about 28 days - the rats become hyperactive and display tremor and ataxia. Subsequently, motor incoordination and muscle tone increase, and the animals die between 70-85 days of age. Since a decrease in the expression of GluR2(B) receptor subunit correlates with increased Ca^{2+} permeability of glutamate-activated channels and may contribute to the pathogenesis of neurodegeneration, we investigated whether chronic administration of the NMDA antagonist 3-(±)-2-carboxypiperazin-4-yl)-propyl-1-phosphonate (CPP) may improve motor performance and prolong survival of mutant rats.

CPP (1 mg/kg/h) or vehicle were chronically administered i.p. by means of osmotic minipumps to rats at the age of 21-28 days and the motor coordination, locomotor activity and electromyographic activity were recorded. Treatment of mutant rats with the NMDA antagonist CPP did not improve the performance of the animals in tests of motor coordination and locomotor activity. Furthermore the onset of neurological symptoms and survival of mutant animals were not altered by the treatment. From these results it may be concluded that NMDA receptor antagonists may not be beneficial in the treatment of chronic neurodegeration seen in spa/spa rats.

11.8

ROLE FOR THE MITOCHONDRIAL GLUTAMINASE IN EXCITOTOXICITY *IN VITRO*. R.Newcomb[1]*, X.Sun[2], L.Taylor[3], N.Curthoys[3], and R. Giffard[2] [1]Neurex Corp. Menlo Park, CA, [2]Dept. Anesthesiology, Stanford Univ. Stanford CA, and [3]Dept. Biochemistry and Molecular Biology, Colorado State University, Fort Collins, CO.

We have been testing the hypothesis that neuronal death results in an exposed form of the mitochondrial glutaminase(Glnase), and that the enzyme subsequently produces significant amounts of glutamate(Glu). Healthy cultures of pure neurons did not generate significant amounts of extracellular Glu (≤.025μM/min) during short term exposure to physiological concentrations of glutamine(Gln) (up to 2 hr at 2 mM). When damaged by 5 hr exposure to NMDA, or by 5-24hr exposure to Gln, the same cultures rapidly generated Glu (0.3-1.0μM/min) from Gln, consistent with an earlier report (Glia 4 91). Glu generation by damaged neurons exposed to Gln was abolished by 30 μM of the membrane impermeant Glnase inhibitor, p-chloromercuriphenylsulphonate (pCMPS). Since pCMPS does not inhibit the Glnase in intact mitochondria (Arch.Biochem.Biophys.243 1), an exposed form of the Glnase is implicated. Western blotting, as well as assay of Glnase activity, showed that the Glnase remained in fragments of neurons after damage. Can Gln neurotoxicity in hypoxic neuronal/glial cultures (Neurosci.Lett.94 57) be accounted for by this Glnase activity? Medium Glu is elevated from 0.3-0.8 μM to 3-4 μM by the combination of 2 mM Gln and 5 hr. hypoxia. This amount of Glu is consistent with 1)The toxicity of Glu in pure neuronal cultures (IC50 2-10μM and 2)The neuronal death caused by 2 mM Gln in hypoxic mixed cultures(c.20%, or 2X the neuronal death with 0.05-0.1mM Gln). The rate of Glu production by Glnase in damaged neurons, when compared with the rate of clearance of Glu from hypoxic mixed cultures, is consistent with this 3 μM concentration of Glu. Finally, pCMPS protects neurons from damage caused by prolonged exposure to Gln. Funded by Neurex and grants from the NIH to N.C.(DK37124) and R.G. (GM49831)

11.9

NEURONAL DEATH IN CYTOKINE-ACTIVATED PRIMARY HUMAN CNS CELL CULTURE: ROLE OF NITRIC OXIDE SYNTHASE 2, TNFα AND EXCITOTOXINS M. Downen*, T. Amaral and S.C. Lee. Dept. Pathology, Albert Einstein College of Medicine, Bronx, NY 10461.

Recent studies of rodent neurons and PC12 cells have demonstrated an important role for nitric oxide (NO) in neuronal toxicity. Currently debated issues include the role of different nitric oxide synthase (NOS) isoforms and the relationship of NO-mediated toxicity to that mediated by excitotoxins and superoxide radicals. In humans, the expression of NOS in CNS cells and its role in neuronal cytotoxicity have not been clearly defined. We examined mediators of neuronal death in primary mixed cell cultures prepared from human fetal cerebrum. In these cultures, astrocytes, but not microglia, selectively express inducible NOS (NOS2) after exposure to IL-1β and IFNγ. Neuronal death is prevented by inhibitors of NO production (N^G-monomethyl L-arginine or L-nitroarginine methyl ester), but not by glutamate receptor antagonists (2-APV, MK-801, or CNQX), or superoxide dismutase. An antibody to TNFα partially inhibits neuronal toxicity and production of NO in cultures treated with IL-1β and IFNγ, while TNFα alone (1-100 ng/ml) has no effect. In combination with IL-1β, TNFα mediates neuronal toxicity via induction of NOS2 in astrocytes. These results clearly demonstrate that in primary human CNS cell cultures, NO released by activated astrocytes mediates neuronal toxicity through pathways that are independent of glutamate receptor stimulation or formation of peroxynitrite. Furthermore, TNFα's role in neuronal toxicity in primary human neuroglial cultures is indirect and is mediated through production of NO. (Supported by NIMH 55477, AECOM BRSG, and a departmental seed fund.)

11.10

MAGNESIUM (Mg) PROTECTS AGAINST COCAINE-INDUCED HEMORRHAGIC STROKES IN A RAT MODEL: A ^{31}P-NMR IN-VIVO STUDY. B.M. Altura*, A. Gebrewold, B.T. Altura and R.K. Gupta, SUNY Health Sci. Ctr. at Bklyn, NY 11203 and Albert Einstein Col. Med., Bronx, NY 10461.

Cocaine (COC) abuse results in an increased incidence of aneurysmal subarachnoid hemorrhages, intracerebral hemorrhages, and occlusion-type strokes in humans. We have reported that COC administration to anesthetized rats can produce hemorrhagic strokes (HS), which are associated with loss of whole brain intracellular free Mg ions ($[Mg^{2+}]_i$), falls in intracellular pH (pHi), progressive falls in phosphocreatine (PCr) and elevation of inorganic phosphate (Pi) up until death. In-vivo ^{31}P-NMR studies were undertaken with anesthetized rats to determine whether systemic administration of $MgCl_2$ (10μmol/min for 45 min) could protect animals against COC-induced HS; and 2. A relationship exists between basal levels of brain $[Mg^{2+}]_i$ and stroke risk. ^{31}P-NMR spectra were obtained at various intervals of time (3-120 min, or up until death) after COC (5, followed by 30 mg/kg at 2 hr). Ion selective electrodes were used to measure plasma Mg^{2+}, K^+, Na^+ and Ca^{2+}. 40% of animals died in the absence of Mg^{2+} infusion following COC. Only 18% died with Mg^{2+} infusion ($p < 0.05$). In the Mg^{2+}-protected animals, neither $[Mg^{2+}]_i$, pHi, [PCr]/[ATP], nor [Pi]/[ATP] fell when toxic and lethal doses of COC were given. Low basal brain $[Mg^{2+}]_i$ (275 $\pm$ 24 vs. 466 $\pm$ 75 μM) and low basal brain [PCr] (3.36 $\pm$ 0.35 vs. 4.26 $\pm$ 0.25 mM) were found to result in a 2-fold increased incidence of stroke. A positive correlation ($r = 0.31$, $p < 0.03$) between brain $[Mg^{2+}]_i$ and [PCr]/[ATP] was found. It is possible that basal brain $[Mg^{2+}]_i$ and [PCr] may be useful as important predictors of susceptibility to COC HS. (Supported in part by NIAAA Grant AA-08674).

INVERTEBRATE LEARNING AND BEHAVIOR I

12.1

INDUCTION OF LTD OF CULTURED *APLYSIA* SENSORIMOTOR SYNAPSES IS BLOCKED BY POSTSYNAPTIC BAPTA AND BY NIFEDIPINE, BUT NOT BY D-APV OR BY OKADAIC ACID. S. Chen, X. Y. Lin, and D. L. Glanzman*. Dept. Physiol. Sci., UCLA, Los Angeles, CA 90024.

Aplysia sensorimotor synapses in cell culture exhibit long-term depression (LTD) following 2-Hz activation of presynaptic sensory neurons (Lin and Glanzman, *Soc. Neurosci. Abstr.* **21**:1023, 1995). To determine whether this form of invertebrate LTD is mechanistically similar to hippocampal LTD, we tested the effect on LTD of sensorimotor synapses of several compounds reported to block the induction of LTD of CA1 hippocampal synapses (Mulkey and Malenka, 1992; Mulkey et al., 1993; Bolshakov and Siegelbaum, 1994). Injection of the Ca^{2+} chelator BAPTA (200 mM in the recording electrode solution, $n = 9$) into the postsynaptic motor neuron prior to 2-Hz presynaptic stimulation blocked the induction of LTD of sensorimotor synapses, as did postsynaptic injection of the L-type Ca^{2+} channel blocker nifedipine (500 µM in the electrode solution, $n = 11$). By contrast, LTD of sensorimotor synapses was not blocked by the NMDA receptor antagonist D-APV (100 µM in the perfusion medium, $n = 8$), nor by the protein phosphatase inhibitor okadaic acid (500 nM in the perfusion medium, $n = 7$). The lack of an effect of D-APV on LTD of sensorimotor synapses is particularly interesting, because this NMDA receptor antagonist does block the induction of long-term potentiation (LTP) of *Aplysia* sensorimotor synapses (Lin and Glanzman, 1994). We conclude that LTD of cultured *Aplysia* sensorimotor synapses requires Ca^{2+} entry into the motor neuron through L-type channels, but does not require activation of NMDA-related receptors nor activation of protein phosphatases 1 and 2A. The cellular mechanisms underlying LTD of *Aplysia* sensorimotor synapses in culture appear to resemble those underlying LTD of synapses in the CA1 region of the neonatal mammalian hippocampus (Bolshakov and Siegelbaum, 1994).

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12.2

NONASSOCIATIVE ENHANCEMENT OF *APLYSIA* SENSORIMOTOR SYNAPSES IS NOT BLOCKED BY D-APV. G. G. Murphy* and D. L. Glanzman, Interdepartmental Program in Neuroscience, School of Medicine, and Department of Physiological Science, UCLA, Los Angeles, CA 90024.

Enhancement of sensorimotor synapses in the cellular analog of classical conditioning in *Aplysia* requires a postsynaptic rise in Ca^{2+} (Murphy and Glanzman, *Soc. Neurosci. Abstr.* **20**:1072, 1994). This essential rise in postsynaptic Ca^{2+} appears to be mediated, in part, by training-induced activation of NMDA-related receptors, because the NMDA receptor antagonist D-APV significantly reduces the synaptic enhancement due to conditioning (Murphy and Glanzman, *Soc. Neurosci. Abstr.* **21**:1023, 1995). An alternative explanation for this result, however, is that D-APV interfered with the activity, or efficacy, of facilitatory interneurons thought to mediate classical conditioning in *Aplysia*. To investigate this possibility, we tested whether D-APV disrupts nonassociative enhancement of sensorimotor synapses following unpaired presentations of the US and CS in the cellular analog of classical conditioning of *Aplysia*'s siphon withdrawal reflex (Hawkins et al., 1983). Both the preparation and experimental protocol were similar to those of our previous experiments (Murphy and Glanzman, 1995), except that the CS (brief activation of a siphon sensory neuron) and US (pedal nerve shock) were delivered in unpaired, rather than paired, fashion during training. Unpaired training resulted in nonassociative enhancement of the monosynaptic sensorimotor EPSP measured 60 min after the end of training (mean EPSP = 136 ± 17% of the pretest EPSP, $n = 3$). Unpaired training in the presence of the D-APV (100 µM in the bathing medium) produced similar enhancement of the monosynaptic EPSP (mean EPSP = 160 ± 30% of the pretest EPSP, $n = 3$). Our results therefore suggest that D-APV's effect on associative synaptic enhancement in the conditioning analog is not due to disruption of the activity, or efficacy, of facilitatory interneurons recruited by the US.

Supported by funds from NSF, NIH, NIMH, and the State of California.

12.3

DEGENERATION OF IDENTIFIED PEPTIDERGIC NEURONS IN THE BRAIN OF A MOLLUSC AND CESSATION OF EGG LAYING BEHAVIOUR. C. Janse, M. van der Roest, E. J. G. Dubelaar (SPON: European Neuroscience Association). Graduate School of Neurosciences Amsterdam, Research Institute Neurosciences Vrije Universiteit, Faculty of Biology, Vrije Universiteit, De Boelelaan 1087, 1081 HV Amsterdam, The Netherlands.

In *Lymnaea stagnalis* caudodorsal cells (CDCs) selectively degenerate at the age of cessation of egg laying behaviour. The initial stages of degeneration consist of reduction of branching patterns of CDCs (Janse et al., J. Neurobiol. 29: 202, 1996). CDCs control egg laying behaviour and are located in the cerebral ganglia in two clusters totalling 100 electrically and chemically coupled cells. Preceding egg laying, CDCs become electrically active and release peptides at the cerebral commissure (CC) into the blood. CDCs have an ipsilateral axon that branches into the CC. Ventral CDCs are important for activation of the entire system; they have special synaptic input sites and have in addition to the ipsilateral axon an axon that crosses the CC and makes a contralateral loop (electrical and chemical contacts with CDCs) to branche again in the CC. Cessation of egg laying may be due to degeneration and electrical dysfunction of increasing number of CDCs of different types. Alternatively, ventral CDCs might deterioriate first. In the present study neurophysiological properties and gross morphology were studied of CDCs. Electrical and chemical coupling were studied with hyperpolarizing stimuli and by induction of depolarizing after potentials (DAP) respectively, into one CDC while recording another. DAPs and afterdischarges were induced with electrical stimuli (2 Hz). Subsequently one of the recorded CDCs was filled with LY. Reduced branching patterns especially occurred in ventral CDCs, other CDCs seemed to be less reduced. This indicates that ventral CDCs start to degenerate first. Reduced ventral cells still had an axon crossing the CC and still were electrically coupled to contralateral CDCs. Moreover, most cells produced a DAP and an afterdischarge which could be transmitted to other CDCs. Obviously, electrical and chemical transmission between CDCs stays present even during considerable morphological reduction of the ventral CDCs. Possibly, cessation of egg laying is due to the input sites of ventral CDCs being turned off. The latter may be part of the degeneration process in the CDCs.

12.4

NONASSOCIATIVE SYNAPTIC PLASTICITY AT TYPE B TO A PHOTORECEPTOR CONNECTIONS IN *HERMISSENDA*. L.M. Schultz*, E.S. Lee, and G.A. Clark, Program in Neuroscience, Psychology Department, Princeton University, Princeton, NJ 08544.

The positive phototactic response of *Hermissenda* is suppressed following classical conditioning with a light conditioned stimulus (CS) and a rotation unconditioned stimulus (US). A lesser-understood, weaker suppression of phototaxis results from US- or CS-alone training. To determine possible neuronal mechanisms for these nonassociative effects, we investigated whether activation of the US or CS pathways would induce synaptic facilitation at the inhibitory type B to A cell connections. We found that activation of the US pathway by mechanical statocyst stimulation enhanced the inhibitory postsynaptic potentials (IPSPs) evoked in type A cells by intracellular stimulation of type B cells. This enhanced synaptic inhibition may represent a means by which US-alone training leads to suppression of phototaxis. We next examined the effects of activating the CS pathway by measuring changes in the synaptic strength of type B to A cell connections both during and after evoked type B cell activity. We found that *during* stimulus trains (interstimulus intervals of 0.3, 3, or 30 sec), there was a monotonic decrease in IPSP amplitude, with greater synaptic depression occurring at higher stimulation frequencies. In contrast, the IPSP amplitude was increased *following* high frequency activation of type B cells by either light or intracellular stimulation, which may represent a mechanism for CS-elicited suppression of phototaxis. Given that the type B to A photoreceptor synapse displays both depression and facilitation, this synapse may serve as an important control point in the neural circuitry for phototaxis.

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12.5

NEURAL CORRELATES INTRINSIC TO PRE-SYNAPTIC AND POST-SYNAPTIC NEURONS IN THE CS PATHWAY OF CONDITIONED *HERMISSENDA*. R. J. Frysztak and T. Crow*. Department of Neurobiology and Anatomy, University of Texas Medical School, Houston, TX 77030

Identified type A photoreceptors of *Hermissenda* express differential effects of classical conditioning. Lateral type A photoreceptors exhibit an increase in excitability to both the conditioned stimulus (CS; light) and extrinsic current (Frysztak & Crow, 1993). In contrast, medial type A photoreceptors do not express enhanced excitability, but do show synaptic enhancement expressed by an increase in the amplitude of spike-elicited IPSPs following conditioning (Frysztak & Crow, 1994). Therefore, both enhanced excitability and changes in synaptic strength may contribute to long-term plasticity underlying classical conditioning. The activation of protein kinase C (PKC) has been implicated in the expression of enhanced excitability in B photoreceptors following classical conditioning. We have examined a possible role for PKC in the expression of enhanced excitability in lateral type A photoreceptors and synaptic enhancement in medial type A photoreceptors following classical conditioning. Spike frequency elicited by multiple extrinsic current steps in lateral type A photoreceptors of conditioned animals (N=10) was significantly reduced following intracellular injection of the PKC inhibitor peptide PKC(19-36) as compared to random controls (N=8) ($p<.001$). Injection of the non-inhibitory analog peptide ([glu^{27}]PKC(19-36) did not affect excitability measured over multiple current steps (N=5). Conditioning resulted in an enhancement of IPSP amplitude recorded from the medial A cells as compared to the random controls (N=13) ($p<.05$). However, injection of PKC(19-36) into the medial B cells did not significantly affect IPSP amplitude in conditioned animals or the random controls. We next examined the effects of PKC(19-36) on 5-HT induced facilitation, a PKC dependent phenomenon. 5-HT produced an enhancement of IPSP amplitude in the medial A ($p<.05$). Enhancement was blocked in a group that received PKC (19-36) injections before 5-HT application. Thus, enhanced excitability in the lateral A photoreceptor of conditioned animals may involve a different mechanism from synaptic enhancement of the connection between the medial B and medial A photoreceptor detected after conditioning. Supported by a grant from NIMH.

12.6

MAY CYTOSOLIC FREE Ca^{++} AFFECT NON ASSOCIATIVE LEARNING PROCESSES IN *H. MEDICINALIS*? M. Brunelli*, R. Frandi, R. Mozzachiodi, R. Scuri and M. L. Zaccardi. Dip.to Fisiologia e Biochimica "G. Moruzzi", Università di Pisa. 56127 Pisa. Italy.

In the leech *H. medicinalis* it is possible to obtain short term habituation of swim induction by low rate application of electrical stimuli onto the body wall. In T sensory neurons (the first station of the neuronal swim network) the low rate intracellular stimulation elicits an increase of the after-hyperpolarization (AHP) amplitude: this AHP potentiation weakens the synaptic efficacy of the connection between T neuron and its followers.

Behavioral experiments have shown that Nifedipine, a L-type Ca^{++} channel blocker, remarkably inhibits the habituation process. By using an electrophysiological approach we have observed that Nifedipine prevents the potentiation of AHP amplitude. These data suggest an important role of extracellular Ca^{++} in this form of non associative learning.

In order to test whether also cytosolic free Ca^{++} is involved in short term habituation, we have injected into the animals a blocker of Ca^{++} release from intracellular stores, TMB-8 (100μM). We have observed a clear-cut reduction of the habituation process.

Preliminary electrophysiological data, at cellular level, show that TMB-8 affects the AHP amplitude. Investigations are in progress in order to clarify whether TMB-8 inhibits the AHP potentiation following low rate repetitive stimulation.

By using other specific antagonists and agonists of intracellular Ca^{++} release we will further enlighten, at behavioral and electrophysiological level, the role of cytosolic Ca^{++} in short term habituation.

12.7

TYROSINE KINASE INHIBITORS BLOCK CONDITIONING-PRODUCED CHANGES IN *Hermissenda* TYPE B CELLS I. Jin* and J. Farley, Program in Neural Science, Indiana University, Bloomington, IN 47405.

Bath-application of 100 uM genistein, a protein tyrosine kinase (PTK)-inhibitor, reduced *in vitro*-conditioning (5 pairings of light and electrical stimulation of statocyst hair cells) produced cumulative depolarization of type B photoreceptors by 79%. Leakage of genistein or lavendustin A (another PTK inhibitor) from the recording electrode into B cells prior to conditioning reduced depolarization by 78% and 51%, respectively. To explore possible interactions between PTKs and PKC in B cells, the preceding experiments were repeated following exposure of B cells to specific activators or inhibitors of PKC. Exposure of the nervous system to the PKC activator, PMA, occluded B cell depolarization by ~50%. The remaining PMA-resistant depolarization was blocked by lavendustin A. Iontophoresis of the PKC pseudosubstrate inhibitor, PKC [19-31], into a type B cell reduced cumulative depolarization by 42%. Bath application of genistein completely prevented the development of PKC [19-31]-resistant depolarization. Voltage-clamp analysis of the effects of genistein upon the K^+ currents of B cells (that are reduced by both conditioning and PKC-activation) revealed a selective increase in the A-type current of untrained specimens by ~25 %. The delayed-currents ($I_{K,v}$ and $I_{K\text{-}Ca}$) were not affected. Our results suggest that PTK and PKC signal-transduction pathways both contribute to the occurence of short-term, learning-produced changes in B cell excitability. Supported by grants from NIH.

12.8

Receptor-Mediated Activation of Phospholipases and Arachidonic Acid Contribute Independently to Sensory Transduction and Activity-Dependent Neuronal Facilitation in *Hermissenda* Type B Photoreceptors

Andrew C. Talk*, Isabel A. Muzzio, and Louis D. Matzel
Program in Biopsychology and Behavioral Neuroscience, Department of Psychology, Rutgers University, New Brunswick, New Jersey 08903

During contiguous pairings of light and rotation, B photoreceptors in the *Hermissenda* eye undergo an increase in excitability (e.g. input resistance and evoked spike rate) that requires a light-induced rise in intracellular Ca^{2+} in the photoreceptor concomitant with stimulation of G-protein coupled receptors as a result of presynaptic hair-cell activity. Here, we report that arachidonic acid (ArA) and its metabolites are involved in independent signal transduction pathways that underlie both receptor function and activity-dependent facilitation in the B photoreceptor. 4-Bromophenacyl bromide (BPB), an inhibitor of phospholipases A_2 (PLA_2) and C (PLC), blocked the generation of light-induced depolarizing generator potentials, but had no effect on the inhibitory postsynaptic responses in the B cell in response to hair-cell stimulation. Quinacrine, which predominantly blocks PLA_2 in neurons, had no effect on either the light response or the IPSP, but did block the increase in cell excitability (i.e. increased input resistance and spike rate) caused by pairings of light and presynaptic hair-cell stimulation. Neither nordihydroquaiaretic acid (NDGA), which inhibits metabolism of ArA by cyclooxygenase, nor indomethiacin, which inhibits lipoxygenase metabolism of ArA, had effects on the light response or IPSP, but both blocked the activity-dependent increase in excitability. In light of prior results indicating that ArA activates PKC in a synergistic fashion with Ca^{2+} and DAG in the B cell, the present experiments strengthen the possibility that PLA_2 induced ArA release is a critical component of the signal convergence that produces associative learning in the system.

12.9

GABA RECEPTOR STAINING IMPLIES FUNCTIONAL SUBDIVISION OF MUSHROOM BODIES. T.M.Brotz* and A.Borst. Friedrich-Miescher-Laboratory of the Max-Planck-Society, Spemannstr. 37-39, D-72076 Tuebingen, Germany.

The mushroom bodies of dipteran and hymenopteran insects, a paired central brain region, are involved in olfactory learning. Until recently, the mushroom bodies have been thought to consist of a mainly isomorphic array of Kenyon cells. This view has been changed by recently published enhancer trap expression patterns in the mushroom bodies of *Drosophila* (Yang et al. 1995, Neuron, 15: 45) suggesting a subdivision of the Kenyon cell population. We have conducted antibody stainings against the Rdl-subunit of the insect GABA receptor in the blowfly *Calliphora erythrocephala*. Compared to all other neuropiles, immunoreactivity is by far strongest in the mushroom bodies. Furthermore, the regions of highest immunoreactivity are confined to the axonal part of the Kenyon cells. Cross sections of the pedunculus, the α– and β-lobes show one or two (depending on the section plane) unstained core structures surrounded by strong concentric immunoreactivity. The posterior part of the pedunculus appears to be subdivided into discrete immunoreactive fascicles which segregate clearly before they project into the calices. Although the γ-lobes are also significantly labeled they do not show the concentric core-surround structure but a characteristic, complex subdivision into a number of compartments. This Rdl-immunoreactivity pattern has striking similarities with the enhancer trap lines mentioned above. Some of these lines, thus, could well be associated with GABA receptor expression. Additional staining against GABA revealed the presence of a number of GABA-immunoreactive neurons that are more or less homogeneously interspersed in the Kenyon cell array and that do not form such characteristic patterns as the Rdl-immunoreactive fibers. Taken together, these results reveal that (i) the population of Kenyon cell fibers is not homogeneous and (ii) inhibitory inputs onto Kenyon cell axons might play an important role for signal processing in mushroom bodies.

This work was supported by the Max-Planck-Society.

13.1

PRESERVATION OF MOTRICITY IN MACAQUE MONKEYS AFTER UNILATERAL NEONATAL LESION OF THE HAND AREA OF THE PRIMARY MOTOR CORTEX (M1). E.M. Rouiller(1), V. Moret(1), A. Tempini(1), X.H. Yu(1) and M. Wiesendanger(2)* Dept. of Physiology, University of Fribourg, 1700 Fribourg(1), Dept. of Neurology, University Hospital, 3010 Bern(2), Switzerland.

Unilateral lesion of the hand representation of M1 in adult monkeys has devastating effects on the finely controled motricity with digits of the opposite hand, such as the precision grip ability. However, following such a lesion at the neonatal stage, monkeys when adult exhibited a significant preservation of manual skill. The aim of the study was to investigate the plastic changes possibly underlying this preserved skill. Previous experiments in rats provided neuroanatomical evidence that the intact opposite hemisphere may contribute to the functional preservation of motricity, by re-directing some corticospinal (CS) axons to the denervated cervical cord. In monkeys, we found little evidence for axonal redirection from the intact hemisphere. First, as assessed by tracing experiments, the CS projection originating from the intact M1 hand representation was not significantly modified. Second, transient inactivation (by infusion of lidocaine) of the intact M1 did not change the behavioral score (precision grip task) of the hand partly deprived of its contralateral pyramidal input, while the performance of the "normal" hand (contralateral to the intact inactivated M1) was dramatically reduced. As assessed by transient inactivation, the supplementary motor area (SMA) on both sides did not contribute to the functional preservation of motricity. However, a comparison of the effects of IntraCortical MicroStimulations (ICMS) in the two hemispheres showed that the cortical territory adjacent to the lesioned M1 zone has been re-organized: in a zone normally representing proximal muscles, ICMS elicited clear movements of the fingers of the pyramidally deprived hand. The functional contribution of this peri-lesion territory was supported by the observation of a dramatic decrease of the behavioural score of the "affected" hand when this peri-lesion territory was transiently inactivated. In conclusion, structural cortical reorganization appears to contribute significantly to the partly maintained functional motricity after neonatal cortical lesion of the hand area of M1.

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13.2

MOTOR CORTICAL ACTIVITY DURING TARGET MOTION IN THE PRESENCE AND ABSCENCE OF MANUAL TARGET INTERCEPTION. N. Lindman Port*, D. Lee, W. Kruse, & A. P. Georgopoulos. Brain Sciences Center, VAMC, Minneapolis, MN 55417

We investigated whether neurons in the motor cortex are involved in processing temporal properties of a target to be manually intercepted. The impulse activity of 202 cells was recorded in the arm area of the motor cortex of a monkey trained to use a 2D articulated manipulandum in both a "Go" and a "No-Go" task. On a computer screen, a target accelerated, decelerated or traveled at a constant velocity. For each motion type, targets traveled for 0.5, 1.0 and 1.5 s (target motion time). A target appeared randomly in either the right or the left lower corner of the screen, and traveled along a 45° path until it crossed the vertical meridian. In both tasks the monkey was required to maintain stable fixation. In the "Go" task, the monkey made an upward movement (12 cm) to intercept, within 130 ms, the moving target as it crossed the vertical meridian. In the "No-Go" task, the monkey was required to maintain fixation and hold the manipulandium steady, within a positional window.

In the "Go" task 57% and in the "No-Go" task 47% of the cells showed a significant difference in impulse frequency between the pre-target motion period and the target motion period (paired t-test, $p<0.05$). During the target motion period in the "Go" task there was a significant effect of target motion type, target motion time and direction of target motion on the frequency of discharge in 10%, 32% and 7% of cells, respectively (ANOVA, $p<0.05$). For the "No-Go" task these percentages were 8%, 30% and 8%, respectively. These results indicate that the motor cortex is involved in processing temporal characteristics of the moving target. (Supported by USPHS grant 1-PSMH48185-01).

13.3

EVIDENCE FOR VISUO-MOTOR PROCESSING IN THE DIRECTIONAL FIRING OF MOTOR CORTICAL NEURONS M.T.V. Johnson*, J.D. Coltz, M.C. Hagen, T.J. Ebner. Depts. of Neurosurgery and Physiology, University of Minnesota, Minneapolis, MN 55455.

It is well known that both visual and movement information are combined in cortical regions surrounding the superior parietal sulcus. However, the degree to which motor cortical neuronal firing is influenced by visual information is less clear. To define the extent of visual processing in the primate motor cortex, a two-dimensional tracking paradigm requiring equal time for the observance and the tracking of a constant velocity target was used. A target appeared at the periphery of the workspace and moved to a central start box in which a cursor corresponding to the position of the animal's hand was waiting (Cue period). As the target moved away from the start box, the primate tracked so that the cursor remained within the target box (Tracking period). This task sequence was performed over 8 directions and 4 constant velocities.

The directionality of neuronal firing during the Cue and Tracking periods was evaluated using linear regression. A significant fit to a cosine function was found for 133/160 neurons (82 both in **C** and **T**, 15 in **C**, and 36 in **T**) in two monkeys. Neurons with significant directionality in both **C** and **T** periods had preferred directions which differed by a mean of 70°. Both **C** and **T** were then halved into early and late periods. Relative to the late Tracking period, the mean change in preferred direction was greatest for the early **C** (78°), less for the late **C** (46°), and least for the early **T** (22°) periods. The distributions differed significantly (Watson's F-test).

Individually and collectively, the preferred direction of neurons differed between the Cue and Tracking periods. The convergence of the "visual" preferred direction to that of the late tracking phase occurred gradually over the earlier periods. The large shifts in preferred direction between the Cue and Tracking periods suggest that substantial processing of visual target information takes place in the motor cortex. Supported by NIH grant NS07361.

13.4

NEURAL CORRELATES OF STATIC AND DYNAMIC PROCESSES DURING ISOMETRIC FORCE OUTPUT IN THE MONKEY. J. Boline*, J. Ashe, Brain Sciences Center, VAMC, and Departments of Physiology and Neurology, University of Minnesota, Minneapolis MN 55417

The activity of cells in the motor cortex has been shown to relate to both static and dynamic force output. However, there is no consensus as to the proportion of cells that relate exclusively to static or to dynamic force, or to both. We examined this issue in monkeys trained to use the arm to exert force on an isometric manipulandum in two different tasks. In the *dynamic* task the animal produced pure dynamic force pulses in eight different directions, while in the *static* task the animal maintained a steady force in the same eight directions. The tasks were carefully matched such that the direction and magnitude of force output was similar in each; they differed only to the extent that one task was purely dynamic and the other static. We recorded the activity from each of 299 neurons in the motor cortex of the monkey during the performance of both tasks. There were significant ($p<0.05$) changes in activity in 180 (60.2%) cells during both the static and dynamic tasks. Forty four (14.7%) cells changed activity exclusively during the dynamic task, while 34 (11.4%) cells were active only during the static task. These findings suggest the following: (1) within the motor cortex there are three functionally distinct groups of cells that relate to static and dynamic processes during isometric force output, (2) the proportion of cells that relate exclusively to static or dynamic force output is small relative to the total population of cells engaged during either process. (Supported by a Merit Review Grant from the Department of Veterans Affairs)

13.5

DIGITAL VIDEO: A TOOL FOR CORRELATING ELECTROPHYSIOLOGY WITH BEHAVIOR. E.P. Gardner* and J.Y. Ro, Department of Physiology and Neuroscience, NYU Medical Center, New York, NY 10016.

The ability to correlate motor behavior with neurophysiological activity has been limited by disparities in technologies used to monitor activity. We demonstrate how digital multimedia techniques allow simultaneous imaging of hand movements and spike trains recorded from the cerebral cortex while monkeys perform a prehension task. Monkeys are trained to grasp, lift and lower knobs (spheres, cylinders, and rectangular blocks) using visual cues. Hand movements are videotaped with two Hi8 cameras, digitally mixed and recorded on a Hi-8 computer video deck; spike trains are stored as an audio signal on the videotape. Hi-8 tapes are striped with RC time code, an electronic tag that numbers each frame, allowing precise synchronization of audio and video when they are digitized. QuickTime (QT) movies are made off-line using digital editing software; video and neuronal signals in 5 min clips are digitized simultaneously using a hardware video capture board interfaced to a Macintosh 840AV computer. Digital video provides a snapshot of the hand position every 33 ms, and precise calibration of the synchronously recorded spike waveforms digitized at 44 kHz. The spike train is displayed as a strip chart in a separate window. QT movies allow a frame-by-frame analysis of hand movements and the corresponding spike activity by matching the precise time codes in the video and the audio windows of the editing program. Subtle changes in hand position from frame to frame are evident in these pictures when they are placed in motion. We show that the kinematics of prehension and specific characteristics of the spike train are reproducible from trial-to-trial, and over several months of study. These imaging methods permit non-invasive, nontraumatic monitoring of behavior in experimental animals, and provide high quality records of synchronized neuronal spike trains. *(Supported by Research Grant NS11862 from NINDS).*

13.6

CUTANEOUS MECHANORECEPTORS IN HAIRY SKIN SIGNAL HAND KINESTHESIA. J.Y. Ro*, S. Ghosh+ and E.P. Gardner, Dept. of Physiology & Neuroscience, NYU Medical Center, New York, NY 10016, and +Dept. of Physiol. & Pharmacol., Univ. Queensland, St. Lucia, Brisbane, Australia.

To understand the role of active touch in hand motor function, we correlated firing patterns of S-I cortical neurons with the kinematics of hand movements during prehension. Three monkeys were trained to grasp, lift and lower knobs of different size and shape (spheres, cylinders, cubes and rectangular blocks) in response to visual cues. S-I cortical neurons with tactile receptive fields on the hand dorsum modulate their firing patterns during prehension even when no direct contact is made with the dorsal hairy skin. Most are tonically active, with steady firing rates proportional to the posture of the joints below the tactile fields. 24 of 27 cells respond to active hand and finger movements, whether or not the movement involves contact with an object. Neurons activated during hand movement (MA) fire most vigorously when the hand posture changes, as the grasp is initiated or released. Some also signal grip force during prehension. Movement inhibited (MI) neurons are silenced during sustained grasp and lift; they respond to steady hand posture when the hand rests quietly on the objects. The similarity of responses to tactile stimuli and to active movements suggests that skin stretch during prehension provides important postural and movement information about the hand kinematics when the fingers are used to perform skilled tasks. In contrast to glabrous skin receptors that signal the physical surface parameters of the object, the hairy skin receptors integrate information about the coordinated movements of the fingers that provides feedback to the motor areas of the brain, facilitating skilled manipulation of the environment with the hand. The principal function of tactile hairy units in the hand dorsum is postulated to be cutaneous kinesthesia, rather than tactile exploration. *(Supported by Research Grant NS11862 from NINDS).*

13.7

TEMPORAL COHERENCE PATTERNS BETWEEN SIMULTANEOUSLY RECORDED MOTOR CORTICAL NEURONS DURING PLANAR REACHING MOVEMENTS. Nicholas G. Hatsopoulos*, Catherine L. Ojakangas, & John P. Donoghue. Box 1953, Dept. of Neuroscience, Brown University, Providence RI 02912.

A major problem in motor control is the manner in which muscles or motor primitives are combined to generate coordinated behavior. Correlation in the firing of motor cortical neurons may provide a way by which motor elements are bound together in a flexible manner. To examine this hypothesis, we recorded from up to 21 neurons simultaneously in motor cortex as a macaque monkey performed a radially-directed hand movement to one of eight possible peripheral targets. Using cross-correlations corrected using a shift predictor (CCR) and normalized joint peri-event time histograms (JPETH's), we found that the coherence between neurons modulates sharply around movement onset and could not be predicted by the firing rate alone. Moreover, both coherence measures (CCR and JPETH) appear to reflect interactions between neurons above those due to common firing modulation. We observed either phasic increases or decreases in coherence or tonic increases which continued to the end of the trial despite phasic modulation in firing rate of neurons. Of 112 neuron pairs examined, about 70 percent showed changes in correlation at around movement onset. The sign of these coherence changes seemed to depend partly on the tuning properties of the constituent neurons. Cells with opposite preferred directions exhibited a transient (100-250 ms) de- or anti-correlation at movement onset followed by a positive correlation. On the other hand, most cells with similar preferred directions showed a strong positive correlation around movement onset. These modulation in coherence may reflect the formation of flexible synergies among motor elements by motor cortical cell ensembles. Supported by NIMH MH19118 & NIH NS25074.

13.8

CHANGES IN SYNCHRONOUS ACTIVITY OF SIMULTANEOUSLY RECORDED NEURONS IN THE MOTOR CORTEX DURING EVOLVING MOVEMENTS. Catherine L. Ojakangas*, Nicholas G. Hatsopoulos, and John P. Donoghue. Dept. of Neuroscience, Box 1953, Brown University, Providence, RI 02912 email: cojakang@brownvm.brown.edu

We have recently begun using a Utah multiple-electrode array to record synchronous activity of motor cortical (MI)populations in the monkey. As described in Hatsopoulos et al. (this meeting), during simple, targeted arm movements the activity of MI neurons becomes correlated, particularly around movement onset. These correlations may reflect binding of motor components into functional synergies. What happens to temporal correlations when movements are adapted over time? The activity of 21 MI neurons was recorded simultaneously while the monkey moved a cursor from a central hold zone to one of three targets. In an adaptation phase, one target was extinguished after movement initiation and a second target was illuminated, requiring the animal to redirect its movement. With the adaptation requirements held constant, movements changed gradually until they resembled direct movements to the second target. Cross correlations and joint peri-event histograms between 504 pairs of neurons revealed that activity was highly correlated before adaptation in 58% (294) beginning around movement onset. During adaptation, however, significantly correlated neuronal activity was weak or absent in 63% of these (186/294) and reduced in the majority of the remainder. With time, as the movements began to resemble the control movements, significant synchronous activity again emerged in 94% (175/186) of the neuronal pairs. This desynchronization and resynchronization of neuronal activity may reflect reorganization of motor cortical circuitry necessary to adapt new movement patterns to changing behavioral requirements. Supported by:NS25074.

13.9

SYNCHRONIZATION BETWEEN FIELD POTENTIALS OF OLFACTORY BULB, SOMATOMOTOR, VISUAL, AUDITORY AND ENTORHINAL CORTICES OF AWAKE CATS

G. Gaál*, P. German, S. Oshtory, A. Smart and W. J. Freeman, University of California, Berkeley

Widespread intermittent synchrony in oscillations has been observed in neural and muscle activity in primates and cats. It has been debated whether it was related to attention (Kreiter & Singer, 1992; Murthy & Fetz, 1992; Gaál et al., 1992; Sanes & Donoghue, 1993, Roelfsema et al., 1995, Bressler & Nakamura, 1995, Menon et al., 1996). We have recorded EEGs from OB and surface epidural arrays over the SM, AI, VI and EC cortices from four awake cats, trained in a visuomotor or in an audiomotor task using CS+ /CS- stimuli. OB oscillations in the 50-60 Hz range were observed on the crests of the respiratory waves and in the 35-45 Hz range in the troughs of the slow waves both at rest and in the tasks. The OB bursts centered on 40 Hz in the task were often noted within 500 msec preceding the sensory stimuli (either CS+ or CS-) and before bar release (CR). They were regularly preceded or accompanied by bursts of similar frequency in SM and occasionally also in EC but rarely in AI or VI. Gamma bursts in AI became more apparent when the animals were retrained in the auditory task following visual discrimination. The spectra from AI, EC and VI showed an increase in beta but no change in the gamma range between task conditions and rest. The spectra from OB and SM did not change significantly in various behavioral conditions and across subjects. Jacobian matrices of nonlinear functions of EEG activity recorded simultaneously in the five brain areas (Gaál et al., 1995) revealed time segmentation of EEGs, relating to behavior, demarcated by valleys of low error. *Supported by NIH R37 MH06686-31 and ONR-N00014-93-1-0938.*

ALZHEIMER'S DISEASE: PATHOLOGICAL MECHANISMS

14.1

BRAIN β-SPECTRIN IS AN INTEGRAL COMPONENT OF AMYLOID PLAQUES IN ALZHEIMER'S DISEASE. R.K.Sihag* and A.M.Cataldo. Mailman Research Center, McLean Hospital, Harvard Medical School, Belmont, MA 02178.

Spectrin, an αβ heterodimer, is a major constituent of the neuronal membrane skeleton. Increased proteolysis of spectrin has been identified as an early neurodegeneration marker following glutamate excitoxicity, denervation, hypoxia and ischemia. In this study, we carried out immunohistochemical studies to examine whether or not spectrin was abnormally processed in Alzheimer's disease (AD). We raised antibodies to the N-terminal actin-binding region of β-spectrin$_{R\&G}$, C-terminal region of β-spectrin$_G$, and α-spectrin$_G$. We report here that the ß-subunit of spectrin is an integral component of ß-amyloid plaques. When tissues from postmortem AD brains were immunostained with either the N-terminal or the C-terminal domain-specific affinity-purified ß-spectrin antibodies, ß-amyloid plaques were specifically stained in the cortical parenchyma in approximately one third of the cases. The immunostaining was unaffected by preadsorption of ß-spectrin antibodies with A_4/β_{1-40} peptide. Pretreatment of the tissue sections from AD brains displaying β-spectrin negative amyloid plaques with formic acid for antigen retrieval did not show immunostaining with either the N-terminal or C-terminal domain-specific β-spectrin antibodies. The SDS-insoluble amyloid plaques were also stained by the ß-spectrin antibodies. The anti-α-spectrin antibody stained neuronal processes, but not amyloid plaques. The presence of ß-spectrin in the amyloid plaques in a subset of AD cases suggests that distinct biochemical pathways are involved in the formation or deposition of ß-amyloid plaques and that an abnormality of ß-spectrin structure or function may be involved in the formation or deposition of ß-amyloid in plaques in a subset of AD cases. Supported by AHAF.

14.2

METABOLISM OF PRION PROTEIN IN THE AMBER CODON 145 MUTATION N. Singh*, G. Zanusso, P. Gambetti, R.B. Petersen. Division of Neuropathology, Case Western Reserve University, Cleveland, Ohio 44106.

Several mutations in the prion protein gene (*PRNP*) segregate with the phenotype of Gerstmann-Straussler-Scheinker disease (GSS): a chronic inherited prion disorder characterized by the presence of prominent amyloid plaques containing prion protein (PrP). One of the mutations associated with GSS phenotype is the substitution of a tyrosine (TAT) to a stop codon (TAG) at position 145 (Y145*Amber*) of *PRNP* leading to the production of a truncated PrP isoform. We have generated an *in vitro* model of the disease using human neuroblastoma cells transfected with normal or Y145*Amber* *PRNP* construct in order to study synthesis and processing of Y145*Amber* mutant PrP (PrP^M). Our findings show that the PrP^M is highly unstable and is rapidly degraded in an intracellular compartment. The degradative process is likely to take place in the endoplasmic reticulum (ER) or in an ER-associated compartment since it is maintained even at 15 °C, a temperature which blocks vesicular transport from the ER. However, PrP^M degradation can be partially inhibited by ATP depletion or by inhibitors of ER proteases. Y145*Amber* provides the first example of PrP^M that is completely degraded in the ER. This finding raises questions concerning the mechanism of PrP conversion in the Y145*Amber* human disease. Contrary to the accepted models of PrP conversion that presuppose the transit through the cell surface for the PrP to be converted, Y145*Amber* PrP^M might convert into the amyloidogenic conformer inside the cell or in the extracellular space following secretion or release when with aging the ER degradation system becomes less effective. Sponsored by NIH grants AG08992 and AG08155 and the Britton Fund and AG08012.

14.3

EXPRESSION OF INFLAMMATION RELATED CYTOKINES (CHEMOKINES) IN BRAINS OF ALZHEIMER'S DISEASE. MQ. Xia, SQ. Qin, M. McNamara, C. Mackay, O. Berezovskaya* and B. T. Hyman. Mass General Hospital and LeukoSite, Inc, Boston, MA 02114.

Growing evidence suggests that brain may have the capacity of synthesizing its own repertoire of inflammatory cytokines. One class of cytokines, chemokines, have chemotactic activity for leukocytes. We examined the expression of a number of chemokines and their receptors in AD brain. Strong IL8RB immunoreactivity was present in neurons, dendrites, and axons in the normal hippocampal formation. In AD, IL8RB immunoreactivity was present in swollen dystrophic neurites surrounding plaques in the hippocampal formation and temporal neocortex. By confocal microscopy the IL8RB neurites did not co-localized with PHF-1 or AT8 (hyperphosphorylated tau) immunoreactive neurites. Gro α (or Nap2), another IL8RB ligand, was detected in astrocytes. Other chemokines, IP-10 and MIP1ß, were present in both normal and (in elevated amounts) in AD astrocytes. Chemokine expression did not vary consistently with duration of illness. These data add to growing evidence that inflammatory cytokines are expressed in brain as well as in the periphery. The presence of IL8RB in neuritic plaques and the elevated expression of other chemokines in astrocytes of AD suggest that inflammatory related responses may be involved in the development of AD pathology.

Supported by NIH grant 3P50AG05134

14.4

MICROGLIAL AND ASTROGLIAL ACTIVATION IN DIFFUSE AND NEURITIC PLAQUES IN ALZHEIMER'S DISEASE. D. R. Thal[1*], R. Schober[1], W. Härtig[2]. [1]Department of Neuropathology, University of Leipzig, 04103 Leipzig, Germany, [2]Department of Neurochemistry, Paul-Flechsig Institute for Brain Research, University of Leipzig, 04109 Leipzig, Germany.

Neuritic and diffuse plaques are hallmarks of Alzheimer's disease (AD). Diffuse plaques are known as amyloid deposits without a core and devoid of associated neuritic and neurofibrillary changes which are features of neuritic plaques. In both types of plaques microglial cells are found. Microglial cells in diffuse plaques are identified by IL-1α expression (Griffin et al., J Neuropath Exp Neurol 54: 276-281; 1995) whereas microglial cells in neuritic plaques additionally show MHC class II- and IL-6-immunoreactivity. To further differentiate microglial and astrocyte activation, we studied diffuse and neuritic plaques in AD cases using double labeling with antibodies directed against β-amyloid peptides (Aβ), MHC class II complex (HLA-DR), IL-6, CD 68, GFAP, apolipoprotein E (polyclonal and monoclonal), APP-695 and hyperphosphorylated τ (AT-8). Moreover, immunohistochemistry for such markers was combined with PAS and Gallyas counterstaining. Furthermore, plaques were screened electron microscopically for Aβ, APP-695 and apolipoprotein E. In microglial cells located within APP-695-positive neuritic plaques large amounts of PAS-positive lipofuscin pigments were found. GFAP-immunoreactivity was present in activated glial cells of the outer part of these plaques and in astrocyte processes of their inner part. In diffuse plaques no accumulations of activated astrocytes or activated MHC class II and IL-6 positive microglial cells were observed. These results suggest that microglial activation is not primarily necessary for the formation of Aβ-positive diffuse plaques but plays an important role in classical plaques.

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14.5

PATHOLOGICAL OBSERVATIONS IN STRUCTURES ASSOCIATED WITH PUPILLARY FUNCTIONS: EDINGER-WESTPHAL NUCLEUS. L.F.M. Scinto*, C-K. Wu, D. Saroff, & C. Geula, Harvard Medical School, Boston, MA 02115

Studies have demonstrated that hypersensitive pupil dilation to dilute tropicamide might serve as a marker for Alzheimer's disease (AD). Neuropathological data from patients with AD and normal elderly individuals suggest a possible mechanism for the observed hypersensitivity. Analysis of the distribution of AD pathology in the brainstems of normal individuals revealed rare amyloid deposits and almost no Alz-50 or PHF1 positive dystrophic neurites and no tangles. The oculomotor complex (CN3), including the EW, was completely devoid of any pathology. In contrast, AD cases exhibited many amyloid plaques and Alz-50 and PHF1-positive dystrophic neurites and neuropil threads. The highest concentration of brainstem amyloid deposits occurred within the central gray (CG). Consistent with its location near the CG, the EW nucleus also contained numerous amyloid plaques. In contrast the somatic portion of CN3 was virtually devoid of any amyloid deposits. The EW also contained collections of Alz-50-positive dystrophic neurites indicative of neuritic abnormalities. Occasional tangles were seen in the EW nucleus. A surprising finding was that most neurons of CN3, including the EW nucleus, contained immunoreactivity for APP and ß-amyloid. The axons of these neurons were particularly intensely stained. This pattern of staining was present in both normal and AD cases. Changes in the amount of APP or β-amyloid within these neurons remain to be investigated. Such changes might contribute to pathology in CN3, and particularly the EW nucleus. Our observations suggest that the presence of AD pathology might be specific to the EW nucleus and not represent general pathology within CN3. Selective pathology in the EW nucleus might lead to neuronal loss and eventually upregulation of cholinergic receptors at the iris, thus accounting for the observed hypersensitivity of AD patients. *This work was supported by a grant from Johnson & Johnson.*

14.6

HIERARCHICAL PATTERNS OF NEURONAL LOSS IN ALZHEIMER'S DISEASE. **T Gómez-Isla, J.L. Price, D.W. McKeel, Jr., J.C. Morris, S. Greenberg*, R.C. Petersen, J.E. Parisi, J.H. Growdon, and B.T. Hyman.** Dept. of Neurology, Massachusetts General Hospital, Boston, MA, Depts. of Anatomy, Pathology and Neurology, Washington University, St. Louis, MO & Depts. of Neurology and Pathology, Mayo Clinic, Rochester, MN.

One of the most consistent alterations in the Alzheimer's disease (AD) brain is the development of neurofibrillary tangles (NFTs) in layers II and IV of entorhinal cortex (EC) early in the disease process, likely contributing to memory impairment. Later on, as the cognitive deficits become broader, NFTs are also found in increasing number in feedforward and feedback projection neurons in layers II, III and V in high order association cortices. We have applied stereological principles to determine whether neuronal loss in AD brains follows similar patterns of selective vulnerability. We studied 53 individuals who had been evaluated at Massachusetts General Hospital, Washington University or the Mayo Clinic. Of 53, 10 were cognitively normal at death, and 43 had a diagnosis of definite AD with different degrees of cognitive impairment at death. Stereologically based neuron counts were carried out in the EC and in a high order association cortex, the Superior Temporal Sulcus (STS). In the cognitively normal individuals, no significant neuronal loss in either of the regions studied was present across age. In the very mildly impaired group the number of neurons in layer II EC decreased by half, in layer IV by 40%, and in the entire EC by 25%. No significant neuronal loss in the STS was found in the same group compared to cognitively normal individuals. In the group with severe cognitive impairment the loss in layer II was about 90%, and about 60% in the entire EC. The number of neurons in the STS was reduced by 50% in this group compared to controls. Neuronal loss paralleled NFT formation, but was independent of Aβ deposition. These results indicate that neuronal loss in AD brains does not occur in a random or widespread fashion but instead follows a hierarchical selective pattern of vulnerability. Support: NIH AG08487, AG03991, AG05681, AG08031, AG06786.

14.7

OXIDATIVE INJURY PREFERENTIALLY AFFECTS PROJECTION NEURONS IN ALZHEIMER CORTEX. **Neil W. Kowall*, Robert J. Ferrante, Robert H. Brown, Joseph S. Beckman, Ladislav Volicer, M. Flint Beal.** GRECC, Bedford VA Medical Center, Bedford MA; Dept. Neurology and Pathology, Boston University School of Medicine and Massachusetts General Hospital, Boston MA.

Oxidative injury has been implicated in the pathogenesis of Alzheimer's disease (AD) and other neurodegenerative disorders. Recent evidence suggests that both beta amyloid neurotoxicity and NFT formation are mediated by oxidation reactions. Consistent with this theory, biochemical and/or histochemical studies of AD brain have shown increased amounts of 8-hydroxydeoxyguanosine (8-OHDG), a marker for DNA oxidation; malondialdehyde (MDA), a marker of lipid peroxidation; heme oxygenase-1 (HO), an antioxidant enzyme, and protein carbonyls, a marker of protein oxidation. We used specific antibodies that recognize 8-OHDG, MDA, HO, 3-nitrotyrosine (3NT), a marker of protein nitration mediated by peroxynitrite, and thiol-specific antioxidant protein (TSA), a ubiquitous free radical scavenger present in human brain, in order to determine the histochemical distribution of oxidative injury. Postmortem samples of hippocampus and neocortex from seven patients with AD and four control patients with other neurological diseases were studied. The general pattern of immunoreactivity seen with 8-OHDG, MDA, 3NT, and HO were highly concordant. Control brains showed little or no immunoreactivity. In all AD cases layers III and V projection neurons in the neocortex and pyramidal cells in the hippocampal formation were immunoreactive. Prominent vascular staining was also observed with HO and MDA antibodies. TSA was concentrated in nonpyramidal neurons in AD. Occasional astrocytes were seen with all antibodies. Senile plaque neurites were prominently stained with NT. Our results indicate that the selective pattern of neuronal degeneration and NFT formation in AD cortex may be a direct consequence of oxidation reactions that preferentially damage pyramidal projection neurons. Supported by grants from the Department of Veterans Affairs and the NIH.

14.8

ELECTROPHYSIOLOGICAL EVIDENCE INDICATES THAT SENILE DEMENTIA OF THE ALZHEIMER TYPE IS AN AGE-RELATED AGING-INDEPENDENT PROCESS: A. L. Politoff* and N. Monson. Neuropsychiatric Research Institute, Fargo VA Medical Center and Dep. Neurosc., Univ. of North Dakota School of Med., 1919 Elm Street, Fargo, N. D. 58102.

A crucial question regarding senile dementia of the Alzheimer type (SDAT) is whether it is the end result of the natural aging of the brain, as postulated by the serial, intrinsic or aging-related model, or the result of some other mechanism that runs in parallel to normal aging, as postulated by the parallel, extrinsic or age-related model. This question can be answered by comparing variables that measure biological aging (aging-dependent variables, ADVs) of normal individuals and of SDAT patients. If the serial model applies, the values of the ADVs of SDAT patients should be at the upper end of the normal ADV curves. If the parallel model applies, the values of the ADVs of SDAT patients might be incompatible with the normal ADVs values. When we analyzed the electrical power contained in the different bands of the electroencephalograms (EEG) of normal adult individuals (n = 44) between 19 and 78 years of age we found several ADVs. In normal control subjects the power of the alpha band in the 2 Hz flash stimulated EEG at the posterior head regions increased with age, while the power of the delta band in the resting EEG at the anterior head regions decreased with age. In SDAT patients (n = 16) the magnitudes of the ADVs were significantly different from normal and opposite to the trend of normal aging. The predicted ages of SDAT patients, as measured by their ADVs, ranged between 37 and 41 years of age while their chronological ages ranged between 58 and 94. The present observations support the parallel model and show that SDAT involves a pathogenic mechanism that is different from normal aging.

Supported by a grant to ALP from the Neuropsy Research Institute of Fargo.

14.9

A TEMPORALLY GRADED NEURAL STRESS-TEST DEMONSTRATES ABNORMAL VISUAL FUNCTION FROM STRIATE THROUGH FRONTAL CORTEX IN ALZHEIMER'S DISEASE (AD) DURING PET.
M.J.Mentis*, G.E.Alexander, M.L. Furey, P. Pietrini, T. Strassburger, J. Krasuski, A. Dani, M.B.Schapiro, S.I.Rapoport. Lab. Neurosci., Natl. Inst. on Aging, NIH, Bethesda, MD 20892.

To determine the response to a temporally graded visual stress-test in AD, at all severity levels, we compared regional cerebral blood flow (rCBF) in 21 AD patients (71±9yr, MMS 15.6 range 0-28) and 19 controls (65±11yr) using PET. The visual stimulus was administered by goggles that flashed light alternately into each eye. All subjects had 5 scans, one at each of 0, 1, 4, 7, and 14 Hz frequencies. In controls, the stimulus evokes differential rCBF responses: biphasic in striate, monotonic increasing in posterior, and monotonic decreasing in frontal association areas. Compared to controls the AD group showed: smaller high frequency striate rCBF response, failure to activate V5 at 1 Hz, and smaller rCBF response in posterior and anterior association areas. rCBF response size was proportional to disease severity. Striate high frequency failure implied magnocellular greater than parvocellular failure at that level. Magnocellular failure was supported by AD inability to activate V5 (motion area with predominantly magnocellular input) in response to apparent motion at 1 Hz. As the percept changes were complex (form, luminance, motion, color), association area rCBF was interpreted as the integrated neural response to simultaneous visual processing of multiple visual modalities. Smaller responses in these areas in AD was interpreted as impairment of multi-modality visual processing. Funded by NIA intramural program.

14.10

PICK'S DISEASE AND ALZHEIMER'S DISEASE HAVE DIFFERENT COGNITIVE PROFILES.
G. Binetti*[1,2], I.J. Locascio[1,2], S. Corkin[2], and J.H. Growdon[1,2]. [1]Department of Neurology, Massachusetts General Hospital, Boston, MA 02114; [2]Department of Brain and Cognitive Sciences, Massachusetts Institute of Technology, Cambridge, MA 02139.

Pick's disease (PcD) is a rare neurodegenerative disease characterized pathologically by focal cerebral atrophy, and clinically by progressive dementia. No agreement exists on the clinical diagnostic criteria for PcD; it is often confused with Alzheimer's disease (AD). The aim of this study was to describe the clinical presentation of PcD and to compare the cognitive deterioration in PcD with that in AD. The subjects were 33 PcD patients (21M/12F; mean age, 66.2; mean education, 14.2; 10 with autopsy confirmed diagnosis) and 121 AD (46M/75F; mean age, 70.7; mean education, 13.1; 8 with autopsy confirmed diagnosis) from the patient population assessed longitudinally between 1985 and 1996. All took a set of cognitive tests that assessed memory, language, visuospatial abilities, abstract reasoning, and executive functions. The tests were administered from 1 to 6 times to PcD patients, and from 2 to 8 times to AD patients. Analysis of the first cognitive assessment (mean time after symptom onset: PcD, 2.8 years; AD, 3.3) showed significantly better performance by the PcD group than by the AD group on NYU Story Recall ($p<.001$) and the Luria Mental Rotation Test ($p<.04$). In order to characterize the rate of progression of the cognitive decline, we performed a nonlinear regression analysis, corrected for age, education, and duration of disease. Over time, PcD patients declined significantly more than AD patients on the Boston Naming Test ($p<.05$) and the Verbal Fluency Test ($p<.001$). The groups did not differ significantly in rate of decline on the other cognitive tests ($p<.1$). A relative preservation of episodic memory and visuospatial ability characterizes the early phase of PcD when compare to AD. The sharper decline of language abilities in PcD sharpens the clinical distinction from AD, and reflects the underlying frontal-lobe and temporal-lobe atrophy.

14.11

ESTROGEN REPLACEMENT THERAPY AS A PROTECTIVE FACTOR FOR ALZHEIMER DISEASE: INTERACTIONS WITH OTHER RISK FACTORS.
A.J. Lerner*, E. Koss, S. Gilham, R. Cole, S. Debanne, C. Esteban - Santillan, , G. J.Petot, D.Y. Rowland, K.A. Smyth, P.J. Whitehouse, R.P. Friedland, Alzheimer's Center and Laboratory of Neurogeriatrics, Case Western Reserve University School of Medicine, Cleveland, OH 44106.

Estrogen Replacement therapy (ERT) has been proposed to be a protective risk factor for the development of Alzheimer's disease (AD). This effect may be mediated by estrogen receptors in brain regions important in AD, such as basal forebrain and hippocampus, and effects of estrogen on cholinergic neurotransmission, modulating levels of neurotrophic growth factors, and maintaining synaptic density. Previous clinical studies of ERT on AD risk have yielded conflicting results.We studied ERT as part of an epidemiologic study on AD risk factors, and assessed the interaction of ERT with cigarette smoking, also postulated to be a protective risk factor for AD. Our female study population consisted of 78 AD cases and 177 controls. AD patients were less likely to have received ERT during each two-decade surveyed. Comparing cases and controls, the odds ratio for ERT alone was 0.41 (95% confidence interval 0.12 - 0.69). When ERT and smoking were combined, the odds ratio was 0.18 (95% C.I. 0.13 - 0.23;). These preliminary data suggest that estrogens interact with other risk factors, such as smoking, in a synergistic fashion to protect against the development of AD. Other risk factors such as Apolipoprotein E genotype need to be evaluated for their interaction in this regard.
Supported by NIA, and Philip-Morris, U.S.A.

14.12

LEFT-HANDEDNESS AND THE RISK OF ALZHEIMER'S DISEASE.
V.W. Henderson,* W.A. Kukull, J.G. Buckwalter, E.B. Larson. Depts. of Epidemiology and Medicine, Univ. of Washington, Seattle, WA; Depts. of Neurology and Psychology and Sch. of Gerontology, Univ. of Southern California, Los Angeles, CA.

Left-handed persons are thought to differ from right-handers in terms of cerebral organization and environmental exposures. Conflicting contentions that left-handers may face an altered risk of Alzheimer's disease (AD) have not been previously evaluated in a defined population. We studied incident AD cases (n = 265, mean age of 79 years) and frequency matched non-demented controls (n= 331), where all subjects were enrolled in a regional health maintenance organization. 13% of AD cases and 8% of controls were reported to be left-handed (a group that included ambidextrous subjects). In multivariate analyses that adjusted for age, education, and gender, cases were more likely to be left-handed (odds ratio = 1.8, 95% confidence interval = 1.0 - 3.0). For left- and right-handers, dementia severity was similar at the time of study enrollment, but left-handers were significantly younger when their dementia symptoms first appeared (74 versus 77 years). These data are consistent with a modestly increased risk of AD among left-handers.
Supported in part by NIH grants AG06781, AG07584, AG05136, AG05142.

INGESTIVE BEHAVIOR: REGULATORS OF INGESTION

15.1

FOURTH VENTRICULAR ADMINISTRATION OF GLUCAGON-LIKE PEPTIDE-1 (7-36) amide (GLP-1) INHIBITS FOOD INTAKE IN RATS.
T.H. Moran*, A. Wohn, G.J. Schwartz and E.E. Ladenheim. Department of Psychiatry, Johns Hopkins University School of Medicine, Baltimore, MD 21205.

To identify sites of action through which the brain/gut peptide GLP-1 inhibits food intake, we examined the effect of 4th ventricular infusions of GLP-1 on 30 min glucose (0.125 g/ml) intake in rats following a daytime six hour food deprivation. Rats equipped with a chronic 4th ventricular cannula were adapted to daily access to a glucose solution. Cannula placement was assessed by examining the ability of 4th ventricular bombesin to inhibit food intake. On test days, GLP-1 in doses of 0.1, 0.32, 1.0 and 3.2 nmoles/rat or saline vehicle were infused five minutes prior to glucose access in a volume of 3 µl and subsequent glucose consumption was measured. Begining at the lowest dose (0.1 nmol), injections of GLP-1 significantly reduced intake relative to vehicle control. At a GLP-1 dose of 0.1 nmol, intake was reduced by 32 %. The effect appears to be maximal at a GLP-1 dose of 1 nmol which resulted in a 60 % suppression of glucose intake. The ability of 4th ventricular GLP-1 to inhibit intake, and the potency with which this occurs, suggests that the hindbrain is a site for GLP-1's actions in the control of food intake. (Supported by DK19302 and DK46448).

15.2

Cholecystokinin acts on the duodenum to reduce sucrose intake.
J.E. Cox*. Dept. Psychology, Univ. Alabama at Birmingham, Birmingham, AL 35294.

This experiment investigated the proposal that CCK-A receptors mediating cholecystokinin satiety are located within the duodenum. Adult, male Sprague-Dawley rats received bolus infusions of the CCK-A antagonist devazepide (20 µg/kg) into the jugular vein (IV group; N=6) or superior pancreaticoduodenal artery (SPD group; N=6). This artery supplies the proximal duodenum. Following 6 h food deprivation, rats received devazepide or vehicle followed immediately by intraperitoneal injections of CCK-8 (2 µg/kg) or saline. Intake of 30% sucrose was measured over the next 15 min. In the SPD group, devazepide significantly antagonized the actions of both exogenous and endogenous cholecystokinin: (1) When devazepide was infused, CCK-8 injections reduced sucrose intake less (36.5%) than they did when rats received vehicle infusions (71.8%; $P<.01$); (2) On tests without CCK-8 administration, devazepide increased intake by 27.4% above baseline ($P<.01$). By contrast, IV infusions of devazepide were ineffective in both respects (P's$>.25$). The enhanced potency of devazepide when administered into an artery perfusing the duodenum supports the hypothesis that endogenous cholecystokinin acts locally to reduce intake. Moreover, these results are consistent with exogenous cholecystokinin having the same mechanism of action.

15.3

LICK PATTERN AND INTAKE AS FUNCTIONS OF GLUCOSE CONCENTRATION AND LICK VOLUME. J.P. Baird, H.J. Grill, & J.M. Kaplan*. Dept. Psychology, Univ. of Pennsylvania, Phila., PA, 19104.

We showed previously in rats ingesting 12.5% glucose from a drinking spout that meal size was actively defended despite explicit manipulation of the amount delivered per lick (drop size). For the current experiment we evaluated the generality of this finding by varying glucose concentration. Daily, rats were permitted 1 h to ingest one of five concentrations of glucose (0.0, 3.2, 6.25, 12.5 or 25%) at one of three drop sizes (2.5, 5.0 or 7.5 ul), until all combinations were tested for each rat. A typical inverted-U concentration-intake function was obtained. There was no overall effect of drop size on amount consumed, however. The defense of intake against the drop size challenge was accomplished through broad adjustments in the licking pattern. Within each concentration, the number of licks varied over a 3-fold range. Meal duration generally increased with increasing concentration but decreased with increasing drop size. Initial (first minute) lick rates increased with concentration, as expected. Drop size also affected initial lick rates, with significantly lower values obtained with the largest relative to the two lower drop size conditions. There were considerable individual differences in the extent to which the increased number of licks with drop size increase was mediated by adjustments in burst number versus licks/burst. We conclude that rats exhibit considerable flexibility in the licking structure of their meals, apparently in order to preserve what may be reasonably regarded as an intake goal specified for each concentration. Supported by NIH DK42284.

15.4

THE WEIGHT-REDUCING EFFECTS OF FLUVOXAMINE IN ZUCKER RATS ARE MEDIATED BY CORTICOTROPIN-RELEASING HORMONE (CRF). I.Wieczorek, C.Schulz, M.Grözinger[1], H.Lehnert. Dept. of Internal Medicine - Endocrinology, Otto-von-Guericke University, 39120 Magdeburg, Germany; [1]Dept. of Psychiatry, University of Mainz, 55101 Mainz, Germany

Previous studies have demonstrated an inhibitory effect of i.p. injection of fluvoxamine, a selective serotonin reuptake inhibitor, on food intake and body weight in rats. The present study was designed to examine whether the effect of fluvoxamine on food intake is centrally mediated by CRF. A fused silica capillary (144µm od) was implanted under chloralhydrate anesthesia into the lateral ventricle of genetic obese Zucker rats fa/fa (12 weeks old) for i.c.v.- injections. The femoral artery was catheterized for blood sampling. On the first day post surgery, animals received an i.p. injection of fluvoxamine (25 mg/kg) and ∝-CRF (25µg/kg) i.c.v., a CRF receptor antagonist. Blood samples were taken before and in 30 min intervals over a 2 hour period after injection. The i.p. and i.c.v. treatments were repeated on 7 consecutive days. In the control group, saline injections were administered i.p. and i.c.v., respectively. Body weight and food intake were monitored daily. During the experiment, animals treated with fluvoxamine i.p./saline i.c.v. exhibited a weight loss of 6-7 g/day, differing significantly from the control group (saline i.p./saline i.c.v.) with a daily weight gain of 5 g. A weight gain of 2 g/day was observed in the group treated with fluvoxamine i.p. and ∝-helical CRF i.c.v.. Food intake was not affected by the different treatments. The results of our study very clearly demonstrate, that the inhibitory effects of fluvoxamine on weight gain are mediated by CRF and are independent of food intake. The apparent discrepancy between unaltered food intake and reduced weight gain can possibly be explained by stimulation of the autonomic nervous system and enhanced thermogenesis. This work was supported by Upjohn.

15.5

POTENT SUPPRESSIVE EFFECTS OF THE PUTATIVE SATIETY AGENT GLP-1 ON SOCIAL-EMOTIONAL BEHAVIORS. J. Panksepp*, M.Y.V.Bekkedal and M. Walter, Dept. of Psychology, Bowling Green State University, Bowling Green, OH 43403

With an ever increasing list of putative satiety peptides that are being postulated, new methods need to be developed to sift natural satiety mechanisms from the many nonspecific ways, such as emotional changes, that appetite can be temporarily suppressed. Glucagon-like peptide-1 (GLP-1) has recently been proposed to be a normal satiety factor (Turton, M.D. et al., Nature, 1996, 379, 69-72), and here we evaluated the effects of this peptide on various social behaviors including separation-induced distress vocalizations (DVs) in young chicks and rough-and-tumble play in juvenile rats, as well as the ability of this peptide to restore normal play behavior in food-deprived animals.

Intraventricular GLP-1 (1 ug) in chicks not only reduced food intake but very markedly and dose-dependently reduced DVs. At 0.5-1.0 ug DVs were eliminated for almost an hour, while at the 2.0 ug the effect lasted almost 2 hrs. The effect was not naloxone reversible as it is with opioids. The 1 ug dose reduced play (as monitored by dorsal contacts and pins) by about 50% in juvenile rats. A day of food deprivation also markedly reduced play which was returned to normal by a half hour meal. This satiety effect was not simulated by the administration of 1 ug of GLP-1 to the food-deprived animals. Rather, play was even further reduced by the peptide. The data indicate powerful emotion modulating effects of GLP-1, with no clear indication that the peptide can produce play effects resembling normal satiation. Accordingly, our data do not support the idea that GLP-1 is a normal satiety factor of the body.

Supported by NIH grant HD 30387.

15.6

GLUCAGON-LIKE PEPTIDE-1 (7-36 AMIDE) ELICITS BEHAVIORAL SATIETY IN SHAM FEEDING RATS. L. Asarian, A.M. Cuomo, E. Corp, B. Hrupka, and N. Geary*. Bourne Laboratory, Dept of Psychiatry, Cornell Medical College, White Plains, NY 10605.

Glucagon-like peptide-1 (7-36 amide) (GLP-1) and its receptors occur in several brain regions and may play a role in the physiological control of feeding behavior (Turton *et al., Nature 379*:69, 1996). To test whether GLP-1 is sufficient to inhibit feeding and elicit satiety in the absence of postingestive food stimuli, 10 rats were implanted with gastric sham-feeding cannulas and lateral ventricle infusion cannulas. Rats sham fed 0.8 M sucrose for 45 min beginning 5 min after icv injection of 2.5 µl aCSF with 0-30 µg GLP-1. Behaviors were measured each minute using a time sampling technique. Rats initiated sham feeding as rapidly after GLP-1 as after aCSF and sham fed similar amounts for 6 min, but subsequent sham feeding was potently inhibited by each GLP-1 dose (Table).

Sham intake	aCSF	3 µg	10 µg	30 µg
1-3 min	3.7 ± 0.5	3.8 ± 0.9	3.5 ± 0.8	4.8 ± 0.5
4-6 min	4.5 ± 0.4	3.8 ± 0.4	3.0 ± 0.6	3.2 ± 0.4
7-9 min	5.1 ± 0.4	3.7 ± 0.5*	2.8 ± 0.7*	2.7 ± 0.5*
1-45 min	61.0 ± 4.3	40.1 ± 5.4**	29.5 ± 5.8**	31.6 ± 5.9**

Intakes are ml, m ± sem; *Less than aCSF, $p < 0.05$; ** $p<0.001$.

No abnormal behaviors were observed, and 30 µg GLP-1 elicited resting, the terminal item in the behavioral sequence of postprandial satiety in real feeding rats (median latency to rest, 33 min vs. 46 min after aCSF, $p<.02$). These data indicate that icv GLP-1 (i) does not affect rats' initial orosensory response to sucrose and (ii) is sufficient to inhibit feeding and induce satiety even in the absence of gastric and postgastric food stimuli. (NIH MH51135)

15.7

A REEXAMINATION OF THE ROLE OF THE GLUCOCORTICOIDS ON GLUCONEOGENESIS AND THEIR IMPACT ON FOOD INTAKE AND BODY WEIGHT. Ali Moshirfar, L. Terranella, O Trocki, K. Kamara, and T.W. Castonguay* Department of Nutrition and Food Science, University of Maryland-College Park, MD 20742

The effects of adrenal steroids on several of the neural controls of food intake and body weight have been described. By contrast, little is known about how the glucocorticoids, via their control of hepatic gluconeogenesis, might also participate in the control of intake. In a series of studies, we have demonstrated that dietary factors (intragstric load of L-tryptophan (TRP)) can dramatically and quickly alter gluconeogenesis. TRP increased assayable phosphoenolpyruvate carboxykinase (PEPCK) activity, but in vivo catalysis was not observed. Further, we have demonstrated that either adrenalectomy (ADX) or RU 486 reduced PEPCK activity as well as food intake and body weight gains. Corticosterone replacement failed to restore PEPCK activity to control levels. Fructose 1,6 bis-phosphotase (FBP) activity was also reduced by ADX, TRP or RU 486. Corticosterone replacement following ADX prevented the decrease in FBPase activity as well as the decreases in food intake and body weight gain. These results suggest that normal control of food intake is achieved when glucose production remains unimpaired. However, if hepatic glucose production is attenuated (as in the case of ADX's simultaenous effects on both FBP'ase and PEPCK) then food intake and body weight gain is suppressed.

15.8

MERCAPTOACETATE ENHANCES INDEPENDENT INGESTION IN RAT PUPS. S. E. Swithers*. Dept. of Psychological Sciences, Purdue University, West Lafayette, IN 47907-1364

In adult rats, administration of a blocker of fatty acid oxidation, mercaptoacetate (MA), increases ingestion (e.g. Langhans & Scharrer, *J. Auton. Nerv. Sys., 18:13-18*, 1987). Previous work has suggested MA does not enhance suckling intake in preweanling rats (Leshem, Flynn & Epstein, *Am. J. Physiol., 258:R365-R375,* 1990). However, rat pups will also display ingestive behavior that is independent of the mother and suckling. The present study investigated the development of effects of MA on such independent ingestion. Rat pups were tested once at 6, 9, 12 or 15 days of age. Prior to testing, pups were injected i.p. with 100, 200, 400 or 800 µmol/kg, (100 µmol/ml) MA or 0.135 M saline. One hour following the injection, pups were placed into test containers lined with paper towels soaked with commercial half and half and allowed to ingest for 30 minutes. In 6- and 9-day-old rats, MA did not affect intake at any dose. However, in 12- and 15-day-old rats, doses of 100, 200 and 400 µmol/kg MA enhanced intake, while a dose of 800 µmol/kg suppressed intake. These results demonstrate that ingestive responsiveness to MA develops between 9 and 12 days of age in rats.

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15.9

IDENTIFYING ROLES FOR INTESTINAL SIGNALS IN THE SATIATING EFFECTS OF FATS IN HUMAN ADULTS. D.A. Booth*, L.A. Dibsdall & N.W. Read. Food & Nutrition Lab., Sch. Psychology, Univ. Birmingham, Edgbaston B15 2TT, U.K.

Foods and their macronutrient contents have no inherent palatability or satiating efficacy. Suppression of food intake by fats depends on physical form as well as chemical structure, and on the timing of the meal's sensory and postingestional actions to coincide with access to foods. We hypothesised that fat which floats above aqueous contents of the stomach is delayed in its action on intestinal chemoreceptors that suppress appetite for food, while fat in aqueous emulsion begins to empty immediately and so may induce an early satiety. This was tested by a method for identifying post-oral control of intake: appetite ratings are corrected for effects on appetite the rater predicts after sensing the load menu and its immediate postingestional effects. The current-minus-expected amount of food wished for eating was -ve at 1 h after high-fat breakfasts with emulsified fat; it remains to be seen if this satiety effect of cream can be prompt and strong enough to condition down the size of meals on that menu, as rapidly digested carbohydrate does. In contrast, for about 2 h after a high-fat breakfast, cur.-exp. hunger-rating differences were +ve with (separating) spread fat. Yet, near lunchtime, post-oral satiety was just as strong after either form of fat as after the carbohydrate in high-starch or high-sugar breakfasts. [Supported by U.K. Government M.A.F.F.]

15.10

CCK-33 INHIBITS FOOD INTAKE AFTER INTRAPORTAL AND INTRAVENOUS ADMINISTRATION. G.P. Smith*, D. Dorré, and L. Melville. Bourne Lab, NY Hospital-Cornell Medical Center, White Plains, NY 10605.

When CCK-8 is administered to rats, its satiating potency depends on the route of administration: Intraperitoneal (ip) CCK-8 is much more potent than hepatic-portal (hp) or intravenous (iv) administration (Greenberg et al., 1987; Strubbe et al., 1989). We interpreted this differential potency to mean (1) that endogenous CCK-8 had a paracrine, not an endocrine, mode of action, and (2) that the reduced potency of CCK-8 after hp administration was due to hepatic uptake and degradation of peptides of less than 10 amino acids (Gores et al., 1986).

To test this interpretation, we administered CCK-33 ip, hp, and iv to male rats (n=5-9) given access to a high-carbohydrate liquid diet for 30 minutes after 16 hours of food deprivation. CCK-33 (6.6, 13.2, and 27.4 ug/kg) was injected ip 5 minutes before the test meal or infused hp or iv (0.39 ml/ min for 12.8 min). Infusions began 5 minutes before the test meal and ended about half-way through the meal so that the total dose was delivered prior to the termination of the meal. The rank order of satiating potency of CCK-33 was ip>hp>iv. The fact that the satiating potency of CCK-33 was largest after ip administration suggests that CCK-33, like CCK-8, acts locally on CCK_A receptors in the abdomen, presumably in the upper small intestine and pyloric sphincter. The larger potency of CCK-33 after hp infusion than after iv infusion is consistent with an additional site of action of CCK-33 in the liver and with CCK-33 being more resistant than CCK-8 to uptake and degradation by the liver.

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15.11

DUODENAL NUTRIENT EXPOSURE ELICITS ORGANIZED GASTROINTESTINAL MOTILITY AND GUT VAGAL AFFERENT SIGNALS IN RAT. G.J. Schwartz* and T.H. Moran. Dept. of Psychiatry & Behav. Sci., Johns Hopkins Univ. Sch. Med., Baltimore, MD 21205, USA

Upper gastrointestinal exposure to meal-related nutrient stimulation contributes to the negative feedback control of food intake in a variety of feeding paradigms, and a role for the afferent vagus in this negative feedback control has been demonstrated. We have been investigating the gastrointestinal and vagal afferent neural events arising from the upper gut in response to duodenal nutrient exposure in rats. Luminal stimulation of the upper 4 cm of the small intestine with 0.2 kcal/ml glucose, maltose or peptone, induces dramatic, short-latency alterations in the pattern of gastric and duodenal motility, compared to isotonic saline. This changed pattern of motility is characterized by an increase in the strength and duration of discrete gastric and duodenal contractile events. These organized changes in motility are paralleled by increases in the frequency and intensity of discrete bursts of activity in gastric and duodenal vagal mechanosensitive afferents. The motility and vagal afferent responses following peptone administration significantly outlasted those elicited by either equicaloric maltose or glucose. These data reveal nutrient chemosensitivity in the duodenal lumen, and demonstrate that neurophysiological activity in vagal afferent fibers supplying gastric and duodenal targets reflect the ability of the duodenum to discriminate its contents. Supported by the Whitehall Foundation and DK 47208.

15.12

EFFECTS OF TACHYKININS AND MAMMALIAN BOMBESIN-LIKE PEPTIDES ON SALT APPETITE: ROLE OF POST-ORAL FACTORS. M.E. SMITH*, L. TANGEMAN AND F.W. FLYNN. Dept. of Psychology and Graduate Neuroscience Program, Univ. of Wyoming, Laramie, WY,

The tachykinins (TK) and the bombesin (BN)-like peptides have commonalities in both function and distribution. Both of these families of peptides are effective in suppressing need-free and sodium need-induced NaCl intake (salt appetite). The possibility that these neuropeptides have a common mechanism of action and suppress salt intake by potentiating post-oral signals was evaluated by testing the potency of TK and BN-like peptides in sodium deficient, sham drinking rats. The effects of lateral intraventricular injections of saline, or selective NK_3 receptor agonists (200 ng SENK, 200 ng $MePhe^7$ NKB, or 200 ng NH_2-senktide) on salt intake (0.5 M NaCl) by sodium deficient rats in the gastric fistula open and closed conditions were tested. A second group of rats was tested in the identical manner except they were administered intraperitoneal injections of saline, 4, 8, and 16 µg/kg BN, gastrin releasing peptide (GRP), or neuromedin B (NMB). *RESULTS*: Intraventricular injections of SENK suppressed both sodium deficiency induced salt intake in both the fistula open and closed conditions. NH_2-senktide suppressed intake in only the open condition, and $MePhe^7$ NKB had no effect in either fistula condition. Peripheral injections of BN and GRP were also potent in suppressing sham drinking of salt by sodium deficient rats. NMB had no effect on salt intake in either fistula condition. The results show that the effects of SENK, BN and GRP on salt intake are largely independent of ingestion-contingent feedback. (Supported by NIH NS24879 to F.W.F.)

SOMATOSENSORY CORTEX AND THALAMOCORTICAL RELATIONSHIPS I

16.1

NMDA RECEPTOR MEDIATED HORIZONTAL CONNECTIVITY IN LAYER IV OF MOUSE BARREL CORTEX, M.J. Gutnick*, I.A. Fleidervish, A.M. Binshtok, Zlotowski Center for Neuroscience and Faculty of Health Sciences, Ben-Gurion University of the Negev, Beersheva, Israel.

Although the coronal plane of section for neocortical slice experiments entails less damage to pyramidal cell dendrites and leaves intra-laminar circuitry intact, it disrupts much of the horizontal connections within a layer. We report a new neocortical brain slice preparation which is cut tangential to the pial surface; we apply it to the study of Layer IV stellate neurons in somatosensory cortex of juvenile (P18 - P22) mice.

Tangential slices (400 µm thick) were obtained by cutting in a plane parallel to the pial surface. In the one slice from each hemisphere that included layer IV, the typical barrel organization was readily visualized at low magnification. With higher magnification IR-DIC video microscopy, patch pipettes were directed to individual stellate neurons for whole cell recording. During the experiments, spiny and smooth neurons were tentatively distinguished on the basis of soma size; cells were filled with biocytin for subsequent morphological study. In current-clamp, 80% of neurons were regular spiking cells with varying degrees of spike adaptation, and the rest were fast-spiking; none were bursters, though we can identify intrinsic bursters in coronal slices using the same techniques. Bicuculine (10µM) induced paroxysmal events that propagated from barrel to barrel. Although 2 mM Mg^{2+} was present in the bath, the synchronous discharge was mediated by NMDA receptors, since it was insensitive to 10 µM CNQX, and reversibly blocked by 50 µM APV. In whole cell recordings, individual spiny stellate neurons showed two types of NMDAR-mediated evoked EPSC which were distinguished on the basis of different Mg^{2+} sensitivities. The data suggest that long after the early critical period of development, Layer IV cells express more than one NMDAR subunit composition, and that a relatively voltage-insensitive receptor is prevalent in the local excitatory synaptic circuitry.

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16.2

$GABA_B$-DEPENDENT BURST-FIRING IN THALAMIC NEURONS: A DYNAMIC CLAMP STUDY. D. Ulrich* and J. R. Huguenard. Dept. of Neurology and Neurological Sciences, Stanford University School of Medicine, Stanford CA 94305.

Synchronized network responses in thalamus depend on phasic inhibition originating in the thalamic reticular nucleus (nRt), and mediated by the neurotransmitter γ-amino-butyric acid (GABA). The suggested role of intra-nRt connectivity in phasing its output remains controversial. Recently functional $GABA_B$ receptors were demonstrated on nRt cells and the slow time course of the $GABA_B$ synaptic response seems ideally suited to deinactivate low threshold calcium channels. This would lead to burst firing, which is a characteristic feature of synchronized responses. In the present study we investigate $GABA_B$ mediated rebound burst firing in thalamic cells. Whole-cell current-clamp recordings were obtained from nRt cells and from thalamocortical (TC) relay cells of somatosensory thalamus in brain slices obtained from Sprague-Dawley rats at P11-13. An artificial hybrid computer-neuron synapse (dynamic clamp) was activated in each cell type. Dynamic clamp $GABA_B$ IPSPs triggered rebound low threshold calcium spikes in both nRt and TC cells, if the peak hyperpolarization of the IPSPs was greater than -92 mV. The threshold IPSP conductance for rebound burst generation was similar in the two cell types (nRt: 7 ± 1 nS ($n=13$); TC: 5 ± 1 nS ($n=8$); mean ± s.e.m). However, burst onset in nRt cells (1 ± 0.07 s) was considerably delayed compared to relay cells (0.6 ± 0.08 s).

These results indicate that while $GABA_B$ IPSPs are capable of generating low threshold calcium spikes in both relay and nRt neurons, the resultant output frequency in TC cells would be in the range of 3 Hz but would be much slower in nRt cells. Furthermore, the minimal synaptic conductance required in nRt cells was much larger than the maximal $GABA_B$ conductance inferred from physiological measurements. Together, these findings suggest that intra-nRt $GABA_B$ mediated events contribute little to synchronization of thalamic oscillations in the frequency range > 2 Hz. (Supported by NS 06477, the Pimley Research Fund, and the Roche Research Foundation.)

16.3

NORADRENERGIC AND CHOLINERGIC MODULATION OF AUGMENTING RESPONSES IN THALAMOCORTICAL PATHWAYS. M.A. Castro-Alamancos* and B.W. Connors, Department of Neuroscience, Brown University, Providence RI 02912.

The augmenting response is a form of short-term plasticity that is elicited in sensorimotor neocortex by applying two or more stimuli to the ventrolateral nucleus of the thalamus at a frequency of 7-14 Hz; the response to the second and subsequent stimuli in a train are enhanced, reaching a steady state by the third stimulus. Augmenting responses are very reliably generated during anesthesia, slow-wave sleep and awake immobility, but are inactivated during certain awake behavioral states, such as exploration of an open field and skilled motor performance.

In anesthetized rats, using 16-channel silicon probes with recording sites equally spaced (100 μm) along the cortical depth, and a microdialysis probe to infuse drugs directly into the area of recording, we investigated the role of the noradrenergic and cholinergic systems in modulating the augmenting response. Application of nicotine enhanced similarly the response to the first pulse and the augmenting response. Application of muscarine depressed similarly both the response to the first pulse and the augmenting response. In contrast, application of norepinephrine produced a selective inactivation of the augmenting response with little effect on the response to the first pulse. The results suggest that a selective intracortical modulation by noradrenergic afferents may be responsible for the behavioral state-dependent inactivation of augmenting responses.

Supported by the NIH and ONR.

16.4

VB THALAMIC AFFERENTS HAVE LITTLE INFLUENCE ON LAYER-5 DESCENDING CORTICOFUGAL NEURONS OF RABBIT S1 BARREL CORTEX. H. A. Swadlow*, Dept. of Psychology, University of Connecticut, Storrs, CT 06269

In previous work in the awake rabbit (Swadlow, J. Neurophysiol., 1995) three lines of evidence converged to indicate that VB thalamic barreloid afferents exert a potent monosynaptic excitatory influence on suspected inhibitory interneurons (SINs) of the topographically corresponding barrel: (1) the great majority of SINs in layer 4, and some in layer 5 showed brief, significant peaks in shift-corrected cross-correlograms with VB afferents, (2) each of these SINs responded at very low thresholds to microstimulation of VB neurons (mean threshold = 4.9 μA), (3) SINs in the vicinity of layers 4 and 5 were among the first cells in cortex to respond to peripheral stimulation, responding at a median latency of 6.6 ms, only 0.63 ms greater than the responses of VB axon terminals entering the cortex.

The present work extends this analysis to descending corticofugal neurons of layer 5 (CF-5 neurons). In contrast to the result for SINs, the same converging lines of evidence indicate that VB barreloid afferents have little influence on CF-5 neurons of the corresponding barrel: (1) no significant peaks were seen in the shift-corrected cross-correlograms of VB barreloid afferents and CF-5 neurons of the corresponding barrel (17 VB-CF-5 pairs tested), (2) None of 11 CF-5 neurons tested were synaptically activated by microstimulation of the corresponding barreloid at intensities of < 10 μA. At higher intensities some CF-5 neurons were activated, but at much longer latencies that was seen in SINs and (3) CF-5 neurons responded to peripheral stimulation at much longer latencies than was seen in SINs, and none responded at latencies of < 8 ms (Swadlow and Hicks, J. Neurophysiol., 1996).

Some of the SINs receiving monosynaptic VB input were clearly in layer 5, at the same depth or deeper than CF-5 neurons found in the same microelectrode penetrations. This result indicates that both cortical depth and cortical cell type are important factors determining the synaptic inputs to a cortical neuron. Previous work has documented that the corpus callosum provides a powerful excitatory synaptic input to CF-5 neurons of rabbit S-1 (Swadlow, J. Neurophysiol., 1990). This result, combined with the failure to observe a physiologically significant input from VB afferents suggests that CF-5 neurons may be selectively involved in the integration of corticocortical inputs. Supported by NS32021.

16.5

CORTICAL SOMATOSENSORY OSCILLATORS AND THE DECODING OF VIBRISSAL TOUCH Ehud Ahissar,* Gabriel Arkon, Miriam Zacksenhouse, Sebastian Haidarliu, Dept. of Neurobiology, The Weizmann Institute of Science, Rehovot 76100, Israel.

Many rodents utilize rhythmic vibrissal movements of 5-11 Hz to localize and identify objects. We suggest that vibrissal temporal information is decoded by thalamocortical phase-locked loops (PLLs). In the PLL model, cortical oscillators develop expectations concerning the timing of the next input, and thalamic cells compare these expectations with the actual input. PLL operation depends on the cortical oscillating frequencies (i) being in the range of peripheral frequencies, and (ii) being controllable by synaptic input. We characterized cortical oscillators in the somatosensory cortex of the guinea pig. Of 1119 units recorded from 11 anesthetized guinea pigs 546 (49%) showed signs of oscillations, and of them 189 (17%) were clearly oscillating. The oscillation frequencies were distributed in a tri-modal fashion with modes at ~1, 10 and 100 Hz. In many cases the 1 Hz oscillations appeared as synchronous activity of a group of cells, while those of 10 and 100 Hz were independent single-unit oscillations. Oscillating frequencies were increased, usually in a dose-dependent manner, by local applications of glutamate in 12/20 cases and were not affected in 7/20; one cell was inhibited. The locally-controllable oscillators in the 10 and 100 Hz fit the requirements of PLL circuits that decode object location and texture, respectively.

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16.6

SIMULTANEOUS NEURONAL ENSEMBLE RECORDINGS AT MULTIPLE TRIGEMINAL SYSTEM LEVELS: SELECTIVE CORTICAL RESPONSIVENESS TO ACTIVE DISCRIMINATIVE WHISKING. John K. Chapin*(1), Ronald S. Markowitz(1) and Miguel A.L. Nicolelis(2), (1)Dept. of Neurobiol. & Anat., Med. College of PA & Hahnemann Univ., Phila., PA. (2)Dept. of Neurobiol., Duke Univ., Durham, NC

To investigate the mechanisms of somatosensory information processing during active tactile discrimination, ensembles of single neurons were simultaneously recorded through microelectrode arrays chronically implanted in up to 5 levels in the rat trigeminal somatosensory system, including the trigeminal ganglion (Vg), principal (PrV) and spinal (SpV) trigeminal nuclei, ventral posteromedial (VPM) thalamus, and primary somatosensory (SI) cortex. Blindfolded rats were trained to stand on a narrow platform and project their mystacial vibrissae across a wide gap to actively discriminate between a variety of randomly arranged tactile objects. The "correct" tactile object marked the position of a narrow slot through which the rat could jump and obtain a water reward. Active whisking movements were detected through videotaped records and EMG recordings in whisker protractor muscles. Marked differences were observed in the responses of neurons at lower vs. higher levels of this system during discriminative vs. non-discriminative whisking. Spontaneous non-discriminative whisker movements "through the air" produced robustly increased activity of neurons at lower levels of this system (e.g. Vg and PrV), but these responses were successively suppressed through higher levels of this system (VPM and SI). In contrast, neurons at the higher levels were generally more responsive than those at lower levels to discriminative whisker contact on the tactile objects, though the patterns of neuronal ensemble response varied widely across the range of objects. SI cortical neurons became particularly active when the correct tactile object was discriminated, but were then strongly suppressed as the rat began to jump across the gap and through the slot. To conclude, as tactile input from mystacial whiskers ascends to the cortex, neuronal responses to it become increasingly reflective of the sensorimotor and behavioral "significance" of the sensory information. *Supported by NIH grant NS23722 and ONR grant N00014-95-1-0246 to JKC.*

16.7

MULTIPLE REPRESENTATIONS OF FERRET FOREPAW REVEALED BY CORTICAL EVOKED POTENTIALS: NORMAL AND REORGANIZED CORTEX. D.F. McLaughlin*, R.V. Sonty and S.L. Juliano, Department of Anatomy and Cell Biology, USUHS, Bethesda, MD 20814.

Our recent multi-unit data indicate that ferret somatosensory cortex contains multiple representations of forepaw and digits. To obtain a global view of the cortical representation of the forepaw, we recorded cortical surface potentials evoked by electrical stimulation of isolated ulnar and median nerves. We also recorded the receptive field of the individual nerves, and confirmed the peripheral cutaneous median and ulnar distribution. Topographic maps of the initial cortical negativity reveal that each nerve is represented multiple times in somatosensory cortex. In maps derived from stimulation of ulnar or median nerve, two foci consistently appear, and correspond to activity in areas 3a and 3b. In ulnar nerve maps, a third, less intense focus appears at the rostral crown of the coronal sulcus in area 1. Because much of the median nerve representation is deep within the coronal sulcus, surface recordings reflect both superficial generators for digit 3, and deep neural sources for digits 1 and 2. Deep activity appears at the surface as two foci, one caudal to the coronal sulcus and the other rostral to the sulcus. We also conducted experiments lesioning either the median or ulnar nerve when the animals were young (<15 days) or adults. These manipulations markedly alter the spatial distribution of the response. In experiments in which the ulnar nerve was lesioned in young (P5-7) animals (but the study conducted as an adult), median nerve stimulation evoked activity in regions normally occupied by ulnar nerve stimulation. Two clear peaks of evoked activity occurred within cytoarchitectonic area 3b, one corresponding to the normal site of median nerve activation, and the other extending into cortical territory normally activated by ulnar nerve. Further analyses of these data will characterize how adult reorganization is affected by interruption of afferent input at different ages of development. Supported by PHS RO1 NS24014.

16.8

LINEAR AND NON-LINEAR PROCESSING OF SPATIAL FORM IN AREA 3b OF THE AWAKE MONKEY. I.A. Twombly, J.J. DiCarlo, K.O. Johnson, and S.S. Hsiao*, Mind/Brain Institute, Johns Hopkins Univ., Baltimore, MD 21218

Single and multi-layer neural networks were used to model the spatiotemporal processing functions of 114 neurons in area 3b from five awake, behaving monkeys. The neural network architectures were loosely constrained to mimic the convergence of tactile information from the peripheral afferents to neurons in primary somatosensory cortex. The network input layer is an array of nodes analogous to the primary afferent population on the finger pad. The output layer is a single node analogous to a single neuron in SI cortex. The connectivity between the input and output is either direct (single layer models) or mediated through a layer of hidden units (multi-layer models). Models were trained using backpropagation with the average peripheral SAI response to embossed letter stimuli (8mm height, 500μ relief) as inputs and the cortical response to the same stimuli as the output.

Single layer models were used to determine the variance (r^2) of each cortical response that could be accounted for by a linear processing function (including threshold rectification). The distribution of r^2 values for the population of cortical responses is approximately normal (mean $r^2 = 0.58$), indicating that no discrete sub-populations of "linear" and "non-linear" processing functions are present. The receptive fields (RFs) predicted from the single layer models are small, well defined regions of excitation and inhibition for models with high r^2, and larger, more complex regions of excitation and inhibition for models with low r^2. Multi-layer models typically explain 20% more variance than single layer models trained on the same data, and predict similar RF structures. These results show that both linear and non-linear processing contribute to the responses of cortical area 3b neurons, and that the predicted RFs vary from good to poor approximations of the true cortical processing functions in area 3b.

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16.9

LAMINAR DIFFERENCES IN SPATIOTEMPORAL RECEPTIVE FIELD STRUCTURE OF NEURONS IN AREA 3b OF THE AWAKE MACAQUE. J.J. DiCarlo*, I.A. Twombly, S.S. Hsiao, and K.O. Johnson. Krieger Mind/Brain Inst., Johns Hopkins Univ., Baltimore, MD 21218.

The spatiotemporal receptive field (RF) structure of 617 area 3b neurons was studied in three awake, behaving monkeys (*Macaca mulatta*). All RFs were centered on one of the distal finger pads. Stimuli consisted of a pattern of randomly-distributed (uniform, 10 dots/cm^2) embossed dots (500 μm dot diam., 400 μm relief) and a pattern of embossed letters of the alphabet (8 mm height, 500 μm relief). Stimuli were scanned over the neuron's RF at eight scanning directions (45 deg. increments) and three velocities (20, 40, 80 mm/sec). Single-unit recordings were made from neurons in all layers of area 3b and the laminar position of each neuron was histologically determined using a fluorescent marking method (1).

The linear spatiotemporal RF of each neuron was estimated by correlating the neural response to the random dot stimulus with the response of peripheral SA1 afferents to the same dot stimulus. An RF estimate was also obtained from a neural network model trained to reproduce the neural response to the letter stimulus (2). For each neuron, both estimation methods produced similar spatiotemporal RF structure.

Results show a variety of RF structures. Neurons with RFs dominated by a single, small, excitatory region tend to be located in and around layer IV. Neurons with larger RFs containing spatially separate regions of excitation and inhibition tend to be found in the supra- and infragranular layers. These results demonstrate the existence of an isomorphic representation of spatial form in layer IV which is significantly transformed in the supra- and infragranular layers.

Supported by: NIH R01 NS18787
NIH R01 NS34086

1. DiCarlo et al., *J. Neurosci. Meth.*, **64**, 74 (1996)
2. Twombly et al., *Soc. Neuro. Abs.* (1996)

16.10

SOMATOSENSORY STIMULATION MAPPING IN RAT BRAIN: A FUNCTIONAL MRI STUDY. JJA Marota, JB Mandeville, BE Kosofsky, JB Keltner, J Berke, L LaPonte, R Weissleder, B Rosen, R Weisskoff, SE Hyman*. Dept. Anaesthesia, Laboratory of Molecular and Developmental Neuroscience, and NMR Center, MGH, Harvard Medical School, Boston, MA 02114.

Because cerebral blood flow (CBF) is coupled to metabolism within the central nervous system (*Ann Neurol. 22:289-297,1987*), changes in local CBF can identify regions with altered neuronal activity. Since CBF is regulated by local cerebral vasodilatation, cerebral blood volume (CBV) also varies with regional metabolic activity. Using an electrical stimulation model in α-chloralose anesthetized, paralyzed (pancuronium) rats, we have used MRI techniques to map brain regions activated by periperal somatosensory stimulation. We employed high speed echo planar imaging with gradient and spin echo imaging sequences sensitive to the paramagnetic state of deoxygenated hemoglobin to quantitate 1) blood oxygen level dependent (BOLD) changes as a measure of local cerebral metabolism, and 2) CVB changes by use of a superparamagnetic iron oxide contrast agent. Electrical stimulation of the forepaw (3Hz, 5V, 0.5ms duration) resulted in an increase in BOLD signal in a discreet area of contralateral somatosensory cortex (SI). The signal took 30s to attain maximal change and required 30s to return to baseline after discontinuation of the stimulus. In the same volume of cortex, CBV increased with a time course which paralleled the BOLD signal change. Increases in both BOLD and CBV were also present in the ventral medial thalamus bilaterally, with a timecourse similar to that of SI neocortex. Although either right or left SI neocortex could be activated (depending upon paw stimulated), there was no change in either BOLD or CBV in SI neocortex ipsilateral to stimulation. In addition, both BOLD and CBV signal decreased in dorsal striatum bilaterally, with a timecourse similar to that of SI neocortex. These findings confirm and extend data previously obtained with other modalities correlating cerebrovascular changes and brain activation during peripheral somatosensory stimulation. (supported by DA09467).

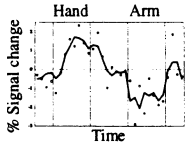
16.11

FMRI OF SOMATOSENSORY CORTEX: SOMATOTOPY AND LATERAL INHIBITION WITHIN THE POSTCENTRAL GYRUS A. Gehi[1], C. I. Moore[1], S. Corkin[1], B. R. Rosen[2]& C. E. Stern*[2]. [1] Dept. of Brain & Cog. Sci., M.I.T., Cambridge, MA 02139; [2] MGH-NMR Center, Harvard, Charlestown, MA 02129.

Macro and microelectrode recordings have revealed detailed somatotopic maps in the mammalian postcentral gyrus (PCG). We used echo-planar fMRI to examine these representations within the PCG in 10 human subjects (age 20-25). Each subject received 6-8 4:00 min scans, with 10 7-mm slices aligned parallel to the PCG (1.5T: TR = 2500; TE = 70). Stimulation of the sole of the foot, the palm, or the volar forearm was alternated with periods of no stimulation. Stimulation consisted of 3-5Hz contact with a 5.88($\log_{10}$.1mg) von Frey pressure aesthesiometer. Significant regions of activation were defined using a Kolmogorov-Smirnov statistic ($p < 0.05$).

Basic somatotopy. In all subjects, we observed a somatotopic map in the PCG. In 4 subjects who received palm and sole stimulation, the PCG was activated in the appropriate regions (i.e., the foot representation was medial to the hand representation, and both stimulations activated regions of the PCG localized to the corresponding positions on the Penfield and Rasmussen homunculus (1950)). In 6 other subjects who received palm and forearm stimulation, we observed palm activation in the expected region of the PCG in 6/6, and forearm activation in 3/6. In all subjects, the superior bank of the insula, an area containing several somatosensory maps (e.g., SII), was successfully activated by tactile stimulation.

Lateral inhibition within the PCG. We observed inhibition of the fMRI signal in the hand representation during arm stimulation (n=6/6: fig.). By contrast, inhibition following arm stimulation was not observed in the insula, suggesting that the different neural representations in the PCG and insula may be characterized by differential responses of the fMRI signal. *Support: SCRF-1416.*



16.12

ACTIVATION OF HUMAN MESIAL CORTEX DURING SOMATOSENSORY ATTENTION TASK. N. Forss*, I. Merlet, S. Vanni, M. Hämäläinen, F. Mauguière and R. Hari. Brain Research Unit, Low Temperature Laboratory, Helsinki University of Technology, 02150 Espoo, Finland, and Hôpital Neurologique, 59 Boulevard Pinel, 69003 Lyon, France.

We recorded somatosensory evoked fields (SEFs) from 10 healthy subjects with a whole-scalp neuromagnetometer to ulnar and median nerve stimuli, presented at random intervals of 2.4–21.6 s. The subjects either counted the stimuli or ignored them by reading a book. The stimuli activated in both conditions the contralateral SI cortex, the ipsi- and contralateral SII cortices, and the posterior parietal cortex, in line with earlier observations. In addition, a novel response was observed in nine subjects at 120–160 ms. It was clearly enhanced by attention and was originated in the mesial paracentral lobule, close to the end of the central sulcus. The observed signals may reflect attention-dependent activation which would be needed to prime the SMA in case a stimulus-related movement would be needed.

Supported by the Academy of Finland and by the EC's HCM Programme through Large-Scale Facility BIRCH at LTL, HUT.

16.13

CORTICAL REORGANIZATION IN ARM AMPUTEES: ALTERATIONS OF SOMATOSENSORY REPRESENTATION IN THE HEMISPHERE CONTRALATERAL TO THE INTACT SIDE T. Elbert*[1], A. Sterr[1], H. Flor[2], B.Rockstroh[1], C. Pantev[3], S. Knecht[3], N. Birbaumer[1], E. Taub[4]. Universities of Konstanz[1], Berlin[2], Münster[3], and Alabama, Birmingham[4]; [1] 78464 Konstanz, Germany.

Magnetic source imaging has provided evidence that extensive cortical reorganization following somatosensory deafferentation in monkeys also occurs and persists in humans following upper extremity amputation. Here we demonstrate that the somatosensory map in amputees is altered also in the hemisphere contralateral to the intact side

Six upper arm amputees and six controls served as subjects. Somatosensory stimulation was delivered separately to the first and the fifth digit of either hand and to the lower lip at the corners of the mouth. Within the range of 30-75 msec a first major peak was identified in each of the magnetically evoked waveforms and its generator was located using a single equivalent current dipole model. In amputees the cortical representations of D1 and D5 of the intact hand encompassed a greater area (Euclidean distance: 12.4 ± 5.5 mm) than in controls (6.9 ± 1.6 mm; $t = 2.4$, $p<.05$). The average difference in inferior-superior direction was 5.5 mm larger in amputees than in controls ($t=3.2$, $p<.01$). Compared to controls, only D1-representation had shifted: along the central sulcus in inferior (2.6 mm, n.s.) and lateral (7.7 mm, $t=2.1$, $p<.1$) direction. However, the distance between the lip and D5-location was 5.5 mm greater in the amputees than in control subjects ($p<.05$). There was no difference in the magnitude of the dipole moments of the digits. In amputees dipole moments were greater for the lip-stimulation on the amputated side than on the intact side.

The present comparison is the first evidence, that cortical reorganization after upper arm amputation occurs in both hemispheres. The alterations observed in the hemisphere contralateral to the intact side may be explained by the increased use of the remaining hand by amputees. The shift was greatest for D1, an observation that is consistent with the relative frequency of use of the different digits.

17.1

EVIDENCE OF APOPTOSIS IN AN ACUTE SPINAL CORD INJURY MODEL. T.K. Swoboda*, Y. Li, R.P. Nockels, M. Chopp. Departments of Neurosurgery and Neurology, Henry Ford Hospital, Detroit, MI 48202.

Apoptosis differs from necrosis particularly in the manner of chromatin breakdown. Utilizing a commercial molecular histochemical marker (Apoptag kit; Oncor, Gaithersburg, MD) that labels the 3'-OH DNA ends generated by DNA fragmentation occurring during chromatin breakdown, apoptotic cells can be individually marked. To determine the extent of apoptosis in acute spinal cord injury, eight S.D. rats were anesthetized with pentobarbital and received laminectomies at the T_{10} vertebral level. In four rats, a ten gram rod was dropped 25mm onto the exposed dura corresponding to the T_{12}-T_{13} spinal cord level utilizing the NYU contusion device. The wound was closed, and all rats were reanesthetized 24h after injury and perfused with heparinized saline and 10% buffered formalin solution. 5µm formalin-fixed paraffin-embedded cross-sections of spinal cord were cut from the T_9-L_3 spinal levels. Sections were stained with the Apoptag kit and counterstained with hematoxylin. Adjacent spinal cord sections were stained with H&E to identify necrotic cells. 5µm serial coronal brain sections, with 2mm spacing, were also stained with H&E and the Apoptag kit for each animal. In the four lesioned animals, widespread necrosis and surrounding apoptosis were seen within the dorsal tracts at the lesion site from T_{11}-L_1. Apoptotic cells were noted in the dorsal region of lesioned spinal cords at the T_9-T_{10} and L_2 levels at numbers significantly higher than in the sham animals. No significant difference was found in the number of apoptotic corticospinal tract neurons in the cortices of lesioned and sham animals.

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17.2

APOPTOSIS IN INJURED SPINAL CORD OF RAT, C. Yong, P. M. Arnold*, M.N. Zoubine, B.A. Citron and B.W. Festoff. Neurobiology Research lab, VA Medical Center, Kansas City, MO 64128; Departments of Neurology and Surgery (Neurosurgery), University of Kansas Med. Center, Kansas City, KS 66160.

We studied neuronal cell death with Hoechst 33342 staining and the *in situ* TUNEL technique in the NYU weight-drop injured rat spinal cord. Functional deficit was assessed at day 1, 3, 7, 14 and 28 after injury by scores on a modified Tarlov scale and the inclined plane test. Rats were perfused with normal saline followed by 10 % formalin and then spinal cords were sectioned (7 µm) and stained with H&E or processed for *in situ* TUNEL with the TDT-mediated dUTP-biotin nick-end labeling method (ApopTag kit, Oncor, Gaithersburg, MD). For Hoechst 33342 staining, rats were perfused with normal saline followed by 4% paraformaldehyde/PBS prior to sectioning (20 µm). As early as one day after injury, multifocal petechiae appeared in all regions of the gray matter surrounding small capillaries, veins, and arterioles, and, to a lesser extent, the white matter. Both apoptosis and necrosis of neurons began 1 day after injury, where nuclei with condensed chromatin were mainly found in the dorsal funiculi, and less in dorsal and ventral horns. Selective stains identified apoptotic cells as both neurons and glia. By 3 days after injury, necrosis was prominent . Gliosis was evident on day 7 with glial like cells having small condensed chromatin nuclei on day 14. On day 28, the lesioned area was cystic with a wall made up of collagen, fibroblasts and astrocytes. This study suggests that apoptosis is evident early and up to 14 days after spinal cord injury. Which factors participate in apoptosis are subjects of current studies. Supported by Medical Research Service., DVA and ALS/Spinal Cord Research Fund.

17.3

UPREGULATION OF CPP32 CYSTEINE PROTEASE ASSOCIATED WITH APOPTOSIS IN RAT CORTEX FOLLOWING TRAUMATIC BRAIN INJURY. A.G.Yakovlev*, S.M.Knoblach, D.Rosenthal, M.Smulson, and A.I.Faden. Georgetown Institute for Cognitive and Computational Sciences and Dept. of Biochemistry, Georgetown Univ.Med.Ctr., Washington DC 20007.

This study delineated the apoptotic component in delayed neuronal death following traumatic brain injury (TBI) in rat cortex and determined the effect of such injury on the expression of the gene encoding ICE-like cysteine protease CPP32. An early biochemical event that accompanies apoptosis in many cell types is the proteolytic cleavage of poly(ADP-ribose)polymerase (PARP), a nuclear enzyme involved in DNA repair and protection of fragmented DNA. PARP is cleaved by a mammalian equivalent of ced-3, cysteine protease CPP32. Apoptosis was monitored by the analysis of associated internucleosomal DNA fragmentation in sham controls or cortex isolated after 1, 4, 12, 24 hrs and 3 days after moderate (2.0 atm) fluid-percussion induced TBI. DNA fragmentation was observed in injured but not in contralateral cortex starting from 4 hrs after trauma. The intensity of DNA "ladders" increased progressively and peaked at 3 days. To address the issue of CPP32 gene expression in traumatized brain we isolated the corresponding rat cDNA. Based on the obtained sequence we used RT-PCR technique for semi-quantitative analysis of this gene expression. Results showed that the levels of CPP32 mRNA were induced in the injured tissue at 4 hrs (4-fold increase) and peaked at 24 hrs (about 5 fold increase). Levels of CPP32 mRNA remained elevated up to 72 hrs after trauma. At the same time, these levels were not affected by trauma in rat cortex contralateral to injury site at any time point tested. The posttraumatic elevation of CPP32 mRNA was consistent with an increase of PARP-cleavage activity in cytosolic extracts of injured cortex, suggesting that TBI also leads to posttranslational activation of a PARP-cleaving protease, presumably CPP32. Taken together, these results suggest that programmed cell death contributes to secondary neuronal loss after fluid-percussion injury, and that early upregulation and activation of CPP32 may play an important role in the induction of apoptosis following TBI.

17.4

DELAYED DEGENERATION OF DISTANT NEURONS FOLLOWING NEOCORTICAL BRAIN CONCUSSION IN INFANT RATS C. Ikonomidou, M. Ishimaru*, P. Bittigau, T. C. Der, Y. Q. Qin, and J. W. Olney. Depts. of Psychiatry and Pediatric Neurology, Washington University Medical School, St. Louis, MO 63110

Previously we described a method for inducing concussive head trauma in infant rats and described the acute lesion which evolves over a 6 hr period subjacent to the neocortical site of impact. We demonstrated that the acute lesion closely resembles the excitotoxic type of neuronal injury caused by glutamate and that NMDA glutamate receptor antagonists can prevent the acute lesion from expanding locally. We have now examined the brains of infant rats at more extended postconcussive time intervals and have found that cytopathological changes appear in a disseminated pattern at distant loci over a 6 to 24 hr period. The distant degenerative reaction was particularly conspicuous at all rostrocaudal levels of the ipsilateral cingulate and retrosplenial cortices, and foci of damage occurred ipsilaterally in the subiculum, dentate hippocampal gyrus, various thalamic nuclei and head of the caudate nucleus. In some cases, a circumscribed lesion was present in the contralateral medial geniculate nucleus. The distant lesions are being evaluated by light and electronmicroscopy in thin plastic sections, by de Olmos cupric silver staining and by the TUNEL staining method (for evaluating the possible involvement of an apoptosis mechanism). At 6 hrs post concussion these several methods show very little evidence of lesions anywhere except at the local site of impact, but in the ensuing 24 hrs the distant lesions become evident by all of these methods. Some of the degenerating neurons in the distant foci are robustly stained by the TUNEL method. It is not clear what this signifies regarding cell death mechanisms in that hypothalamic neurons killed by an excitotoxic mechanism (subcutaneous monosodium glutamate) also show TUNEL positivity while failing to meet other criteria for apoptosis. Supported by DA 05072, AG 11355 and RSA MH 38894 (JWO).

17.5

BCL-2 PROTEIN EXPRESSION AFTER SEVERE CONTUSIVE BRAIN INJURY IN RATS. R.S.B. Clark*, P.M. Kochanek, S.C. Watkins, M. Chen, D.S. Turner, J. Chen, and S.H. Graham. Depts. of Anesth/CCM, Neurology, and Cell Biol. and Physiol., and the Safar Center for Resuscitation Research, U. Pittsburgh, PA 15213.

Delayed neuronal death occurs after traumatic brain inury (TBI). The exact mechanism is unknown but apoptosis may play a role. Bcl-2 is a highly conserved apoptosis-suppressor gene that is induced in brain after ischemic and kainate-induced injury[1,2], and may serve to inhibit apoptotic delayed neuronal death. The purpose of this study was to determine if bcl-2 protein is also induced after severe TBI.

Anesthetized adult rats were subjected to severe controlled cortical impact injury to the left parietal cortex immediately followed by 30 min of hypoxia (PaO_2=35-45 torr). Rats were killed at 8, 24, 72, or 168 h after trauma (n=3-5/group). Naive rats were used as controls. Brains were removed and Western analysis was performed using a mouse MoAb against human bcl-2 (Dako, Carpinteria, CA). Bcl-2 was induced at 8, 24, and 72 h in ipsilateral traumatized cortex and hippocampus. Minimal baseline bcl-2 was detected in control brains and in ipsilateral cortex distal to the contusion. By 7d bcl-2 expression decreased vs. earlier time points, but remained increased vs. controls. To determine what cell types expressed bcl-2 immunocytochemistry was performed on brain sections from separate rats 24 h after trauma (n=3). Immunocytochemistry was performed using fluorescent detection methods and the same primary antibody. Bcl-2 expression was seen in cells with the distinct morphologic appearance of neurons and endothelial cells. Other cells expressed bcl-2 and may have been leukocytes and/or glia. Bcl-2 expressing cells were seen only in the penumbra surrounding the contusion. Nonspecific staining was not seen in sections incubated without primary antibody.

Bcl-2 protein is expressed after severe TBI in cell-types that include neurons and endothelium. Because bcl-2 suppresses apoptosis, its induction in neurons in the penumbra surrounding the contusion may be an attempt to reduce delayed neuronal death. Supported by NIH/NINDS 2P50 NS30318-04A1 [1]Chen, et al, Neuroreport 1995;6:394-8, [2]Chen, et al, Soc Neurosci Abstr 1995;21:302

17.6

ROLE OF GROUP I METABOTROPIC GLUTAMATE RECEPTORS IN POSTTRAUMATIC NEURONAL INJURY. A. G. Mukhin*, L. Fan and A. I. Faden. Georgetown Institute for Cognitive and Computational Sciences, Washington DC 20007

Increased glutamate release and activation of ionotropic glutamate receptors have been implicated in the pathophysiology of traumatic brain injury (TBI). The role of metabotropic glutamate receptors (mGluR) in posttraumatic central nervous system injury has been largely unexplored.

In vitro experiments were performed on 17-21 DIV rat cortical neuronal/glial cocultures, growing in 96-well plates. Traumatic injury to cells was delivered using a punch, that produces 28 parallel cuts 1.2 mm in length uniformly distributed throughout the cell layer at 0.5 mm intervals in each well. Cell death was detected 16 h after injury by measuring LDH release. For *in vivo* studies, male Sprague-Dawley rats were anaesthetized with sodium pentobarbital (70 mg/kg, ip) and subjected to moderately severe (2.8 atm) lateral fluid percussion-induced TBI. Outcome variables included motor recovery and histology at 2 weeks after TBI.

(+)-MCPG (500µM) — an antagonist of both group I (phospholipase C-coupled metabotropic receptors subtypes $mGluR_1$ and $mGluR_5$) and group II (subtypes negatively linked to adenylate cyclase activity) — reduced posttraumatic increases in phosphoinositide hydrolysis and neuronal cell death following mechanical trauma in mixed neuronal/glial cultures. (S)-4CPG (30µM), which is an antagonist at group I mGluR but an agonist at group II mGluR, showed a similar neuroprotective effect in tissue culture. In the *in vivo* studies, (RS)-MCPG administered either icv (0.5 µmol 15 min before, plus 0.5 µmol 60 min after, trauma) or iv (48 µmol 15 min after trauma) improved neurologic recovery and limited the cell loss in ipsilateral hippocampus following TBI. Administration of antisense oligodeoxynucleotides (2µM over 5 days) directed to $mGluR_1$, but not those directed to $mGluR_5$, reduced posttraumatic neuronal cell death in tissue culture.

Together, these findings suggest that activation of $mGluR_1$ contributes to posttraumatic neuronal injury, and that $mGluR_1$ antagonists may have therapeutic potential . Supported by NIH grant NS27849 and CDC grant CCR306634.

17.7

EVIDENCE FOR ULTRA-EARLY CYTOSKELETAL CHANGE AND ITS RELATION TO THE PATHOBIOLOGY OF TRAUMATIC AXONAL INJURY. J. MOROI*[1], J. TROJANOWSKI[2], and J. POVLISHOCK[1]. [1]Depts. of Anatomy and Neurosurgery, Med. Col. of VA.,VA Commonwealth Univ, Richmond, VA 23298, and the [2]Dept. of Neuropathology, Univ. of PA, Philadelphia, PA 19104.

Recently, we have suggested that the axonal injury associated with traumatic brain injury has two forms of genesis, one involving compaction of the neurofilaments (NF), with the loss of sidearms and the other involving NF misalignment (Pettus et al., J. Neurotrauma, 11(5), 1994). In the current investigation, we rigorously explore the genesis of these two different forms of reactive axonal change, using antibodies specific for each of these events. For this purpose, we used RMO 14 antibody, targeted to the rod domain which is only visualized once the associated NF sidearms are lost, together with antibodies targeting the 68 kD subunit, a marker for reactive axons showing NF misalignment. Anesthetized rats were subjected to severe impact acceleration insult, and at 15 min to 6 h postinjury were prepared for LM and EM visualization of both antibodies using double-labelling with immunogold and HRP-DAB. Antibodies targeting the rod domain readily identified damaged/reactive axons in various regions of the neuraxis. These sites were associated with NF compaction and side arm loss reminiscent of that previously described. Over time, these sites became more prominent, and the immunoreactivity was associated not only with sites of compaction, but also with the onset of reactive axonal swelling. In contrast, the 68 kD immunoreactivity was never associated with the site of NF compaction. Rather, it appeared to label an independent population of axons not showing overt NF compaction and sidearm loss. These double-label immunocytochemical findings clearly suggest that two distinct populations of reactive axons exist. It is posited that these distinct populations reflect differences in pathogenesis which may be linked to the severity of the insult sustained by individual axons. (Supported by NS 20193).

17.8

BONE MORPHOGENETIC PROTEINS AS MEDIATORS OF PLASTIC CHANGES FOLLOWING TRAUMATIC BRAIN INJURY A. Lewén, S. Söderström, L. Hillered* and T. Ebendal Departments of Neurosurgery , Clinical Chemistry and Developmental Neuroscience, Uppsala University Hospital and Biomedical Centre, S-751 85 Uppsala, Sweden

Members of the transforming growth factor ß superfamily act on the cell via induction of a heteromeric complex of type I and type II serine/threonine protein kinase receptors. Recently, a new type II receptor was identified as the human bone morphogenetic protein receptor II (BMPR-II). The expression of BMPR-II in the brain has until now not been studied. It is of interest to elucidate which receptors may act in concert *in vivo*, to mediate neurotrophic interactions, during brain development, during normal brain function and after brain injuries.

To investigate how serine/threonine protein kinase receptors are expressed in the brain after an acute brain injury, a focal cerebral contusion injury was produced over the right parietal cortex by the weight drop technique in artificially ventilated rats. The expression of BMPR-II-, ActR-IA-, ActR-IB-, ActR-IIA-, trkB- and *c-fos*-mRNA was studied by *in situ* hybridization 0.5 h, 2.0 h, 6.0 h and 18.0 h after injury. We found that, BMPR-II and ActR-IA were simultaneously upregulated in neurons in the dentate gyrus 6h after a mild cerebral contusion injury, a finding which seemed to be specific for these two receptors. This study suggests that type I and type II receptors act together in signal transduction *in vivo* and that BMPs may be involved in neuronal plasticity after traumatic brain injury.
Supported by the Swedish Medical and Natural Science Research Councils, public US federal funds and the Swedish Brain Society.

17.9

POST-TREATMENT WITH INTRAVENOUS BASIC FIBROBLAST GROWTH FACTOR REDUCES HISTOPATHOLOGICAL DAMAGE FOLLOWING FLUID-PERCUSSION BRAIN INJURY. W.D. Dietrich*, O. Alonso, R. Busto, and S.P. Finklestein. Neurotrauma Res. Center, Dept. Neurology, Univ. Miami Sch. of Med., and the CNS Growth Factor Res. Lab., Dept. Neurology, Massachusetts General Hospital and Harvard Med. Sch., Boston.

We determined whether treatment with basic fibroblast growth factor (bFGF) would protect histopathologically in a rat model of traumatic brain injury (TBI). Fasted Sprague-Dawley rats were anesthetized with 70% nitrous oxide, 1% halothane and 30% oxygen. Under controlled physiological conditions and normothermic brain temperature (37-37.5°C), rats were injured with a parasagittal fluid-percussion pulse ranging from 1.6-1.9 atm. Rats were randomized into two groups where bFGF (45 ug/kg per hour) in vehicle (n = 7) or vehicle alone (n = 7) was infused intravenously for 3 hr, beginning 30 min after TBI. In vehicle-treated animals, necrotic neurons were observed throughout the lateral cerebral cortex remote from the impact site 3 days after TBI. An intracerebral contusion was present in all rats at the gray-white interface underlying the injured cortical areas. Post-traumatic administration of bFGF significantly reduced the numbers of damaged cortical neuron profiles at several coronal levels and reduced the total number of damaged neurons (696 ± 148 vs. 1,248 ± 198, means ± SEM), $p < 0.05$ ANOVA). In addition, contusion areas at several coronal levels and total contusion volume was significantly reduced (1.13 ± 0.39 mm^3 vs. 3.18 ± 0.81 mm^3, $p < 0.05$). These data demonstrate neuroprotection with intravenous bFGF infusion in the post-traumatic setting.

17.10

INTERLEUKIN-6 (IL-6) AND INTERLEUKIN-10 (IL-10) IN CEREBROSPINAL FLUID (CSF) FOLLOWING SEVERE TRAUMATIC BRAIN INJURY (TBI) IN CHILDREN M. Bell, P. D. Adelson*, L. Doughty, J. Carcillo, R. Clark, S. DeKosky and P. Kochanek. Safar Center for Resuscitation Research, Departments of Anesthesiology/CCM, Neurosurgery, Univ of Pittsburgh, Pittsburgh, PA 15213.

Inflammation may play an important role in the pathophysiology of TBI in children. IL-6 and IL-10 are markers of inflammation that are pro- and anti-inflammatory in nature, respectively, and have been measured in serum and CSF as an index of the degree of inflammation in diseases including sepsis and meningitis. We hypothesized that both IL-6 and IL-10 would be increased in the CSF of children after severe TBI. Eleven children who sustained severe TBI (GCS ≤ 7) were studied. Standard neurointensive care was provided. Ventricular CSF collected each of the first three days after TBI was analyzed for IL-6 and IL-10 concentrations (pg/ml) by ELISA. IL-6 was markedly increased on d 1 vs. d 2 and 3 (mean ± SE, 2179.8 ± 412.2 vs. 933.0 ± 273.2 and 883.4 ± 226.3, $p = 0.004$, repeated measures ANOVA). IL-10 concentrations tended to be increased on d 1 compared to d 2 and 3 but did not reach statistical significance (48.4 ± 17.1 vs. 25.3 ± 8.54 and 21.3 ± 8.14). IL-6 and IL-10 concentrations were correlated on d 1 and 2 ($r = 0.62$ and 0.86, $p < 0.05$). IL-6 and IL-10 concentrations were not associated with initial GCS, age (< 4 y) or death. We conclude that CSF IL-6 was increased early after TBI in children. CSF IL-6 concentrations in children are comparable to those found in CSF of adults after TBI[1] and serum of children with septic shock[2]. The associated increase in serum IL-10 seen in sepsis, however, was not observed in CSF after TBI. This suggests that the balance between pro- and anti-inflammatory cytokines in brain after TBI is different than that observed in serum during sepsis. [1]McClain et.al., J: Clin Med 1991, 118: 225-31. [2]Doughty et. al., Crit Care Med 1996, 24:A33. Supported in part by an SCCM Established Investigator Grant, 2P50 NS30318-04A1 from NINDS and the Laerdal Foundation.

17.11

DOUBLE LABELING STUDIES USING ANTIBODIES TARGETED TO THE NEUROFILAMENT ROD AND SIDEARM DOMAINS IN THE STUDY OF THE GENESIS OF TRAUMATICALLY INDUCED AXONAL INJURY. J. POVLISHOCK*[1], J. TROJANOWSKI[2], AND J. MOROI[1]. [1]Depts. of Anatomy, Med. Col. VA, VA Commonwealth Univ., Richmond, VA 23298 and [2]Dept. of Neuropathology, Univ. of PA, Philadelphia, PA 19104.

We have recently emphasized the utility of antibodies targeted to the rod domain for recognizing the initiating steps in the pathobiology of axonal injury. It was posited that these antibodies were capable of recognizing the neurofilament (NF) rod domain segments whose sidearms had been altered by processes related to the traumatic insult. In this study, we attempt to confirm this suggestion through the use of double-labeling strategies targeting the NF rod and sidearm domains independently. Rats were subjected to impact acceleration traumatic brain injury and then, at 5 min to 6 h postinjury, were transcardially perfused and prepared for LM and EM visualization of antibodies targeting the NF rod (RMO14) and sidearm domains (RMO270, phosphorylation independent). The RMO14 antibody was visualized through the use of immunogold, whereas the RMO270 antibody visualized through the use of HRP-DAB. Consistent with previous reports, the RMO14 antibody served as an early marker of reactive axonal change, demonstrating sites of NF compaction which led to local cytoskeletal dysfunction, axonal swelling and detachment. When the antibody targeted to the NF sidearms was employed, it labeled all intact axons, and to our surprise, was also co-localized at the same sites showing RMO14 activity. This finding was contrary to our expectations, as we had posited that the unmasking of the NF rod domain was associated with the functional disruption of the NF sidearms. This suggests that these sidearms may be only partially cleaved by the traumatic insult. Alternatively, the constituent epitopes may not have been removed from the site of injury. This suggests that future studies directed at longer survival times may be necessary to fully reveal this NF sidearm alteration. (Supported by NS20193)

17.12

INCREASED IMMUNOREACTIVITY OF *c-JUN* AND *JUN-B* BUT NOT *JUN-D* IN DYING AND DEAD NEURONS AFTER PHYSICAL INJURY. R.Raghupathi*[1], L.J. Rosenberg[2], T.K. McIntosh[1], and J.H. Lucas[2]. Div. of Neurosurgery, Univ. of Pennsylvania, Philadelphia PA 19104 and Dept. of Physiology, Ohio State Univ., Columbus OH 43210.

To evaluate the immediate-early gene (IEG) response of CNS neurons to mechanical injury, cultured mouse spinal cord (SC) neurons were subjected to UV laser microbeam transection of a primary dendrite 100µm from the soma (n = 109 neurons in 14 cultures) and immunostained with c-*jun*/AP-1, a pan-*jun* antibody (AB). Unlesioned control neurons (n = 112) showed light AB staining of the somal cytoplasm and nucleus. Increased immunoreactivity in the cytoplasm and nucleus was observed in 19 of 27 cells at 30 min. Phase contrast microscope evaluations of viability at 2h revealed that 53% of lesioned neurons were either dead or dying; intense nuclear AB staining was observed in 52% of these cells. At 24h after lesioning, 15 of 39 lesioned neurons were still alive and stained similar to unlesioned cells. Among the dead cells at 24h, 30% were too deteriorated to stain (debris); however, 100% of the remaining dead cells exhibited intense nuclear staining. In separate studies, a similar pattern was revealed for c-*jun*/p39 (specific for c-*jun*; 43 lesioned/39 control) and *jun*B (51 lesioned/48 control) with increased somal staining in lesioned cells at 30 min and 2h, return to the control pattern in surviving cells at 24h, and intense nuclear staining in dead cells at 24h. Increased *jun*D immunoreactivity was observed in 17 of 19 lesioned neurons 30 min after dendrotomy, but not in viable, dying or dead lesioned neurons at 2h or 24h (20 lesioned/20 control per time). c-*jun* has been implicated in neuronal death during development and after hypoxia/ischemia or seizures. These data suggest that c-*jun* and *jun*B may play a role in neuronal death following physical (membrane disruption) injury of mammalian SC neurons. Supported by PHS grants 29683 to JHL at OSU and NINDS grants 26818 and 08803 to TKM at UPenn.

18.1

BRAINSTEM CYSTS, CEREBRAL ANEURYSMS AND HYDROCEPHALUS IN A HIGHLY FUNCTIONING MALE: A POSSIBLE EXAMPLE OF DEVELOPMENTAL FIELD DEFECT.

B. W. Chopko*, C. Ames, A. Brant, D. Barba. Division of Neurological Surgery, UCSD Medical Center, San Diego, CA 92103.

A 19 year old Caucasian male presented with new onset severe headaches and episodic bouts of unresponsiveness. Past medical history was noncontributory, including a normal prenatal, perinatal and developmental course. Physical exam was significant for gait ataxia; no other neurologic abnormalities were evident. Magnetic resonance imaging revealed obstructive hydrocephalus, multiple cysts of the midbrain, pons and cerebellum, and a left middle fossa mass lesion. Cysts were nonenhancing, without edema, contained fluid isodense with cerebrospinal fluid, and extensively involved the midbrain to the point of apparent total replacement. Cerebral angiography demonstrated a left internal carotid artery (ICA) giant (2.8 cm maximal dimension) paraopthalmic aneurysm, a second 8 mm left ICA paraopthalmic aneurysm, and fetal origin of the left posterior cerebral artery. Cysticercosis studies were negative. A midbrain biopsy revealed gray matter with mild diffuse microglial hyperplasia, scattered reactive lymphocytes, and a few chronic inflammatory cells. Neurons were of normal appearance, and no evidence of neoplastic or active infectious processes was observed. Hydrocephalus was treated with ventriculoperitoneal shunting, and the aneurysms were treated by carotid ligation. This case is an association of two unusual findings in a young, highly functioning male: ICA aneurysms and brainstem cysts of unknown etiology. The constellation of findings may represent a developmental field defect, with disruption of neighboring ectodermal and mesodermal derivatives culminating in vascular and parenchymal abnormalities.

Supported by University of California intramural funding.

18.2

DECREASED KAINATE RECEPTOR BINDING IN THE ARCUATE NUCLEUS IN THE SUDDEN INFANT DEATH SYNDROME. A Panigrahy*, JJ Filiano, LA Sleeper, F Mandell, M Valdes-Dapena, H Krous, LA Rava, WF White, HC Kinney. Division of Neuroscience, Children's Hospital, Boston, MA 02115.

The sudden infant death syndrome (SIDS) is the leading cause of postneonatal mortality in the United States; its etiology is unknown. The arcuate nucleus (ARC), located on the ventral medullary surface (VMS), is postulated to be homologous to chemosensitive respiratory fields in the cat, leading to the hypothesis that a defect of the ARC in SIDS results in an impaired arousal response to hypercarbia or asphxyia during sleep and sudden death. Recently we reported a decrease in muscarinic receptor (mAChR) binding in the ARC in SIDS victims (Kinney et al, Science 269:1446-50;1995). Glutamate via kainate (KA) receptors has been implicated in mediating the ventilatory response to carbon dioxide. KA binding is heavily localized in the human infant ARC (Panigrahy et al, Neuroscience 67:441-445;1995). To test the specificity of the mAChR binding deficit in SIDS victims, we used the same database to test the hypothesis that KA binding is decreased in the ARC in SIDS victims. We assessed ^{3}H-KA binding with quanitative tissue receptor autoradiography in 17 brainstem nuclei. Cases were classified as SIDS, 47; acute control, 15, chronic control with oxygenation disorders, 17. The ARC was the only nucleus in which there was a significant difference in the age-adjusted mean KA binding between the SIDS group (37$\pm$2 fmole/mg tissue) and both the acute controls (77$\pm$4) ($p<0.0001$) and chronic group with oxygenation disorders (69$\pm$4) ($p<0.0001$). There was a positive correlation between the density of mAChR and KA binding in the SIDS cases ($R^2=0.212$;$p=0.0025$). These data indicate that the putative neurochemical defect in the ARC in SIDS victims involves more than one neurotransmitter receptor, and that VMS binding deficits put infants at risk for SIDS. Supported by SIDS Alliance (HCK), HD-20991 (HCK), and a Howard Hughes Medical Student Research fellowship (AP).

18.3

COGNITION AND LOCOMOTOR ACTIVITY IN THE DEVELOPING RAT: COMPARISONS OF HISTAMINE H_3 RECEPTOR ANTAGONISTS AND ADHD THERAPEUTICS C. E. Tedford*, G. P. Pawlowski, M. A. Khan, J. G. Phillips, and S. L. Yates, Gliatech, Inc., 23420 Commerce Park Road, Cleveland, OH 44122

The localization and physiological role of the Histamine H_3 receptor subtype in the CNS has been recently described by several investigators. Ontogenetic studies have described independent and heterogeneous developmental patterns of histamine receptor subtypes (Ryu et al., 1995) and autoradiographic studies have identified unique CNS localization patterns for the H_3 receptor (Pollard et al., 1993). Behaviorally, arousal or vigilant properties of H_3 receptor agents have been indicated in EEG studies in several species. Additionally, modulation of learning and memory has been demonstrated with H_3 receptor subtype selective compounds.

The present studies characterized the behavioral development of cognitive and motor patterns in juvenile rat pups and evaluated the influence of histamine H_3 receptor blockade in both immature and adult rats. Comparisons with (methylphenidate, pemoline and d-amphetamine) were made for cognitive and motor effects. Immature rat pups up to postnatal days 15-16 displayed sustained locomotor activity profiles and acquisition deficits in a 10-trial passive avoidance response (PAR) paradigm. Improvements in the step-through latency were most prominent with either pre-treatment of methylphenidate (Ritalin) or the histamine H_3 receptor antagonists. Sustained SLA profiles were seen in the pups while habituation to novel environments developed with maturation. Psychostimulant as well as H_3 antagonist pre-treatment produced distinctly different SLA patterns in the immature rat pups versus the adult rats. These studies characterized cognitive and motor behavioral traits in immature rat pups and evaluated the use of this model for developmental disorders such as ADHD. Finally, the use of H_3 antagonists in cognitive and motor disorders is described. Funded in part by NINDS #1R43NS33808-1 and Gliatech, Inc.

18.4

ALTERATIONS IN DOPAMINE AND GLUTAMATE RECEPTOR BINDING AFTER GESTATIONAL PROTEIN DEPRIVATION IN RATS: AN ANIMAL MODEL OF SCHIZOPHRENIA? P.D. Butler[1,2,3]*, D.A. Klugewicz[1,2], L. DeSanti[3], J. Rotrosen[1,2], J.M. Gorman[3], and H.K. Kramer[2]. [1]Psychiatry Service, NYDVAMC and Depts. Of Psychiatry, [2]NYU Medical Center and [3]New York State Psychiatric Institute.

Recent evidence indicates that prenatal nutritional deprivation is one early environmental exposure that may increase the risk of schizophrenia. Previous experiments in our laboratory have shown that female rats protein restricted throughout gestation had a significant increase in amphetamine-induced locomotion and apomorphine-induced stereotypy as adults, but not as adolescents. Because glutamate (GLU) modulates dopamine (DA) functioning, and abnormal DA/GLU interactions have been implicated in the pathophysiology of schizophrenia, we examined whether alterations in glutamatergic, dopaminergic and serotonergic receptor functioning may underlie the changes in DA-mediated behaviors previously found. In these rats at PND 85 we assessed the density of 5-HT (^{3}H-paroxetine) and DA transporter sites (^{3}H-GBR 12935), and D_2 and NMDA-type GLU receptors (^{3}H-haloperidol and MK-801) in various brain regions. In the hippocampus of protein deprived female rats there was a 71% increase in the density of NMDA receptors, while the number of 5-HT uptake sites was unchanged from controls. In the striatum, significant increases in D_2 (43%) and NMDA (35%) receptors were observed while there was a 34% decrease in DA uptake sites. No significant changes were apparent in the cortex. Down-regulation of the DA transporter coupled with the up-regulation of D_2 receptor sites suggest less DA availability in the striatum. The elevated D_2 receptor density in the striatum may in part underlie our previously observed increase in DA-mediated behaviors. One potential mechanism of DA/GLU interaction in these animals could be that an increase in D_2 receptors on GLU terminals would decrease GLU release producing a functional NMDA receptor hypofunction, which could produce the compensatory increase in NMDA receptors seen here. The finding of alterations in NMDA and D_2 receptor binding provide further support for this as an animal model of schizophrenia.

18.5

NEUROANATOMICAL ABNORMALITIES IN PEDIATRIC OBSESSIVE COMPULSIVE DISORDER: IMPLICATIONS FOR ABNORMAL DEVELOPMENTAL MATURATION OF FRONTO-STRIATAL CIRCUITRY.

D. R. Rosenberg*, M. Keshavan, K. O'Hearn, E. Dick, W. Bagwell, A. Seymour and B. Birmaher. Western Psychiatric Institute and Clinic, University of Pittsburgh Medical Center, Pittsburgh, PA 15213

Abnormalities in specific fronto-striatal circuits have been implicated in obsessive compulsive disorder (OCD). Although OCD commonly emerges during childhood or adolescence, few studies have examined fronto-striatal anatomy in psychotropic-naive pediatric OCD patients near the onset of illness to study the possible role of atypical developmental processes in this disorder. Magnetic Resonance Imaging (MRI) scans from 20 psychotropic-naive, non-depressed OCD patients aged 7.2-17.7 years, and 20 case-matched healthy comparison subjects were analyzed to determine the volumes of the following structures: prefrontal cortex, basal ganglia (caudate + putamen), lateral and third ventricles, and intracranial volume. OCD patients had significantly smaller basal ganglia volumes ($F[1,33] = 9.33$, $p = .004$) than controls, but did not differ in prefrontal cortex, lateral and third ventricle or intracranial volume. Basal ganglia volumes were inversely correlated with OCD symptom severity (partial $r = -.65$, $p = .004$) but not illness duration. Volumes of other structures were not significantly correlated with the duration or severity of OCD symptoms. Age was not correlated with basal ganglia volumes, but was inversely correlated with prefrontal cortical gray matter (partial $r = -.53$, $p = .02$) and positively with lateral ventricular volumes (partial $r = .52$, $p = .03$) in OCD patients but not controls. Our findings provide new evidence of pathology of the basal ganglia and frontal cortex in pediatric OCD. Moreover, our data suggest a possible abnormality in the developmental maturation of prefrontal cortical gray matter and lateral ventricular volumes in early-onset OCD patients compared to controls.

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18.6

BRAIN SEROTONIN SYNTHESIS MEASURED WITH α[C-11]-METHYL-TRYPTOPHAN POSITRON EMISSION TOMOGRAPHY IN NORMAL AND AUTISTIC ADULTS D. C. Chugani,* O. Muzik, P. Chakraborty, T. Mangner, H. T. Chugani, Dept. of Pediatrics, Neurology, and Radiology, Children's Hospital of Michigan, Wayne State Univ., Detroit, Michigan

Infantile autism is characterized by disturbances of social interaction, language, and stereotypic behaviors. Increased platelet serotonin is found in one-third of autistics; however, serotonin metabolites in cerebrospinal fluid have not demonstrated consistent abnormalities. Local cerebral serotonin synthesis rate was measured with α[C-11]-methyl-tryptophan ([C-11]AMT) in normal adult brain (n=10; 5 males, 5 females; mean age 28.3 years) and 4 autistic adults (n=4; 3 males, 1 female; mean age 26.5 years) using positron emission tomography (PET). Dynamic brain image data together with arterial plasma input function were used to perform Patlak analysis to determine the K-complex, a macro-parameter which is directly proportional to the serotonin synthesis rate. Values of K-complex for different brain regions corresponded well to the distribution of serotonergic terminals in the brain previously determined in vitro. Values in normal females were 10-20% higher ($p<0.05$, MANOVA) than those measured in normal males. Comparisons made between male autistics and control male subjects showed that mean K-complex values were significantly higher for all brain regions in the autistic group ($p < 0.05$, MANOVA). In conclusion, we have shown gender differences in cerebral serotonin synthesis in normal subjects and increased serotonin synthesis in autistic men as compared to normal males.

Supported by the Mental Illness Research Association of Michigan.

18.7

PET WITH [FLUORINE-18]FLUORODOPA IN AUTISM. M. Ernst *, A.J. Zametkin, J.A. Matochik, D. Pascualvaca, P.H. Jons, K. Hardy, R.M. Cohen. Laboratory of Cerebral Metabolism, NIMH, NIH, Bethesda, MD

Autism is a pervasive developmental disorder of early onset characterized by impairment in social interaction, verbal and nonverbal communication and a restricted range of interests. Dopamine function has been proposed to play a role in the etiology of this disorder. Using positron emission tomography, presynaptic accumulation of [Fluorine-18]Fluorodopa was measured in basal ganglia (caudate nucleus and putamen) ventral tegmental complex (substantia nigra and ventral tegmentum), and frontal and occipital cortices of 14 autistic children (age: 13.1±2.4 years; 8 males / 7 females), and 10 healthy controls (age: 14.9±2.0 years; 7 males / 3 females). Results were expressed as ratios of specific to nonspecific (occipital cortex) radioactive counts ([Fluorine-18]). [Fluorine-18] ratio was significantly lower in the medial frontal cortex of autistic subjects compared to controls (t=2.6, p<0.02, df=22), and was similar in basal ganglia and ventral tegmental regions.

	Anterior medial Frontal Cortex	Basal Ganglia	Ventral Tegmental Complex
Control Ss (N=10)	1.15±0.47	3.18±0.98	1.10±0.63
Autistic Ss (N=14)	0.70±0.37	3.23±0.64	1.10±0.49

These findings support a dopaminergic deficit specific to the medial frontal cortex and are consistent with reports implicating the frontal cortex in the neuropathophysiology of autism.

Supported by the NIH Intramural Research Program

18.8

QUANTITATION OF DOWN SYNDROME BRAIN METABOLITE CONCENTRATIONS USING *IN VIVO* ^{1}H MR SPECTROSCOPY. W. Huang, E. Daly, J. Krasuski, L. Beason-Held*, S. Rapoport, and M. Schapiro. Lab. of Neurosciences, Natl. Inst. on Aging, NIH, Bethesda, MD 20892

Our laboratory has reported elevated cerebrospinal fluid (CSF) *myo*-inositol (mI) concentration, as well as elevated CSF choline concentration, independent of dementia, in Down Syndrome (DS) adults between 22 and 63 years of age. In this study, *in vivo* ^{1}H MR spectroscopy (MRS) was employed to measure absolute *brain* concentrations of the following metabolites in 12 nondemented DS (6 young, 35 ± 4 yr; 6 old, 47 ± 3 yr) and 12 age-matched control (6 young, 31 ± 6 yr; 6 old, 48 ± 6 yr) subjects: N-acetylaspartate (NAA), total creatine (Cr), choline-containing compounds (Cho), and mI.

Using a 1.5 T GE Signa scanner, MRS data were collected from a volume (*ca.* 8 cm^3) in the occipital area, parietal area, and a bottle of standard solution (containing known concentrations of the four metabolites) which was placed next to the subject's head during scanning. Absolute concentrations (mmol/liter wet brain tissue) in the two brain regions were obtained following comparison of spectral signal intensities between standard and brain, and correction for CSF volume fractions.

Brain mI and Cho concentrations were found to be significantly *increased* (mI: 50-60%, $p < 0.005$; Cho: 40-50%, $p < 0.005$) in both locations of both DS age groups. Cr was *increased* (20-30%, $p < .01$) in the old DS subjects in both brain regions, whereas NAA was *decreased* (10-20%, $p < 0.05$) in the occipital region in both DS age groups.

The increase of brain mI in DS is consistent with the CSF mI studies and with a gene dose effect, as the gene for transporting mI is found on chromosome 21. The increase of brain Cho is consistent with the increase of CSF choline in young DS adults and may reflect increased membrane phosphatidylcholine turnover.

18.9

PROFILES OF DENDRITIC PROTEIN EXPRESSION IN RETT AND DOWN SYNDROMES. W.E. Kaufmann*, C.V. Taylor, J.J. Israel. Depts. of Pathology, Neurology, and Pediatrics, Johns Hopkins University and Kennedy Krieger Institute, Baltimore, Maryland.

Rett (RS) and Down (DS) syndromes, as most forms of mental retardation (MR), show a generalized reduction in dendritic arborizations in neocortex. In both disorders, there is temporal correlation between dendritic anomalies and cholinergic deficiency: early in RS and late-progressive in DS. We have studied patterns of dendritic protein expression as an approach to understand the pathogenesis of these dendritic abnormalities. In both RS and a murine model of early neocortical cholinergic denervation, we have demonstrated a selective reduction in microtubule-associated protein (MAP)- 2 immunoreactivity. This investigation characterizes the expression of MAP-2 and other dendritic proteins in RS, contrasting them with DS, by quantitative immunoblotting after SDS/PAGE. Assays were performed on frontal cortical samples from 9 RS and 5 DS patients and 5 normal age-matched controls (NC), using monoclonal antibodies against MAP-2, MAP-5, and high and medium molecular weight (mw) neurofilaments (NFs), and the chemiluminescence method.

ANOVA and multiple regression analyses of optical density values, acquired using the NIH Image software, showed: a decrease in high mw MAP-2, which differentiated RS from NC and DS subjects (p=0.006 and p=0.04, respectively); a relative increase in white matter low mw MAP-2 in RS (p<0.05); and a reduction in the 68 kDa MAP-5 fragment in DS (p=0.016); without significant changes in NF. These findings support the hypothesis that MAP-2 expression is selectively altered in RS. The relative increase in the immature (low mw) MAP-2 isoform, suggest a developmental arrest as the pathogenetic mechanism. MAP-5 reduction in DS may be related to the reported reduction in neuronal density in this condition. Similar studies in the murine model, currently undergoing, will provide information about the role of early cholinergic deficit in the differential protein profile found in RS and DS neocortex. Supported by HD01046 and HD24448 and the Research for Rett Syndrome Foundation.

18.10

In Vivo Imaging of Pre-and Postsynaptic Dopamine Receptors in Rett Syndrome

D.F. Wong,*S.Naidu, F.Yokoi, G.Grunder, S. Szymanski, R.F. Dannals, H.Ravert, M.Kuhar, A.Gjedde, H.Moser. Johns Hopkins U., Baltimore, MD; Aarhus U., DK

Rett Syndrome (RS) is a disease of females associated with stereotypical movements, progressive rigidity, microcephaly and seizures. There is reduced melanin content and tyrosine hydroxylase in substantia nigra pars compacta (SNPC) with decreased tissue levels of dopamine. Post mortem decreases in D_2 dopamine receptors have also been observed.

We have carried out PET studies of both pre-and postsynaptic dopamine (DA) receptors. Twelve patients were studied for D_2 DA receptors using [^{11}C]3-N-methylspiperone (NMSP) and two PET scans, each with high specific activity (HSA) [^{11}C]NMSP, where one was preceded by haloperidol, to estimate B_{max} (Wong et al., 1986). Another 12 pts were studied with a single HSA [^{11}C]WIN 35,428 injection for dopamine transporter binding (DAT). For each scan, 50 frames and simultaneous plasma radioactivity via arterial sampling with HPLC metabolite correction was obtained over 90 min.

These studies have demonstrated low to low normal D_2 receptors compared to age-matched control values for the caudate. For the DAT, a simple tissue ratio method of total to nonspecific binding and a kinetic analysis measuring the binding potential were employed. The cerebellum (CB) K_1/k_2 was assumed in the striatum in fitting k_3/k_4 for B_{max}/K_D for the kinetic approach. When age matched (N=11) or age/gender matched (N=5) the kinetic method, for both the caudate (CA) and putamen (PU) yielded a significant reduction of up to 45% in the k_3/k_4 ratio ($p<0.01$). The CA/CB ratio and the PU/CB ratio were also significantly decreased with age/gender matching. In two subjects that had both pre- and postsynaptic measures, the DAT and D2 receptors were either low or low-normal. These findings together with decreased DA levels postmortem suggest that there may be immature SNPC cells, neuronal loss and/or down-regulation of DAT with no compensatory D2 up-regulation. Supported in part by HD 24448 and HD 24061.

18.11

GENES WITH TRINUCLEOTIDE REPEATS: A ROLE IN NEURODEVELOPMENT. R.L. Margolis[1]*, K.L. Chow[2], A.H. Sharp[1], M.R. Abraham[1], C.A. Ward[1], G. Schilling[1], O.C. Stine[1], C. Callahan[1], S.B. Gatchell[1], H.S. Yoon[1], M.G. McInnis[1], C.A. Ross[1]. Lab. of Molecular Neurobiology, Dept. of Psychiatry, Johns Hopkins Univ., Balto., MD 21205[1]; Dept. of Biology, Hong Kong Univ. of Sci. & Techn., Kowloon, Hong Kong[2].

In an effort to obtain candidate genes for neuropsychiatric disorders, we have used brain cDNA libraries to clone and characterize gene fragments containing trinucleotide repeats. We have recently reported that one of these, CAGR1, contains a highly polymorphic (CAG)n 5' untranslated repeat that varies in length in the normal population from 6 to 31 triplets (Margolis et al, 1996). One individual with bipolar affective disorder type II and a movement disorder has an allele with 46 triplets. CAGR1, located on chromosome 13q13, encodes an amino acid sequence homologous to the *C. elegans* cell-fate determining gene *mab-21*. We have used affinity purified polyclonal antibodies to examine mammalian CAGR1 protein expression. CAGR1 protein is expressed as a doublet migrating at about 40kDa on PAGE. Expression in several regions of the rat brain is developmentally regulated, with adult levels of expression not occurring until after the first postnatal week. The protein was found in both soluble and particulate fractions following subcellular fractionation. To further characterize the CAGR1 protein, we coupled a CAGR1 cDNA encoding the complete human protein to an inducible heat shock promotor. In both transient and integrating transformation of mab-21 mutant nematode lines, induced expression of this construct partially reverses the mutant phenotype of sensory organ deficits. Taken together, these results suggest that CAGR1 may play a role in the development of the mammalian nervous system. We are continuing to search for other cDNAs with polymorphic trinucleotide repeats and differential expression during development. (Supported, in part, by the NIMH, Stanley Foundation, and NARSAD Foundation.)

18.12

GENE THERAPY OF CANAVAN DISEASE. M. During*, Z. Zeng, R. Kaul, F. Sorgi, L. Huang, E. Mee, J. Wang, L. Mayes, L. Ment, P. Leone Auckland Medical School, New Zealand; Yale University, New Haven, CT; Miami Children's Hospital, Florida; University of Pittsburgh, PA.

Canavan Disease (CD) is an autosomal recessive leukodystropy associated with spongiform degeneration of the brain leading to death in the first decade of life. CD is characterized by mutations in the aspartoacylase (ASPA) gene resulting in loss of enzyme activity and elevated levels of N-acetyl-aspartate (NAA). We developed an *in vivo* gene transfer protocol for CD using a novel liposome-polymer-DNA (LPD) complex. LPD is a mixture of the neutral lipid DOPE with a cationic liposome, DC-Chol, together with poly-L-lysine to form unique virus-sized, i.e. 60-100 nm, non-aggregating particles. The expression plasmid consisted of a transcription unit (CMV promoter, human ASPA cDNA, SV40 polyA) flanked by AAV 145 bp ITR. Efficient transduction and enzyme activity was obtained *in vitro* using LPD-AAVaspa. *In vivo* studies in rats (n=120) and primates (n=4) demonstrated safety and stability of gene transfer (to 6 months). Convection, blood-brain-barrier disruption and intraventricular approaches were evaluated for global brain delivery. Using a combination of intraventricular injections with mannitol, widespread expression extending into brain parenchyma was observed. Regulatory approval was given by local ethical and regulatory committees including the FDA for a Phase I safety study. Two children (aged 19 and 24 months) were operated on in early March 1996. No adverse effects occurred apart from a mild transient postoperative fever. At 6 weeks post-gene transfer, there were improvements in both psychometric testing and neurological function. NMR proton spectroscopy demonstrated NAA levels within or close to the normal range in several brain regions including frontal cortex, parietal white matter and the anterior occipital lobe. Levels of NAA remain high in posterior occipital cortex and basal ganglia. This preliminary data suggests that sufficient gene transfer was obtained to result in partial phenotypic correction. (8 month follow-up data will be presented). This study demonstrates that direct intraventricular gene transfer within the human brain can be safe. Moreover, our indirect data on gene expression provides real hope for clinical efficacy of neonatal genetic intervention in neurogenetic disorders.

19.1

DIFFERENCES IN β-AMYLOID DEPOSITION IN TEN DOG BREEDS.

Marketta Bobik, M. J. Russell, R. G. White*, Y. Hou, and J. W. Geddes[1]. Dept. of Anesthesiol., Univ. California, Davis, CA 95616. [1]Center on Aging, Univ. Kentucky, Lexington, KY 40536.

We previously reported differences in the incidence of β-amyloid (Aβ) plaques in beagles and St. Bernards. The occurrence of Aβ in aged laboratory beagles is similar to that seen in Alzheimer's disease (AD), and the aged canine has been suggested as a model for the early stages of AD. Because comprehensive evaluations of dog breeds are limited, we have surveyed ten common breeds of dogs. Brain tissues from dogs that had been euthanized and archived for clinical reasons were evaluated. The breeds included: beagles, boxers, cocker spaniels, Dobermans, German shepherds, golden retrievers, Great Danes, Labrador retrievers, poodles and St. Bernards, the total of 314 dogs with a sample size of 13 -103 in each group. The percentage of Aβ positive dogs was found to be: 49, 65, 67, 56, 54, 67, 77, 65, 74, and 29, respectively for each breed. To determine the presence of Aβ deposits, coronal half-brain sections from the parahippocampal region were stained with: a modified Bielschowsky, Congo Red, Thioflavine-S, and immunohistochemistry (10D5 antibody from Athena). Marked differences were observed in: 1) plaque incidence, 2) plaque type, 3) plaque distribution, 4) plaque density, and 5) the age of onset of plaque deposition. Taken together these data suggest a genetic component in canine β-amyloid deposition. Cerebral Aβ angiopathy was independent of age and severity of amyloid plaques.

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19.2

OVEREXPRESSION OF PERLECAN AND BETA AMYLOID PROTEIN IN TRANSGENIC MICE.

K. Fukuchi*[1], T. Tokunaga[1], J.R. Hassell[2], and A.D. Snow[3]. [1]Dept. of Comparative Medicine, Univ. of Alabama at Birmingham, Birmingham, AL 35294; [2]Dept. of Ophthalmology, Univ. of Pittsburgh, Pittsburgh, PA 15213; [3]Dept. of Pathology, Univ. of Washington, Seattle, WA 98195.

Perlecan, a specific heparan sulfate proteoglycan, accumulates as part of the fibrillar amyloid deposits in neuritic plaques and cerebrovascular amyloid in Alzheimer's disease (AD). Several lines of evidence suggest that perlecan plays important roles in the pathogenesis of amyloidosis including AD. Perlecan also appears to be essential for consistent persistence of brain amyloid deposits in a rat infusion model for β-amyloidosis (Snow et al. 1994, Neuron 12:219). In order to study the roles of perlecan in AD amyloidosis and to possibly create a better animal model to study the neuropathology of AD, we have established transgenic mice that overexpress perlecan. A 12-kb cDNA coding for entire mouse perlecan under the control of a cytomegalovirus enhancer and β-actin promoter was injected into fertilized mouse eggs. Four founder lines of perlecan transgenic mice were identified by Southern blot analysis of DNA isolated from the emerging pups. These transgenic founders carried 1 to 10 copies of the transgene. Phenotypic consequences of overexpression of perlecan by the exogenous promoter will be presented. Perlecan transgenic mice will be mated with transgenic animals that overexpress human β-amyloid protein in order to establish new lines of transgenic mice that overproduce both perlecan and β-amyloid protein.

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19.3

ANALYSIS OF MICE WITH TARGETED APLP2 AND APLP2/APP ALLELES.

C.S. von Koch[1,*], F. Yu[3,4], H. Zheng[5], H. Chen[5], M. Trumbauer[5], L. van der Ploeg[5], D.L. Price[1,2] and S.S. Sisodia[1,2]. Dept. of Neuroscience[1], Pathology[2], Johns Hopkins Univ. Sch. of Medicine, Baltimore, MD 21205; Schepens Eye Research Institute[3], Harvard Medical School[4], Boston, MA 02114 and Merck Research Labs[5], Rahway, NJ 07065

APLP2 is a member of a larger gene family that includes APP and APLP1. In order to examine potential biological roles of APLP2 during development or aging, we have used gene targeting strategies to generate mice devoid of APLP2 expression. Homozygous APLP2 knockout (KO) mice are fertile and without overt abnormalities up to 14 months of age. Recently, *in situ* hybridization and immunocytochemistry analysis have shown that APLP2 is upregulated in migrating corneal epithelial cells following corneal wounding (Guo et al., submitted). To determine if APLP2 is required during corneal wound healing, we lesioned corneas of APLP2 KO and wild-type mice. Interestingly, we observed a ~1 day delay in wound closure compared with control mice. We are currently performing tissue culture experiments, using corneal explants, to more clearly define the role of APLP2 during wound healing.

Further, to study the function of APLP2 and other members of this gene family *in vivo*, we generated mice with targeted APLP2 and APP genes. Double KO mice are born at the expected frequency, but ~80% die within the first week after birth, suggesting a role for APLP2 and APP in early postnatal development. Surviving double KO mice are 20-30% reduced in weight compared with control mice and exibit ataxia, weakness and spinning behavior.

Supported by NIH, NINDS, AHAF and Adler Foundation.

19.4

DEVELOPMENT OF ß-AMYLOID PLAQUES IN HuAPP695.SWE TRANSGENIC MICE

G. M. Cole*[1], F. Yang[1], S.A. Frautschy[1], T. C. Saido[2] and K. Hsiao[3]. [1]Sepulveda VAMC, GRECC 11E, Sepulveda, CA. 91343 and Dept. Med., UCLA., [2]Dept. Mol. Biol.,Tokyo Metropolitan Inst. Science, Tokyo 113 Japan and [3]Dept. Neurol., Univ. Minn., Minneapolis, MN 55455

High copy number Tg(HuAPP695.SWE)2576 transgenic mice bearing the "Swedish" NL double mutation (Tg2576) developed ß-amyloid deposits with age which reacted with antibodies spanning the length of Aß from ß1 to ß42. Deposits were found in frontal, temporal and entorhinal cortex, presubiculum, subiculum, hippocampus, fimbria/fornix, corpus callosum and cerebellum. Deposits occurred between 9 and 12 months in the transgenic, but not in control mice. Dense deposits were thioflavin S positive and showed the amyloid characteristic apple green birefringence with Congo red. High magnification revealed the fibrillar character of thioflavin and Congo red positive amyloid. Deposits range from diffuse to classical. Gallyas silver stain identified abnormal neurites associated with deposits and the loss of fibers in areas with ß-amyloid immunoreactive deposits. Selected plaques were associated with GFAP labeled reactive astrocytes. Microglia were found adjacent plaques and in areas of Aß deposition. Unlike sporadic AD brain, most deposits were immunoreactive for both Aß40 and Aß42 as might be expected with the "NL" mutation which dramatically increases both Aß40 and 42. The areas around plaques showed abundant evidence of cell surface and process staining in the surrounding area where evidence of cellular and neuronal degeneration was evident. Additional punctate neuropil immunostaining was found with some Aß antibodies. Intense APP immunostaining could be found in neurons of the entorhinal cortex and hippocampus. Since this Tg2576 line of mice developed correlative age-related memory deficits and elevated Aß ELISA levels, it appears quite promising as a model for assessing the possible role of region-specific Aß deposits in age-related memory loss in an experimental model. Supported by NIH grants AG11125 (GMC), NS33249 (KH)

19.5

CORRELATIVE MEMORY DEFICITS, Aß ELEVATION AND AMYLOID PLAQUES IN TRANSGENIC MICE

K. Hsiao,[1]* P. Chapman,[2] S. Nilsen,[1] C. Eckman,[3] S. Younkin,[3] G. Cole[4]. [1]Department of Neurology, University of Minnesota, Minneapolis, MN 55455; [2]Physiology Unit, University of Wales, Cardiff CF1 3US; [3]Mayo Clinic Jacksonville, Jacksonville, FL 32224; [4]GRECC, Veterans Administration Medical Center, Sepulveda, CA 91343.

Transgenic mice overexpressing the 695-amino acid isoform of human K670N-M671L Alzheimer amyloid precursor protein (APP) driven by a prion protein cosmid vector have normal learning and memory in spatial reference and alternation tasks at three months of age but show impairment by nine to ten months of age. A five-fold increase in Aß(1-40) and 16-fold increase in Aß(1-42) accompanied the appearance of these behavioral deficits. Numerous congophilic Aß plaques were present in cortical and limbic structures in two mice with elevated Aß levels. The correlative appearance of behavioral, biochemical and pathological abnormalities reminiscent of Alzheimer's disease (AD) affords new opportunities for exploring the pathophysiology and neurobiology of AD in mice.

19.6

THE PDAPP TRANSGENIC MOUSE DEPOSITS ß-AMYLOID42 IN A REGION-SPECIFIC MANNER SIMILAR TO ALZHEIMER'S DISEASE

K. Johnson-Wood, M. Lee, R. Motter, K. Hu, G. Gordon, R. Barbour, M. Gordon, H. Tan, D. Games, I. Lieberburg, D. Schenk*, P. Seubert, and L. McConlogue.
Athena Neurosciences, Inc., So. San Francisco, CA 94080

The PDAPP transgenic mouse, over-expressing the human amyloid precursor protein ($APP717_{V->F}$) has been shown to display amyloid deposition and neuropathology similar to that seen in Alzheimer's disease (AD). This animal provides a convenient system to study the mechanisms whereby different brain regions show selective vulnerability for ß-amyloid (Aß) deposition. Quantitative measures of APP metabolites and Aß were made in hippocampal, cortical, and cerebellar brain regions of heterozygote transgenic mice from 4 months up to 12 months of age. The APP-beta and APP-alpha/full-length levels within each respective brain region remain nearly constant at all ages, whereas total Aß levels in the hippocampal and cortical regions increase in an age-dependent manner that mirrors amyloid plaque deposition observed immunohistochemically. For example, hippocampal Aß levels in 12 month old mice were 106-fold higher than in 4 month old mice. In contrast, cerebellar Aß levels were comparatively low and remained unchanged with age. The $Aß_{42}$ peptide has recently been shown to be the predominant form of Aß in AD. In the 4 month old transgenic mice, it represents ~27% of the Aß present which increased to ~97% in 12 month old transgenes. The selective deposition of $Aß_{42}$ and the spatial distribution of the Aß deposits are further evidence that the pathological processes occurring in the PDAPP transgenic mouse parallel the human AD condition.

19.7

DEVELOPMENT OF AGE- AND REGION- DEPENDENT PROGRESSION OF ALZHEIMER-TYPE NEUROPATHOLOGY IN PDAPP TRANSGENIC MICE.

D. Games*, T. Guido, K. Khan, F. Soriano, A. Justice, M. Mallory, R. Motter, L. McConlogue, D. Schenk and E. Masliah#. Athena Neurosciences, 800 Gateway Blvd., South San Francisco, CA 94080. #University of California, San Diego, Department of Neurosciences, La Jolla, CA. 92093.

Transgenic mice overexpressing mutant beta-amyloid precursor protein $717_{V->F}$ develop several pathological characteristics of Alzheimer's disease (AD), including the deposition of beta-amyloid protein (Aß) into plaques, neurodegeneration and gliosis. In ongoing studies, we are documenting the age and region- dependent development of Aß deposits, Aß plaque-associated proteins, synaptic and dendritic loss, neuritic pathology and cytoskeletal abnormalities. Similar to AD, both diffuse and compacted plaques developed in the affected regions, with the first deposits occurring at approximately 4 months of age. $Aß_{x-42}$ constituted the majority of the plaques and was associated with the early deposits. $Aß_{x-40}$ deposition was less abundant than $Aß_{x-42}$ and was primarily associated with plaque cores and vascular amyloidosis. Plaque deposition initiated in the frontal and retrosplenial cortices and hippocampus, with later involvement of the amygdala, temporal and entorhinal cortex. Aß deposition was well-established by 9 months of age. Ongoing studies are examining the age-related progression of synaptic, dendritic and gliotic alterations. The time course of these changes and a comparison of homozygotic and heterozygotic mice will be presented.

19.8

MOLECULAR AND ANATOMIC CORRELATES IN THE PDGF-hAPP V717F TRANSGENIC MOUSE. M.C. Irizarry*, K. J. Page, F. Soriano, D. Schenk, D. Games, and B. T. Hyman. Neurology, Mass General Hospital, Boston, MA 02114; Athena Neurosciences, South San Francisco CA 94080.

The PDGF-hAPP V717F transgenic mouse displays many neuropathologic features of Alzheimer's disease, most prominently Aß deposition. Anti-Aß stained sections from 12 heterozygote transgenic mice consistently demonstrate initial Aß deposition at 8 months of age in the dentate gyrus, CA1 hippocampal subfield and cingulate. By 12 months of age, Aß deposits are present in entorhinal cortex, but not in subcortical structures or amygdala. In this study we utilized in situ hybridization to correlate these pathological features with mRNA expression of transgene products, synaptic proteins, and bioenergetic proteins. The transgene expresses the mRNAs for the major human APP (hAPP) isoforms hAPP695, hAPP751, and hAPP770. hAPP751 mRNA expression in 4 and 11 month animals (4 transgenic animals each age) displayed a regional gradient, with highest mRNA expression in the dentate gyrus and CA1, followed by the cingulate. There was minimal expression in the amygdala. There was a trend ($p < 0.04$) to a small 8-23% increase in hAPP751 mRNA expression at 11 months compared to 4 months depending on brain region. Relative optical density of hAPP751 hybridization increased 39-74% in homozygote animals compared to heterozygotes. Regional mRNA distribution of synaptophysin and cytochrome oxidase IV did not differ among 4 heterozygote transgenic mice and 4 non-transgenic littermates at 4 months and 11 months of age. Thus, Aß deposition in these mice occurs with regional specificity that partially parallels expression of hAPP mRNA, and is not associated with significant alterations in synaptophysin or cytochrome oxidase IV mRNA in heterozygote animals at 4 and 11 months of age; further analysis of older animals is underway. Support: NIH AG05134-11S1.

19.9

ANIMAL MODELS FOR ALZHEIMER'S DISEASE BASED ON GENETICS AND PATHOLOGY

B. Sommer*, D. Abramowski, K. Bürki, K. Dixon^, M. Duke, P. Kelly, B. Ledermann, P. Paganetti^, A. Probst#, C. Sturchler-Pierrat, K.-H. Wiederhold, M. Staufenbiel. Sandoz Pharma AG, Basle; ^Sandoz Research Institute, Berne; #Inst. of Pathology, Basle; Switzerland.

In an attempt to obtain features of Alzheimer's disease pathology we produced transgenic mice expressing human APP. Several expression constructs were engineered with cDNAs encoding mutated and wt APP_{751} driven by various neuron specific promoters (Rhombotin, NSE, human Thy-1, mouse Thy-1, NF-L). In our hands, only the Thy-1 promoter gave rise to high expression levels of transgene mRNA. The highest levels obtained exceed the endogenous APP message by 1.5 to 5 fold. During aging of the animals, expression levels are maintained. In situ hybridization demonstrated the presence of APP transgene message throughout the brain with minor variations between different mouse lines. Expression studies have been extended to the protein level and confirm the in situ hybridization results. Biochemical analysis revealed considerably elevated levels of Aß peptide in the brains of mice expressing the double mutation 670/671. Histological analysis of these mice at the age of one year showed significant deposits of APP and Aß in cortex. These deposits were thioflavin S positive and showed Congo Red birefringence, significant features of senile plaques in AD pathology. The mice are currently undergoing behavioural assessment.

Furthermore, we used homologous recombination in embryonic stem cells to inactivate the APP gene as a strategy of assessing the natural function of the APP molecule and providing a mouse background lacking endogenous APP. A vector was designed to target the first exon of APP and electroporated into stem cells to generate chimeric mice. Homozygous APP k.o. mice have been generated and are currently under close investigation. (Supported by Sandoz Pharma AG)

19.10

FACTORS PROMOTING Aß DEPOSITION AND TOXICITY IN THE RAT.

S.A. Frautschy*, A. Li, M. E. Harris, F. Yang, G.M. Cole. (GRECC, Sepulveda VAMC, Dept. Med. & Neurol., UCLA)

Non-transgenic rodent models were developed to investigate factors that rapidly accelerate Aß deposition without increasing APP expression. Mini-osmotic pump infusion of soluble Aß40 was co-infused into the lateral ventricle of 9 month old rats in the presence of factors that potentially modulate Aß deposition. Compared to Aß40 infusion alone, co-injection of Aß42 caused a 50% increase in the number of diffuse Aß deposits. Co-infusion with Aß40 with the immunostimulating peptide tuftsin (Thr-Lys-Pro-Arg) increased the number of diffuse plaques, without increasing toxicity at one month after infusion. Infusion of an immunoglobulin-derived tripeptide (Thr-Lys-Pro) that suppresses microglia activity did not increase the number of diffuse plaques. These deposits were frequently associated with lectin-GSA B4 positive ramified microglia, but not astrocytes. Except for the finding that Aß42 infused rats had a diminution of hippocampal creatine kinase, biochemical analysis of brain homogenates did not suggest significant toxicity. We are currently developing more sensitive biochemical and histochemical methods to detect toxicity and whether the diffuse plaques eventually progress to become neuritic. Preliminary evidence suggests that the diffuse plaques are compacted with time by microglia. When apolipoprotein E related carriers were infused with Aß, gross neurotoxicity and enlargement of the ventricles relative to carrier alone was apparent by one month after infusion. These carrier-infused rats had diffuse Aß immunoreactive deposits but not amyloid plaques. These data suggest : (1) Aß neurotoxicity can occur independent of the development of amyloid plaques (2) microglia play an important role in plaque development (3) and Aß chaperones that increase delivery of Aß to the neuropil also increase toxicity. Supported by NS30195, AG10685

19.11

LATERALITY IN THE HISTOLOGICAL EFFECTS OF BILATERAL AMYLOID-β 25-35 INJECTIONS *IN VIVO* INTO THE AMYGDALA OF YOUNG F344 RATS.

E. M. Sigurdsson*, J. M. Lee, X. -W. Dong, M. J. Hejna, and S. A. Lorens. Depts. Pharmacology and Pathology, Loyola Univ. Chicago Medical Center, Maywood IL 60153.

The histopathological characteristics of Alzheimer's disease (AD) include amyloid deposition, reactive astrocytes, neurofibrillary tangles and neuronal cell loss in several brain regions. Previously, we have observed that single amyloid-β 25-35 (Aβ) injections (5.0 nmol) into the right amygdala (AMY) of rats produce progressive (8 vs. 32 d) cytoskeletal and astrogliotic reactions within the AMY, and at distant brain regions that project to the AMY (Neurobiol. Aging, in press). To examine the time course of these effects, and to determine if they are potentiated by bilateral injections of Aβ, we injected Aβ (5.0 nmol) into the right and left AMY of young male F344 rats. Control rats received vehicle infusions. Animals were sacrificed at 8, 32, 64, 96 and 128 d postoperatively (n = 16-29 per timepoint). Aβ induced neuronal tau-2 staining and reactive astrocytosis (GFAP) within the AMY, as well as at distal sites such as the hippocampus and cortical regions. These histological changes were associated with neuronal shrinkage within the AMY and nearby cortical areas but not in the hippocampus. Surprisingly, these changes were predominantly observed within the right hemisphere with the left hemisphere significantly less affected ($p < 0.05$-0.001). The effects of Aβ peaked at 32 d postoperatively ($p < 0.05$-0.001). These data support our previous findings within the right hemisphere at 8 and 32 d post-injection. They suggest, moreover, a laterality in the histopathological effects of Aβ, and that the effects of single injections are in part transient. These findings also suggest a direct association between plaque and tangle formation in AD, and support the use of this rat model to screen drugs that may attenuate or enhance the initial pathological events associated with AD. [Supported in part by an unrestricted research grant from the Institut de Recherches Internationales Servier (France)].

19.12

ß-AMYLOID-INDUCED NEUROTOXICITY AND TAU PHOSPHORYLATION IN THE AGED NON-HUMAN PRIMATE.

C. Geula*, C.-K. Wu, D.M. Saroff, A. Lorenzo and B.A. Yankner. Harvard Medical School, New England Deaconess Hospital and The Children's Hospital, Boston, MA 02215

Neurotoxic effects of intracerebral injections of fibrillar and soluble ß-amyloid protein ($Aß_{1-40}$) dissolved in PBS were assessed in one young adult marmoset, three aged marmosets and two aged rhesus monkeys. Injections of vehicle caused a small area of gliosis confined to the needle tract. In contrast, injections of fibrillar Aß (200 pg) caused marked neuronal loss and gliosis. The loss of neurons extended as far as 0.5-1 mm away from the center of the injection. Soluble Aß produced much less toxicity. Aß injections caused more toxicity in the aged marmosets when compared to the young marmoset. Fibrillar Aß also markedly increased staining for phosphorylated tau epitopes (at Ser^{262} and $Ser^{396/404}$) in neurons, neurites and tangle-like structures. Some neurons were affected at quite a distance away from the center of the injection. Soluble Aß or vehicle, on the other hand, induced low levels of tau phosphorylation in a very small number of neurons. These results show that injection of Aß at a level similar to that in a single senile plaque is capable of causing significant neurotoxicity and inducing tau phosphorylation in the aged monkey cerebral cortex. This model more closely reproduces the pathological changes observed in AD as compared with the rat model, and can be used for screening agents which may alter the neurotoxic effects of Aß *in vivo*. (Supported by NINDS and Sandoz Pharma Ltd)

20.1

POTENTIAL BRAIN NEURONAL TARGETS FOR AMPHETAMINE, METHYLPHENIDATE AND MODAFINIL INDUCED WAKEFULNESS EVIDENCED BY C-FOS IMMUNOCYTOCHEMISTRY IN THE CAT

J.S. Lin, Y. Hou, P.H. Luppi* and M. Jouvet Département de Médecine Expérimentale, INSERM U52, CNRS ERS 5645, Faculté de Médecine, Université Claude Bernard, 8 Avenue Rockefeller, 69373 Lyon, FRANCE

Many experimental and clinical data suggest that the pharmacological profile of modafinil, a newly discovered wake-improving substance, differs from that of amphetamine and methylphenidate, two classical psychostimulants. The brain targets on which modafinil acts to induce wakefulness, however, remain unknown. A double-blind study using the immediate-early gene c-fos as experimental marker in the cat was conducted, therefore, to identify the potential target neurons of modafinil and compare them with those for amphetamine and methylphenidate. Animals were sacrificed 90 and 120 minutes following a single oral administration of methylphenidate, amphetamine or modafinil at their equivalent doses for wake induction (2.5, 1, or 5 mg/kg respectively) and brain sections submitted to immunocytochemistry of c-fos. Both methylphenidate and amphetamine evoked a dense c-fos expression in a large number of neurons in the striatum and whole cortex, especially in the caudate nucleus and mediofrontal cortex known to be dopaminergic targets. In contrast, modafinil elicited no or few labelled cells in these strial and cortical structures, but did induce marked c-fos labelling in neurons of the anterior hypothalamic nucleus and other localised areas such as pontine and periaqueductal gray. These results provide the first evidence for the potential brain targets of modafinil, which differ from those of amphetamine or methylphenidate, and further support the belief that modafinil induces wakefulness by mechanisms distinct from those of the two stimulants. In addition, in the light of these data and the fact that modafinil reduces cortical GABA outflow, we proposed an hypothesis of cortical disinhibition for the mode of action of modafinil in wake induction.

20.2

BILATERAL, PONTOMESENCEPHALIC LESIONS INCREASE MONOCULAR DEPRIVATION (MD)-INDUCED, PLASTIC CHANGES IN LATERAL GENICULATE NUCLEUS(LGN) CELLS. James P. Shaffery*[1], Gerald A. Marks[2], Samuel G. Speciale[2] and Howard P. Roffwarg[1], Depts. of Psychiatry, [1]Univ. of Mississippi Med. Center, Jackson, MS 39216, & [2]Texas Southwestern Med. Center at Dallas, 75235.

Behavioral suppression of rapid eye-movement (REM) sleep in MD-kittens during the critical period of visual system development leads to augmentation of the morphological changes in LGN cells after MD alone. A cardinal electroencephalographic feature, which characterizes the activated nature of REM sleep, the ponto-geniculo-occipital (PGO)-wave, is eliminated with bilateral, pontomesencephalic lesions that sever the ascending pontine cholinergic fibers, thought to be responsible for producing PGO-waves. We predicted that because such lesions eliminate PGO-wave activity in LGN, while having no effect upon total sleep amounts, they would lead to morphological changes in LGN cell sizes in MD-kittens corresponding to what is observed after behavioral REM-sleep deprivation.

An opaque eye-patch was sutured beneath the right eyelid of 35 day old kittens and standard sleep-recording electrodes, including one in LGN, were implanted under IACUC approved conditions. After 6-7 days of recovery, a 24 hr baseline sleep-recording was obtained to detect PGO-waves. Kittens without PGO-waves (SHAM, N=7) were anesthetized (Ketamine and Xylazene) but no current was passed on electrodes implanted at initial surgery (P2, H-1 & either L2.5,3.5, or L1.0,2.0,3.0). Kittens exhibiting PGO-waves (LESION, N=7) received bilateral electrolytic lesions (5mA for 20s, or 30s). Changes in LGN cell sizes were indexed as a ratio of the mean of 100 A1-lamina cells in the LGN ipsilateral to the deprived eye to the mean of 100 A-lamina cells from the same LGN. During the seven day, post-lesion survival period only one LESION kitten still had PGO-waves. The other six LESION kittens, having a mean ratio (0.78, SE 0.05) significantly ($p < 0.05$) smaller than SHAM kittens (0.90, SE 0.3), exhibited a combined effect of MD and PGO-wave suppression similar to that seen after MD and behavioral REM-sleep deprivation. These data lend additional support for our hypothesis that REM-sleep has a distinct role in CNS development. Research supported by NIH/NINDS grant NS31720 to HPR.

20.3

SYNCHRONIZATION AND DESYNCHRONIZATION OF THE EEG AND BEHAVIORAL EFFECTS AFTER CHOLINERGIC STIMULATION OF THE NUCLEUS RETICULARIS PONTIS CAUDALIS. M. Garzón, I. de Andrés* and F. Reinoso-Suárez, Departamento de Morfología, Universidad Autónoma. Madrid, 28029, Spain

Local cholinergic stimulation of the ventral part of the nucleus reticularis pontis oralis (vRPO) produces EEG desynchronization with all polygraphic and behavioral signs of paradoxical sleep (PS). In the present study we have tried to precise the caudal extension of the PS producing area in the pontine tegmentum examining the polygraphic and behavioral effects of small volume carbachol microinjections (20 nl; 0.2M, 0.02M and 0.01M concentrations) delivered in different sectors of the nucleus reticularis pontis caudalis. Eight cats with electrodes for EEG, EOG, EMG and PGO chronic recordings were used. Carbachol microinjections in the rostral part of RPC at the level of the trigeminal motor nucleus (3 cats) resulted in sleep behavior associated with atonia and PGOs, but simultaneously there was a conspicuous synchronization in the cortical EEG. Synchronized EEG and PGOs but not atonia were obtained after RPC microinjections at the level of the abducens nucleus (2 cats). Behavioral and polygraphic wakefulness with desynchronized EEG occurred in the remaining 3 cats which had the most caudally RPC microinjections, situated at the level of the facial nucleus. EEG power spectra showed that the carbachol RPC-induced EEG synchronization after the highest carbachol dose (0.2M) was due to an enhancement of the 4-9 Hz band; while an increase of the 10-14 Hz band took place in EEG synchronization after the two remaining doses of carbachol (0.02M and 0.01M). In spite of the behavioral sleep of the animals which had EEG synchronization after RPC carbachol microinjections, a decrease of the 0.5-3 Hz band was observed. The present results indicate that the rostral RPC may be involved in the generation of some PS events such as PGO waves and atonia, but it seems not participate in PS desynchronization. EEG desynchronization after RPC cholinergic stimulation (in the caudal part) is associated with wakefulness.
Supported by PB94-1545 DGICYT and CI1*-CT93-0002 EC Grants

20.4

LONG-TERM ENHANCEMENT OF REM SLEEP BY MICROINJECTION OF VIP INTO THE ORAL PONTINE TEGMENTUM

P. Bourgin, C. Lebrand, M. Hamon and J. Adrien , (SPON: E.N.A.)
INSERM U288, CHU Pitié-Salpêtrière, 75013 Paris, FRANCE.

Rapid eye movement (REM) sleep can be elicited in the rat by microinjection of the cholinergic agonist carbachol into the oral pontine reticular nucleus. Intracerebroventricular administration of vasoactive intestinal peptide (VIP) during the light period enhances REM sleep in several species. Since this latter peptide is co-localized with acetylcholine in many neurons in the central nervous system, we hypothetized that the oral pontine tegmentum could be one target for VIP to induce REM sleep. After classical implantation of electrodes for sleep monitoring and of a guide tube for microinjections, we studied the effects on the sleep-wakefulness cycles of infusion into the oral pontine reticular nucleus of: 1- VIP (1 and 10 ng dissolved in 0.1 µl saline), and: 2- fragments of VIP (VIP 1-12: 4.3 ng and VIP 10-28: 7 ng) in order to verify the specificity of the VIP effects.
When injected into the posterior part of the oral pontine reticular nucleus, the peptide (17 rats), but not its fragments (9 rats), produced an increase in the amounts of REM sleep. VIP at 1 (n=6) and 10 (n=17) ng induced a two-fold enhancement of REM sleep during 4 hours, at the expense of wakefulness. At the dose of 10 ng, these effects persisted during another 4 hours. Moreover, when the peptide was injected into the center of the positive zone (7 rats), REM sleep was enhanced during 3 to 8 consecutive days. These data provide the first evidence that REM sleep can be elicited at both short and long-term by a single intracerebral microinjection of VIP. Short-term effects would result from simple diffusion of the peptide up to the center zone. The results suggest that this peptidergic mechanisms within the caudal part of the oral pontine reticular nucleus may play a critical role in the long-term regulation of REM sleep. Further investigations will study the possible peptidergic-cholinergic interactions in this effect.

20.5

MICRODIALYSIS PERFUSION OF AN ADENOSINE UPTAKE INHIBITOR IN THE BASAL FOREBRAIN INCREASES SLEEP. Robert E. Strecker*, Tarja Porkka-Heiskanen[1], Mahesh Thakkar, Alvhild Alette Bjorkum, and Robert W. McCarley, Dept. of Psychiatry, Harvard Medical School, Brockton VA Med. Ctr., Brockton, MA 02401, and [1]Institute of Biomedicine, University of Helsinki, Finland.

Increased levels of CNS adenosine (AD) are thought to promote the transition from wakefulness to sleep. Our lab has previously shown that AD inhibits mesopontine cholinergic (ACh) neurons involved in REM sleep regulation, and also that microdialysis perfusion of 300 µM AD in the basal forebrain (BF) produces increased sleep in vivo. The present study tested whether increasing endogenous BF AD levels with an AD uptake inhibitor would also increase sleep. Microdialysis was performed in freely moving cats implanted with electrodes for the recording of behavioral state, and with cannula used to guide the probes to either the substantia innominata region of the BF (rich in ACh neurons), or the VA/VL of thalamus. Behavioral state and extracellular AD levels were simultaneously measured during a 3h control period, and during the subsequent addition of S-(4-nitrobenzyl)-6-thioinosine (NBTI; 1µM), a potent AD uptake inhibitor, to the artificial CSF perfusate. BF perfusion of NBTI (N=6) produced a significant decrease in the total proportion of time spent awake (from 50% to 31%), and a corresponding increase in slow wave sleep (from 40% to 52%). REM sleep increased also (11% to 17%), but not significantly ($p<0.1$). In contrast, NBTI perfusion in the VA/VL (N=4) did not produce a consistent change in behavioral state. NBTI produced a 2 fold increase in extracellular AD levels in 4 of 9 experiments. The finding that NBTI perfusion in BF, but not VA/VL, decreases wakefulness suggests that the AD effect on arousal may be specific to regions containing ACh neurons that are known to modulate CNS arousal. Supported by NIMH grant MH39683; Dept. Vet. Affairs; Fulbright Fellowship to AAB.

20.6

THE EXPRESSION OF C-FOS AND NGFI-A DURING SPONTANEOUS WAKEFULNESS IS DECREASED AFTER LESIONS OF THE LOCUS COERULEUS. G. Tononi*, C. Cirelli, and M. Pompeiano, *The Neurosciences Institute, San Diego, CA 92121*

The expression of the immediate early genes (IEGs) c-fos and NGFI-A is higher after periods of waking (W) than after periods of sleep (S) in many brain regions. Systems with diffuse projections that fire at higher levels during W than during S, such as the noradrenergic locus coeruleus (LC), may coordinate such changes in gene expression. To determine whether LC activity is necessary for the induction of IEGs during W, WKY rats implanted for polysomnographic recordings were injected with 6-hydroxydopamine (6-OHDA, RBI) into the LC of one side (0.5-4 µl; 2.5 µg/µl). Animals were sacrificed after 2 weeks either during the light period after 3h of S, or in the dark period after 3h of W. As expected, in control rats immunocytochemistry for Fos (CRB) and NGFI-A (Zymed) showed no Fos and little NGFI-A protein expression after 3h of S (n=7) but a marked expression of Fos and NGFI-A in cerebral cortex and other brain areas after 3h of W (n=8). However, in rats who had been awake but in which the LC of one side had been lesioned (n=6), as indicated by the disappearance of dopamine B hydroxylase- (DBH, Eugene) and tyrosine hydroxylase- (TH, Boehringer) positive fibers in target cortical regions, Fos and NGFI-A expression was almost abolished on the lesioned side. On the intact side Fos and NGFI-A levels were high, comparable to those observed in normal rats after periods of W. To rule out circadian effects, other rats were kept awake for 3h during the light periods (n=9). As after spontaneous W, Fos and NGFI-A levels were high on the intact side but low or absent in areas depleted of TH-positive fibers. The levels of CREB phosphorylation (anti-P-CREB, UBI), which is involved in the transcriptional activation of both c-fos and NGFI-A, were reduced in correspondence with the disappearance of TH-positive fibers. There were no differences between the electrocorticogram on the intact and on the lesioned side, and power density spectra were comparable. To rule out volume conduction effects, a group of rats (n=5) was injected systemically with DSP-4 (RBI, 50mg/kg) to destroy LC terminals bilaterally. These animals exhibited a bilateral reduction of cortical TH staining associated with a corresponding reduction of Fos, NGFI-A, and P-CREB staining. However, the raw electrocorticogram and its power density spectrum were not significantly different before and after (5 to 8 days) the injection. These results suggest that LC activity during W is necessary for the induction of IEGs during W, while it is not necessary for the activation of the electrocorticogram. (Supported by Neurosciences Research Foundation).

20.7

CAUSALITY ANALYSIS OF RHYTHMIC ACTIVITIES OF DESYNCHRONIZED SLEEP IN THE RAT. [1]K.Sameshima*, [2]L.A.Baccala, [1,3]G.Ballester, [1,3]A.C.Valle and [1]C.Timo-Iaria. Univ. São Paulo [1]School of Medicine and [2]Escola Politécnica; [3]Fed. Univ. São Paulo Medical School (EPM). 01246-903 São Paulo, SP, Brazil.

The degree of synchrony between two neural structures can be assessed through coherence of their electro-oscillograms. Alas, classical coherence proves useless in addressing the physiologically crucial question of causality between signals originating in two or more neural structures. One possible way to overcome this limitation resides in the study of directed coherence which allows one to determine the direction of information flow between structures by testing for the existence of signal feedback between them. Investigations of the theta rhythms during desynchronized sleep (employing electrocorticograms and subcortical field potentials recorded by bipolar electrodes) through this methodology revealed among other things that (i) direction of influences among structrures exibiting theta oscillations fluctuates along the dessinchronized sleep, revealing the rat's dynamic state during the genesis of rhythmic electrical activity; (ii) information flow is essentially unidirectional from denteate gyrus to CA3, and to CA1 hippocampal fields in episodes of irregular oscillation, and the direction reverses or becomes bi-directional during oniric episodes when whiskers are observed to move vigorously; (iii) the usually high influence measured by directed coherence of cortical areas 3 and 17 onto hippocampal structures dies down during episodes of irregular oscillation; (iv) mutual influence between these areas may occur at different frequencies. These preliminary findings suggest that studies of the directed coherence can be useful in dynamically determining the functional as opposed to the anatomical relationships between neural structures.

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20.8

REM SLEEP SELECTIVELY ACTIVATES ANTERIOR PARALIMBIC GLUCOSE METABOLISM. E.A. Nofzinger, R.Y. Moore, M.B. Wiseman, B. Gao*, D.J. Kupfer, M.A. Mintun. Sleep and Chronobiology Center, PET Center, Center for Neuroscience, Univ. Of Pittsburgh, Pittsburgh, PA 15213.

Recent studies have elucidated brainstem mechanisms involved in REM sleep and it is well-known that this sleep state is associated with a general pattern of global cortical arousal similar to waking. Much less is known about other aspects of REM sleep and, particularly, about other forebrain mechanisms that would distinguish REM sleep from waking. This seems particularly important given the apparent importance of REM sleep in learning and memory and affective state regulation and the clear abnormalities of REM sleep associated with mental disorders. For these reasons, we sought to determine the differences between forebrain activity in REM sleep and waking in healthy control subjects using PET. Adult women (n=6) were screened by structured lifetime psychiatric interviews, medical history and examination, then underwent three nights of EEG sleep studies, magnetic resonance imaging scans of the head, and waking and REM sleep-related 18F-fluoro-deoxyglucose PET scans. PET images were spatially normalized using MR-based anatomical resolution and differences between the waking and REM sleep PET scans were determined by means of statistical parametric mapping (SPM, Friston, 1995). Regions showing increased relative glucose metabolism during REM sleep in comparison to waking included the orbital, paraolfactory, and medial prefrontal cortex, the anterior cingulate cortex and the insular cortex. These regions share several important features: 1) projections from midline thalamus; 2) reciprocal connections with the amygdala and other phylogenetically old cortical areas; 3) projections from dopaminergic and cholinergic cell groups. These structures are involved particularly in autonomic and endocrine control, motivated behavior and attention and form an important component of forebrain structures integrating homeostatic regulation with behavioral adaptation. REM sleep, therefore, in addition to activating neocortex, selectively activates an older forebrain network involved in integrating physiological regulatory mechanisms with behavioral adaptation.
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20.9

SLEEP-WAKE ACTIVITY AND THERMOSENSITIVITY OF NEURONS IN THE VENTRAL LATERAL PREOPTIC AREA OF RATS. M.N. Alam, R. Szymusiak*, T.L. Steininger and D. McGinty. Res. Service (151A3), V.A. Medical Center, Sepulveda, CA 91343 and Dept. Psychology, UCLA.

A subpopulation of warm-sensitive neurons in the medial preoptic/anterior hypothalamus of cats display increases in discharge rate at the onset of sleep (Alam et al., *Am J Physiol 269:*R1240, 1995). Warm-sensitive/sleep-active neurons are hypothesized to be a critical component of the rostral hypothalamic sleep regulatory mechanism. Enhanced c-fos expression during sleep has been recently described in a population of neurons located in the adjacent ventral-lateral preoptic area (VLPO) of rats (Sherin et al., *Science 271:*216,1996). Sleep-wake discharge and thermo-sensitivity of VLPO neurons have not been characterized.

We examined sleep-waking discharge patterns of 47 cells in the VLPO region of rats, using chronic microwire technique. The majority of VLPO cells were slow-firing, with 30/47 exhibiting maximum discharge rates of <2Hz and only 6/47 exhibiting peak rates >5 Hz. The most common cell type found were those displaying at least 20% higher discharge rates during nonREM sleep compared to waking (19/47). VLPO/sleep-active cells averaged a 115±29% increase in discharge rate during nonREM sleep compared to waking. Of the remaining cells, 17 exhibited higher discharge rates in waking compared to sleep, and 11 displayed similar rates during waking and sleep. Fifteen of 19 VLPO/sleep-active neurons were examined for responsiveness to local changes in temperature. A majority (10/15) were classified as warm-sensitive (>10% increase in discharge rate/°C); 2/15 were cold-sensitive and 3/15 were temperature insensitive.

These preliminary results demonstrate the presence of warm-sensitive/sleep-active neurons within the rat VLPO. *Supported by the Veterans Administration.*

20.10

ACTIVITY OF REM-OFF CELLS DURING CATAPLEXY IN THE NARCOLEPTIC DOG. M.-F. Wu*; S. Gulyani, E. Mignot[1], and J. M. Siegel. Neurobiol. Res. (151A3), Sepulveda VAMC, CA 92343, Dept. Psychiat., UCLA, CA 91020 and [1]Dept. Psychiat., Stanford Univ. Sch. Med., Palo Alto, CA 94305.

Cataplexy in narcoleptics has been hypothesized to be due to the release of REM sleep components into waking. We have previously reported on cells in the pons and the medulla of narcoleptic dogs that increase activity during cataplectic attacks and also during REM sleep (REMS), as well as cells that increased firing in REMS but not in cataplexy. Now we report on cells in the area of locus coeruleus (LC) that ceased firing in REMS and in cataplexy (CAT).

Cataplexy-off/REM-off cells (n=7) had a slow, regular, firing pattern in quiet waking, greatly reduced activity in non-REM sleep (NREMS), and were mostly silent in REM sleep and cataplexy (mean discharge rates in spikes/s: AW=1.37±0.15; QW=1.43±0.24; NREMS=0.28±0.05; CAT=0.31±0.18; REMS = 0.10±0.01). These cells were determined to be noradrenergic LC cells according to the criteria adapted from previous studies in the cat and rat. Clonidine (2 μg/kg, i.v. or 0.1 mg/kg, p.o.) reduced discharge rate by 50% in waking. Cells ceased firing in cataplexy and REMS after physostigmine (0.05 mg/kg, i.v.) and prazosin (0.5 mg/kg, p.o.), as in pre-drug conditions. Physostigmine, however, did not alter discharge in waking without cataplexy. On the other hand, prazosin reduced cell activity by an average of 56% in waking. While REM-off cells always ceased discharge during cataplexy, discharge cessation followed cataplexy onset and resumption of discharge preceded cataplexy offset, indicating that cessation of discharge in LC cells results from and may regulate cataplexy, but does not initiate the triggering of cataplexy. These studies suggest that cataplexy results from a disruption of REMS state organization, including an altered time course of activity change in REM-on and REM-off cells.
Supported by the Depart. of VA and USPHS grant NS14610.

20.11

BILATERAL LESIONING OF THE FASCICULUS RETROFLEXUS DISRUPTS THE REM STAGE OF SLEEP IN RAT

A. Valjakka[1], M.M. Airaksinen[1], J. Vartiainen[2], J.T. Tuomisto[3], H. Olkkonen[4] and L. Tuomisto[1]*
[1]Dept. of Pharmacol. and Toxicol., University of Kuopio, [2]Nokia Research Center, Tampere, [3]National Public Health Institute, Kuopio, [4]Dept. of Applied Physics, University of Kuopio, Finland

The fasciculus retroflexus (FR) fiber bundle comprises the well delineated bidirectional axonal connection between the habenular nuclei complex of epithalamus and the interpeduncular nucleus (IPN) of limbic midbrain. According to the widespread anatomical connections of the IPN it may function as an essential signal communication crosspoint between the limbic forebrain and the limbic midbrain and the more caudal pontine structures which contain areas intimately involved in the control of sleep-waking stages. Hence, the present study addressed the influences of bilateral electrolytic lesioning of the FR on sleep-waking stages as assessed by six-hour day-time recordings of electroencephalogram (frontal cortex and hippocampus), electro-oculogram and body movement activity, in freely behaving rats. As compared to the control-operated rats, FR lesioning almost completely abolished the time spent in rapid-eye-movement sleep (REM) but had no significant effects on the durations of other types of synchronized sleep patterns (quiet, slow-wave or intermediate states of sleep) or active/quiet waking stages. REM sleep loss was attributable to the reduction of hippocampal theta activity and alterations of REM frequency without significant changes in frontal cortex EEG properties. Our results point to the dissociation of REM sleep control mechanisms comprising FR, IPN and basal forebrain-hippocampal parts of the limbic system from the thalamocortical arousal control.

20.12

A SUBPYROGENIC ENDOTOXIN CHALLENGE INCREASES SWS AND DELTA POWER IN HEALTHY MEN J. Mullington*, D. Hermann, C. Korth, F. Holsboer and T. Pollmächer. Max Planck Institute of Psychiatry, Clinical Institute, 80804 Munich, Germany

The current study was designed to examine nocturnal sleep following endotoxin challenge (*Salmonella abortus equi*) administered close to nocturnal sleep onset. A placebo controlled crossover design was used. Subjects were permitted to sleep ad libitum following injection of either .8 ng/kg (n=5), .4ng/kg (n=5) or .2 ng/kg (n=7) body weight. Blood was sampled through an indwelling forearm catheter, processed for white blood cell counts or centrifuged and frozen for later assay of cytokines and cortisol. Rectal temperature (rT), EEG and EKG were measured continuously. Sleep was analyzed using standardized stage scoring as well as spectral analysis.

Significant ($p<0.05$) increases were found following each dose of endotoxin for TNF-alpha (174.3, 72.1, and 39.8 pg/ml for .8, .4 and .2 ng/kg respectively), IL-6 (235.9, 46.0, and 25.9 pg/ml), IL-1ra (35.1, 20.5, 3.0 ng/ml) and cortisol (192.8, 91.9, 26.2 μg/l). rT was increased following the .8 and .4 ng/kg doses (1.5°C and 0.5°C respectively, $p<0.05$), but not following the .2ng/kg dose (0.1°C, *n.s.*). Sleep was transiently disrupted following the .8 and .4 ng/kg doses and REM sleep was suppressed, but following the .2 ng/kg dose, minutes of SWS were increased in every subject (42.9±5.7 vs 54.4±5.8 SEM, $p<0.05$). In addition, EEG delta power in SWS was increased 476.7±19.0 vs 537.7±24.7 SEM, $p<0.01$), particularly due to changes in the first 3 hours following the .2 ng/kg injection. These results demonstrate complex modulation of the human sleep-wake regulatory system by endotoxin host challenge. While endotoxin was sleep disruptive at 0.8 and 0.4 ng/kg, it led to enhanced sleep at subpyrogenic levels. Dose dependent sleep disruption and enhancement occurred when TNF-alpha and IL-6 were at peak levels, 2 h *before* the highest rT. Because subpyrogenic doses of endotoxin mimic a very early stage of host response, it seems possible that SWS plays an adaptive role particularly during the early stage of infection. -Supported by the Volkswagen-Stiftung, Hanover, Germany.

21.1

SEQUENTIAL SEGREGATION OF TRUNK NEURAL CREST CELL FATES. P. D. Henion, A. Novicki* and J. A. Weston. Institute of Neuroscience, University of Oregon, Eugene, OR 97403

During embryonic development, trunk neural crest cells give rise to peripheral neurons, glial cells, and melanocytes. The process by which neural crest cells diversify, however, is unclear. We tested the prevailing hypothesis that neural crest cell precursors are specified in a stochastic manner from pluripotent precursors. We used *in vitro* clonal analysis to determine the differentiative behavior of individual neural crest cells at different times after segregation from the neural tube. We labelled individual cells of cultured quail neural crest populations with lineage dye, grew the cultures in a homogeneous, nutrient-rich medium, and subsequently determined the phenotype(s) of cells in the resulting clones using cell type-specific markers. We found that the initial population, 1-6h after cells had started to segregate from the neural tube, contains some fate-specified precursors, but is composed primarily of unrestricted or partially restricted precursors. One or possibly two cell divisions (4-7h) later however, we found no cells that gave rise to both neurons and melanocytes. Thus, soon after segregation from the neural tube, neurogenic and melanogenic fates had segregated. One day later, and two days before neuronal differentiation can first be detected, neuronal and glial fates had segregated. These results indicate that neural crest cell fates are specified soon after segregation from the neural tube in a sequential, non-random manner, even in a homogeneous *in vitro* environment. Supported by NS29438 and NS09031.

21.2

CHICKEN EHAND AND DHAND INFLUENCE NEURAL CREST CELL DIFFERENTIATION. M. J. Howard* and P. Cserjesi. Medical College of Ohio, Toledo, OH 43699 and Columbia University NY, NY 10032.

Members of the basic helix-loop-helix (bHLH) family of DNA binding proteins have been implicated as having an important role in the development of subpopulations of peripheral neural crest-derived neurons. In rodents, the expression of MASH-1, an achaete-scute DNA binding protein homologue, has the temporal and spatial expression to indicate a role in peripheral neuron development. In animals that have a targeted null mutation of MASH-1, the development of neural crest-derived sympathetic and enteric serotonergic neurons is greatly reduced (Guillemot et al., 1993, Cell 75:463-476: Blaugrund et al., 1996, Development 122: 309-320). We have cloned the chicken homologues of dHAND (ckdHND) and eHAND (ckeHND), bHLH DNA binding proteins that have a similar but restricted expression pattern compared to MASH-1. Our whole-mount *in-situ* hybridization studies, using radiolabeled riboprobes synthesized from our cloned ckeHND and ckdHND cDNA's, suggest that ckeHND and ckdHND mRNA is present in heart, sympathetic and enteric neurons, beginning as early as two days in the heart and appearing between three and four days in peripheral neurons. No signal was detected in dorsal root ganglia or brain. In order to determine if neuronal differentiation would be affected by blocking expression of these gene products, antisense oligonucleotides (50 μM) were applied *in-vitro*, to segments of quail neural tube from which neural crest cells were migrating. The differentiation of neurons was assessed by morphological criteria and the expression of either neurofilament proteins or the neurofilament associated protein, NAPA 73. Neither ckeHND or ckdHND antisense oligonucleotide applied individually blocked the differentiation of neural crest-derived neurons. However, if both antisense oligonucleotides were applied together (25μM each) over a period of 7 days, neural crest-derived neurons failed to develop even though the differentiation of neural tube-derived neurons was unaffected. These data suggest that chick HAND gene products can act in concert to influence the development of neural crest-derived neurons in culture. This work was supported by NIH-HD 28184 to MJH.

21.3

ROLE OF MASH-1 IN THYROID C-CELL DIFFERENTIATION AND GENE EXPRESSION. T. M. Lanigan[1] and A. F. Russo,*[1,2] [1]Mol. Biol. Program and [1,2]Dept. of Physiology and Biophysics Univ. of Iowa. Iowa City, IA 52242.

We have investigated the role of the developmentally-regulated transcription factor, MASH-1, in thyroid C-cell differentiation. A logical target for MASH-1 action is the calcitonin/calcitonin gene-related peptide (CT/CGRP) gene, since the phenotypic hallmark of C-cells is expression of the hormone calcitonin. We first showed that MASH-1 is present in juvenile rat C-cells. This is an unusual finding, since the only other example of MASH-1 expression in adult tissue is in the presumed neuroblasts of the olfactory epithelium. We have demonstrated that MASH-1 could transactivate the CT/CGRP cell-specific enhancer 3-4 fold in a heterologous cell line. Based on these findings, we predicted: 1) MASH-1 may regulate CT/CGRP gene expression *in vivo* and 2) MASH-1 may be required for thyroid C-cell differentiation. To test these predictions, we obtained homozygous mouse embryos containing a targeted mutation in MASH-1. Using a sensitive RT-PCR assay, we found calcitonin mRNA in the MASH-1 knockout mice. These preliminary data suggests that MASH-1 is not required for either C-cell differentiation or calcitonin expression. These results support the idea that C-cells are derived from a sympathoadrenal progenitor, and positions C-cells within a neuronal developmental pathway. This work was supported by National Institutes of Health (HD25969).

21.4

TRANSCRIPTION FACTOR INVOLVEMENT IN CELL FATE DETERMINATION IN THE RAT PNS, L.M. Donahue* and A.J. Reinhart. Dept. of Cell Biology and Biochemistry, Texas Tech University Health Sciences Center, Lubbock, TX 79430.

We are interested in the molecular mechanisms underlying the ability of multipotential neural crest stem cells to give rise to neurons and glia. We are using the rat PNS-derived cell line family called RT4 as a model of neuronal and glial differentiation. The RT4 stem cell (RT4-AC) converts spontaneously and reproducibly in culture to two neuronal derivatives (RT4-B, RT4-E) and one glial derivative (RT4-D). There is now a large body of evidence to suggest a role for transcription factors in the regulation of cell fate decisions during neural differentiation. We are examining the function(s) of candidate transcription factors expressed in the RT4 cell line family to assess their involvement in the conversion of the stem cell to the derivative cell types. We report here that four previously isolated POU domain-containing transcription factor genes (Oct-1, Oct-2, Brn-5 and SCIP), and REST, a repressor of mature neuronal gene transcription, are expressed in the RT4 family. REST, Oct-1, Oct-2 and Brn-5 are expressed in all of the RT4 cell lines, but SCIP is expressed only in the stem cell and glial derivative. Although Oct-2 is expressed in all of the RT4 cell types, it is preferentially expressed in the neuronal lineages. Work is currently underway to determine the role(s) of SCIP and Oct-2 in the conversion of the RT4 stem cell to the derivative cell types.

Work funded by NIH grant HD29400

21.5

ORIGIN OF INTERSTITIAL CELLS OF CAJAL IN THE MOUSE INTESTINE. H.M. Young*, D. Ciampoli, B.R. Southwell and D.F. Newgreen[1]. Department of Anatomy & Cell Biology, University of Melbourne, Parkville, 3052, VIC, Australia, and [1]The Murdoch Institute, Royal Children's Hospital, Parkville, 3052, VIC, Australia.

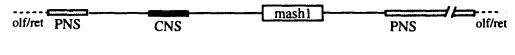
The interstitial cells of Cajal (ICC) are found in a number of different locations in the gastrointestinal tract, where they form close associations with both muscle cells and nerve terminals. They express receptors to a variety of different neurotransmitters and also the receptor tyrosine kinase, c-*kit*. In this study we examined the embryological origin of ICC in the mouse intestine to determine whether they arise from the neural crest or from the mesoderm of the intestinal wall. Segments of intestine were removed from embryos either before or after the arrival of neural crest cells (the precursors of enteric neurons and glial cells) and transplanted under the renal capsule of host (adult) mice and allowed to develop for 3 - 5 weeks. In the mouse intestine, antibodies to c-*kit* selectively label ICC at the level of the myenteric plexus and antibodies to the neurokinin receptor, NK1, selectively label ICC at the inner margin of the circular muscle. The presence of neurons in the explants was examined using antisera to neuron-specific enolase, nitric oxide synthase, substance P and calretinin. In segments of gut explanted after the arrival of neural crest cells, immunoreactive neurons and c-*kit*- and NK1-immunoreactive ICC were present with a similar distribution to that seen in control tissue at a similar developmental age. In segments of gut explanted before the arrival of neural crest cells, neurons were not present, however immunoreactive ICC were present in these aneural explants, indicating that ICC do not arise from the neural crest. The source of ICC in mammals is therefore likely to be the mesenchyme of the gut.

This work was supported by the NHMRC (Australia).

21.6

Evidence for multiple cassettes of regulatory elements for Mash1 expression. S. Verma*, T. Savage, D. Smith, and J. E. Johnson. Cell Biology and Neuroscience, University of Texas Southwestern Medical Center, Dallas, TX 75235-9111

Mammalian achaete-scute homolog, *Mash1*, is a neural differentiation gene of the basic helix-loop-helix class whose expression is restricted to the developing nervous system. Gene knockout experiments have demonstrated that Mash1 is required for the generation of olfactory neurons and neurons of the autonomic nervous system. As a means to identify genes that act upstream of *Mash1* to restrict its expression to the nervous system, we have characterized cis-regulatory elements using *lacZ* as a reporter gene in transgenic mice. We have obtained evidence for the presence of at least three lineage specific regulatory elements. We have tested a 36 kb sequence flanking the Mash1 coding region. Transgenic mice containing this 36 kb region express the *Mash1/lacZ* transgene in the CNS and the PNS. Expression of the *Mash1/lacz* transgene was never observed in the olfactory epithelium or retina suggesting that elements regulating expression in these lineages must lie outside of this (36 kb) region. Further dissection of the 36 kb sequence identified a 1.2 kb region upstream of the Mash1 coding region that regulates CNS expression. Elements controlling PNS expression do not colocalize with the CNS elements and are found elsewhere in the 36 kb sequence. We are currently in the process of identifying proteins that bind these sequences to control Mash1 expression. Supported by NIH RO1-NS32817-02.



21.7

CHARACTERIZATION OF THE EARLY SA CELL LINEAGE IN THE MOUSE USING ANTIBODIES TO PHOX2 AND GAP43. F. Deimling[a], K. Krieglstein[a], K. Unsicker[a], C. Goridis[b] & W.B. Huttner[c]*. (a) Anatomy &Cell Biol./Neuroanatomy Univ. Heidelberg, (b) IBDM, F-13288 Marseille, and (c) Neurobiology Univ. Heidelberg, D-69120 Heidelberg, Germany.

Sympathetic neurons and endocrine chromaffin cells of the adrenal medulla and paraganglia are supposed to share a common precursor, the sympathoadrenal (SA) progenitor cell. Mice deficient for signals that have been considered to be essential for the development of the SA cell lineage (c-RET, MASH1, glucocorticoid, neurotrophins) have been generated, but require more detailed analysis of the lineage. We have employed a set of partially novel markers to immunohistochemically characterize and distinguish putative subsets of SA progenitor cells in wildtype mice. Several of the established rat SA lineage markers including SA1 and B2 could not be adapted to mouse. We find TH- and Phox2-immunoreactivities (ir) co-localized at E12.5 in migrating, MASH1$^-$ SA progenitors in the region of the urogenital crest and mesonephros tubules. Intensity of Phox2-ir in sympathetic paravertebral and preaortal ganglia is maintained between E13.5 and E18.5, while TH-ir declines and becomes undetectable in a large number of SA cells within the sympathetic sublineage. However, within the developing adrenal gland virtually all SA progenitors remain intensely TH- and Phox2-ir. GAP43, a proposed marker for postnatal noradrenergic chromaffin cells, was found from E13.5 to E18.5 in a subset of adrenal TH$^+$ cells, which comprised a substantial proportion of PNMT$^+$ cells. GAP43 is therefore unlikely to be a useful marker for presumptive noradrenergic chromaffin cells. PNA staining did not distinguish between subsets of chromaffin cells. We are now applying these and additional markers to an analysis of the SA cell lineage in glucocorticoid-receptor deficient mice. **Supported by DFG (SFB 317/C8/D4)**

27.8

VISUALIZATION OF VESICULAR MONOAMINE AND ACETYLCHOLINE TRANSPORTERS IN DEVELOPING NEURONS AND NEUROENDOCRINE CELLS. M.-K.H. Schäfer, B. Schütz, J.D. Erickson, L.E. Eiden and E. Weihe*. Dept. Anatomy & Cell Biology, Philipps Univ., D-35033 Marburg, Germany; Sec. Molecular Neuroscience, Lab. of Cell Biology, NIMH, Bethesda, MD 20892.

The cholinergic and monoaminergic vesicular transporters VAChT and VMATs 1 and 2 have been visualized by immunohistochemistry in central and peripheral cholinergic and monoaminergic neurons and neuroendocrine cells during development using specific antibodies.

VMAT2, and not VMAT1, is expressed in neuroepithelial cells in the developing central nervous system at around embryonic day 12 (E12), and in monoaminergic nerve terminals beginning around E14. VMAT1 and VMAT2 are co-expressed during development in sympathoadrenal derivatives of neural crest cells (chromaffin, SIF and carotid body paracrine cells), with VMAT1 expression eventually predominating in endocrine and paracrine cells in the mature animal, and only VMAT2 expressed in peripheral neurons. In the gut, enterochromaffin cells express only VMAT1 throughout development, while enterochromaffin-like (ECL) cells of the stomach appear to express only VMAT2 throughout their development.

Cholinergic peripheral sympathetic innervation arises without the intermediacy of a monoaminergic phenotype as evidenced by parallel and exclusive innervation of developing sweat gland and sweat gland vasculature by VAChT- and VMAT2-positive terminals, respectively. In the CNS and PNS, VAChT-positive terminals are considerably more extensive than previously reported using the cholinergic marker ChAT, in primate as well as rat (J. Mol. Neurosci. 6:225, 1995; PNAS USA 93:3547 1996). Novel cholinergic systems have been identified in the rat hypothalamus and sensory mesencephalic trigeminal nucleus using the VAChT marker. An intrinsic neuronal system in cerebral cortex contains both VAChT mRNA and protein, and has been characterized according to its ontogeny and areal distribution. *Supported by VW-STiftung, DFG, and NIMH IRP.*

21.9

EARLY EXPOSURE TO DEPOLARIZING STIMULI LEADS TO LONG-TERM CHANGES IN TRANSMITTER REGULATION IN PRIMARY SENSORY NEURONS. T. Brosenitsch, and D.M. Katz.*Case Western Reserve University, School of Medicine, Cleveland, OH 44106.

Mechanisms responsible for generating transmitter diversity in developing sensory neurons are not well defined. However, neuronal activity, and factors in the ganglionic microenvironment, may play a role in regulating expression of some traits. For example, depolarizing stimuli promote (Hertzberg, et al., 1995), and ganglionic non-neuronal cells inhibit (Fan & Katz, 1993) expression of catecholaminergic properties in sensory ganglion cells in culture. In the present study we asked whether responsiveness to depolarizing stimuli is developmentally regulated, and whether exposure to such stimuli early in development results in long-term changes in transmitter regulation. Dissociate cultures of embryonic rat petrosal ganglion (PG) sensory neurons were plated at various ages in the presence or absence of a depolarizing concentration of KCl (40mM) for 3 days and then stained for tyrosine hydroxylase (TH) and neurofilament protein. Neurons were most responsive to KCl treatment on embryonic day (E)16.5; at this age, nearly 100% of ganglion cells in treated cultures were TH$^+$, compared to 10% in untreated controls. In contrast, KCl treatment induced TH expression in only 40-50% of E13.5 or postnatal day 1 ganglion cells. This peak in responsiveness on E16.5 corresponds to the stage at which TH normally appears in catecholaminergic ganglion cells in vivo (Katz & Erb, 1990), indicating that sensitivity to depolarizing influences and transmitter development may be linked. In addition, depolarization of fetal neurons had a long term effect on regulation of TH expression. Normally, TH levels in KCl-treated E16.5 PG cultures return to control values one week following removal of KCl. However, PG neurons exposed transiently to high KCl on E16.5 exhibited a 3.5-fold greater response to a second depolarizing stimulus ten days later compared to neurons with no prior history of stimulation [32% TH$^+$ (pretreated) vs 9% TH$^+$ (naive)]. These data indicate that early exposure to depolarizing stimuli may sensitize developing sensory neurons to subsequent activation. Supported by HL-25830 (Proj. 4;DMK) and T32NS07118.

21.10

FUNCTIONAL ROLE OF N-MYC IN EARLY NEURONAL DIFFERENTIATION OF PC12 CELLS. J. Bao*, D.S. McGehee, D. Talmage**, L. Role. Ctr for Neuro & Behav, **Inst of Human Nutr, Columbia Univ, NY, NY 10032.

High expression of N-myc in embryonic neuronal tissue suggests a possible functional role of N-myc in early neuronal differentiation. The functional ability of N-myc in cellular proliferation and differentiation is believed to require dimerization with Max, a newly identified nuclear protein containing a basic helix-loop-helix region, and leucine zipper (bHLH-LZ), which permit dimerization and DNA binding. Despite these findings the neural-like cell line PC12, which undergoes neural differentiation in the presence of nerve growth factor (NGF) does not express Max. This raises the question as to whether N-myc is involved in neuronal differentiation of this cell line.

To address this question, we overexpressed a truncated form of Max that included only HLH-LZ domain as a dominant negative to block N-myc in PC12 cells. Transfected cells had no neurite outgrowth and no detectable voltage gated Ca^{2+} influx even after 3-7 days of NGF treatment. In contrast, wild type PC12 cells sprout prodigious processes and manifest robust voltage-gated Ca^{2+} influx. Our data suggest that N-myc, in the absence of its partner Max, can mediate early neuronal differentiation in PC12 cells perhaps in collaboration with another partner. We are using the yeast genetic screen to investigate the molecular basis of neural differentiation by N-myc (NS29071).

21.11

IN VITRO ISOLATED HUMAN CNS PRECURSOR CELL SHOW MULTILINEAGE CAPACITY. X. Cui, P.M. Almqvist*, A. Kjeallgaard, U. Lendahl, Å. Seiger, and L. Wahlberg, Dept. of Clinical Neuroscience, Section of Neurosurgery, Dept. of Gynecology and Obstetrics, Dept. of Cell and Molecular Biology, and Dept. of Clinical Neuroscience and Family Medicine, Section of Geriatric Medicine, Karolinska Institute, S-171 77 Stockholm, Sweden.

To address the question of lineage commitment of the differentiated progeny of human neural stem cells, embryonic neural tissues derived from elective first trimester abortions were used and precursor cells were isolated and expanded in culture and formed free-floating neurospheres in response to EGF and EGF/bFGF. Tissues from spinal cord, brainstem, and forebrain were collected. In the late developmental stage, the forebrain was further divided into cortex and subcortex. Neurospheres derived from either early (5-8 weeks of gestation) or late (9-11 weeks) first trimester tissue specimens were kept under permissive conditions for 1 - 4 weeks before transfer to a poly-D-lysine substrate and serum containing medium. Whole spheres of precursor cells were allowed to differentiate on the substrate for one week in culture followed by immunohistochemical analysis using cell-type specific markers. Neurospheres were isolated from all areas and were positive for the intermediate filament marker nestin and remained proliferative during the 1 - 4 weeks in free-floating culture conditions as indicated by BrdU uptake. After plating, the spheres differentiated rapidly into neuronal, astrocytic, and oligodendrocytic lineages. Precursor cells derived from the forebrain of early stage embryos differentiated mainly into NF positive neuronal cells (84%). The cortical tissue of the later gestational ages showed a predominance of GFAP positive astrocytes (80%). The subcortex differentiated into 50% NF positive cells and another 30% stained positive for GFAP. The spinal cord showed 55% NF positive neurons and 20% GFAP positive astrocytes.

In conclusion, we find that EGF responsive neural precursor cells can be isolated from all major divisions of the human first trimester CNS and remain proliferative for at least one month. Different regions and gestational ages appear to yield different proportions of the three major phenotypes. MFR grant # 13X-11570

21.12

CONDITIONAL IMMORTALIZATION OF HUMAN NEURONAL, GLIAL AND MULTI-POTENT CNS PROGENITOR CELLS. D. W. Y. Sah[1,*], J. Ray[2], N. Richard[1], J. Leisten[1] and F. Gage[2]. [1]Signal Pharmaceuticals, Inc., San Diego, CA 92121 and [2]Laboratory of Genetics, The Salk Institute, La Jolla, CA 92037.

CNS drug discovery would be facilitated by overcoming the difficulty of obtaining sufficient primary human CNS tissue for research and development, the limited lifespan of primary cultures and the paucity of human CNS lines. Presently, drug development is carried out with rodent cells or cloned human CNS channels and receptors; however, species differences, abnormal cellular environment and artificial subunit combinations are potential caveats. Human CNS lines containing functional native channels and receptors in their normal cellular environment offer significant advantages.

Here, we describe the establishment of conditionally- immortalized human CNS cell lines that can be differentiated in 1-2 weeks. We immortalized cultures of proliferating human CNS progenitor cells by retroviral infection with a tetracycline (tet)-responsive retroviral vector containing the v-myc oncogene and neo^R (Hoshimaru et al., 1996); viable, proliferating cells that tolerated G418 were selected. In proliferative growth conditions, immortalized cells doubled every ~2 days and expressed the oncoprotein. In the presence of tet, the oncogene was suppressed rapidly and virtually completely; cell division stopped, neuronal (MAP2a/b) and glial (GFAP) cell-type specific markers were expressed, and voltage (sodium)- and ligand (NMDA, kainate, and GABA) -gated currents were present. Clonal cell lines could be differentiated rapidly into neurons only, astrocytes only, or both neurons and glia, establishing the existence of neuronal, astrocytic and multi-potent human CNS progenitor cells. Moreover, these cell lines provide powerful tools for drug discovery. [Supported by grant from NIA.]

21.13

MODULATION OF TYROSINE HYDROXYLASE (TH) EXPRESSION IN THE HUMAN CELL LINE N TERA-2 (NT2) R.S.Hartley* and V.M.-Y. Lee Inst. of Neurol. Sci. and Dept. of Path & Lab. Med., Univ. of Penn. Sch. of Med., Phila, PA 19104

In our investigation of factors that affect the neurotransmitter phenotype of differentiated NT2 cells (NT2N), we find that TH immunoreactivity is dependent upon culture conditions. Specifically, mechanical and chemical purification procedures eliminate TH expression. Previously, we noted that purified cultures of NT2N cells did not express significant levels of TH *in vitro* or when implanted into the murine CNS, although untreated NT2 cells differentiated and expressed TH when implanted into the murine striatum. Additionally, TH expression levels increased slightly when NT2N cells were cultured with striatal extracts.

TH expression was monitored by immunofluorescence and western blotting with three different commercially available anti-TH antibodies. Using these techniques, we find that a majority of NT2N cells are strongly TH immunoreactive if the neuronal cells are not dissociated or purified. In these cultures, TH expression increases during the five week retinoic acid differentiation period along with the total number of neuronal cells. In contrast, TH immunoreactivity diminishes with increasing chemical and mechanical manipulation. Abundant presynaptic terminals containing large numbers of small clear vesicles and occasional large dense core vesicles are observed by electron microscopy (EM) in co-cultures of rat cortical astrocytes with NT2N. Future studies with these co-cultures will determine whether the dense core vesicles contain dopamine by immuno-EM and whether astrocytes cultured from different CNS areas can influence the expression of TH in NT2N cells. We are further investigating the NT2N catecholaminergic phenotype using HPLC. This system may be helpful for studying the differentiation of catecholaminergic cells and for therapeutic implantation in diseases such as Parkinson's Disease.

Supported by grants from the NIH.

21.14

RETINOIC ACID INDUCES THE EXPRESSION OF CHOLINERGIC MARKERS IN HUMAN NT2 NEURONS. M. Paterlini, A. Valerio*, F. Baruzzi, M. Boifava, P. Liberini, M. Memo, P.F. Spano. Div. Pharmacol., Dept. Biomed. Sci. & Biotechnol., Brescia University School of Medicine, 25123 Brescia, Italy.

NTtera 2 clone D1 (NT2) cells acquire a neuronal phenotype after induction with retinoic acid (RA) yielding a highly purified population of human postmitotic neurons (hNT2 neurons). hNT2 neurons express cytoskeletal proteins typical of central nervous system (CNS) neurons and are being extensively used for studying neuronal development, degeneration and transplantation. The neurotransmitter(s) phenotype induced by RA treatment in NT2 cells *in vitro* has not been determined yet. RA treatment has been demonstrated to confer cholinergic properties to various types of cells in culture, also inducing the expression neurotrophin receptors. In the present study, we investigated on the neurotransmitter synthesized by hNT2 neurons by measuring the expression of choline acetyltransferase (ChAT), the enzyme responsible for acethylcholine production. *In vitro* maturation of hNT2 neurons was observed during 28 days *in vitro* (DIV) after RA treatment. The neuronal origin of differentiated cells was confirmed by immunoblotting with an anti-neuron-specific enolase monoclonal antibody (mAb) (DAKO). Mouse anti-ChAT (Chemicon) and anti-NGF receptor (p75) (Boehringer) mAbs were used to investigate on ChAT and p75 expression. ChAT immunostaining was undetectable in untreated NT2 cells. A significant ChAT immunoreactivity was found in purified hNT2 neurons starting from the third DIV and increased consistently over the following DIV. The specificity of this result was confirmed by immunoblotting showing the appearance of a 68 KDa ChAT band of increasing intensity from 7 DIV up to 28 DIV. p75 immunostaining and immunoblotting showed the appearance of a specific signal at later stages of development *in vitro* (significant levels at 21 DIV). Our data indicate that markers specific for cholinergic neurons appear during the terminal differentiation of hNT2 neurons, which represent therefore an useful model for the study of the pathophysiology of human CNS cholinergic systems. *This study was supported by funds from Telethon-Italy.*

CELL DIFFERENTIATION AND MIGRATION I

22.1

ISOLATION AND CHARACTERIZATION OF A ZEBRAFISH HOMOLOGUE OF THE CELLULAR RETINOIC ACID-BINDING PROTEIN. A. A. Fluet* and L. M. Roman, Department of Physiology, Johns Hopkins School of Medicine, Baltimore, MD, 21205.

Neural crest cells play an important role in the developing embryo, contributing to such diverse structures as the PNS and the outflow tract of the heart. Perhaps because of the precision in migration and cell interactions required for their proper development, the structures that neural crest cells contribute to are often sensitive to teratogens such as ethanol and retinoic acid (RA). Cellular Retinoic Acid-Binding Protein I (CRABPI) is involved in regulating retinoic acid (RA) availability within cells. CRABPI is expressed during early development in those cells which are sensitive to RA exposure, including a subset of migrating cephalic neural crest cells. The zebrafish (*Danio rerio*) provides many advantages for studying the effects of teratogens on the developing neural crest and related structures. As a potential marker for neural crest and/or RA sensitive cells, we have isolated a clone from a 9-16 h embryonic zebrafish cDNA library that encodes a CRABP. The predicted 137 amino acid protein includes two highly conserved arginine residues known to be involved in RA binding to both CRABPI and II. This protein is 82% and 83% identical to mouse and human CRABPI, respectively and 72% and 70% identical to CRABPII from these same species, suggesting it may be a CRABPI. Northern analysis of 9-10 h and 20 h zebrafish mRNA shows hybridization to a 1.96 kb and 0.85 kb transcript, respectively. Preliminary *in situ* hybridization experiments show the presence of transcripts in 10 h embryos. In 24 h embryos this expression is maintained in the hindbrain region. A more complete characterization of the expression pattern of this CRABP at the mRNA and protein level will provide insight into the role this binding protein plays during zebrafish development and provide a tool for studying the effects of teratogens on neural crest cells. Supported by a NIH NRSA (AA05443).

22.2

VSX-1, A RETINA SPECIFIC PAIRED-CLASS HOMEOBOX GENE IS EXPRESSED IN ZEBRAFISH EMBRYOS. M. Passini, A. Canger, E. Levine, N. Schechter, and A. Francis*. Departments of Biochemistry, Cell Biology, and Psychiatry; SUNY at Stony Brook, Stony Brook, NY 11794.

We are studying the possible roles Vsx-1 has in eye development during zebrafish embryogenesis. Vsx-1 is a paired-class homeobox gene originally cloned from an adult goldfish cDNA library. Its expression is restricted to two regions of the retina: 1) in a subset of neurons occupying the inner nuclear layer (INL) of the mature retina; 2) in a subset of undifferentiated post-mitotic progenitor cells located at the retinal margin. This leads to a hypothesis that Vsx-1 is involved in directing the differentiation and maintenance of INL cells. To test this, we use zebrafish embryos because they are amenable to genetic and functional analysis.

Temporal and spatial analysis is a prerequisite to functional studies. RNAse protection assays in temporal studies show low levels of Vsx-1 mRNA expression at 24hpf, but high levels at 48hpf and throughout embryogenesis. It is interesting to note that INL cells begin differentiating around 2 days post fertilization. The RNAse protection assay in zebrafish embryos indicate that the expression of Vsx-1 may be restricted to the retina. In situ hybridization studies are now in progress to confirm this. Furthermore, in adult zebrafish Vsx-1 is exclusively expressed in the eye.

We have cloned full length goldfish Vsx-1 cDNA into pSP64T to generate full length Vsx-1 mRNA containing 5' and 3' UTR sequences from the Xenopus B-globin gene. The presence of these sequences extend the half-life of injected messages in both Xenopus and zebrafish. In vitro translation in rabbit reticulocytes demonstrate that Vsx-1 mRNA generated from this vector is being translated into the expected full length 38kd protein. Misexpression of Vsx-1 may disrupt the lamination of the retina by an overall increase in INL size in relation to the other layers, or a direct increase in a subset of INL cells that normally express Vsx-1. This phenotypic analysis is now in progress. (EY05212 to N.S.)

22.3

INITIAL CALCITONIN GENE RELATED PEPTIDE EXPRESSION IN RAT DRG NEURONS IN VIVO AND IN VITRO. X. Ai, C. P. Robertson, S. E. MacPhedran, and A. K. Hall*, Dept. Neurosciences, Case Western Reserve University School of Medicine, Cleveland, OH 44106.

The presence of the neuromodulator, calcitonin gene related peptide (CGRP) in some adult rat sensory neurons is correlated with connections to specific peripheral targets. How sensory neuropeptides are matched to targets during development is not known. Embryonic lumbar rat DRG neurons isolated at E14 before peripheral target connections are present in vivo develop appropriate CGRP expression in the presence of serum in vitro (*C.P. Robertson and A.K. Hall, Soc. Neurosci. Abst.21:569, 1995*). Most DRG neurons at E14 are postmitotic, and lack CGRP mRNA as detected by RT-PCR. CGRP immunoreactive (IR) neurons can be detected after 4 days in culture, at a stage equivalent to E18, when CGRP protein and mRNA are detected in vivo. Thus, DRG neurons at E14 have the capacity to express CGRP around the period of target contact. By contrast, CGRP was not detected in DRG neurons isolated at E12 even after prolonged culture. Thus, E12 neurons do not appear competent to express CGRP. One factor that can elicit CGRP expression from E14 DRG in fully defined medium is high [KCl], suggesting that activity or calcium influx may affect initial CGRP expression. Although CGRP develops in isolated embryonic DRG, target tissues may also contribute to the regulation of this neuropeptide. The role of target tissues in the development of CGRP was evaluated by growing E14 DRG cells with rat skin, skelétal muscle, or smooth muscle primary cells or cell lines. Our data suggest that an event occurs in vivo between E12-E14 that confers to some sensory neurons the capacity to express CGRP at the equivalent of E18. A combination of this intrinsic program and environmental cues such as activity or target may contribute to the mature neuronal CGRP phenotype.

This work was supported by the National Institutes of Health NS 30842.

22.4

NGF AND NT-3 MEDIATE NEURITE OUTGROWTH FROM CHICK SENSORY GANGLIA WITHOUT RAISING THE Brn-3a mRNA LEVELS.
J. Lindeberg, A. Bäckström* and T. Ebendal, Department of Developmental Neuroscience, Uppsala University, Biomedical Center, Box 587, S-751 23 Uppsala, Sweden.

We have isolated a member of the Brn-3 family of transcription factors from chicken. Based on sequence and expression pattern data it is homologous to the Brn-3a gene. In mouse, the Brn-3a gene is abundantly expressed in most peripheral and central sensory neurons. It shows gene activating properties and increased levels of expression under differentiation and neurite outgrowth in the ND7 neuronal cell line. In order to study the interactions between the Brn-3a gene and the neurotrophins and the Brn-3a effect on neurite growth we have established a quantitative PCR approach using fluorescent dUTP. This enabled us to measure the levels of chick Brn3a mRNA from small numbers of cells. Our results show that explanted E9 chick peripheral ganglia do not undergo any significant changes in Brn-3a mRNA levels when stimulated with NGF or NT-3. This correlate with earlier studies performed in cultured adult mouse DRG neurons but contrast with the studies done in the mouse ND7 cells since we in our experiments obtain neurite outgrowth without increase in Brn-3a mRNA. To investigate further the relations between Brn-3a and the neurotophin system and to test whether Brn3a is nessesary for neurite growth from sensory neurons in vivo we have isolated a genomic Brn-3a clone from 129SVJ mice. This has been used in a gene targeting experiment aiming at producing mice deficient in functional Brn-3a.

This work was supported by the Swedish Natural Science Research Council (B-BU 4024-317).

22.5

DEVELOPMENT OF INTERSTITIAL CELLS OF CAJAL (ICC) IN THE MOUSE GUT: REQUIREMENT FOR KIT LIGAND (KL) J. Wu, T.P. Rothman*, & M.D. Gershon. Dept. of Anat. and Cell Biol. and Ctr. Neuro. & Beh., Columbia Univ., P&S New York, NY. 10032.

ICCs are small gastrointestinal cells, which act as pacemakers for smooth muscle and are closely associated with enteric neurons. All ICCs express Kit and their numbers are decreased in W/W^v mice, in which there is a *c-kit* mutation. The current study was carried out to determine the relationship of the development of ICCs to that of neurons. Kit immunoreactivity served as an ICC marker. Rare ICCs were detected in foregut as early as E12, but ICCs were abundant and present in networks throughout the bowel by E14. ICCs were found in the aganglionic colon of *ls/ls* mice in which the endothelin-3 gene (*edn 3*) is mutated. ICCs also were also present in the entirely aganglionic bowel of *c-ret -/-* mice. ICC developed in organotypic cultures of gut explanted as early as E11; however, ICCs did not appear in dissociated cultures of bowel explanted at E11 - E16. Addition of EDN-3 to dissociated cultures (0.1-5 μM) increased the numbers of both non-neuronal cells and neurons (PGP9.5-immunoreactive), but did not induce the appearance of ICCs. In contrast, when KL (100 ng/ml) was added to the medium, clusters of ICCs appeared. ICCs always grew on the surfaces of non-neuronal cells, which were smooth muscle actin-immunoreactive. There was no obvious relationship between Kit-expressing ICCs and neurons. These data support the idea that ICCs are mesodermal. Since KL must be supplied when the gut is dissociated, their development probably depends on cell surface KL. ICCs evidently develop independently of neurons and EDN3. Supported by NIH grants HD21032, NS12969, and NS15547.

22.6

DEVELOPMENT OF MIDBRAIN MOTOR NEURONS IN THE ABSENCE OF FLOOR PLATE AND NOTOCHORD. G. S. Sohal* and M.M. Ali. Dept. of Cellular Biology and Anatomy, Medical College of Georgia, Augusta, GA 30912.

The differentiation of motor neurons in the spinal cord is dependent on inductive signals from the floor plate and notochord. Whether the differentiation of motor neurons in the midbrain is similarly dependent was investigated. Almost all trochlear motor neurons are born on E5 & 6 and the nucleus first appears on E7 in normal duck embryos. Surprisingly, extirpation of the floor plate and notochord on E 2.5 (stage 9-10 duck embryos) did not prevent the formation of the trochlear motor neurons. Trochlear motor neurons failed to differentiate after extirpation of an area lateral to the floor plate, termed as the ventromedial region, on E 2.5, despite the presence of the floor plate and notochord. These results suggest that the floor plate/notochord may not be the only source of inductive signals for the differentiation of all motor neurons. The differentiation of midbrain motor neurons may depend on signals from elsewhere. Experiments are in progress to determine if cells associated with the ventromedial region can cause ectopic motor neuron formation. Supported by NIH grant HD 28601.

22.7

SONIC HEDGEHOG IS INVOLVED WITH NEURONAL DEVELOPMENT OF MURINE SPINAL CORD PRECURSOR CELLS *IN VITRO*. R.Dutton, P.Bartlett*, D.Bumcrot#, E.Marti#, R.Takada#, A.McMahon#, & M.Murphy. The Walter and Eliza Hall Institute, Parkville, Victoria, Australia, 3052, and #The Department of Molecular and Cellular Biology, Harvard University, MA, 02138.

Sonic hedgehog (shh) is a vertebrate homologue of the *Drosphila* gene family, hedgehog. Shh is expressed in many regions of the developing chick and mouse, including the ventral midline (Riddle et al., 1993 Cell 75:1401) and has been shown to induce floor plate cells and motor neurons in chick neural tube explant cultures (Roelink et al., 1995 Cell 81:445). The objectives of this study were to determine the effect of shh on the development of isolated murine spinal cord stem cells *in vitro*, to assess if shh may be acting directly on the cells to induce neuronal differentiation; specifically motor neuron differentiation. We found shh significantly increased the number of morphologically mature neurons compared to control cultures. The shh treated neurons also developed longer neurites compared to controls. Thus, under these conditions shh appeared to regulate neuronal differentiation, and may also have a role in neurite outgrowth. The increase in the number of morphological neurons observed with shh was transient, peaking at 18 hours, and therefore it does not appear to act as a survival factor for newly generated spinal cord neurons. However, there was no proportional increase in the number of cells expressing isl-1 (a marker for early motor neurons) in shh treated cultures suggesting shh does not act directly on the precursor cell to induce motor neuron differentiation. Supported by NH&MRC, Australia.

22.8

TARGET DEPENDENCY OF AUTONOMIC MOTOR NEURON DIFFERENTIATION. R. P. Barber*, R. Wetts, and J. E. Vaughn. Division of Neurosciences, Beckman Research Institute of the City of Hope, Duarte, CA 91010. USA.

In developing rat spinal cord, somatic and autonomic motor neurons (SMNs and AMNs) originate from the ventral ventricular zone, cease dividing at the same time, express choline acetyltransferase, and migrate together into a common primitive motor column. At this point, the fate of AMNs diverges: only they migrate dorsally into the lateral horn and express nitric oxide synthase as detected by NADPH diaphorase histochemical activity. In order to identify the cellular events that underlie this differential fate, we performed experiments on organotypic slice cultures of thoracic spinal cord. In control cultures, dorsal migration of AMNs and expression of diaphorase activity occurred as *in vivo*. If sympathetic ganglia were removed after the arrival of AMN axons at E14, dorsal migration and diaphorase expression occurred by 2 days *in vitro* (2 div). However, if target was removed prior to AMN axon arrival at E13, diaphorase expression in the AMNs did not occur by 2 div. Thus, diaphorase expression in AMNs seems to require target contact for its initiation, but apparently can continue autonomously thereafter. Current experiments seek to establish whether the dorsal migration, as well as other aspects of AMN differentiation, depend on target interaction as does the induction of diaphorase expression. *Supported by NIH grant NS25784.*

22.9

NCAM 16, a Monoclonal Antibody Recognizing the Third Immunoglobulin-like Domain of the Neural Cell Adhesion Molecule N-CAM, is Chemotactic for Spinal Cord Neurons and for Neuronal Cell Lines R.L. Ackley, S. S. Roy, R. Madison, J.J. Hemperly* Dept. of Molecular Biology, Becton Dickinson Research Center, P.O. Box 12016, Research Triangle Park, NC 27709 USA.

The proper and dynamic interactions between living cells involve both cell-cell and cell-substrate adhesion molecules. One of the best characterized of these is the neural cell adhesion molecule, N-CAM, which was originally isolated based on its ability to mediate direct cell adhesion among retinal neurons. N-CAM comprises a family of molecules which arise by alternative splicing of RNA derived from a single gene and are extremely well conserved among all species examined. It has more recently become clear that CAMs can not only mediate direct adhesion, but are also capable of cell signaling, perhaps using cellular kinase and phosphatase pathways. We have generated a number of monoclonal antibodies to human N-CAM, and shown that one of these antibodies, NCAM 16, a mouse IgG2b, is able to promote the migration of rat primary spinal cord neurons and a number of human neuronal cell lines. Both the whole antibody and Fab fragments exhibit this effect, suggesting that cross-linking at the cell surface may not be needed. In contrast, another antibody, NCAM 14, also a mouse IgG directed at an extracellular epitope of human N-CAM, is not chemotactic. We have mapped the epitope of NCAM 16 to the third Ig domain of N-CAM, a region shown by others in chickens, to be very important in the homophilic binding of N-CAM on one cell to N-CAM on another. Bacterial fusion proteins comprising this region of the molecule, however, do not stimulate migration of cultured medulloblastoma cells but can inhibit the effect of NCAM 16. Based on these observations, we also tested human brain N-CAM in these assays and demonstrated that it was also chemotactic for spinal motor neurons. Further studies will be required to evaluate the biochemical consequences of the binding of this antibody and how these effects parallel the effects of N-CAM itself in vivo.

22.10

DIVERSITY OF NEURONAL STEM CELLS IN THE ADULT FISH RETINA. Andreas F. Mack * PFl f. Brain Res. Universität Leipzig, Jahnallee 59, 04109 Leipzig, Germany.

The adult fish retina contains stem cells in two locations: dividing cells (called rod precursors) in the outer nuclear layer give rise exclusively to rod photoreceptors and cells in a peripheral germinal zone give rise to all retinal cells types.

I used a reaggregate culture method to test the developmental capacity of stem cells in the peripheral germinal zone and of rod progenitors in the center of the mature fish retina. Peripheral and central retinal areas of juvenile or adult cichlids (Haplochromis burtoni) were separated, dissociatiated, and allowed to reassociate on a rotary shaker. Dividing cells were labeled with bromodeoxyuridine either before or after dissociation. Rod precursors are the only proliferating cells in the central retinal areas. The aggregates were processed for immunocytochemistry.

In both central and peripheral cultures, cells reassociated readily. Cells derived from peripheral tissue formed sphere-like aggregates. Typically, pigmented cells are located in the center surrounded by opsin-positive cells. Cell processes positive for the glial marker protein vimentin showed radial and tangential orientation outlining the spherical arrangement of the aggregate. Cell proliferation continued in these aggregates during the entire culture period of about two weeks. In contrast, the aggregates derived from central retinal tissue showed little structural organization. Opsin-positive and vimentin-positive cells were present but did not reveal any particular arrangements. Continuous cell proliferation was observed only occasionally.

These results show that adult fish retinal tissue can regenerate retina-like structures. They indicate that rod precursors and central differentiated retinal cells are less capable of forming organized aggregates and maintain cell division.

Supported by the Deutsche Forschungsgemeinschaft

22.11

EXPRESSION OF IODOPSIN MRNA IN LOW DENSITY CULTURES OF CHICK EMBRYO PHOTORECEPTOR CELLS GROWN IN LIGHT: DARK CYCLES. S.M. Argamaso-Hernan[1], E. Adler-Graschinsky[2*] and R. Adler[1]. Wilmer Eye Institute, J. Hopkins Univ., Sch. Med., Baltimore, MD 21287[1], ININFA, Buenos Aires, Argentina.[2]

Northern analysis has demonstrated that light regulates iodopsin expression in high density cultures of chick embryo retinal cells (Pierce et al, Neuron, 10:579-84, 1993). We are investigating this phenomenon in single cells using low density cultures in which chick embryo photoreceptors have been shown to react photomechanically to light (Stenkamp and Adler, PNAS 90:1982-86, 1993). Cultures grown in 12:12h light:dark cycles for 6 days were fixed at 4 hr intervals, processed for *in situ* hybridization with digoxigenin-labeled iodopsin probes, and analyzed quantitatively by an investigator unaware of sample identity. The overall frequency of iodopsin-positive photoreceptors remained unchanged at all time points, but there were clear differences in the relative intensity of hybridization signals. The strongest signals were recorded 2-6 hr after light offset, which is somewhat later than the peak of expression reported by Pierce et al (1993). The possibility that these changes in signal intensity may be correlated with photomechanical movements is currently under investigation using other agents that promote photoreceptor elongation, such as dopamine and cytochalasin D. Supported by NIH grants EY04859 and T32 EY07143

22.12

DO TYROSINE HYDROXYLASE- AND PEPTIDE-IMMUNOREACTIVE NEURONS COMPRISE OVERLAPPING CELL POPULATIONS IN THE CHICK SPINAL CORD? P.A. Gunlé, A.Y. Gonzales and J.A. Wallace*. Dept. of Anatomy, Univ. of New Mexico Sch. of Med., Albuquerque, NM 87131.

Tyrosine hydroxylase-immunoreactive (TH-IR) cells occur in large numbers ventral to the central canal and along the outer border of the dorsal horn in the chick spinal cord near hatching. Although such TH-IR neurons in the spinal cord of lower vertebrates synthesize catecholamines, in contrast, comparable TH-IR cells in the chick do not (Wallace et al.,`96). Of interest, though, are reports of various peptide-containing neurons that appear with remarkably similar distributions to both the ventral and dorsally located TH-IR cells in the chick embryonic spinal cord. Therefore, we have examined the potential of co-localization between several neuropeptides and TH within cells of the chick spinal cord near hatching. Using antisera against substance P (SP), somatostatin (SOM), VIP and M-ENK we first verified by nickel-intensified immunocytochemical staining the occurrence and position of cells containing these peptides, examined within the cervical and thoracic cord. Our results were similar to those described by Du and Dubois (`88) with the detection of SP-, SOM- and M-ENK-immunoreactive cells immediately ventral to the central canal and SP- and M-ENK-containing cells along the outer boundary of the dorsal horn. However, when dual-fluorescence immunocytochemistry was employed to visualize TH and individual peptides simultaneously, no TH-IR cells were found to possess any of the peptides studied, although their position and morphology were similar, and the cells were often situated immediately adjacent to one another. Therefore, at least near the age of hatching in the chick, it appears that TH-IR and peptide-containing cells in the spinal cord represent different neuronal populations. The possibility of co-existence of TH with peptides needs to be examined further with additional peptides and at different embryonic ages and cord levels. Supported by DHHS-GM08222 and RR08139.

PATTERNING I

23.1

MORPHOLOGICAL AND IMMUNOCYTOCHEMICAL CHARACTERIZATION OF TRANSIENT NEURONAL POPULATION OF THE INTERNAL CAPSULE IN THE DEVELOPING HUMAN CEREBRUM. K. Letinić and I. Kostović*. Croatian Brain Research Institute, School of Medicine, University of Zagreb, Croatia.

Nissl, Golgi, AChE and immunocytochemical tehniques were used to study pre- and postnatal development of the internal capsule neurons in postmortem brains of human fetuses ranging from 12 weeks of gestation to adult age. Surprisingly large population of cells lying among the fibers of the internal capsule is found during the fetal period. These cells resemble reticular thalamic neurons and are particularly frequent in areas of the internal capsule near the ventral parts of the reticular nucleus. Their frequency declines during early infancy and very few cells are apparent in the one year old infant as in adult. Internal capsule neurons are multipolar and polymorphous with well pronounced dendritic arborization and sometimes many spine-like protrusions covering their cell bodies and dendrites. Furthermore, they have properties typical of mature neurons showing intense immunoreactivity for MAP2 (microtubule associated protein 2), and they also contain one of neuropeptids, namely SRIF (somatostatin). Subpopulations of neurons are immunoreactive for calcium-binding protein calbindin-D_{28K} and parvalbumin and some of them show AChE activity. Finally, low-affinity p75 NGF (nerve growth factor) receptor immunolabelled neuronal elements were observed within the internal capsule during the fetal period. A group of neurons described in this study corresponds to the perireticular thalamic nucleus found in certain mammalian species (Mitrofanis and Guillery, Trends Neurosci16:240, 1993), hitherto unidentified in the primate brain. Recent study in the developing rat suggested that the perireticular cells send transient, "pioneer" projection to cortex appearing before thalamocortical projection (Adams and Baker, J Comp Neurol 359:613, 1995). The transient nature, distinct antigenic properties and early cortical projection of the perireticular nucleus suggest developmental roles comparable with those of the cortical subplate zone. By analogy with the subplate zone of the cortical anlage, the region lateral to the reticular thalamic nucleus can be regarded an essential compartment of the thalamic anlage and appropriately termed the "perithalamic zone". *Supp. Ministry of Science, Republic of Croatia.*

23.2

A SOLUBLE OLIGODENDROCYTE-DERIVED SIGNAL INDUCES REGULARLY-SPACED SODIUM CHANNEL CLUSTERS ALONG CNS AXONS IN VITRO. M. R. Kaplan*, A. Meyer-Franke, S. Lambert, V. Bennett, S. R. Levinson, B. A. Barres. Dept. of Neurobiology, Sherman Fairchild Science Building, Stanford Univ., Sch. of Med., Stanford, CA 94305-5401.

Saltatory conduction depends on the formation of regularly-spaced clusters of sodium channels at nodes of Ranvier. In the CNS, these clusters occur at axonal intervals of about 100 times the axon diameter. It is not known whether the regular spacing of nodes results from regularly-spaced glial contacts or is instead intrinsically specified by the axonal cytoskeleton. In the PNS, Schwann cell contact induces sodium channel clustering along the axons of DRG neurons in vitro and in vivo. Similarly, it has been suggested that astrocyte contact induces sodium channel clustering along CNS axons.

In this study, we have used an antibody specific to sodium channels to determine their distribution in purified rat retinal ganglion cells cultured either in the absence or presence of purified glial cell types. Using this system we have found that the induction, but not the maintenance, of sodium channel clustering depends on a soluble oligodendrocyte-derived signal. Surprisingly, the glial-induced clusters are regularly spaced at the predicted interval in the absence of glial-axonal contact. Thus, whereas oligodendrocytes produce soluble signals that are necessary to induce sodium channel clustering in vitro, the spacing of these clusters appears to be dictated by the axon.

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23.3

DEVELOPMENT OF DIFFERENTIAL LOCALIZATION OF MAP2 AND CaMKII mRNAs IN NEUROPILS OF THE RAT HIPPOCAMPAL FORMATION. M.A. Paradies* and O. Steward, Dept. of Neuroscience, University of Virginia School of Medicine, Charlottesville, VA 22908.

The presence of mRNA in dendrites is now well established. Moreover, different mRNAs are associated with different dendritic sub-domains. While the mRNAs for both microtubule associated protein 2 (MAP2) and the alpha subunit of calcium/calmodulin dependent protein kinase II (CAMKII) are present in the dendrites of hippocampal neurons, MAP2 mRNA is concentrated in proximal dendrites whereas CAMKII mRNA is distributed throughout the dendritic arbor. This pattern of mRNA distribution suggests a relationship with the laminar distribution of afferents. The present study uses in situ hybridization to evaluate the distribution of these two dendritic mRNAs during the period of afferent synaptogenesis, between postnatal day 1(P1) and P28. MAP2 mRNA was present in cell body layers and proximal dendrites as early as P1. By P7, MAP2 mRNA extended into the proximal 1/2 of the neuropil of the dentate gyrus and hippocampus. In contrast, CaMKII mRNA was evident in cell body layers at P1 but was barely detectable in neuropil layers until P7. Levels of CaMKII mRNA increased substantially at P15 in both cell body and neuropil layers. By this time, CaMKII mRNA was visible throughout the proximo-distal extent of the neuropil. These observations reveal that the differential subcellular distribution of MAP2 and CaMKII mRNAs develops in concert with the growth of dendrites and the formation of synapses. Supported by NS12333. MAP is the recipient of NRSA NS09752.

23.4

INTRACORTICAL AND THALAMOCORTICAL PROJECTIONS IN ADULT RATS THAT SUSTAINED NEONATAL FORELIMB REMOVAL. D.L. Howell, A. Stojic, R.D. Lane* and R.W. Rhoades. Dept. of Anatomy and Neurobiology, Medical College of Ohio, Toledo, OH 43699.

Neonatal forelimb removal results in innervation of the cuneate nucleus by sciatic nerve primary afferents and development of cuneothalamic neurons with receptive fields that include both the hindlimb and the forelimb stump (Proc. Natl. Acad. Sci. 92:4264-4268, 1995). However, this brainstem reorganization is almost completely unexpressed in the primary somatosensory cortex (S-I). Blockade of cortical GABAergic inhibition by bicuculline and phaclofen produced a large increase in hindlimb-responsive sites (44.2% versus 6.2% prior to blockade) in the cortical stump representation of neonatally manipulated rats. GABA blockade produces significantly ($p<0.01$) fewer (11.7% versus 2.7% prior to blockade) hindlimb-responsive sites in the forelimb representation of normal rats. The present study asked whether hindlimb information reached the stump representation via altered intracortical connections from the hindlimb representation in S-I or via thalamocortical innervation from the hindlimb representation in the thalamus. Anterograde and retrograde tracing with biotinylated dextran amine and *Phaseolus vulgaris* leukoagglutinin demonstrated no significant effect of neonatal forelimb removal on projections between the hindlimb and stump/forelimb representations in granular S-I. Retrograde labeling of the stump and hindlimb thalamocortical neurons with true blue and diamidino-yellow resulted in normal segregation of these two neuron populations. These results suggest that hindlimb information may reach the cortical stump representation of the amputated rats via thalamocortical neurons that respond to stimulation of both the stump and the hindlimb. Supported by NS 28888 and DE 07734.

23.5

MORPHOLOGY OF TRIGEMINAL PRIMARY AFFERENT AXONS AFTER BLOCKADE OF AXOPLASMIC TRANSPORT DURING POSTNATAL DEVELOPMENT. S. Zhang*, F.P. Goldstein, N.L. Chiaia, and R.W. Rhoades. Dept. Of Anatomy and Neurobiology, Medical College of Ohio, Toledo, OH 43699

We previously reported that attenuation of axoplasmic transport in the developing infraorbital nerve (ION) with vinblastine does not abolish the normal vibrissae-related patterning of the ION primary afferents that supply the vibrissae follicles, but does result in a loss of all other central vibrissae-related patterns of cells and fibers. The present study was undertaken to determine the effects of this manipulation on the morphology of single trigeminal primary afferents that terminated within the vibrissae representation of subnucleus interpolaris (SpI). Vinblastine-impregnated implants were placed over the ION on the day of birth (P-0). On P-6, rats were anesthetized and the trigeminal ganglion was exposed by aspirating the overlying cerebrum and diencephalon. Neurobiotin was then injected into the trigeminal ganglion and animals survived for 8-12 hours. The tracer was demonstrated histochemically and labelled fibers within SpI were reconstructed using a computer-microscope system. Reconstruction of 35 axons from normal rats aged P-6 and 50 fibers from rats whose IONs were exposed to vinblastine demonstrated no significant between-group differences in morphological characteristics including total fiber length, arbor area, arbor volume, number of branch points, and number of boutons. These results, like those from our previous study, indicate that a relatively normal primary afferent pattern is insufficient for maintenance of vibrissae-related patterns by brainstem, thalamic, and cortical neurons. Supported by DE 07734, DE 08971, NS 28888.

23.6

EFFECTS OF ABNORMAL ACTIVITY ON DEVELOPMENT OF THALAMOCORTICAL AND INTRACORTICAL PROJECTIONS IN THE RAT'S PRIMARY SOMATOSENSORY CORTEX. N.L. Chiaia*, C.A. Bennett-Clarke and R.W. Rhoades. Dept. Of Anatomy and Neurobiology, Medical College of Ohio, Toledo, OH 43699

Reduction of cortical activity during early postnatal development by blockade of voltage-dependent sodium channels with tetrodotoxin (TTX) has no significant effect on the patterning of either thalamocortical projections to the vibrissae representation in the primary somatosensory cortex or intracortical projections in lamina IV of this region. The present study asked whether inducing abnormal patterns of cortical activity during development by placement of penicillin-impregnated implants could alter either thalamocortical or intracortical projections. Implants were placed over the SI cortex on the day of birth (P-0) and resulted in abnormal bursting cortical activity in recordings made on P-5-9. Assessment of thalamocortical projections by staining for AChE in animals killed on P-6 indicated no change in the patterning of these fibers. Rows of dense patches corresponding to the vibrissae follicles remained visible in the treated cortices. Assessment of intracortical connections in layer IVon P-40-60 using anterograde and retrograde transport of biotinylated dextran amine also failed to reveal any changes from normal organization. A fenestrated pattern complementary to the vibrissae-related patches of thalamocortical afferents was present in both the normal and treated animals. These results, like those from the experiments in which activity was blocked with TTX, indicate that the postnatal development and maintenance of thalamocortical and intracortical projections in rat's primary somatosensory cortex are not influenced by marked changes in activity levels in this region. Supported by NS 28888, DE 08971, DE 07734.

23.7

PRENATAL EXPOSURE TO COCAINE ALTERS SEROTONIN LEVELS AND THE REPRESENTATION OF THE VIBRISSAE FOLLICLES IN THE RAT'S PRIMARY SOMATOSENSORY CORTEX. C.A. Bennett-Clarke*, J. Garcia, N.L. Chiaia, and R.W. Rhoades. Dept. Of Anatomy and Neurobiology, Medical College of Ohio, Toledo, OH 43699

Previous studies have shown that prenatal cocaine exposure reduces the density of high-affinity serotonin (5-HT) uptake sites in the cerebral cortex and that altered levels of 5-HT can change primary somatosensory cortical development. The present study was undertaken to determine the effects of prenatal cocaine exposure on 5-HT levels in the primary somatosensory cortex and the organization of the patches of thalamocortical afferents representing the vibrissae follicles in this region. Fetal rats were exposed to cocaine between E-13 and birth by implantation of osmotic minipumps that delivered 15.5 mg/day of cocaine to the internal carotid artery of pregnant dams. Pups were delivered by cesarean section, cross-fostered until P-6 and then used in either anatomical or neurochemical experiments. Levels of 5-HT in cortex were measured by HPLC. The average value for 25 normal rats was 133.8 pg/mg and that for 48 cocaine-treated animals was 194.4 pg/mg ($p<0.05$). Measurements of the areas of the AChE-stained patches of thalamocortical afferents corresponding to the three most caudal vibrissae in rows A,C and D indicated an increase in average patch size of 7% ($p<0.0001$). Increases ranged between 5% (D-3 and A-1 patches) and 9% (A-2 and D-2 patches) for individual clusters of thalamocortical axons. These changes in the vibrissae representation were not associated with significant alterations in either brain or cortical weight. These results thus indicate that brief prenatal exposure to cocaine significantly influences both 5-HT and thalamocortical development. Supported by EY 08861 and DE 07734.

23.8

Searching for Tenascin-C receptors with a library of recombinant domains

A. Scholze[1], A. Niehaus*[1], U. Rauch[2], H. Zimmermann[3], and A. Faissner[1]
[1] Department of Neurobiology, INF 364, University of Heidelberg, D-69120 Heidelberg; [2] MPI for Biochemistry, Protein Chemistry, Am Klopferspitz 18a, D-82152 Martinsried, [3] Biocenter of J. W. Goethe-University, Zoological Institute, Neurochemistry, Marie-Curie-Str. 9, D-60439 Frankfurt am Main; Germany

Tenascin-C (TN-C) glycoproteins are transiently expressed by astrocytes and distributed in discrete patterns in some areas of developing neural tissues. TN-C mediates neuron-glia interactions and contributes to the control of neuron migration and neurite outgrowth and to the formation of transient boundaries. The glycoprotein is composed of a serial arrangement of EGF-like and Fibronectin type III repeats, and a carboxyterminal region homologous to fibrinogen β and γ. Several lines of evidence suggest that the different functions are encoded by distinct domains, which implies the existence of separate neural receptor systems. In order to characterize these further, an approach based on affinity chromatography with a library of recombinant domains encompassing the FNIII repeats of TN-C was designed. Affinity columns were constructed by coupling recombinant proteins to sepharose and loaded with detergent extracts of membrane preparations or physiological saline extracts from postnatal day 6 to 14 mouse brains. Subsequently, the columns were washed and eluted at different stringencies. The resulting eluates were analysed by SDS-page, ligand blots with appropriate recombinant TN-C domains and ELISA or Western blot approaches using defined antibodies to known components. Thereby, NCAM, 5' nucleotidase and neurocan could be identified as potential ligands. Currently, these hypothesized interactions are being tested with binding assays using the purified molecules.
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23.9

LOCALIZATION OF APLP2 AND APP AT THE TECTAL MIDLINE IN DEVELOPING HAMSTERS A.M. Confaloni*, K. L. Moya, A. W. Lyckman, and S. Jhaveri. Ist. Sup. Sanità, 00161 Rome, Italy; CNRS URA 2210 and INSERM U334, S.H.F.J., C.E.A., 91401 Orsay, France; Dept. Brain & Cognit. Sci., M.I.T., Cambridge, MA 02139, U.S.A.

Radial glial cells present along the midline of the developing tectum are thought to be involved in retaining the laterality of the retinotectal projection (Jhaveri, Persp. Dev. Neurobiol., 1994). Retinal axons cross the midline abnormally if the integrity of these raphe glia is compromised during early postnatal life (Schneider, Br. Behav. & Evol. 1973). Sulfated proteoglycans, expressed along the tectal midline, may mediate the barrier function of the tectal glia (Hoffman-Kim et al., Neurosci. Abst., 1995). Since APP and APLP2 found in glial cells reportedly are CSPG core proteins (Pangalos et al., J. Neurochem. 65: 762, 1995), it was of interest to determine whether they are present at the tectal midline.

Postnatal hamster pups, were anesthetized, perfused with 4% paraformaldehyde, and their brains processed for immunostaining with MAb 22C11 (which targets epitopes on both APP and APLP2), CT-15 (which is directed against the cytoplasmic C-terminal of APP) and D2-II (which specifically recognizes APLP2). All three Abs stain the midline glia, but do so differentially. In P0 and P2 animals, CT-15 is localized near the ventricular surface only, whereas D2-II gives immunostaining along the apical processes of the radial glia. In contrast, immunoreactivity with MAb 22C11 is present along the full length of the glia from the ventricular surface to end feet at the pial surface. This is the first report of spatial segregation of specific (putative) PGs in different cellular compartments of the tectal glia.

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23.10

CO-CULTURES OF TECTAL ASTROCYTES AND RETINAL NEURONS: AN IN VITRO MODEL SYSTEM FOR STUDYING AXON-GLIA INTERACTION. A. Sower*, M.J. Young, and S. Jhaveri, Dept. Brain & Cognitive Sciences, M.I.T., Cambridge, MA 02139.

Studies from our lab indicate that a group of radial glia located at the tectal midline is involved in retaining the laterality of the retinotectal projection; these cells begin to differentiate just prior to the arrival of retinal axons in the superior colliculus. Intertectally-projecting fibers, on the other hand, are able to successfully cross the tectal midline, but they do so before the midline glia have differentiated (S. Jhaveri, *Persp. Dev. Neurobiol.*, **1**: 237-243, 1993). As a prelude to examining the interactions of individual retinal axons and intertectal fibers with the raphe glia, we have developed a co-culture preparation in which astroglia are harvested from the dorsal midbrain of hamsters at different ages (E13-P0), grown in culture for varying amounts of time, and seeded with dissociated retinal cells. Cultures were immunostained with antibodies directed against neuron-specific enolase and GFAP to characterize the cell populations and quantify retinal neurite outgrowth.

Excellent neurite outgrowth is obtained from retinal cells plated on tectal glia: this growth is vulnerable to the effects of age of the donor astrocytes, and also to the time the glia have been in culture before retinal neurons are plated onto them, with greater extension obtained on younger astrocytes. We are presently investigating whether lateral vs. midline tectal astrocytes have differential effects on supporting retinal neurite outgrowth. These studies will provide new information on whether the age of the neurons or the maturity of the glial substrate determines the growth potential of afferent systems.

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23.11

REGIONAL DIFFERENCES IN TECTAL CHONDROITIN SULFATE EXPRESSION REFLECT DIFFERENTIAL RATES OF GLYCOSAMINOGLYCAN BIOSYNTHESIS

Diane Hoffman-Kim[1]*, Arthur D. Lander[2], and Sonal Jhaveri[1].
[1]Dept. of Brain and Cognitive Sciences, MIT, Cambridge, MA; [2]Dept. of Developmental and Cell Biology, University of California, Irvine, Irvine, CA.

Chondroitin sulfate proteoglycans (CSPGs) are expressed throughout the developing brain, both in regions where axons preferentially grow and in areas that axons distinctly avoid. *In vitro* experiments also suggest that some CSPGs enhance while others inhibit neurite outgrowth. The expression of CS along the midline of the developing tectum and the inhibition of retinal axon growth *in vitro* by CS substrates have given rise to the hypothesis that midline CSPG(s) act as barrier molecules, preventing retinal axons from crossing the tectal midline. Earlier biochemical analysis of PGs in homogenates from midline and lateral tectum revealed that the concentration of CS at the midline does not reflect increased expression of any particular PG core protein. In the present study, we address the possibility that overall CS synthesis may be increased at the midline. Briefly, coronal tectal slices of 300 µm thickness were labeled, either for one hour or overnight, with ^{35}S-sodium sulfate +/- ^{3}H-leucine. Sulfate incorporation specifically into glycosaminoglycans was quantified for midline and lateral regions. After overnight labeling, midline cells incorporated approximately 1.5 times the sulfate (normalized to protein) that lateral cells did, approximately 70% of which could be accounted for, in both regions, by CS and heparan sulfate. In contrast, midline cells did not incorporate significantly more ^{3}H-leucine than lateral cells, indicating that midline cells are not simply more metabolically active. Experiments in which metabolic labeling was limited to one hour also showed increased ^{35}S-sodium sulfate incorporation by midline (vs. lateral) cells, comparable to that seen with overnight labeling. These data suggest that high level expression of CS at the tectal midline is the result of an increased rate of CS biosynthesis by tectal midline cells.

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23.12

SPINOCEREBELLAR MOSSY FIBER AFFERENTS FORM SEGREGATED COLUMNS IN JUVENILE *STAGGERER* MUTANTS. Z. Ji, Q. Jin, and M. W. Vogel*. MPRC, Univ. MD Med. School., Baltimore, MD 21228,

Cerebellar afferents form segregated longitudinal terminal fields within individual cerebellar lobules, and Purkinje cells are hypothesized to be critical organizing elements for the connectivity patterns. In support of this hypothesis, a previous study has shown that mossy fiber projections are diffuse in P25 *staggerer* (*sg/sg*) mutants (Arsénio Nunes 1988 J. Comp. Neurol. *273*: 120-136). *Staggerer* mutants have severe Purkinje cell deficits, but at P25 granule cells are also depleted. To test the hypothesis that mossy fiber distribution is disrupted because virtually all granule cells die after three weeks of development we have analyzed the distribution of WGA-HRP labeled spinocerebellar mossy fiber terminals in *sg/sg* mutants towards the end of the period of granule cell genesis (P12-P13) and before massive granule cell death (P16). The distribution of HRP labeled mossy fiber terminals was analyzed in 8 homozygous *sg/sg* mutants (P12-P16) and 5 controls (+/*sg* and +/+) following the injection of 2% WGA-HRP into the lower thoracic/upper lumbar region of the spinal cord. Spinocerebellar mossy fibers distribute in segregated terminal fields in the anterior vermal lobules of P12 to P16 *sg/sg* mutants. Although the pattern of labeled terminals is different from controls, the results indicate that topographic cues are expressed in the early postnatal *sg/sg* cerebellum (perhaps by *sg/sg* Purkinje cells) and mossy fiber terminals may become disorganized or retract as granule cells die in the older *sg/sg* mutant. The results suggest that granule cells play a role in stabilizing the pattern of mossy fiber projections. Supported by NIH grant NS29277 to MWV.

23.13

PURKINJE CELL PATTERNING IN THE EMBRYONIC CEREBELLUM OF MICE EXPRESSING AN OLFACTORY MARKER PROTEIN-LacZ TRANSGENE. M.G.Nunzi* and E.Mugnaini, Institute of Neuroscience, Northwestern Univ., Chicago, IL 60611.

The cerebellar cortex is a unique system to study neuronal patterning in the CNS, as it is organized into compartments defined by the distribution of Purkinje cells (PCs) and afferent projections into a series of parasagittal domains. While it is known that PC compartmentalization occurs in embryonic development, the cellular events underlying segregation of different PC phenotypes and topographic matching are still obscure. In a previous study, we have shown that in a mouse line (HpY-1) carrying a *lacZ* transgene containing a 0.3kb of upstream and 0.35kb of downstream sequence from an olfactory marker protein (OMP) gene, the *lacZ* reporter is expressed by PCs arranged within parasagittal bands spanning the posterior lobe. We suggested that regulatory elements near the transgene integration site could be involved in specification of PC rostrocaudal compartments. To investigate early histogenetic events involved in segregation of *lacZ* -expressing PCs, embryonic cerebella were analyzed with X-gal staining and immunocytochemistry to PC - specific antigens. *LacZ* expression was first detected by E13 in a cluster of cells outlining the ventricular neuroepithelium. By E15, *lacZ* defined subsets of PCs settled in the cortical region. These observations suggest that PC heterogeneity occurs in or near the ventricular neuroepithelium and that segregation into on-off clusters very likely involves migrating PCs. Studies in progress may reveal whether the *lacZ* -expressing cells in the subventricular zone are mitotically active or represent a subpopulation of newly differentiated PCs. (Supported in part by NIH Grant NS 09904).

23.14

REGIONAL VARIATION IN THE DEVELOPMENT OF THE DEEP CEREBELLAR NUCLEI: A TALE OF TWO MUTANTS

B. Kuemerle, S.M. Maricich and K. Herrup*. Alzheimer Research Laboratory, Case Western Reserve University, Cleveland, Ohio 44106

Neurons of the deep cerebellar nuclei (DCN) are the major targets of the Purkinje cells of the cerebellar cortex. In the mouse, the DCN are organized into three clusters located in the cerebellar white matter, just above the fourth ventricle. Whereas many neurological mutations have profound effects on cerebellar cortical cell number, there is only one reported instance of change in the number of DCN neurons. As part of our ongoing study of two cerebellar mouse mutations, *Engrailed-2* (*En-2*) and *weaver (wv)*, we have found that both suffer a 30% loss of neurons in the DCN. Interestingly, this loss is non-uniform; specific mediolateral subdivisions of the DCN are uniquely targeted. In the *Engrailed-2* mutant, the cells of the nucleus medialis are preferentially reduced, while in *wv/wv* mice, it is the nucleus interpositus that suffers the greatest loss. In both cases, the relationship between the cortical defect and the DCN loss suggests intriguing new facets of the phenotypes of the two mutations and the provide insight into the processes of cerebellar compartmentation.

Supported by the NIH (NS18381 and NS20591)

23.15

3-D RECONSTRUCTIONS OF FIBER PARTITIONING AND LOBULATION IN THE CEREBELLAR VERMIS AND HEMISPHERE, S. A. Bayer*, and J. Altman, Department of Biology, Indiana University, Purdue University in Indianapolis, Indianapolis IN 46202-5132.

The developing cerebellum was reconstructed in rats from embryonic day (E) 15 to postnatal day (P) 20. Up to E20, the cerebellar cortex has no fissures. By E21, four fissures appear that separate five lobes. The anterobasal (vermal lobules I-III) and the anterodorsal (vermal lobules IV-V) lobes have little or no extensions into the hemisphere. The central lobe contains nearly all hemispheric lobules and vermal lobules VI, VII and VIII. The posterior lobe (paraflocculus and vermal lobule IX) and inferior lobe (flocculus and vermal lobule X) are pulled apart and the cortex is fragmented between P1 and P20. The medullary layer was separately reconstructed in the central lobe and lobulation in the vermis and hemisphere was linked to partitioning of its fiber panels. Boundaries between lobules are more distinct in the hemisphere than in the vermis on both P1 and P5. By P20, systematic branching of just two fiber panels in the medullary layer expand to service the majority of the hemispheric lobules. It is postulated that specific subsets of Purkinje neurons link up with tagged incoming fibers during migration to produce the characteristic lobules in the vermis and hemisphere. (supported by CRC Press, J. Altman, and S. A. Bayer)

24.1

METALLOPROTEINASES ARE EXPRESSED BY SENSORY NEURONS. T. Ferguson, D. Neubauer, and D. Muir* Depts. of Neuroscience & Pediatric Neurology, University of Florida College of Medicine, Gainesville, FL 32610

Matrix metalloproteinases (MMPs) are implicated in wound healing, tumor metastasis, and other matrix remodeling events. We find that neurite outgrowth in 3D extracellular matrix gels is MMP-dependent and that induction of MMP-2 (type IV collagenase) by cultured dorsal root ganglia (DRG) neurons occurs in response to NGF (Muir, 1994 Exp. Cell Res. 210:243). To further examine the roles of MMPs in axonal outgrowth, we examined the immunocytochemical distribution of several MMPs within the rat nervous system. We find MMP-1, MMP-2 and MMP-3 are expressed by normal DRG neurons. In subsequent studies we focused on MMP-2 expression by DRG neurons during active axonal growth. All neurons within embryonic day 11 DRG immunolabeled for MMP-2. In contrast, only 50% of the neurons in adult DRG showed punctate, cytoplasmic and axonal MMP-2 staining. The majority of MMP-2-positive soma were 700-1300 μm^2 in area.. However, seven days after sciatic nerve crush, the staining of cognate neurons (L4/L5 DRG) increased to over 75% largely due to de novo expression of MMP-2 by regenerating small DRG neurons. In spinal cord, dorsal horns were more intensely stained than surrounding gray matter, suggesting the presence of MMP-2 associated with centrally projecting DRG processes. MMP-2 expression appears unique to peripheral neurons since spinal cord neurons were not immunolabeled, including motor neurons which project axons along side DRG axons. In cultured DRG neurons, activated MMP-2 was shown to be transported to the growth cone and activated enzyme was enriched in a membrane fraction. Together, these findings suggest MMPs may play a role in peripheral nervous system matrix remodeling during axonal outgrowth. Presently, we are exploring whether MMPs are differentially expressed during neuronal development and regeneration. Supported by NIH/NINDS.

24.2

ISOLATION OF NERVE GROWTH CONE PROTEINS AND CHARACTERIZATION WITH PEPTIDE ANTIBODIES. R. Kuwano *, H.Tanaka, H. Saitoh, and K.T. Abe. Research Laboratory for Molecular Genetics, Niigata University, 1 Asahimachi, Niigata 951, Japan

The nerve growth cone plays an important role in pathfinding and recognition of the axon for synapse formation. To elucidate molecular mechanisms of neural network formation and maintaining neuronal functions we analyzed proteins on the growth cone isolated from early postnatal mouse brain. We prepared growth cone particle-enriched (fraction A, the interface between 0.32M and 0.75M) and -non-enriched (fraction C, the interface between 1.0 M and 2.66M) fractions by sucrose density gradient centrifugation. Membrane proteins of the fractions A and C were analyzed by lectin-affinity chromatography, ion exchange chromatography, 2-dimensional gel electrophoresis and SDS-PAGE. Partial amino acid sequences of several proteins concentrated in the fraction A were obtained and compared with those in the protein and nucleotide sequence databases.

We identified GAP-43, NAP-22, gp93, MARCKS and M6 in the fraction A which were reported previously to present on the growth cone. In addition we found several proteins with unique sequences in the fraction A. We synthesized peptides corresponding to the partial amino acid sequences and inoculated rabbits for the peptides conjugated with KLH. Antibodies against some of these proteins are used for characterization of these proteins by western blotting and for determination their intracellular localization in differentiated murine neural precursor cells that were isolated from 10.5 days fetus head and cultured for several days.

24.3

CONTINOUS RENEWAL OF THE AXONAL PATHWAY SENSOR APPARATUS BY INSERTION OF NEW SENSOR MOLECULES INTO THE GROWTH CONE MEMBRANE L. Vogt, U. Ziegler, R. J. Giger, B. Kunz, P. Streit*[1] and P. Sonderegger. Institute of Biochemistry and [1]Brain Research Institute, University of Zurich, CH-8057 Zurich, Switzerland

Growth cones are capable of probing their environment for local growth stimulating or inhibiting cues during neurite outgrowth. Guidance information residing in the molecular terrain of cell surfaces is thought to be recognized by cell adhesion molecules of the growth cone membrane. Using adenoviral vectors we heterologously expressed two functionally active chicken cell adhesion molecules, axonin-1 and Ng-CAM, in rat dorsal root ganglia (DRG) neurons, in order to determine whether cell adhesion molecules are inserted either into the membrane of the growth cone, the axolemma of the axon shaft, or the neuronal cell body. We found that axonin-1 and Ng-CAM are exclusively inserted at the growth cone. Interestingly, neither the glycosylphosphatidylinositol-anchored protein axonin-1 nor the transmembrane protein Ng-CAM exhibited evidence for retrograde diffusion along the axonal shaft. Therefore we concluded that these molecules are immobilized in the axon by interaction with other proteins with which they form the sensor apparatus of the growth cone.
(Supported by the Swiss National Science-Foundation and the Théodore Ott Foundation).

24.4

THE GOLGI IS LOCALIZED TO THE BASE OF THE INITIAL UNIPOLAR PROCESS IN CEREBELLAR GRANULE CELLS IN VITRO. J. F. Zmuda and R.J. Rivas*, Dept. of Zoology, U. of Maryland, College Park, MD 20742.

Cerebellar granule neurons differentiating in situ pass through unipolar and bipolar axonal stages before glial-guided neuronal migration and subsequent dendrite extension. The migrating granule cell is highly polarized in the direction of migration, positioning the nucleus to the cell posterior and localizing membranous organelles such as the Golgi to a rostral juxtanuclear area (Rivas and Hatten, 1995. *J. Neurosci.* 15: 981-989). To determine whether Golgi positioning plays a role in specifying the sites of initial unipolar and bipolar axon extension, purified P4-P6 mouse granule cells were cultured for 1-5 days in vitro (DIV). To mark the position of the Golgi at various times after plating, granule cells were labeled with the fluorescent lipid analog, BODIPY-ceramide (C_5-DMB-Ceramide; Pagano et al., 1991. *J. Cell Biol.* 113: 1267-1279), which preferentially accumulates at the Golgi. At 1-2 DIV, most granule cells extended a single neurite; in 97% (287/296) of these unipolar cells, the Golgi was localized to a juxtanuclear area at the base of the unipolar process. By 2-3 DIV, many unipolar cells began to extend a second process from the opposite pole of the cell. In these cells, the Golgi was localized to the base of the newly emerging neurite in 89% (62/70) of cells observed, suggesting that the Golgi may have re-oriented from the initial position at the base of the first process. By 3-4 DIV, nearly all the cells had adopted bipolar or more complex multipolar morphologies. In bipolar cells, two well established neurites extended from opposite poles of the cell; the Golgi was observed to be positioned randomly between the two processes in 66% (40/61) of observed cells and toward one process or the other in the remaining cells. Since the Golgi is critically involved in the modification and targeting of newly synthesized proteins and lipids, these results suggest that the Golgi may re-orient to facilitate the emergence of newly forming neuronal processes at specific sites on the plasma membrane. Supported by American Paralysis Association RB1-9501.

24.5

CHARACTERISATION OF MAP1B PHOSPHORYLATION AT A SITE RECOGNISED BY MONOCLONAL ANTIBODY SMI-31. M. Johnstone, R. G. Goold, I. Fischer† and P. R. Gordon-Weeks*. D.B.R.C., King's College London, 26-29 Drury Lane, London WC2B 5RL, U.K. †Dept. Neurobiol. & Anatomy, Med. College of PA and Hahnemann University, 3200 Henry Ave., Philadelphia, PA 19129, U. S. A.

MAP1B is a microtubule-associated protein that is expressed early in neurons and plays a role in axonal outgrowth. MAP1B has at least two types of phospho-isoforms, one of which is developmentally down-regulated following neuronal maturation and one of which persists into adulthood. Since phosphorylation is likely to regulate MAP1B activity we characterised the different sites of phosphorylation using a series of recombinant GST-fusion proteins. These have been phosphorylated in an *in vitro* kinase assay in which the recombinant proteins were incubated with a high speed supernatant from neonatal rat brain in the presence of ATP. Phosphorylation of recombinant fusion proteins was detected on Western blots using a panel of phosphorylation-dependent mAb. The analysis showed that mAb SMI-31, which recognises a developmentally-regulated phospho-isoform of MAP 1B, recognises a recombinant protein equivalent to residues 1110-1361 of rat MAP1B after *in vitro* phosphorylation. This phosphorylation site has also been characterised by inclusion into the *in vitro* kinase assay pharmacological agents, peptides and antibodies based on candidate regions of the MAP1B sequence. We aim to use tools identified in these studies to probe the function of MAP1B in cultured neurons.

This work is supported by the M.R.C. and N.A.T.O.

24.6

TAXOL INHIBITS PAF-INDUCED SHAPE CHANGE IN NEURITES. **R.S. McNeil, J.W. Swann and Gary D. Clark*. Cain Foundation Laboratories, Baylor College of Medicine, Houston, TX 77030.**

Platelet-activating factor (PAF) is a potent phospholipid that has been implicated in the human brain malformation disorder Miller-Dieker Lissencephaly. We have previously shown that activation of the neuronal, G-protein linked PAF receptor by nanomolar concentrations of PAF and of a nonhydrolyzable PAF, methyl carbamyl PAF (mc-PAF), lead to the collapse of neuronal growth cones and to neurite withdrawal in rat hippocampal culture. Associated with this withdrawal, PAF produced morphological changes in neurites, which led to the formation of bead-like structures. In these studies, we have examined the effects of culturing hippocampal neurons in media containing 1μM taxol, which has been shown to favor tubulin polymerization and stabilize microtubules, prior to PAF agonist application. The withdrawal of neurites associated with 1μM mc-PAF was -17.1 ± 6.3 (Mean ± SE, N=5) in naive cultures and .27 ± .58 length units (N=5) in cultures treated with taxol. The bead-like morphologic changes induced by mc-PAF in nontreated neurites were not seen in taxol treated neurites. We conclude that pre-incubation with taxol significantly inhibits the neurite shape changes produced by PAF receptor activation, thus suggesting that microtubule depolymerization is a component of PAF-induced morphological changes in neurites.
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24.7

EFFECTS OF MICROTUBULE AGENTS ON GROWTH CONE FILOPODIA. G. Gallo* and E. D. Pollack. Dept. of Biological Sci., University of Illinois at Chicago, Chicago, IL 60607.

Microtubule polymerization and transport underlie nerve fiber elongation and have been shown to be involved in the regulation of growth cone lamellar remodeling (Gallo and Pollack, 1995, Mol. Biol. Cell, Suppl. 103a). The response of growth cones of spinal nerve fibers, growing from lumbosacral spinal cord explants from larval Rana pipiens, to microtubule agents were studied using video-microscopy. During microtubule agent induced growth cone collapse (50nM - 1 mM vinblastine and 0.1 mg/ml nocodazole, n=29), some but not all filopodia were observed to reach lengths up to 75% greater than the maximal lengths attained by filopodia in control growth cones. Also, filopodia were noted to give rise to small transient lamellae along their shafts. We have previously shown that 20 nM vinblastine affects the remodeling of growth cone lamellae without affecting nerve fiber integrity, microtubule arrays or the size of growth cones. Following a 24 hr exposure to 20 nM vinblastine, filopodial length was increased but filopodial number was not affected. The increase in filopodial length was not due to the elongation of filopodia to abnormal lengths, but resulted from a greater percentage of filopodia reaching greater lengths. These data show that microtubules are involved in the regulation of filopodia as well as lamellae at the neuronal growth cone. (Supported by the UIC Campus Research Board)

24.8

GROWTH CONE CYTOSKELETAL DYNAMICS. M. Steketee and K.W. Tosney*. Neuroscience Program and Biology Department, The University of Michigan, Ann Arbor, MI 48109.

We are examining how guidance alters cytoskeletal dynamics by studying an invariant response to contact with a physiologically relevant guidance cue. Sensory growth cones from the DRG normally encounter and always avoid posterior sclerotome (PS) in the embryo. In culture, filopodial contact always inhibits veil protrusion along contacting but not non-contacting filopodia. Since the response is invariant, we are using it to assess the dynamic cytoskeletal changes that mediate the avoidance response.

We see reproducible changes in the state of filamentous actin (f-actin) polymerization that correlate with the response to contact. The growth cone cytoskeleton is composed of f-actin in dynamic equilibrium with free globular actin (g-actin). Filopodial contact with PS inhibits f-actin polymerization locally while non-contacting filopodia support polymerization. The contacting, quiescent regions contain high [g-actin] and low [f-actin] while active protrusions contain high [f-actin] and low [g-actin]. Therefore, veil protrusion can be directed by guidance cues that locally promote or inhibit f-actin polymerization. We are also evaluating the roles of several actin binding proteins and second messenger systems in veil protrusion. For example, tyrosine phosphorylation increases at the leading edge of actively protruding veils. To determine if tyrosine phosphorylation is a prerequisite for f-actin polymerization, we are using inhibitors of tyrosine phosphorylation. These data support the hypothesis that environmental cues guide neurites to their targets by modulating the cytoskeletal dynamics of the growth cones. Supported by NIH #NS21308.

24.9

DIFFERENTIAL DISTRIBUTION OF CAMs ON EMBRYONIC AND ADULT MOUSE OPTIC AXONS *IN VITRO*. C.A. Bates* and R.L. Meyer. Dev. and Cell Biology, Univ. Calif. Irvine, Irvine, CA, 92717.

Embryonic axons grow through the adult CNS, while injured adult axons can not. Since cell adhesion molecules (CAMs) can regulate axonal growth, the differential expression of CAMs may underlie regenerative failure. In this study, we compared embryonic and adult optic axons growing *in vitro* for the presence of CAMs involved in axon outgrowth.

Embryonic (E15 or later) and adult mouse retinas were removed and placed on a laminin substrate under serum free conditions where they extended optic axons within 2 days. Antibodies to the CAMs, L1, Thy-1, N-cadherin, NCAM and polysialylated NCAM (PS-NCAM), were used.

We found that embryonic optic axons were positive for L1, normally found on developing axons, while adult axons were negative. Similarly, N-cadherin was present in embryonic neurons, but was not detectable in adults. Thus, adult regenerating optic axons are not able to re-express the early CAMs, L1 and N-cadherin. Conversely, embryonic axons had no detectable Thy-1, while adult optic axons were Thy-1 positive. In this case, growing adult axons persisted in the expression of the mature CAM, Thy-1. NCAM, as expected, was present in both embryonic and adult optic axons. Unexpectedly, PS-NCAM, normally found only in embryonic axons, was also detectable in adult axons although possibly at lower levels. With the exception of NCAM, regenerating adult axons express different CAMs than embryonic axons.

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24.10

RETINAL GROWTH CONE MOTILITY CONTROLLED BY MYOSIN LIGHT CHAIN KINASE. John T. Schmidt*, and Xiaoying Jian, Dept. Biol. Sci., SUNY Albany NY 12222

Actin-myosin interactions in non-skeletal muscle cells are regulated by the Ca^{++}-calmodulin-dependent myosin light chain kinase (MLCK), which phosphorylates and activates myosin II. We previously showed that pharmacological inhibitors of MLCK block motility in retinal growth cones (Jian et al., 1994, J. Neurobiol. 25:1310), and that MLCK mRNA is both expressed in neurons and is upregulated during axon outgrowth in goldfish retinal ganglion cells (Jian et al, 1995 Neurosci. Abstr. 21: 1773). Here we have taken a peptide from the autoinhibitory domain of MLCK (aa743-758, KKYILRRKWQKTGHAV-NH_2) and coupled it to stearate at its N-terminal to allow it to cross the plasma membrane, a technique shown to work with PKC inhibitors. The homologous 16aa sequence from chicken gizzard MLCK has an IC50 of 0.4μM and inhibits MLCK independent of calmodulin (Foster et al, 1990, Arch. Biochem. & Biophys. 280:397). Application of this peptide at 10μM to retinal growth cones first blocked motility and then collapsed the lamellipodia and filopodia just as the pharmacological inhibitors did. These structures are actin based and we previously showed that inhibiting MLCK eliminated F-actin staining by rhodamine-phalloidin. The time to effect is concentration dependent, and at low concentrations the effect is a reversible inhibition of motility. Control application of stearate alone is without effect. These results verify the conclusion from pharmacological inhibitors that MLCK controls growth cone motility via regulation of its actin cytoskeleton. We have also generated a polyclonal antibody against an MLCK peptide from the C-terminal domain that stains bands on western blots at 135kDa and 190kDa (MLCK isoforms) and at 35kDa (smaller kinase related protein--telokin), consistent with findings in chick and rat. It also stains cultured retinal neurons and their processes. Supported by NIH grant EY-03736.

24.11

ASSOCIATION OF MAP2C WITH ACTIN IN NEURONS. M. Morishima-Kawashima* and K. S. Kosik. Center for Neurologic Diseases, Brigham and Women's Hospital and Harvard Medical School, Boston, MA 02115.

MAPs were originally found as microtubule-associated proteins. There are many reports which showed that some MAPs interact with actin in vitro. This observation suggests additional functions for MAPs in vivo. We examined the interaction between actin and MAPs in cultured rat cerebrocortical neurons. Immunoprecipitation experiments showed the association of actin with MAP2C, but neither with HMW MAP2 nor tau. This association was detected during the first week in culture while neurites developed; thereafter the association decreased. MAP2C and actin co-immunoprecipitated from detergent-resistent cytoskeletal fractions, which indicates that MAP2C is not associated with free actin but with actin filaments. Treatment of cultures with cytochalasin D and colchicine shifted a part of the association to the cytosolic fraction. When cortical neurons were treated with neurotrophin-3 which enhanced neurite extension and branching, the association of MAP2C with actin increased. These results raise the possibility that one function of MAP2C is to interact with actin as well as microtubules during process formation. (supported by NIH grants.)

24.12

LOCALIZED PHOTOLYSIS OF CAGED CALCIUM INDUCES ACCUMULATION OF F-ACTIN, AND TRANSIENT PROTRUSION OF FILOPODIA FROM NASCENT NEURONS IN SITU. P.M. Lau, R.S. Zucker and D. Bentley*. Dept. of Molecular and Cell Biology, Univ. Calif., Berkeley, CA 94720.

A key element in growth cone steering is control of F-actin in filopodia. To regulate cytoskeletal changes within growth cones following contact with extracellular guidance cues, intracellular signaling cascades are activated. In many systems, calcium has been implicated as a critical intermediate in these cascades. To isolate effects of calcium ion concentration from upstream signaling elements, we injected caged calcium compounds, nitr-5 and nitrophenyl-EGTA, into grasshopper pioneer neurons in situ. Using brief (100 ms) flashes of UV through a 20-30 µm diaphragm, selected regions of the growth cone and/or nascent axon were illuminated. Within 1-2 min after illumination, filopodia emerged from the selected region. Following a single flash, filopodia reached a maximum length (5-30 µm) in about 10 min. Multiple flashes increased the persistence of filopodia, and initiated protrusion of additional filopodia. Using in vitro cultures of afferent (femoral chordotonal organ) neurons loaded with caged calcium and Calcium Green, elevation of calcium ion concentration within a few seconds of a flash was confirmed. Neurons in situ were co-injected with caged calcium and rhodamine-conjugated phalloidin. Within 20 seconds of illumination, bright spots of rhodamine appeared in the selected region. These spots dispersed in about 15 min. These results indicate that direct, local elevation of calcium ion concentration can initiate localized foci of actin polymerization, and subsequent protrusion of filopodia.

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24.13

EXPRESSION OF A TRUNCATED NEUROFILAMENT PROTEIN DISRUPTS INTERMEDIATE FILAMENTS AND ALTERS EARLY PERIPHERAL NERVOUS SYSTEM DEVELOPMENT IN *XENOPUS LAEVIS*. B.G. Szaro* and W. Lin. Department of Biological Sciences, University at Albany, SUNY, Albany, NY 12222.

Intermediate filament protein expression is both tissue-specific and developmentally regulated. This property has implicated intermediate filaments, a principal component of the cytoskeleton, in modulating cell structure during development. To explore the role of intermediate filaments in neural development, we injected RNA encoding a truncated form of the *Xenopus laevis* middle molecular weight neurofilament protein (NF-M) into embryonic frog blastomeres at the 2-cell stage. Similar truncated forms of mammalian NF-M disrupt both neurofilaments and vimentin intermediate filaments in transfected fibroblasts. Thus, expression of truncated NF-M in frog embryos should interfere with the organization of their intermediate filaments. In cultures made from dissociated neural tubes and their adjacent myotomes, expression of truncated NF-M disrupted neurofilaments in neurons and desmin filaments in muscle cells. In intact embryos, peripheral nerves were severely stunted as late as stage 37/38. Also, in these embryos, neurons appeared ectopically along the flank. Whereas the stunted peripheral nerves were identical to those seen previously with injected neurofilament antibodies, the ectopic neurons were novel. This suggested that the latter resulted from disruption of intermediate filaments other than the neurofilaments. Thus, these experiments suggest that intermediate filaments serve multiple functions important for normal neural development.

This work was supported by NIH grant NS-30682.

24.14

NITRIC OXIDE SYNTHASE IN REGENERATING AXONS IS CONCENTRATED IN GROWTH CONES. D.B. Wayne* and J.H.P. Skene, Department of Neurobiology, Duke University, Durham, N.C. 27710.

Nitric oxide synthase (NOS) is expressed in dorsal root ganglion (DRG) cell bodies during development and regeneration (Verge et al., PNAS 89: 11617, 1992). Inhibition of NOS with N^G-methyl-L-arginine does not affect basal rates of neurite extension in vitro, but does attenuate calcium-induced inhibition of neurite extension. To better understand the role of NOS in neuronal responses to calcium, we determined the cellular distribution of the calcium-sensitive neuronal isoform of NOS (nNOS) in acutely isolated DRG neurons. Cells were primed with a nerve crush 4-14 days prior to dissociation, and stained after one day in vitro with an antibody specific for nNOS (generously provided by Piers Emson). nNOS is present in many but not all regenerating DRG neurons. In those cells that have NOS, the enzyme is present in all processes and particularly concentrated in growth cones. The presence of NOS in regenerating growth cones, as well as its ability to alter cellular responses to a calcium influx, suggest that NO may modulate neuronal growth as an *intra*cellular signal molecule. [Supported by NIH grant EY11475]

24.15

MEMBRANE PHOSPHOLIPID AND NEURITE OUTGROWTH: EVIDENCE THAT PHOSPHOLIPID SYNTHESIS INCREASES IN GROWING NEURONS. W. Araki*, J. Breu and R. J. Wurtman. Dept. of Brain and Cog. Sci., Mass. Inst. of Technol., Cambridge, MA 02139.

Phospholipids are major constituents of cell membranes and are required to sustain neuronal growth. However, their regulation in growing neurons is not well understood. We are examining phospholipid synthesis and metabolism in growing neurons, using PC12 cells in which neurite outgrowth is induced by treatment with nerve growth factor (NGF). When PC12 cells are plated on collagen-coated dishes and cultured with or without 50 ng/ml NGF for 4 days, total phospholipid levels in the NGF-treated cells, normalized for DNA content, increase by 30% and 120% on the 2nd and 4th day respectively, as compared with untreated control cells. Analysis of individual phospholipids showed that the increases in phosphatidylcholine (PtdCho), phosphatidylethanolamine and sphingomyelin were more pronounced than those in phosphatidylserine and phosphatidylinositol. [^{14}C]Choline incorporation into PtdCho was examined in cells labelled for 2 hr with [methyl-^{14}C]choline. The amounts of radiolabelled PtdCho per DNA in NGF-treated cells were about 5-fold and 14-fold greater than in control cells, on the 2nd and 4th days respectively. These results indicate that (i) phospholipid synthesis in PC12 cells is enhanced by induction of neurite outgrowth with NGF; (ii) it continues to increase during induced neuronal growth; and, (iii) the enhancement involves the CDP-choline pathway (Kennedy cycle). The activities of particular enzymes in the CDP-choline pathway are being examined to identify the loci at which neuronal phospholipid synthesis is regulated. (Supported by NIMH 28783 and The Center for Brain Sciences & Metabolism Charitable Trust.)

24.16

MEMBRANE DOCOSAHEXAENOIC ACID CONTENT INFLUENCES A-TYPE PHOSPHOLIPASE ACTIVITY IN PC12 CELL NERVE GROWTH CONES. Rex E. Martin* and J. Quyen Wickham. Anatomical Sciences, Univ. Oklahoma College of Medicine. Oklahoma City, OK 73106.

As the murine central nervous system develops, the essential fatty acid metabolite, docosahexaenoic acid (DHA) accumulates in membranes of nerve growth cones (NGC). At maturity, the brain's synapses retain high levels of DHA. Especially during development, specificity of function for DHA is indicated because visual impairment and cognitive deficits are noted when diets are deficient in DHA or its precursors. Testing the hypothesis that DHA decreases the activity of phospholipase A_2 (PLA_2), we incubated nerve growth factor-differentiated PC12 cells with 33nM DHA for 4 days. DHA incorporation in NGC membranes was confirmed in parallel incubations with 33nM [^{3}H]DHA. Control and DHA-enriched NGC particles were isolated and incubated with exogenous L-α-1-stearoyl-2-[^{14}C]arachidonyl-phosphatidylinositol ([^{14}C]AA-PI). Immediate incorporation of [^{3}H]DHA into phospholipids including phosphatidylinositol was noted and the 4-day supplementation of PC12 cells with DHA clearly induced changes in the phospholipase activity of NGC. No radiolabeled phosphatidic acid was formed during the assays indicating limited PI-specific phospholipase D activity. [^{14}C]arachidonyl-diacylglycerol formation indicated C-type phospholipases could be present, but they were not apparently DHA-sensitive. The A-type phospholipases were active in the PC12 NGC as radiolabeled free arachidonic acid and lyso-phosphatidylinositol were formed immediately. One or more of these enzymes was apparently sensitive to DHA because 40% less [^{14}C]AA-PI was hydrolyzed by NGC extracts from DHA supplemented cells. Perhaps DHA's role in the nerve ending is to mediate activity of the A-type phospholipases. (OUHSC Alumni Assn. grant to REM and OCAST HN4-018).

24.17

DIGITAL ELECTRON MICROSCOPIC ANALYSIS OF REGENERATING AXON CLUSTERS IN HUMAN SURAL NERVE BIOPSIES. K.A. Sullivan*, J.H. Lillie[1] and D.A. Greene. Dept. of Int. Med. and Anat. & Cell Biol.[1], Univ. of Michigan, Ann Arbor MI 48109.

Diabetic neuropathy is characterized by distinct morphological features including thickening of basement membranes, axon degeneration and regeneration. Axon sprouting leads to an increased number of regenerative axon clusters; however, as the disease progresses there is an overall loss of axons. We have employed the most current digital imaging techniques in order to examine the number of regenerating axon clusters in whole nerve fascicles. Human sural nerve biopsies were assessed for EM suitability based on the degree of fixation, absence of mechanical artifacts and size (100,000 - 425,000 μm^2). Based on these criteria, the largest fascicle was selected. Thin sections were collected onto formvar coated slot grids and coated with a layer of carbon. The sections were then viewed on a Phillips CM100 transmission electron microscope equipped with a CompuStage. Digital images were captured with a Kodak digital Megaplus 1.6 camera and stored as individual tiff files. A montage of the entire fascicle was constructed and the resulting virtual image was viewed by trained EM readers. Computer assisted analysis included confirming the identification of individual axons and labeling regenerating axon clusters. A regenerating cluster was defined as two or more myelinated axons within a continuous basement membrane. Quality control was maintained by randomly assigning a sample to different readers and comparing the results. The EM readers were consistent in their analysis of the samples. It is now possible to aquire an accurate count of the total number of axons within an entire nerve fascicle and to further classify those axons into discrete populations based on relevant criteria.

Supported by Hoffmann-LaRoche, Canada.

25.1

CELLULAR MECHANISMS GOVERNING RETROGRADE SIGNAL TRANSDUCTION AT IDENTIFIED NEUROMUSCULAR CONTACTS IN CELL CULTURE. J. C. Poyer* and M. J. Zoran. Department of Biology, Texas A&M University, College Station, TX 77843.

Helisoma motoneuron B19 undergoes significant presynaptic changes in vitro following target contact and these changes are restricted to neuritic sites of muscle contact. The percentage of B19-muscle preparations with excitation-secretion (E-S) coupling, detected by acetylcholine sensitive reporter cells, has been shown to be significantly increased in cocultures of appropriate partners as compared to inappropriate pairs. E-S coupling is never detected in neurites without target contact. In addition, the induction of secretory competence requires transsynaptic activity. Together, these results suggest that B19 acquires functional secretory properties through activity-dependent interactions with specific muscle targets.

In the present studies, acquisition of functional secretion was blocked in muscle-contacted neurons (n=14) following incubation in medium containing 10 mM suramin. Spontaneous transmitter release rates were similar in control neurons with (n=11) and without (n=18) muscle contact and in suramin-treated neurons with (n=14) and without (n=8) muscle contact. Suramin disruption of E-S coupling suggests that the induction of presynaptic changes in B19 might be regulated by a receptor/ligand interaction. Muscle cells that were stripped of extrinsic proteins by a 1 hr digestion in 100 ng/ml trypsin failed to induce E-S coupling when cocultured in contact with neurons (n=13) for 48 hr. Similarly, neurons (n=9) that were cultured in medium conditioned by sustained muscle incubation, but lacked direct appropriate muscle contact failed, to acquire functional secretion. Furthermore, muscle cells digested with phosphatidylinositol-specific phospholipase C (5 mU/ml) for 1 hr lost the ability to induce E-S in B19 (n=27). Collectively, these data indicate that retrograde signal transduction governing presynaptic modifications in B19 involves cell-surface associated proteins. Whether activity-dependent release of diffusible cues are also involved remains unclear. This research was supported by NSF Grant IBS-9421372

25.2

TARGET-DEPENDENT INDUCTION OF NEURONAL SECRETION INVOLVES NEW PROTEIN SYNTHESIS. M.B. Turner* and M.J. Zoran
Department of Biology, Texas A&M University, College Station, TX 77843

Synapse formation between Helisoma neuron B19 and muscle targets in culture involves a target-dependent induction of secretory properties. This induction requires many hours of B19-muscle contact. Neuron B5, however, forms functional synapses in minutes. These different latencies in formation of functional transmission suggests that B5 possesses secretory function prior to target contact, while B19 acquires target derived signals that upregulate secretory function. These intrinsic differences lead us to test whether target-dependent induction of B19 secretion involves new gene expression and protein synthesis.

Neurons were treated with a 1h pulse of either a transcriptional inhibitor, actinomycin D or α-amanatin, or a translational inhibitor, anisomycin or cyclohexamide. After removal of the inhibitor, muscle targets were cocultured with B19 for 2 days prior to electrophysiological recording. These inhibitors significantly decreased secretory efficacy of B19, but had no effect on B5. To insure that B19 was able to recover from the translational inhibitors, ^{3}H-leucine uptake was monitored using autoradiography. Within hours of treatment, B19 showed uptake of radioactive leucine comparable to untreated neurons. In addition, treatment with anisomycin had no longterm detrimental effects on the initiation or rate of neuritic outgrowth. In further studies, B19 was treated with anisomycin 2 days prior to muscle contact. In this paradigm, upregulation of secretory function was not inhibited.

These results indicate establishment of functional synaptic transmission in B19, but not B5, requires new gene expression and protein synthesis. The finding that the disruption of secretion upregulation in B19 occurs only when protein synthesis inhibition is imposed at the earliest stages of neuron-muscle contact suggests that a critical period of new gene expression may exist for target-induced acquisition of functional secretory properties by B19.
This work was supported by NSF Grant IBN-9421372.

25.3

unc-55*, A NUCLEAR HORMONE RECEPTOR, PLAYS A ROLE IN THE DIFFERENTIATION OF THE VD MOTOR NEURONS IN *C.ELEGANS M. H. Zhou, W. W. Walthall*, Biology Dept, Georgia State University, Atlanta, GA 30303

Six DD and 13 VD motor neurons (mns), collectively called the D mns, form a cross-inhibitory network and are involved in the forward and backward movement of the nematode *C. elegans*. The D mns share many features related to their structure, function and neurochemistry. However, they differ in their synaptic patterns. Mutations in the gene *unc-55* (uncoordinated) cause VD mns to adopt the same synaptic pattern as DD mns resulting in a reduction of ventral inhibition, an enhancement of dorsal inhibition, these two changes sum to create a ventral asymmetric locomotory pattern. Genomic sequence analysis suggested that *unc-55* encodes a nuclear hormone receptor that shares high sequence similarity with members of the COUP gene family. Both a P box and a D box, critical domains in the this family, have been identified in *unc-55* genomic sequence. Mutations in five alleles are closely associated with the P Box, the DNA binding domain. To confirm the role of *unc-55* in the regulation of the VD mn synaptic choice we are attempting to: (1) Isolate *unc-55* cDNAs through library screening; (2) Characterize the mutated sites in *unc-55* alleles; and (3) Construct an *unc-55-lacZ* fusion to determine its expression pattern.

25.4

SHAKING-B (NEURAL) IS ESSENTIAL FOR FUNCTIONAL GAP-JUNCTIONS IN A SUBSET OF *DROSOPHILA* SOMATIC MUSCLES. Jonathan P. Bacon*, Richard A. Baines, Martin G. Todman, Lucy A. Stebbings and Jane A. Davies. Sussex Centre for Neuroscience, University of Sussex, Brighton, BN1 9QG. UK.

The *shaking-B* locus of *Drosophila* encodes at least two, highly homologous, membrane-spanning proteins, shakB(neural) and shakB(lethal). Our previous work has shown that shakB(neural) is essential for electrical synapse formation in the Giant Fibre System (Phelan et al (1996) J Neurosci 16:1101-1113). *In situ* hybridisation shows that, in addition to CNS expression, *shakB* transcripts are present in some embryonic muscles. We compared membrane properties of first instar somatic muscle in larvae carrying mutations that disrupt the function of shakB proteins. The ventral obliques (muscles 15-17) that stain with the *in situ* probe and the ventral longitudinals (muscles 6 and 7) that do not, were investigated by whole-cell voltage clamp in a calcium free saline. Under these conditions, only two voltage-activated potassium currents are visible - a fast (I_A) and a delayed rectifier (I_K) current. These currents are significantly reduced in magnitude in muscles 15 and 16 of *shakB(neural)* mutant larvae compared to either their Oregon-R or *shakB(lethal)* mutant counterparts. The same currents are, however, normal in muscles 6 and 7 of *shakB(neural)* mutant larvae. This apparent increase in input resistance in muscles 15 and 16 of *shakB(neural)* mutant larvae could, hypothetically, be explainable by an absence of functional gap-junctions that serve to dye-couple these muscles to their neighbors during embryogenesis. This was tested: carboxyfluorescein injected into muscle 6 in Oregon-R stage 16 embryos spreads to adjacent muscles including muscles 15 and 16. In *shakB(neural)* mutant embryos, dye fails to invade muscles 15 and 16 indicative of a loss of functional gap-junctions in these muscles. Expression of a UAS-*shakB(neural)* construct using a muscle enhancer-GAL4 line (24B) in *shakB(neural)* mutants fully rescues both the dye-coupling and magnitude of whole-cell currents of the ventral obliques (15 and 16). These observations provide support for the hypothesis that *shakB(neural)* is essential for the normal functioning of some gap-junctions. Supported by the BBSRC, UK.

25.5

SYNAPSE-SPECIFIC ELECTRICAL COUPLING BETWEEN IDENTIFIED NEURONS REFORMS IN CELL CULTURE. T.M. Szabo* and M.J. Zoran. Department of Biology, Texas A&M University, College Station, TX 77843.

Electrical synapses are crucial to the functioning of complex nervous systems and have been shown to play an important and integrative role. Electrical connections between identified motoneurons in the buccal ganglia of the snail, *Helisoma*, have been characterized based on electrophysiological analysis of motor patterns and image analysis of cellular morphology. In the present studies, we have investigated the nature of electrical coupling between phase and anti-phase components of the snail's feeding neural circuit. These neurons are active at different stages in the feeding cycle and receive antagonistic inhibitory and excitatory inputs from the central pattern generator. In dual electrophysiological recordings, neurons were penetrated by intracellular electrodes and assayed for electrical coupling under isopotential conditions. In reduced muscle-ganglion preparations, neuron B19 was electrically coupled to phase neurons, contralateral B19 and motoneuron B18, and possessed average coupling coefficients (cc) of 0.22 (n=30) and 0.16 (n=19), respectively. In contrast, B19 was coupled weakly, if at all, to anti-phase neurons VB10 and VBx, with an average cc less than 0.02.

To test whether phase and anti-phase neurons are competent to couple electrically, we examined them in cell culture. Isolated B19s plated into pairs for 6 days formed neuritic contacts and had a cc of 0.36 (n=32), which was significantly greater than the cc of 0.19 (n=38) measured between B19-VB10 pairs ($p<0.001$). In addition, B19-VBx cell pairs had a cc of 0.01 (n=6). Neurons forming electrical connections with inappropriate targets may selectively eliminate these connections as development proceeds. Preliminary results indicate that inappropriate connections in culture decrease in coupling strength during sustained periods of cell-cell contact.

25.6

Adult pattern and embryonic development of synaptic connections between three identified neurons in the leech. F. James Eisenhart[1] and William B. Kristan, Jr.*[1,2] Group in Neurosciences[1] and Department of Biology[2], UCSD.

A central question in neurobiology is, how do neurons find the right synaptic partners during development? We are addressing this question by studying the development of the synaptic connections between three identified neurons in the leech: cells 1, 102, and 3. These cells are motor neurons found in each segmental ganglion. Cells 1 and 102 inhibit the dorsal longitudinal muscles and also inhibit cell 3 centrally through graded synapses that are distributed across multiple synaptic sites. Previous work showed that cell 1 makes its entire connection on either the first (in 70% of ganglia) or second (30%) major branch of cell 3, suggesting that there may be competition between either pre- or postsynaptic processes within the neuropil.

To investigate this possibility, we used photoablation to isolate the site of the cell 102's connection onto cell 3 and determine its relationship to the cell 1 connection. We filled cell 3 with a fluorescent dye and photoablated successive postsynaptic branches while monitoring the size of IPSPs from cells 1 and 102. Our results suggest that cell 102 also makes its entire connection on a single major branch of cell 3 and that it occupies the same branch as cell 1. One interpretation of this result is that the postsynaptic cell 3 branches compete for the right to synapse with the two presynaptic inhibitors, who may then compete for space on the winning branch. To test this hypothesis, we are studying the development of these connections directly. As a first step, we filled cell 3 with a fluorescent dye in embryos from E9 to E13. We found that cell 3 initially produces many fine central branches from its main axon. Most of these are lost, and the survivors grow thicker and generate secondary branches. This oversprouting and retraction is consistent with the idea that the postsynaptic branches of cell 3 compete among themselves for presynaptic connections.

Supported by NIH grant NS 25916 (WBK) and a Lucille P. Markey fellowship (FJE).

25.7

TIMING OF NEURITE OUTGROWTH AND SYNAPSE FORMATION BETWEEN IDENTIFIED NEURONS IN THE EMBRYONIC LEECH CNS.
D. Reese* and P. Drapeau, Center for Research in Neuroscience, McGill University and Montreal General Hospital, Montreal, PQ, Canada, H3G 1A4.

We are interested in the physiological events leading to the formation of synapses between central neurons during embryonic development. Because of the accessibility of identified neurons in segmental ganglia of the leech (*Hirudo medicinalis*) embryo, we have been able to combine morphological and physiological techniques in order to correlate neurite extension and contact with synapse formation. In particular, we have examined the presynaptic Retzius (R) neurons and their targets the pressure-sensitive (P) neurons. In embryos at different stages of development, R neurons were injected with Lucifer Yellow and P neurons with Rhodamine-dextran. The embryos were fixed and examined by confocal microscopy in order to determine the timing of neurite outgrowth and contact between the neurons in the central neuropil. Neurites came into contact by embryonic day 12 (40% of development), at which stage whole-cell patch clamp recording first detected spontaneous synaptic activity in the P neurons. We have also been able to record simultaneously from both the R and P neurons in order to determine the timing of evoked release and changes in transmitter (serotonin) sensitivity.
Supported by the FRSQ and MRC of Canada.

25.8

SYNAPTIC PLASTICITY IN THE CEREBRAL GANGLION OF *APLYSIA* HAS A DEVELOPMENTAL ONSET. S.M. Fredman*. Dept. of Physiology, Meharry Medical College, Nashville, TN 37208.

The monosynaptic connection between the A and B neurons in the cerebral ganglion of *Aplysia* exhibits two prominent forms of activity-dependent synaptic plasticity. We now report that they have a different developmental onset. In adult *Aplysia*, stimulation of individual presynaptic A neurons at <0.01 Hz results in low frequency synaptic depression (LFSD), a progressive reduction in the amplitude of EPSPs evoked in postsynaptic B neurons. Brief high frequency stimulation (2 sec @ 20 Hz) produces slow developing potentiation (SDP), an ~250 % increase in EPSP amplitude with slow kinetics. SDP reaches a peak 5 min after the tetanizing train and lasts ~30 min. Both forms of plasticity appear to be due to changes in transmitter release by the A neurons and are mediated by distinct second messenger/ signal transduction pathways. These forms of plasticity appear at different times during development, with LFSD being present before SDP.

In juvenile *Aplysia*, 155 days after hatching and weighing ~7.5 g, A-B neuron synapses exhibit LFSD but not SDP. LFSD also appears to be more pronounced in juveniles than in adults, with EPSPs decaying more rapidly. As development progresses, two changes take place. First, a weak form of SDP appears, but only in <50 % of the A neurons. Following the tetanizing train, EPSP amplitudes increase, but rarely exceed the initial amplitude, and decay back to control levels in <10 min. Second, more A neurons begin to display SDP. In slightly older and/or larger *Aplysia* (165-175 days, 12-14g) the potentiation is greater in amplitude, reaching ~150 % of the initial amplitude, and has a longer duration, ~15 min. By the time animals reach an age of ~200 days and a weight of ~18g, SDP is indistinguishable from that seen in the adult. We are presently trying to determine the cellular mechanisms underlying this developmental plasticity.
Supported by NINDS grant NS28199 to SMF and the Meharry NSF/MRCE in Cell and Molecular Biology, HRD9255157.

25.9

DEVELOPMENT AND PLASTICITY OF REGENERATING APLYSIA SENSORIMOTOR SYNAPSES IN CELL CULTURE. R.L. Coulson and M. Klein*. Lab. of Neurobiology & Behavior, Clin. Res. Inst. of Montreal, Montreal, Quebec, Canada H2W 1R7.

We used soma-to-soma preparations of Aplysia mechanoreceptor sensory neurons and LFS motor neurons in culture in order to investigate the time course of synapse formation and of 3 forms of short-term synaptic plasticity associated with simple forms of learning. The soma-to-soma configuration allowed us to examine synaptogenesis independently of the processes of neurite outgrowth and pathfinding.

We found that 25% of correct pairs had detectable synaptic connections at 1 h after pairing, and that this percentage increased to an asymptotic value of 82% by 20 h. The amplitude of the EPSPs increased from an average of 2.36 ± 0.52 (SEM) mv at 2 h to 11.01 ± 1.62 mv at 16-35 h. In some cases no EPSP was detected until after application of the facilitatory transmitter serotonin (5HT). If these pairs are taken into account, then the percentage of connections was 50% at 1 h, while there was no change in the maximal percentage.

Homosynaptic depression had similar kinetics in pairs at early (1-10 h), intermediate (17-35 h) and late (>40 h) times after pairing. Furthermore, synaptic augmentation induced by 5HT after 2 test stimuli and synaptic restoration induced by 5HT after 15-17 stimuli were not statistically different among the 3 groups.

We conclude that regenerating synapses form rapidly upon cell contact and that the plastic capabilities of the newly-formed synapses are similar to those of mature synapses. Our results suggest that recruitment of new functional synapses may not be limited to long-term forms of learning or other long-term phenomena, but could play a role in the induction of intermediate-term and perhaps even short-term forms of plasticity. Supported by NIMH and NSERC.

25.10

A MEMBRANE STRIPE ASSAY FOR DETERMINING NEUROMUSCULAR POSITIONAL SELECTIVITY. A.S. Norton[1], R.L. Graves[1], H. Wang[1], J.G. Cloud[2], W.R. Trumble[1], L. Ziskind-Conhaim[3], and M.B. Laskowski[1]*. WAMI Medical Program[1] and Dept. of Biological Sciences[2], Univ. of Idaho, Moscow, ID 83843 and Dept. Physiology[3], Univ. Wisconsin Medical School, Madison, WI 53706.

Previous studies from our laboratory have shown that spinal cord motoneuron pools map onto the surface of the rat diaphragm and serratus anterior muscles, and that this map is partly reestablished after denervation. In an effort to define the mechanisms whereby developing and regenerating neurites seek out and synapse upon positionally matched muscle cell partners, we have developed an in vitro stripe assay system modelled after Bonhoeffer et al (Devel. 101:685, 1987). Rostral or caudal muscle groups were isolated including axial and proximal limb musculature of E-18 embryonic rats. Crude membrane fractions of muscles were prepared using sucrose gradient density centrifugation. Laminin-coated nitrocellulose filters were placed on a lane-forming matrix yielding alternating stripes of rostral and caudal membranes. Cervical and lumbosacral spinal cord (SC) slices (300 µm) of E-15 embryos were positioned on the nitrocellulose filters with the ventral regions facing the lanes, and the co-cultures were incubated at 37°C in Costar^{R} dishes. Preliminary results show that after 4 days in culture, growth cones of neurites from cervical SC slices were positioned with equal frequency over rostral or caudal membranes. Similarly growth cones from lumbosacral SC were distributed with equal probability over both lanes. Although no gross differences were observed in rostral or caudal selectivity, this assay provides a novel method to identify more subtle growth patterns of developing SC neurites in our search for neuromuscular positional cues.
(Supported by NIH #NS-27024).

25.11

INNERVATION OF FOREIGN CENTRAL TARGETS BY 1A AFFERENTS IN THE EMBRYONIC CHICK SPINAL CORD. A. M. Ritter* and E. Frank Dept. Neurobiology, U of Pittsburgh Sch. of Med., Pittsburgh PA 15261

Sensory neurons innervating muscle spindles (1a afferents) use cues derived from their peripheral target muscles to form connections with the correct motoneuron pools in the spinal cord (Wenner and Frank 1995). Here we ask whether the target muscles of motoneurons play a similar role in specifying which inputs motoneurons receive from 1a afferents, by looking at 1a inputs onto thoracic motoneurons which have been forced to innervate hindlimb muscles. Neural tube from segments LS1-3 in St 17-18 chick embryos was removed and replaced with thoracic neural tube from donor embryos St 16-17; embryos were then allowed to develop until St 39-40 for electrophysiological experiments. The transplanted motoneurons innervate hindlimb targets but still maintain features characteristic of their thoracic origin (Yin and Oppenheim 1992). In contrast to the weak 1a synaptic input that thoracic motoneurons normally receive, both ventral root and intracellular recordings revealed that hindlimb 1a afferents had established short latency, fatigue-resistant connections with the transplanted motoneurons. Further experiments will reveal whether the pattern of 1a inputs is appropriate for the novel peripheral targets of these thoracic motoneurons. Supported by NINDS to EF
Wenner and Frank (1995) J Neurosci 15(12):8191-8198
Yin and Oppenheim (1992) J Neurobiol 23(4):376-395

25.12

INNERVATION SPECIFITY OF ENTORHINAL PROJECTION NEURONS CANNOT BE OVERRIDDEN BY ALTERING TARGET TISSUES IN 1 WEEK OLD POSTNATAL RAT SLICE CO-CULTURE.
P. M. Field, D. Li and G. Raisman.*
The Norman and Sadie Lee Research Centre, Laboratory of Neurobiology, National Institute for Medical Research, The Ridgeway, Mill Hill, London NW7 1AA, UK

The entorhinal cortex *in situ* forms a projection from layers II and III to the subiculum, a projection exclusively from layer II to the dentate gyrus, and a projection from layer IV to the adjacent perirhinal cortex. We have carried out organotypic slice co-culture experiments to present postnatal day 7 (P7) rat entorhinal cortex with different types of P7 target tissue. (1) When entorhinal slices were co-cultured with target tissues that contained only the subiculum and adjacent part of the hippocampal field CA1, but not dentate gyrus, extracellular uptake of biotin dextran from crystals placed on the target slice retrogradely labelled cells in both layer II and layer III of the entorhinal cortex. (2) When entorhinal slices were co-cultured with target slices containing only dentate gyrus, the cells of the entorhinal layer II were retrogradely labelled, but those of layer III were not. (3) When entorhinal slices were co-cultured with target slices containing perirhinal area, only the cells of entorhinal layer IV were labelled. Thus the exclusive relationship of the layer II cells to the dentate gyrus target tissue has already been established by 1 week of age, and is maintained by the regenerating entorhinal axons. Layer III entorhinal cells cannot be induced to project to the dentate gyrus even when deprived of their own target, and layer IV cells are specified to project only to the perirhinal area. Deprivation of the normal target tissue and presentation of an incorrect target tissue is not sufficient to over-ride the specificity of the entorhinal projection neurons.

25.13

An Analysis Of The Synaptic Input From The Parafascicular Thalamic Nucleus Onto Parvalbumin Striatal Interneurons In The Normal Adult Rat And The Effect Of Neonatal Decortication Upon Its Organization, T.Rudkin* and A.F. Sadikot. Montreal Neurological Institute, McGill University, Montreal, Canada.

We use the rat striatum as a model system to address the question of how striatal afferents acquire their ordered synaptic relationship. Both the motor cortex and the lateral segment of the parafascicular thalamic nucleus *(Pf)* in the rat send massive, glutaminergic input to the dorsolateral striatal area. Distributed within this striatal area is a population of interneurons which are identified by their immunoreactivity for the calcium binding protein parvalbumin (PV). PV-dendrites are the postsynaptic target of cortical terminals (Lapper et al '92). Our preliminary evidence suggests that thalamic boutons do not form synapses with PV immunoreactive elements in the normal, adult rat brain. To test whether competition between cortical and thalamic terminals during development contributes to the sparse thalamic innervation onto PV- interneurons, we performed anterograde tract-tracing experiments on bilaterally decorticated P3 neonatal rats. The rats were allowed to survive into adulthood and were stereotactically injected into the *Pf* with the anterograde tracer *biotinylated dextranamine*. Double-labeling immunohistochemistry revealed overlapping thalamic fibres and PV-immunoreactive elements. If competitive mechanisms do indeed participate in the formation of the normal striatal synaptic organization, we would expect that unlike normal adult rats, thalamic terminals of neonatally decorticated rats will allow *Pf* to form synapses with PV-interneurons. Although more analysis is required, preliminary observations of electron micrographs have yet to demonstrate thalamic innervation of PV-immunoreactive elements. This suggests that mechanisms other than competition between afferents underlie the establishment of stable synaptic connections between cortical terminals and PV interneurons within the rat neostriatum. (Supported by the Canadian MRC and a studentship to T.R. from FCAR.)

25.14

PLASTICITY IN THE RETINOTECTAL PATHWAYS: NITRIC OXIDE SYNTHASE BLOCKADE ENHANCES REORGANIZATION OF THE UNCROSSED AXONS FOLLOWING RETINAL LESIONS C.A. Serfaty*, P. Campello-Costa & R. Paes de Carvalho. Departamento de Neurobiologia. Universidade Federal Fluminense. Niteroi. Brasil . 24001-970.

Nitric Oxide is a free radical that has been implicated in synaptic plasticity in the hippocampus, neocortex and cerebellum. We studied the ontogeny of tectal nitric oxide synthase and the effect of systemic blockade of NO-synthase on the rearrangement of the uncrossed retinotectal axons following retinal lesions. Homogenates from the superior colliculus of pigmented rats ranging from post natal day 5 (P5) to P42 were prepared for the assay of NADPH-diaphorase activity or ^{3}(H) citrulline formation. Another group received a temporal retinal lesion which induces rearrangements in the uncrossed pathway of the intact eye. Unoperated animals were used as controls. Experimental and control groups were treated either with L-Nitro-Arginine (NARG, 50mg/kg) or vehicle, IP, during 2-7 days. Retinotectal projections were traced with intraocular injections of Horseradish Peroxidase (HRP). NO-synthase activity was detected at P5, peaked at P21 and declined at P42 in either diaphorase or ^{3}(H) citrulline assays. The systemic blockade with NARG revealed a conspicuous effect on the reorganization of retinotectal axons which invaded the visual layers of the superior colliculus in both normal and lesioned P10-P21 rats. In the later group (lesion/P21), an enhanced expansion of the uncrossed axons were observed towards the surface of stratum griseum superficiale (SGS) after NARG treatment. P5 rats did not show any significant effect on the axonal distribution after NARG injections and P42 rats showed only a slight expansion of retinal axons in the tectal neuropil. Both the time course of NO-synthase expression and the effects of systemic blockade suggest that NO plays a role on the stabilization of the retinotectal synapses after the period of topographic refinement, indicating a critical period of plasticity, dependent on retrograde messengers around the second and third post natal week.
Supported by CNPq, PROPP-UFF.

25.15

Disruption of nitric oxide synthesis and signalling prevents the establishment of proper retinal connections in the optic lobe of *Drosophila melanogaster*. S. M. Gibbs and J.W. Truman,* Dept. of Zoology, University of Washington, Seattle, WA 98195.

During the first half of metamorphosis in *Drosophila*, the photoreceptors express a soluble guanylate cyclase (sGC), the activity of which can be visualized through stimulation with exogenous nitric oxide (NO) and immunodetection with a cyclic GMP antibody. This period of NO sensitivity directly precedes the appearance of functional synapses within the optic lobe and ceases after 50% of pupal development. The laminar and medullar layers of the optic lobes display diaphorase activity during this time, indicating the possible presence of nitric oxide synthase (NOS). Using an *in vitro* brain-eye disc culture system, we have pharmacologically manipulated components of the NO/cGMP pathway during the period of NO sensitivity and observed the effects on retinal patterning in the medulla. The application of the NOS inhibitor L-NAME or the NO scavenger hemoglobin resulted in overall disruption of the retinal projection pattern and the failure of individual PR axons to terminate growth in the medulla; similar concentrations of D-NAME or methemoglobin had no effect. When low levels of methylene blue were added to inhibit the sGC, disruption was observed only in the posterior projections, those having grown into the medulla after the start of inhibitor treatment. Finally, flies mutant for sGC show an increased sensitivity to blocking NO synthesis with L-NAME, displaying posterior but not anterior disruption with lower concentrations having no effect on wild type flies. Together these experiments suggest strongly that NO and cGMP may be important components of a system used to maintain contacts between the retinal axons and their postsynaptic targets in *Drosophila*.
Supported by NS Grant#13079.

25.16

INTIMATE CONTACT BETWEEN NEURONS AND GLIA DURING CARBOHYDRATE MEDIATED TARGETING. M.-H. Tai*, D.M. Autio, M. B. Rheuben and B. Zipser. Depts. Physiol. and Anat., Michigan State University, E. Lansing, MI 48824.

On embryonic day (E) 9-10, the initial defasciculation and dispersal of leech sensory afferents into the neuropil of CNS ganglia is mediated by a surface carbohydrate, the constitutive marker CE0. At E11-12, subsets of sensory afferents are distinguished by their subset markers CE1, CE2 and CE3, which now mediate the consolidation of sensory afferent subsets into unique target regions. Using HRP-labeled antibodies to these markers, we examined their function at the ultrastructural level. Sensory afferents project through roots and connectives in cohesive bundles in intimate assocation with glial processes. In the neuropil, dispersed sensory afferents are associated with both glial cells and central axons. Sensory afferents express CE0 over the entire surface. Contacted neurons and glial cells only express CE0 on membrane surfaces in the immediate vicinity of the sensory afferent. Possibilities include: (1) CE0 is expressed by central neurons and glial cells in a locally restricted way, or (2) CE0 is secreted or transferred after intimate contact from the sensory afferent to the cell that it contacts. Synapse formation coincides with the expression of subset markers in which sensory afferents can be presynaptic on small central profiles or postsynaptic to spine evaginations from large axons.
NIH NS25117

NEURONAL DEATH I

26.1

TIMING OF GRANULE CELL DEATH IN THE CEREBELLA OF STAGGERER AND WILD-TYPE MICE. J. M. Soha* and K. Sunter-Mann. Dept of Surgery, Yale Medical School, New Haven, CT 06520.

Numerous studies have documented an episode of cell death among cerebellar granule cells in the mouse that occurs postnatally and depends on the size of the target Purkinje cell population. In the staggerer mutant, virtually all granule cells die owing to developmental failure of their target. We have undertaken an autoradiographic study to determine if significant granule cell death occurs before the majority of parallel fiber growth, and if the timing of death depends on the level of target support.

Groups of mouse pups, both staggerer and wild-type, were injected with ^{3}H-thymidine to label newly generated granule cells at one of three ages spanning the interval of granule cell genesis: P5, P9 and P13. Animals in each group were killed at a series of subsequent ages and their cerebella sectioned and processed for autoradiography. Pycnotic cells, including a fraction labeled with ^{3}H-thymidine, were identified and counted in the internal granular layer. The frequency of pycnotic cells is analyzed by age for each group, and by date of injection for given ages.

In staggerer, granule cell death is first observed but is rare at 3-4 days after injection. Labeled pycnotic cells are common in the IGL by 5 days post-injection. Granule cell death peaks at 5-10 days post-injection and is virtually complete by 15 days post-injection. Comparison with DiI labeling experiments suggests that granule cells axons (parallel fibers) attain nearly their mature length before the cell death decision occurs. Preliminary data supports a similar timing in wild-type, suggesting that the timing of granule cell death does not depend upon the level of trophic support.

Supported by NIH grant NS-32613.

26.2

CELL PROLIFERATION AND APOPTOSIS IN THE POST-NATAL HUMAN CEREBELLAR CORTEX. L.Lossi[1], S.Ghidella[1], M.A.Greco[3], D.Zagzag[3], G.C.Panzica[2] and A.Merighi[1]*. Dept. [1]Morfofisiologia Veterinaria and [2]Anatomia e Fisiologia Umana, University of Turin, I-10126 Turin, Italy and [3]Dept. of Pathology, New York University Medical Center, New York, NY 10016, USA.

In situ techniques for the detection of DNA fragmentation reveal the existence of a number of apoptotic cells in the post-natal cerebellum of rodents (Wood *et al.*, **Neuron**, 11:621, 1993; Kruger *et al.*, **J.Neurosci.**, 15:3366, 1995). However, the nature of these apoptotic cells is controversial. In the mouse they have been considered to be granule cells, while in the rat they were identified as astrocytes. We have studied here cell proliferation and apoptosis in the human cerebellum from birth up to 400 days post-natally. Proliferating cells were immunocytochemically labeled using antibodies against the proliferating cell nuclear antigen (PCNA) and the Ki-67 nuclear antigen. Apoptotic cells were identified by the TUNEL and T7 DNA polymerase methods. Finally, we detected the *bcl-2* protein and type-III β tubulin by immunocytochemistry to further analyze the mechanism of molecular control and the nature of apoptotic cells.

PCNA/Ki-67 positive nuclei were observed from birth up to 4 months and were most concentrated in the external granular layer (EGL). Nuclei with fragmented DNA were visible from P1 to P90. During the first month they were mainly detected in the EGL, while at later stages tended to accumulate in the inner granular layer (IGL). Expression of the *bcl-2* protein was spatially and developmentally regulated in parallel with the presence of apoptotic cells. *Bcl-2* immunoreactivity was highest immediately after birth and localized to the external proliferative layer of the EGL and outer part of the IGL. The intensity of the reaction progressively decreased and was completely abolished by P120. Certain type-III β tubulin positive cells contained an apoptotic nucleus, thus they likely corresponded to neuroblasts and/or postmitotic granule cells.

Supported by grants of the Italian MURST (60%) and CNR.

26.3

DEVELOPMENTAL NEURONAL DEATH IN THE SUBSTANTIA NIGRA PARS CONPACTA OF THE MOUSE. Vernice Jackson-Lewis*, Vladimir Kostic, and Serge Przedborski. Dept. Neurology, Columbia University, New York, NY 10032.

Neuronal death with the morphology of apoptosis occurs during development of the central nervous system. Recently, apoptosis was described in the substantia nigra pars compacta (SNpc) during development in rats. To examine the possibility that a similar phenomenon occurs in mice, we quantified the number of apoptotic SNpc neurons in developing mice. Counts were performed through the entire extent of the SNpc using Nissl and tyrosine hydroxylase stained sections from postnatal day 2 to postnatal day 28. We found that apoptosis does occur in the developing mouse SNpc. The vast majority of the apoptotic neurons, retained their tyrosine hydroxylase phenotype, indicating their dopaminergic nature. The number of apoptotic neurons decreased with age. However, a significant number of apoptotic neurons were still evident as late as postnatal day 28. This study demonstrates that development neuronal death in SNpc occurs in mice as in rats and thus strengthens the belief that development apoptosis is a phenomenon common among mammalian species.

This work is supported by the NINDS, the Parkinson's disease Foundation, and the Lowenstein Foundation

26.4

TIME COURSE OF DEVELOPMENTAL CELL DEATH IN PHENOTYPICALLY-DEFINED DOPAMINERGIC NEURONS OF THE SUBSTANTIA NIGRA. T.F. Oo and R.E. Burke*, Department of Neurology, Columbia University, New York, NY 10032.

We have previously shown that natural cell death, with the morphology of apoptosis, occurs within the cytoarchitectonic boundaries of the substantia nigra pars compacta (SNpc) postnatally in the rat (Mol Cell Neurosci, 1993). However, the occurrence of natural cell death within phenotypically-defined dopaminergic neurons of the SN has not previously been identified, nor has its time course been defined in pre- as well as postnatal development. To achieve these goals, we prepared complete sets of sections through the SN in prenatal (E19, 20, 21) and postnatal (P2 through P28) rats for tyrosine-hydroxylase (TH) immunostaining, to identify the dopaminergic phenotype, and for Nissl counterstain, to identify characteristic intranuclear apoptotic chromatin clumps. Sections were analyzed at 600x. We quantified the number of TH-positive cells with apoptotic chromatin clumps (i.e., TH-positive by cellular criteria) and the number of apoptotic profiles that were TH-negative, but within 15 microns of 2 TH-positive neurons (i.e., within the dopaminergic cell group by regional criteria). The counts of apoptotic profiles identified by cellular and regional criteria were analyzed separately, but were highly correlated ($r = 0.939$). Both sets of data revealed two peaks of natural cell death within the SN, a major, broad peak occurring from E21 to P6, and a second minor peak at P14. These two peaks were apparent in data expressed as apoptotic cells/section or as apoptotic cells/SN. We conclude that natural cell death occurs within defined dopaminergic neurons of the SN, with a biphasic time course. The basis of the biphasic time course is unknown, but it is notable that P14 is a period of marked synaptogenesis within the target, striatum, so the P14 peak may represent a period of competition for target-derived trophic support. NS26836, PDF.

26.5

CHARACTERIZATION OF DOPAMINERGIC MIDBRAIN NEURONS IN A DBH:BDNF TRANSGENIC MOUSE. Alonso-Vanegas M.A*, Sadikot A.F, Causing C, Miller F. Cone Laboratory for Neurosurgical Research and Centre for Neuronal Survival, Montreal Neurological Institute, McGill University, Montreal, PQ, Canada.

Neurotrophic factors including BDNF have been implicated in neuronal survival, differentiation, and plasticity in the developing and adult nervous system. Dopaminergic (DA) neurons of the mouse midbrain develop according to a strict neurogenetic timetable, with birth of neurons between E10-13 (Bayer et al. '95) and adult spatial configuration largely achieved by the early postnatal period. BDNF displays neurotrophic actions on DA neurons in cell culture (Hyman et al, '91). As a model system for determining the role of BDNF in mid-brain development, we utilize a transgenic mouse that overexpresses brain-derived neurotrophic factor (BDNF) in adrenergic and noradrenergic neurons from the dopamine-ß-hydroxylase (DBH) promoter (DBH:BDNF) (Causing et al. '95). We have performed a quantitative analysis of dopaminergic neurons at the mid-substantia nigra level comparing heterozygote DBH:BDNF animals to wildtype control mice. Our preliminary results based on 4 transgenic animals and 4 controls indicate an increase in mean cell number of 63% in transgenic animals. This data suggests that BDNF protects dopaminergic neurons from developmental cell death. Such protection may be mediated by anterograde transport and release of neurotrophins by locus coeruleus. The precise subpopulations of dopaminergic midbrain neurons that are the target of this trophic effect remain to be determined. (Supported by the MRC of Canada and the Network of Centres for Excellence).

26.6

STRIATAL INJECTION OF 6-HYDROXYDOPAMINE INDUCES APOPTOTIC CELL DEATH OF NIGRAL DOPAMINERGIC NEURONS IN NEONATAL RATS. M.J Martí*, C.J James, T.F Oo, W.J Kelly, R.E Burke, Department of Neurology, Columbia University, New York, NY 10032.

Experimental manipulations that diminish target-derived support often result in an augmented regressive event in developing neurons. There is evidence that such support is also important for the development of substantia nigra (SN) dopaminergic system. We hypothesized that if developing dopaminergic neurons depend on target-derived trophic factors for their viability, then destruction of their intrastriatal terminals with 6-OHDA could lead to programmed death of these neurons. An intrastriatal injection of 6-OHDA (15µg/µl) was made at postnatal day (PND)7. Rats were sacrificed at different post lesion days (PLDs): 0 to10. Sections were processed for silver staining and for tyrosine hydroxylase (TH) immunostaining with Nissl counterstain to identify and quantify cell death in SN and for *In situ* end labeling (ISEL). Intrastriatal 6-OHDA injection in PND7 rats resulted in augmented cell death in SN and the morphology of degenerating cells was apoptotic. Apoptosis was identified by the characteristic formation of rounded, distinctly bounded chromatin clumps on both Nissl and silver stain, and by ISEL. TH stain demonstrated that the apoptotic cells were dopaminergic. The induced apoptosis became apparent at PLD3 ($E= 2.8 \pm 0.6$, $C=0.5 \pm 0.2$), was maximal at PLD6 ($E= 7.2 \pm 1.0$, $C= 0.3 \pm 0.1$) and was almost complete by PLD10. In conclusion, destruction of intrastriatal dopaminergic terminals is associated with augmented apoptotic cell death in the SN of immature rat brains. This result support the hypothesis that developing dopaminergic neurons are dependent on target-derived supports. (NS26836) (PDF) (HCP).

26.7

CEP-1347 PROTECTS NIGROSTRIATAL DOPAMINERGIC NEURONS FROM DEGENERATION AFTER MPTP-INDUCED LESIONS IN VIVO. M.S. Saporito*, E.R. Brown, C. Murakata#, M. Miller and S. Carswell. Cephalon Inc. 145 Brandywine Parkway, West Chester, PA 19380, #Kyowa-Hakko Kogyo, Tokyo, Japan.

We have identified a class of small organic compounds that protect CNS neurons from degeneration. These compounds are represented by CEP-1347, a structurally novel indolocarbazole that lacks protein kinase C and Trk-inhibitory activities, yet, enhances the survival of cultured basal forebrain and spinal cord cholinergic neurons. Moreover, CEP-1347 promotes the survival of neurons in *in vivo* models of motoneuron and basal forebrain cholinergic degeneration. For example, systemic administration of CEP-1347 to rats subjected to an excitotoxic lesion of the nucleus basalis prevents the loss of cortically-projecting cholinergic neurons, suggesting that this compound may be useful in the treatment of diseases involving cholinergic degeneration. In the present studies, it was of interest to determine whether CEP-1347 prevents the degeneration of other neuronal types that reflect the pathology of additional CNS neurodegenerative disorders. In that regard, CEP-1347 was assessed in the MPTP mouse model, an *in vivo* model of Parkinson's disease. MPTP administration (20 mg/kg; s.c.) produced an approximate 50% loss of striatal tyrosine hydroxylase (TH) activity that was maximal 3 days post-injection. Administration of CEP-1347, at daily doses ranging from 0.01 to 10 mg/kg (s.c.), to MPTP-treated mice reduced the loss of striatal TH activity to approximately 25%. CEP-1347 did not inhibit monoamine oxidase B or block uptake of catecholamines into synaptosomes, indicating that this compound does not prevent the metabolic conversion of MPTP to MPP^+ or inhibit the active uptake of MPP^+ into dopaminergic neurons. These studies demonstrate that CEP-1347 protects dopaminergic terminals from the neurotoxic effects of MPTP and suggest that Parkinson's disease may be an additional therapeutic target for this class of indolocarbazoles.

26.8

SYSTEMIC 3-NP PRODUCES STRIATAL APOPTOSIS IN RATS BUT NOT BALB/C BYJ MICE. T. Alexi*, P.E. Hughes, H.Y. Kim, B. Knüsel and A.J. Tobin. Dept. Physiological Sciences, UCLA and Andrus Gerontology Ctr, Univ. of Southern Calif., Los Angeles, CA.

Metabolic compromise of Sprague Dawley rat striatum with 3-nitropropionic acid (3-NP) results in its preferential degeneration, thus mimicking the pathology of Huntington's disease. We now show a surprising species difference in the susceptibility to systemic 3-NP, with BALB/c BYJ mice being resistent to striatal neurotoxicity. Both rats and BALB/c BYJ mice showed comparable levels of depletion of succinate dehydrogenase activity, the target of 3-NP inhibition. However, only rats developed striatal lesions, as revealed by TUNEL staining of chromatin condensation and apoptotic bodies. TUNEL-positive nuclei were widespread throughout the striatum in rats at 1-7 days after 3-NP injection. While mice did not show any striatal lesions, they did show CA1 hippocampal damage, at 3-7 days post-injection. Treated rats with striatal lesions also showed similar CA1 lesions.

Our results show an unexpected species difference in striatal neurotoxicity to systemic 3-NP despite equivalent levels of metabolic inhibition. In addition, systemic 3-NP in rats produces widespread striatal apoptosis. These results suggest that the link between the selective vulnerability of striatal cells to energy compromise is not as straightforward as previously believed.

This work is supported by a NINDS Postdoctoral Fellowship. PEH is supported by a Health Research Council of New Zealand Overseas Postdoctoral Fellowship.

26.9

LOSS OF CHOLINERGIC CELLS IN THE DEVELOPING SEPTOHIPPOCAMPAL SYSTEM OF RATS EXPOSED TO LOW LEVELS OF LEAD. N. A. Bourjeily* and J. B. Suszkiw. Dept. of Molecular and Cellular Physiology, Univ. of Cincinnati College of Medicine, Cincinnati, OH 45267-0576.

We previously reported a decrease in cholineacetyltransferase (ChAT) activity and HC-3 binding sites in the developing septohippocampal system of rats exposed to low levels of Pb. The similar reduction in ChAT and HC-3 binding sites suggests that Pb either compromises functional development of septal cholinergic neurons or causes their death. In this study, we sought to determine whether the Pb-induced cholinergic deficit is a result of partial loss of septal cholinergic neurons and whether this cell loss leads to a reduced cholinergic innervation of hippocampus. Timed-pregnant Sprague-Dawley rats received 0.2% lead acetate in drinking water starting one week before parturition and continuing until pups were 21 days old (P21). Brains from control and Pb-exposed pups were sectioned through the extent of MS/vDB and dorsal one-third of hippocampus. Septal sections were stained immunocytochemically using a polyclonal anti-ChAT antibody and number of ChAT-immunoreactive (ChAT-IR) cells was determined. Hippocampal sections were stained using an AChE-histochemical method and density of AChE-positive fibers was assessed. In Pb-exposed P7 and P21 rats, there was a 24% decrease in number of ChAT-IR cells in MS/vDB. In addition, density of AChE-positive fibers was reduced (14-18%) in four regions of hippocampus known to be innervated by cholinergic cells in MS/vDB. Three months after termination of Pb exposure, density of AChE-positive fibers in the four hippocampal regions returned to normal. We conclude that perinatal low level Pb exposure results in death of cholinergic cells in the developing septohippocampal system and reduction in cholinergic innervation of hippocampus. The latter effect is evident as early as P7 and is followed by a delayed period of compensatory cholinergic fiber sprouting after termination of Pb exposure. The findings of this study implicate the involvment of the cholinergic septohippocampal pathway in the cognitive and learning impairments associated with developmental Pb exposure. (Supported by NIH grant ES06365).

26.10

DISTRIBUTION OF GIRK2 mRNA IN THE TESTIS AND HYPOTHALAMUS OF THE NORMAL MOUSE: IMPLICATIONS FOR THE WEAVER MOUSE. S.M.W. Harrison[1,*], M.J. Hilderbrand[2], J.C. Niu[3], D.D. Hunter[1,3], and S.K. Roffler-Tarlov[1,2,3]. [1]Dept. of Anatomy and Cell Biology, [2]Program in Genetics, [3]Dept. of Neuroscience, Tufts Univ. Sch. of Med., Boston, MA 02111.

A point mutation has recently been identified in the *Girk2* gene of the weaver mutant mouse (Patil et al. 1995. Nature Genetics 11:126) suggesting that mutated Girk2 may lead to the neurological and fertility deficits found in the weaver mouse. We have begun to investigate the possible role of mutant Girk2 in weaver male sterility by defining the distribution of *Girk2* mRNA in normal mouse testis and hypothalamus. We have used *in situ* hybridization (ISH) and RT-PCR to determine the spatial and temporal pattern of *Girk2* gene expression in these tissues.

Weaver male germ cells complete meiosis but are arrested and die during differentiation in mid-spermiogenesis. This pathology appears in the first spermatogenic cycle, after postnatal-day (P) 21. It is not known whether germ cells or another hypothalamic-pituitary-testicular axis cell type are the primary target of the mutation. We have studied *Girk2* expression in pubertal wild-type testis and hypothalamus with respect to the development of the weaver's male-sterile phenotype.

We were unable to detect *Girk2* expression in P7 wild-type testis. In the P7 wild-type hypothalamus, RT-PCR did not identify *Girk2* mRNA, however, a few cells in the arcuate nucleus were *Girk2* positive by ISH. At all other ages studied, *Girk2* message was present in testis and in several hypothalamic cell populations, including in a limited number of cells of the arcuate nucleus. The first testicular expression of *Girk2*, between P7 and P14, is coincident with the onset of meiosis in the first spermatogenic cycle. ISH revealed *Girk2* message specifically in mid-meiotic germ cells at P14, as well as in early post-meiotic germ cells in older mice. *Girk2* expression, therefore, precedes the weaver's testicular pathology by at least one week. These patterns of *Girk2* expression suggest that male germ cells may be a direct target of weaver, but do not eliminate involvement of the hypothalamus. NS20181, NS31673.

26.11

DISTRIBUTION OF *GIRK2* RNA IN THE MIDBRAIN, CEREBELLUM, AND OLFACTORY BULB OF POSTNATAL MICE. J.C. Niu*, S.M.W. Harrison, Y. Xu, D.D. Hunter and S.K. Roffler-Tarlov. Dept. of Neuroscience, Tufts Univ. Sch. of Med., Boston, MA 02111.

Weaver (gene symbol *wv*) is a murine autosomal recessive mutation that affects the postnatal development and viability of several types of cells. The effects of *weaver* on dopamine-containing neurons in the substantia nigra in the midbrain and the granule and Purkinje cells in the cerebellum can be seen during the first postnatal week. There is now compelling evidence to show that a point mutation in the *Girk2* gene, which encodes a G protein-coupled inwardly rectifying K^+ channel, is *weaver* (Patil et al. 1995, Nature Genetics 11:126).

Using *in situ* hybridization, we have begun to examine the expression of *Girk2* transcripts in the developing brain of wild-type mice, paying special attention to sites where cell death occurs in weavers. Specific digoxigenin-labeled RNA probes directed to the 3' UTR of *Girk2* RNA revealed wide distribution of *Girk2* on postnatal days (P) 7 and 24. In the ventral midbrain, several groups of nuclei express *Girk2*. Neurons in the substantia nigra, vulnerable to cell death in weavers, express *Girk2*, as do several cell groups that are retained in the weaver's midbrain. Granule cell death in weaver is most prominent in the postmitotic region of the external germinal layer in the vermis; however, in the P7 cerebellum, *Girk2* is expressed throughout the external germinal layer and in the internal granular layer. *Girk2* expression in Purkinje cells showed regional differences with expression high in the vermis and low in the cerebellar hemispheres. The olfactory bulb, not thought to be a target of the *weaver* mutation, shows strong *Girk2* expression in the mitral/tufted cells and in some periglomerular cells. Our data suggest that the *Girk2* mutation is a necessary but not sufficient cause of cell death in weaver mice. NIH NS 20181 and NS 31673.

26.12

COORDINATED EXPRESSION OF FAS/APO (APOPTOSIS)-1, RIP (RECEPTOR INTERACTING PROTEIN), AND FAS-L (FAS LIGAND) IN THE DEVELOPING RAT EMBRYONIC AND POSTNATAL CEREBRAL CORTEX. W. C. Conner, J. S. Wieters, Z. F. Cheema, and R.C. Miranda*, Department of Human Anatomy and Medical Neurobiology, Texas A&M University, College of Medicine, College Station, TX 77843

Cells within the developing rat cerebral cortex undergo apoptotic cell death within the first postnatal week. Our previous research demonstrated that the Fas/Apo-1 receptor is transiently expressed during this period of cell-suicide. The Fas receptor is activated by a transmembrane ligand, Fas-L. Additionally, Fas itself associates and interacts with other transmembrane proteins, RIP and FADD. In this study, we examined the spatio-temporal coordination of the expression of RIP and Fas-L with the Fas receptor. We used double *in situ* hybridization to examine the colocalization of RIP mRNA to Fas mRNA-expressing cells. Cells expressing both types of mRNA colocalized within the ventricular zone and cortical plate. In addition, combined immuno-histochemistry was used to examine the relationship between Fas and Fas-L-immunoreactive cells. Fas-L-immunoreactive cells appeared to be localized proximate to Fas-immunoreactive cells, suggesting that cell-cell interactions may activate the Fas pathway. Our current research is focused on an examination of the interaction between these elements of the Fas cell-suicide signaling pathway. *Supported by funds from Texas A&M University.*

26.13

MK-801-INDUCED EXPRESSION OF BCL-2, FOS AND HSP72 PROTEINS IN THE RAT CEREBRAL CORTEX.

X. Zhang, X. Fan, P.H. Yu* and A.A. Boulton*
Neuropsychiatry Research Unit, Department of Psychiatry, University of Saskatchewan, Saskatoon, Canada S7N 5E4.

The NMDA receptor antagonist (+)MK-801 (Dizocilpine)(5 mg/kg, sc) has been shown to induce a rapid and long-lasting (2 hr to 2 weeks) reduction in the expression of *bcl-2* proteins in the retrosplenial granular cortex (RSG), and layers IV and VI of the somatosensory cortex (SS), although dying neurons were only identified in the RSG. An induction of Fos and/or Fos-related antigens (Fra) was observed in the RSG and layers II, III, V and VI in SS adjacent sections from 1 hr to 1 week post-injection of (+)MK-801. The majority of HSP72-immunoreactive neurons in RSG were also Fra-immunoreactive. Western blot analysis revealed a rapid, transient and substantial expression of 69 kDa Fra as well as a rapid, prolonged and moderate expression of 46 kDa and 35 kDa Fra in the RSG. Such (+)MK-801-induced responses are quite unique. It has been reported that glucose utilization was significantly decreased in layer IV of SS and increased in RSG. Our results are consistent with the hypothesis that psychomimetic (+)MK-801 causes a functional disconnectivity in the SS (i.e. external signals cannot be properly perceived, while inter- and intra-cortical communications as well as self-defined outputs are increased). In addition the reduction in *bcl-2* expression is not necessarily related only to neuronal apoptosis. (Supported by Saskatchewan Health and HSURC)

27.1

MUTATIONS IN *BENCHWARMER*, A NOVEL MEMBER OF A TRANSPORTER-LIKE PROTEIN FAMILY, INDICATE THAT A SMALL SUBSET OF GLIA PREVENT NEURONAL APOPTOSIS IN THE *DROSOPHILA* BRAIN A. Kania, J.T. Littleton, J.D. Sweatt* and H.J. Bellen. Howard Hughes Medical Institute and Division of Neuroscience, Baylor College of Medicine, Houston, TX 77030.

Programmed cell death (apoptosis) is a fundamental feature of animal development and is under the control of similar effector molecules in evolutionarily divergent species. Apoptosis has been proposed to be the default pathway when vertebrate cells are deprived of necessary growth signals. The role of glia in neuronal survival and as a source of growth factors in vertebrates has been well documented. Glia are known to provide fully developed neurons with survival factors that prevent them from undergoing cell death. However, the role of glial derived signals in promoting survival of immature neurons and the ability of immature neurons to undergo programmed cell death is still being studied. We have screened for P-element enhancer detector mutations that affect nervous system development in *Drosophila* and have identified an essential gene which we have termed *benchwarmer*. Benchwarmer is the first member of a novel family of transporter-like proteins that includes a human homolog and that is expressed in a small subpopulation of glia throughout development. Mutations in *benchwarmer* cause pupal lethality with rare adult escapees. Mutant flies display behavioral and locomotor defects, and exhibit abnormal neuronal communication. These defects are accompanied by a severe loss of neurons in the adult brain. This loss of neurons results from extensive abnormal apoptotic cell death in early pupal development, suggesting lack of an essential non-cell autonomous signal from *benchwarmer* expressing glia. Furthermore, this apoptotic death indicates that newly born neurons have the capability for undergoing programmed cell death early in their life. These results, combined with previous studies, suggest that the default developmental pathway for neurons in the absence of glial derived survival signals is apoptosis. H.J.B. is an Associate Investigator of the Howard Hughes Medical Institute.

27.2

MOLECULAR MECHANISMS OF DEVELOPMENTAL NEURON DEATH, **J. Saltis and P.G. Noakes*, School of Anatomy, University of New South Wales, Sydney 2052 NSW, Australia.**

Neuron death is a normal developmental phenomenon that coincides with the formation of peripheral synapses. Peripheral tissues are thought to provide trophic factors to innervating neurons. A limited supply or access to these factors results in neuron death (1). Our aim was to investigate whether aberrant synapse formation affects motor and sensory neuron survival. Deletion of rapsyn, a specific post-synaptic membrane molecule, was achieved by gene knockout in mice (2). Rapsyn colocalises with acetylcholine receptor (AChR) clusters during the formation of the neuromuscular synapse. In its absence, AChRs and cytoskeletal elements associated with the postsynaptic membrane fail to concentrate beneath the motor nerve terminal. By measuring neuron numbers in these mutant mice, we hope to reveal whether a viable neuromuscular synapse is required for neuronal survival. We examined the number of motor, sensory and sympathetic neurons in rapsyn-deficient and wild type mice at birth. Animals were overdosed with 100% CO_2. 10µm thionin stained paraffin sections were examined by light microscope. We found a significant reduction in both motor ($p<0.03$, t-test; n=3) and sensory ($p<0.002$; n=3) neuron number at the brachial level of mutant mice compared to wild type. There was no effect on sympathetic neuron number. Motor neuron number was reduced from 3055 ± 334 to 1873 ± 455 and sensory neuron number from 7155 ± 733 to 3483 ± 73. Sympathetic neuron number remained at around 13,700. These results indicate that a deficiency in rapsyn leading to an aberrant synapse interferes with the survival of motor and sensory neurons. The possibility remains that the disruption of the synapse may either reduce neurotrophic factor production or hamper the accessing of target-derived trophic factor.

(1) Oppenheim RW, Prevette D *et al* (1991) Science **251** 1616-1618
(2) Gautam M, Noakes PG, Mudd J *et al* (1995) Nature **377**, 232-236

This work was supported by the Motor Neuron Disorder Foundation of Australia and the Australia Research Council.

27.3

A TEMPORAL RELATIONSHIP BETWEEN MOTONEURON BIRTHDATE AND ONSET OF PROGRAMMED CELL DEATH M.J. Burek*, D. Prevette, and R.W. Oppenheim, Dept. Neurobiol. and Anat., Bowman Gray School of Medicine, Winston-Salem, NC 27157

We are interested in whether there is a fixed temporal interval between the genesis of developing neurons and when they undergo programmed cell death [PCD] (Galli-Resta and Ensini, 1996). One excellent model system to experimentally address this issue is the large-scale death of lumbar motoneurons (MNs) of the chick embryo spinal cord. MNs are born between embryonic days (E)2 and 4.5 and compete for access to target muscle-derived trophic factors between E6 and E11. This culminates in the death of approximately one-half of the original cell population. Since the earliest born MNs are the first to die in vitro in the absence of target muscle-derived trophic factors (Mettling et al., 1995), we hypothesized that a similar correlation between motoneuron birthdate and onset of cell death occurs in vivo. To test this we injected bromodeoxyuridine (BRDU) in ovo to label MNs born both early and late in the proliferative period and determined when, during the cell death period, these two neuronal cohorts undergo PCD. We report here that at E5.5 when motoneuron PCD commences, the latest-born MNs (E4-E4.5) (labeled by BRDU injections on E4) are not yet in the lateral motor column. Interestingly though, the absolute number of BRDU-labeled MNs increases between E5.5 and E8.5, but then declines by 50% coincident with a dramatic increase in the number of pyknotic BRDU-labeled cells. Since MNs born after E4 die only after E8 it suggests these cells have a fixed 4 day interval between their birth and onset of target dependence. We are currently testing whether, conversely, the earliest-born MNs are the first to die in vivo, and if MNs require cellular interactions with their target muscles to establish target dependence. Finally, we will determine a fixed temporal interval between cell birth and cell death is evident among other neuronal populations that undergo PCD in the developing chick embryo. Supported by NIH grants NS 20402 (R.W.O.) and NS 09838 (M.J.B.).

27.4

A NOVEL TYPE OF PROGRAMMED NEURONAL DEATH IN THE CERVICAL SPINAL CORD OF THE CHICK EMBRYO. H. Yaginuma*[1], M. Tomita[1], N. Takashita[1], S.E. McKay[2], C. Cardwell[2], Q.-W.Yin[2] and R. W. Oppenheim[2] [1]Inst. of Basic Medical Sci., Univ.of Tsukuba, Tsukuba, 305 Japan. [2]Dept. of Neurobiol. and Anat., and Neurosci. Prog., Bowman Gray School of Medicine, Wake Forest University, Winston-Salem, NC27157

We examined the massive early cell death that occurs in the ventral horn of the cervical spinal cord of the chick embryo between embryonic day (E) 4 and E5. Studies with immunohistochemistry, *in situ* hybridization and retrograde tracing methods revealed that many of dying cells expressed Islet proteins and *Lim-3* mRNA (motoneuron markers) and send their axons to the somatic region of the embryo before cell death. These data strongly suggest that the dying cells are somatic motoneurons. Counts of motoneuron number between E3.5 and E10 revealed that, in addition to cell death between E4 and E5, motoneuron death also occurs between E6 and E10 in the cervical cord. Studies with ^{3}H-thymidine autoradiography revealed that the cells dying between E4 and E5 become post-mitotic before E3.5, whereas genesis of motoneurons is still ongoing between E4 and E5. Increasing the size of peripheral targets, treatment with neuromuscular blockade and treatment with partially purified muscle or brain extracts, and defined neurotrophic agents, such as NGF, BDNF, NT-3, CNTF, bFGF, PDGF, S100-β, activin, CDF/LIF, BMP-2, IGF-I, IL-6 and TGF-β1 were all ineffective in rescuing motoneurons dying between E4 and E5. Early cell death also occurs in a notochord-induced ectopic supernumerary motoneuron column in the cervical cord. Transplantation of cervical neural tube to other segmental regions failed to alter the early death of motoneurons. These results suggest that the mechanisms that regulate motoneuron death in the cervical spinal cord between E4 and E5 are independent of interactions with targets. Rather this novel type of cell death seems to be determined by signals that are either cell autonomous or that are derived from other cells *within* the cervical neural tube.

27.5

DIFFERENTIAL LEVELS OF APOPTOSIS IN DEVELOPING RAT SOMATIC AND AUTONOMIC MOTOR NEURONS. R. Wetts* and J. E. Vaughn. Division of Neurosciences, Beckman Research Institute of the City of Hope, Duarte, CA 91010. USA.

In contrast to numerous studies of apoptosis in developing chick CNS, few details are known about this process in mammalian species. To label apoptotic motor neurons in rat thoracic spinal cord, we used terminal transferase-dUTP nick end labeling (TUNEL). Among somatic motor neurons (SMNs), peak levels of TUNEL-positive cells (1.2 - 1.6 SMNs/section) during the E14 - E18 period were much higher than baseline levels (0.0 - 0.4 SMNs/section) from E20 - P15. However, TUNEL-labeled autonomic motor neurons (AMNs) were present only at baseline levels (0.0 - 0.4 AMNs/section) over the entire period studied (E15 - P15). In order to determine whether apoptosis of motor neurons was target dependent, we conducted experiments on cultured slices of E15 specimens. SMNs survived in control cultures, but disappeared within 4 days after removal of their peripheral target. AMNs, on the other hand, survived for 8 days without their target.

Considering the developmental and phenotypic similarities between SMNs and AMNs, it is surprising that SMNs undergo apoptosis to a much higher degree than AMNs. The phenotypic trait(s) that underlies this developmental difference might also be critical in amyotrophic lateral sclerosis, in which SMNs degenerate and AMNs do not. *Supported by NIH grant NS25784.*

27.6

ETHANOL EFFECTS ON MOTONEURONS OF THE CHICK EMBRYO SPINAL CORD D.M. Bradley*, F. Beaman, D.B. Moore, and M.B. Heaton. Dept. of Neuroscience, Univ. of Florida Coll. of Med., Gainesville, FL 32610.

Ethanol is known to exert a variety of teratogenic effects in the developing central nervous system. The current studies are a continuation of earlier work in this laboratory which found embryonic chick motoneurons to be significantly reduced in number following chronic ethanol treatment (CET) from embryonic day 4 (E4) to E11. We hypothesized that ethanol could be exacerbating naturally-occurring cell death (NOCD), which is from E6 to E9 in the chick spinal cord, or exhibiting direct toxicity to this population. Further studies revealed that neuromuscular blockade by curare in conjunction with CET results in motoneuron numbers similar to control embryos and significantly lower than curare only embryos. Since NOCD is suspended in the presence of curare, we theorized that cell death resulted from directly toxic effects of ethanol. In an additional experiment, we wished to determine whether ethanol was toxic to spinal cord motoneurons during a later developmental period in which cell numbers were relatively stable. The time period of E10 to E15 was chosen because it follows the period for NOCD and motoneuron number is somewhat stable. For this study, chick embryos were divided into ethanol and control groups, and the lumbar section of the lateral motor column was counted on E16. Additional studies will explore whether exogenous neurotrophic factors can modulate the toxic effects of ethanol *in vivo* in a manner similar to previous *in vitro* experiments performed in this laboratory.

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27.7

THE RESCUE OF AVIAN MOTONEURONS BY ACTIVITY BLOCKADE AT THE NEUROMUSCULAR JUNCTION. R.W. Oppenheim*, D. Prevette and S.W. Wang. Dept. Neurobiology and Anatomy, Bowman Gray Sch. of Med., Winston-Salem, NC 27157.

Blockade of neuromuscular (NM) transmission rescues developing avian motoneurons (MNs) from programmed cell death. Prevention of cell death has been attributed to increased axonal branching and synapse formation elicited by activity blockade. However, according to a recent study, neither activity blockade nor increased axonal branching are necessary for the ability of NM blocking agents (e.g. curare) to prevent MN death (Hory-Lee and Frank 1995 J. Neurosci.) These investigators also report that curare potentiates the effects of muscle extract (MEX) in promoting MN survival *in vitro*. They conclude that NM blocking agents are more likely to rescue MNs by acting centrally at neuronal nicotinic receptors rather than by blocking peripheral NM activity. Although we have been able to repeat their *in vitro* results, our *in vivo* studies, by contrast, indicate that: (1) Curare rescues MN in a dose-dependent manner; (2) NM activity is similarly reduced by curare in a dose-dependent fashion; (3) Doses of curare that fail to affect NM activity also fail to rescue MNs; and (4) Blockade of neuronal nicotinic receptors with methyllcaconitine fails to alter MN survival. Studies are in progress to examine axonal branching following treatment with different doses of curare. Supported by NIH grants NS20402 and NS31380

27.8

EFFECTS OF NEUREGULINS ON DEVELOPING NEURONAL AND NON-NEURONAL CELL POPULATIONS IN THE CHICK EMBRYO. V.L. Turgeon*, D. M. Prevette, J. Calderó, J. Esquerda, S. Wang, J. Hong, L.J. Houenou, and R.W. Oppenheim. Department of Neurobiology & Anatomy, Bowman Gray School of Medicine, Wake Forest University, Winston-Salem, NC 27157, USA.

The neuregulin (NG) gene gives rise to a number of alternatively spliced transcripts [heregulin (HG), glial growth factors (GGF) and acetylcholine receptor inducing activity (ARIA)] whose protein products are expressed in various neuronal and non-neuronal cells during development. NG is a member of the EGF gene family and receptors for NG (ErbB) are also members of the EGF receptor gene family. Treatment of chick embryos *in vivo* during the period of programmed cell death (PCD) with HG had no effect on the survival of spinal sensory or motor neurons, but did increase the growth (cell size) of motor neurons, while there was no effect on cell size of sensory neurons. Addition of HG to purified populations of chick motoneuron cultures reduced their survival 48 hours after plating; whereas HG had little if any effect on the survival of cultured spinal sensory neurons. Experiments are in progress to examine the effects of HG on Schwann cell and trigeminal ganglion cell survival *in vivo*, and to examine GGF's effects on the survival of sensory and motor neurons and Schwann cells *in vitro* and *in vivo*. (We thank Genentech for the generous gift of rHG and Cambridge Neuroscience for GGF. Supported by NIH grant NS20402.)

27.9

REGULATION OF SCHWANN CELL DEATH IN DEVELOPING SPINAL CORD NERVE ROOTS. J. Calderó*[1], D. Ciutat[1], R.W. Oppenheim[2], J. Ribera[1], A. Casanovas[1], O. Tarabal[1], J. Lladó[1] and J. E. Esquerda[1]. [1]Dept. C. M. B., Facultat de Medicina, Universitat de Lleida, Av. Rovira Roure 44, 25198 Lleida, Catalonia, Spain. [2]Dept. of Neurobiology and Anatomy, Bowman Gray School of Medicine, Wake Forest University, Winston-Salem, NC 27157.

In the chick embryo, Schwann cells (SCs) die by apoptosis both during normal development and following axonal degeneration induced with neurotoxins (Ciutat et al., 1996). Chronic blockade of neuromuscular activity during development with pharmacological agents such as (+)-tubocurarine (Tc) in the chick embryo rescues motoneurons from naturally occurring cell death. Treatment of chick embryos with Tc from E3 results in a reduction of SC apoptosis on E9-E10, but not in earlier stages. By contrast, limb-bud ablation performed on E2, which leads to degeneration and death of up to 90% of motoneurons, determines an important increase in SC apoptosis coincident with the period of maximal motoneuron loss. Sciatic nerve transection on neonatal rats also induces massive apoptotic cell death on SCs in distal nerve stump. These results suggest that the survival of SCs during development is transiently dependent on axonal derived trophic signals. Whether or not the massive SC death accounts for motoneuron death after neonatal axotomy is currently investigated. (Supported by Ministerio de Educación y Ciencia, PB93-0642, Ajuntament de Lleida and NIH, NS 20402 [RWO], grants).

Ciutat D., Calderó J., Oppenheim RW., Esquerda J.E. (1996). Schwann cell apoptosis during normal development and after axonal degeneration induced by neurotoxins in the chick embryo. *J. Neurosci.*, in press.

27.10

THE EFFECTS OF ADENOSINE AND 2-DEOXYADENOSINE ON CHICK EMBRYO SYMPATHETIC GANGLION CELLS IN VIVO. W.J. Crossland*, J. Kulkarni, and A.R. Wakade. Departments of Anatomy and Pharmacology, Wayne State Univ. Sch. Med., Detroit, MI 48201.

Nucleosides, such as adenosine and 2-deoxyadenosine, are known to produce cell death in cultured neurons. We wanted to know if these nucleosides also have toxic effects *in vivo*.

Fertile chicken eggs were opened and 300 µl aliquots of adenosine, 2-deoxyadenosine (both containing the adenosine deaminase inhibitor 2-deoxycoformycin) or saline were pipetted onto the chorioallantoic membrane or yolk, the eggs resealed, replaced in the incubator until sacrifice. Sympathetic ganglia were dissociated and counts of ganglion cells carried out.

When injected on embryonic day (E) 3 both nucleosides were lethal to the embryos but by E6/7 only the 2-deoxyadenosine was toxic. Following sublethal doses of 2-deoxyadenosine injected at E7 and observed at E9, the normal development of the sympathetic chain ganglia at the lumbosacral level was grossly reduced in size compared to that of saline controls. Furthermore, the number of sympathetic neurons/ml was reduced from a control value of 198 X 10^3 to 23 X 10^3 and average wet weights of whole spinal cords decreased from 23.6 mg to 15.0 mg. These results demonstrate that the nucleosides are capable of inducing abnormalities in the nervous system *in vivo* and provide a substantiation of the effects of adenosine and 2-deoxyadenosine seen *in vitro*.
(Supported by a grant from Midwest Eye Bank to W.J.C.)

27.11

CELL DEATH IN THE AVIAN CILIARY GANGLION IN VIVO IS MODULATED BY CILIARY NEUROTROPHIC FACTOR. T.P. Finn and R. Nishi*. Department of Cell and Developmental Biology, Oregon Health Sciences University, Portland, Oregon 97201.

Chicken CNTF (chCNTF), also known as growth promoting activity (GPA), is a potent neurotrophic factor in vitro and a strong candidate for a true target derived neurotrophic factor in vivo for ciliary ganglion (CG) neurons. ChCNTF and chCNTF receptor messages are present during the time window of development when CG neurons are dependent on their targets in the eye for survival and chCNTF-like immunoreactivity is present in the target cells the neurons innervate. To establish whether chCNTF is a true target derived neurotrophic factor for CG neurons we altered chCNTF levels in vivo. Recombinant chCNTF (rchCNTF) injected into the eye socket did not, however, rescue any additional neurons over that normally seen, although it was able to rescue the majority of neurons which apparently died as a consequence of a lesion generated inadvertently from multiple injections. Multiple injections of rchCNTF into the vitreous of the eye under non-lesion conditions also did not result in a significant rescue of additional neurons. As a better means of making exogenous chCNTF available to CG neurons we infected E2.75 or E4 chick embryos with a replication competent retrovirus (RCAS) expressing full length chCNTF. In vitro, fibroblasts or choroid smooth muscle cells infected with the RCAS-chCNTF construct expressed high levels of chCNTF immunoreactivity and greater than a 1000 fold increase in chCNTF-like biological activity was present in choroid conditioned medium compared to controls. Western blots of cell extracts verified the full length protein was being made. In vivo, viral gag protein as well as ectopic chCNTF-like immunoreactivity could be detected throughout the two targets in the eye and extracts of infected eyes had at least 20 fold more chCNTF-like biological activity than control eyes. 21 - 93% of the neurons which would otherwise have died were rescued from cell death in RCAS-chCNTF infected embryos compared to embryos infected with vector alone. We are also infecting chick embryos with a RCAS construct expressing a reverse orientation (anti-sense) chCNTF construct as a means of blocking endogenous CNTF. The rescue of CG neurons in chCNTF overexpressing embryos strongly suggest that chCNTF is a neurotrophic factor for CG neurons in vivo and is a limiting factor in determining the size of the surviving neuronal population.

This work was supported by NS25767

27.12

NATURALLY-OCCURRING AND DEAFFERENTATION-INDUCED CELL DEATH DURING POSTNATAL DEVELOPMENT OF THE VENTRAL COCHLEAR NUCLEUS. T. S. Tierney*, F. A. Russell and D. R. Moore. University Laboratory of Physiology, Parks Road, Oxford OX1 3PT, UK.

Ever since Hamburger and Levi-Montalcini (1949), most studies of naturally-occurring cell death (NCD) have emphasized the role of target tissue in governing neuron number. These studies have established the "neurotrophin hypothesis", in which competition for target-derived growth factor is thought to be the major regulator of neuron number. However, the role of afferents has received significantly less attention. In this study, we compared the critical period for deafferentation-induced cell death (DCD) to the time course of NCD in the developing gerbil cochlear nucleus. To investigate DCD, unilateral cochlear removals were performed on gerbils at two day intervals from postnatal day (P)3 to P11, and the brains were examined at 3 months. To investigate NCD, normal gerbil brains were examined between P0-P15 or at 3 months. In Nissl stained sections, stereological methods were used for estimating neuron number. From P5 on, the boundary of the cochlear nucleus was clearly circumscribed, and neuronal somata were relatively large, mononucleate and distinct from other non-neural elements. From P10-P140, NCD significantly reduced neuron number by 28% (Student's t-test, t_4=3.18, p=0.003). However, cell death was markedly increased (to >50%) following deafferentation during (but NOT after) the first postnatal week, clearly showing that an abrupt transition in the ability of cochlear nucleus neurons to survive in the absence of afferent input occurs before the period of NCD is complete. While afferent input is an important factor regulating neuron survival during early postnatal development, the critical period for DCD is not concomitant with NCD.

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27.13

DEAFFERENTATION-INDUCED NEURONAL CELL DEATH IN CHICK COCHLEAR NUCLEUS INVOLVES MITOCHONDRIAL UNCOUPLING
D. Durham* and L. Crenshaw , Dept. of Otolaryngology and the Smith MRRC, Univ of Kansas Med Center, Kansas City, KS 66160

Cochlea removal results in death of 30% of neurons in chick cochlear nucleus (n. magnocellularis, NM) within several days. Prior to death, NM neurons show a dramatic proliferation of mitochondria. Blockade of this mitochondrial upregulation with chloramphenicol (CAP) increases the number of dying NM neurons, suggesting that increased oxidative capacity enhances neuronal survival. We evaluated the role of mitochondrial dysfunction in the death of NM neurons using a histochemical stain for a Mg^+-stimulated ATPase whose activity increases with mitochondrial uncoupling (Meijer and Vloedman, 1980, Histochem 69:217).

Hatchling broiler chicks were anesthetized and one cochlea was removed. Birds were sacrificed 6 hr (n=8), 15 hr (n=10) or 3 days (n=6) after surgery. An additional set of animals received hourly injections of CAP (50 mg/kg, n=12) or saline (n=6) after cochlear ablation and were sacrificed at 15 hr. For all birds, frozen sections through NM were stained for ATPase activity. Optical density measurements of individual NM neurons were used to estimate ATPase activity, comparing neurons ipsilateral (deafferented) and contralateral (control) to cochlea removal in each bird.

In normal animals, all NM neurons show a low level of ATPase staining. At 6 hr after cochlea removal, all deafferented NM neurons show increased ATPase staining. By 15 hr, a subset of deafferented neurons showed markedly increased staining intensity. The number of these darkly staining neurons doubled in animals receiving CAP. No darkly staining neurons were evident 3 days after cochlea removal.

The percentage of neurons displaying great increases in ATPase staining matches the number of neurons that die after cochlea removal. Thus we conclude that mitochondrial uncoupling is involved in neuronal cell death in NM.

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27.14

ACTIVITY-DEPENDENT REGULATION OF AVIAN COCHLEAR NUCLEUS NEURONS: ROLE OF METABOTROPIC GLUTAMATE RECEPTORS.
R. L. Hyson*. Program in Neuroscience, Florida State University, Tallahassee, FL 32306.

Elimination of auditory nerve activity results in atrophy and death of neurons in the cochlear nucleus, n. magnocellularis (NM), of the chick. One of the early events believed to lead toward cell atrophy and death is a disruption of ribosomes in the postsynaptic NM neurons. Rapid activity-dependent changes in ribosomes can be visualized by immunolabeling using an antibody, called Y10B, that recognizes ribosomal RNA. The signal by which auditory nerve activity maintains ribosomal integrity in NM neurons is unknown. One possibility is that the important trophic signal is the neurotransmitter, glutamate. This experiment examines the role of glutamate receptors in activity-dependent regulation of ribosomes.

Brain slices containing the auditory nerve and NM on both sides were obtained from 7-10 day old chickens. The auditory nerve on one side of the slice was stimulated for 1 hr. Slices were maintained in medium containing either ionotropic glutamate receptor antagonists (20 µM CNQX and 50 µM APV) or a metabotropic receptor antagonist (250 µM MCPG). Ionotropic antagonists eliminated EPSPs evoked by auditory nerve stimulation, but NM neurons on the stimulated side of the slice still showed greater Y10B labeling than the neurons on the unstimulated side. The metabotropic antagonist did not block EPSPs but did block the activity-dependent difference in Y10B labeling. These data suggest that glutamate may play a trophic role in the young auditory system through activation of metabotropic glutamate receptors.

(Supported by NIH grant DC 00858).

27.15

PERIODIC BLOCKADE OF AMPA/KAINATE RECEPTORS BEFORE THE ONSET OF SYNAPTIC TRANSMISSION ENHANCES NEURONAL SURVIVAL IN THE CHICK BRAINSTEM AUDITORY SYSTEM.
D. Solum, D. Hughes and T. N. Parks*. Dept. of Neurobiology & Anatomy, University of Utah School of Medicine, Salt Lake City, UT 84132.

In ovo administration of CNQX (a competitive antagonist of AMPA and kainate receptors) to chick embryos between embryonic days (E)8 and 15 significantly reduces normal neuronal death in nucleus laminaris (NL) and prevents the deafferentation-induced death of neurons in nucleus magnocellularis (NM) caused by unilateral otocyst removal on E3. Because similar effects are produced by the AMPA/kainate receptor antagonist GAMS, but not by the NMDA receptor antagonist CPP, non-NMDA receptors appear responsible for these effects. White Leghorn embryos (with or without unilateral otocyst removal at E3) were given daily 200 µl injections of saline, or saline containing 200 µg CNQX, onto the CA membrane on E8, E9 and E10. At E17, neurons in NL of unoperated animals were counted in thionin-stained paraffin sections. The CNQX-treated group had 2798±122 (mean±S.E.M, n= 4) neurons in NL and the saline-treated control group had 2410±49 (n=7); this 16% difference was statistically reliable. Treatment with CNQX from E8-10 also appears to prevent the 33% cell loss in NM that follows otocyst removal. Thirteen normal E10 embryos were given single injections of CNQX or saline and the number of spontaneous movements counted at 0.5, 1, 2, 4, 8 and 24 hrs after the injection. CNQX significantly suppressed spontaneous motility from 0.5-4 hrs but motility returned to control levels by 8 hrs. Therefore, CNQX administered on E10 should no longer be effective in blocking non-NMDA receptors by E11, when electrical stimulation of afferents can first elicit postsynaptic field potentials in NM and NL. We conclude that brief periods of non-NMDA receptor blockade prior to the onset of synaptic transmission can increase neuronal survival in central auditory neurons, both during normal development and following early deafening.

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TRANSPLANTATION I

28.1

POTENTIAL CONTRIBUTIONS TO NEURAL TRANSPLANT SURVIVAL BY REACTIVE HOST ASTROCYTES.
J.M. Krum* and J.M. Rosenstein. The George Washington University Medical Center, Washington, DC 20037.

Neural transplants consisting of solid pieces of fetal neocortex placed within host neocortex or striatum are potential models for fetal hypoxia/ischemia, as transplants do not receive a regulated blood flow for 3 - 10 days. Since relatively short periods of hypoxia/ischemia in vivo result in destruction of neuronal elements in neonatal rat brain, a mechanism must exist which allows transplanted neurons to survive the initial post-operative (p.o.) period. The reactive gliosis that occurs adjacent to transplants may enhance the grafts' survival, since astrocytes in vitro can increase their levels of anaerobic glycolysis and use their glycogen stores to produce lactate which can be exported for use as a neuronal energy substrate. Astrocytes also metabolize glutamate via glutamine synthetase (GS) to form glutamine, another neuronal energy substrate. The spatial and temporal patterns of glycogen content (GLY), glutamate transporter (GLT 1), and GS expression in astrocytes associated with neocortical transplants were qualitatively determined and compared to GFAP expression. E19 neocortex was placed within the parietal cortex of adult hosts and allowed to survive from 3 days to 4 months. Serial sections from each transplant were processed for either GLY histochemistry, GLT 1, GS or GFAP immunohistochemistry. In the first week, a strong GFAP + astrocytic reaction was localized around the transplants which also exhibited intense GLT 1 and GS immunoreactivity and dense accumulations of GLY. Within the transplant, GLT 1 and GS immunoreactivity was negligible, but GLY + astrocytic processes were present throughout the grafts. Both GS expression and GLY content in the surrounding host tissue gradually decreased after 3 weeks. By 4 months p.o., GS and GLY were no longer distinguishable within the gliotic area. GLT 1 and GS immunoexpression within the transplants began to increase during the second week p.o., while GLY remained prominent around the vasculature. From the third week on, transplant GS expression increased and was comparable to that of normal cortex by 4 weeks p.o., while GLY content diminished. The temporal and spatial patterns of GLT 1, GS and glycogen suggest that astroglia have the potential to support transplant survival at early postoperative times. Supported by NS-17468.

28.2

GRAFTING OF GENETICALLY TRANSDUCED ASTROCYTES INTO ADULT RAT BRAINS: THE HOST RESPONSE TO TRANSPLANTATION
S Ausim Azizi*, Movement Disorders Center, Dept Neurology, Med College of Pennsylvania and Hahnemann Univ, Philadelphia, PA 19129

Astrocytes, because of their natural properties of migration, integration into the Central Nervous System (CNS), elaboration of trophic factors as well as availability may serve as ideal vehicles for gene transfer into the CNS. Using conventional culture techniques and gentle proteolytic enzymes, Dispase and Collagenase, we obtained primary cultures of astrocytes from adult rats. Astrocyte cultures were transduced using viral and non-viral vectors containing a reporter gene beta-galactosidase. The transduced astrocytes were grafted into the brains of adult rats. *Lac-Z* positive cells could be found in the brains for several weeks after transplantation. Currently, we are in the process of cataloguing the *in vivo* patterns of migration, integration and long term survival of the transduced grafted astrocytes. The host response to grafting in the brain is different from that elsewhere in the body, delineated by a phase of graft success followed by rejection. Our studies suggest that the initial reaction to grafting in the brain is marshaled by the glia; indeed the host astrocytes surround and 'wall-off' the graft in the first 24 hours post-transplantation. This 'gliosis' may have a protective role for the host and the graft, isolating one from the other. This process may initially protect the graft from detection and destruction by the peripheral immune system. We believe that this model has great overall promise for the study of autologous transplantation, the host CNS immune reaction and for *ex vivo* gene therapy. *(supported by The Dept of Neurlogy, MCPHU)*

28.3

EFFECT OF NEOCORTICAL TRANSPLANTATION AND ENVIRONMENTAL ENRICHMENT ON HOST BRAIN DEVELOPMENT. J. Jones and A.J. Castro*
Department of Cell Biology, Neurobiology and Anatomy, Loyola University Stritch School of Medicine, Maywood, IL 60153.

Plates of presumptive sensorimotor (homotopic) or occipital (heterotopic) neocortex obtained from fetal rats 14-16 days of gestation were grafted into large lesion cavities made immediately prior to grafting in the right sensorimotor cortex of newborn rats. Unoperated normal and lesion only littermates served as controls. Postoperatively, half the litters were raised in standard (Std) home cage conditions and the remaining litters were raised in enriched (Enr) conditions until 5-6 months of age. Body weight, pinna detachment and eye opening were evaluated during rearing to ensure that there were no group differences in general somatic growth. Animals were sacrificed by anesthetic overdose and perfused with 4.0% paraformaldehyde solution. Brains were weighed, post-fixed, frozen and stored at -70°C until serial sectioning at 30μ using a cryostat. Adjacent Nissl and acetylcholinesterase sections were used to measure brain length and cortical thickness. Statistical analyses were made on the brains of Std versus analogous Enr animals using ANOVA and Fisher post-hoc analysis ($p < 0.05$). A statistically significant enrichment induced increase in brain weight was observed in normal animals (1.976g ± 0.034) as well as in animals with homotopic transplants (1.927g ± 0.031) when compared to normal (1.834g ± 0.030) and homotopic (1.816 ± 0.031) Std animals. Brain length, measured by counting the number of sections between the initial crossing of the corpus collosum rostrally and the posterior commissure caudally was also found to be increased in normal and homotopic Enr animals. Dorsal and lateral cerebral cortical thickness, measured bilaterally using NIH Image Software at three levels: rostral to the corpus collosum, at the level of the anterior commissure and at the level of the posterior commissure, showed increases at all levels in normal and homotopic Enr animals. These results indicate that environmental enrichment can promote the growth of host brains receiving homotopic neocortical transplants. Such an effect was not observed in animals with heterotopic transplants. Comparisons of subcortical thalamic area are currently in progress.

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28.4

INTEGRATION OF MOUSE POSTNATAL HIPPOCAMPAL PROGENITOR CELLS GRAFTED ONTO ORGANOTYPIC HIPPOCAMPAL SLICES. K. Bulsara*, M. T. Austin, A. K. Shetty, Y. D. Philips and D. A. Turner. Neurosurgery, Neurobiology, Duke Univ. Med. Ctr., VAMC, Durham, NC 27710.

We hypothesize that uncommitted neuronal progenitor cells will show enhanced migration and neuronal differentiation when grafted into an *in vitro* host after a lesion. We grafted EGF-derived progenitor neurospheres cultured from neonatal mouse hippocampi into normal and kainic acid (KA)-treated rat organotypic hippocampal slice cultures. We assessed migration daily using DiI labeling to measure cell outgrowth (confirmed using M6 anti-mouse antibody) and differentiation using MAP-2 antibody.

Neonatal mouse hippocampus was cultured in EGF using methods described by Svendsen et al. (1995). Following 7 days *in vitro* and 7 days post-passaging, numerous free-floating neurospheres were obtained. Organotypic hippocampal slices from postnatal rats (P10) were prepared (Stoppini et al, 1991), with preservation of principal hippocampal cell layers. Slice cultures exposed to 3 μm KA for 14 hr demonstrated selective loss of the CA3 pyramidal cell layer. Hippocampal neurospheres were explanted onto the surface of the hippocampal slice in the CA3 st. radiatum region. These explants onto both normal and KA-treated hippocampal slices demonstrated good cell survival. Migration was measured as the surface area of the graft (assessed by phase contrast, DiI and M6). The neurosphere grafts (n=5 in each group) showed initially a small area (control = 0.045±0.008 mm^2; KA-treated = 0.032±0.006 mm^2; NS) but enlargement by day 4 (control = 0.7±0.22 mm^2; KA-treated = 0.49±0.13 mm^2; NS), occupying most of the CA3 region of the slice cultures. Only a few MAP-2 positive cells were noted in the neurosphere grafts in control or KA-treated slices.

These initial results suggest that the KA lesion *in vitro* does not lead to enhanced dispersion or differentiation of progenitor cells beyond that already provided by the control slice culture environment. Thus, additional undefined factors may be critical to achieve enhanced neuronal differentiation. Supported by NIA AG13165, VAMC.

28.5

AGE OF THE TARGET TISSUE INFLUENCES NORADRENERGIC INNERVATION. N. Srivastava*[1], G.A. Gerhardt,[2,3] and A.C. Granholm,[1,3]
Departments of Basic Science[1], Psychiatry[2] and Pharmacology[3], University of Colorado Health Sciences Center, Denver, CO 80262.

The dependency on the age of the target tissue for the regenerative ability of central noradrenergic neurons (NE) was investigated using intraocular double transplants of fetal brainstem tissue together with young (3 weeks) or aged (16 months) iris tissue. Pieces of fetal brainstem tissue (E17), containing locus coeruleus (LC) neurons, and old or young irides from Fischer 344 (F344) rats were transplanted adjacent to each other in the anterior chamber of the eye of 5-6 week old F344 host rats. The double transplants were allowed to mature for 5 weeks, after which the intraocular grafts and the host irides were dissected out and were either frozen for HPLC measurements of NE, or stretch prepared for Falck-Hillarp monoamine histochemistry. All iris transplants from young donors received a dense innervation of noradrenergic fibers from the LC transplant, arranged as single varicose fibers or thick axon bundles. However, a mossy and sparse innervation pattern with few, if any, axon bundles could be seen in the aged iris transplants, as compared to the young. Preliminary studies with HPLC also suggest that NE levels were reduced in aged transplanted irides. These data indicate that the age of the target tissue can influence the fiber outgrowth from young LC neurons supporting, at least in part, the hypothesis that extrinsic factors in the target tissue can influence age-related changes in NE innervation. Supported by USPHS grants MH49661 and AG06434.

28.6

CONDITIONALLY IMMORTALIZED NEURAL PROGENITOR CELLS GRAFTED TO THE STRIATUM EXHIBIT SITE-SPECIFIC NEURONAL DIFFERENTIATION AND ESTABLISH CONNECTIONS WITH THE HOST GLOBUS PALLIDUS. C. Lundberg*, C. Winkler, S.R. Whittemore# and A. Björklund.
Wallenberg Neuroscience Center, Dept. Physiol. and Neurosci., University of Lund, Sölvegatan 17, 223 62 Lund, Sweden# The Miami Project and Dept of Neurological Surgery, University of Miami School of Medicine, Miami FL 33136

The cell line RN33B has been reported to differentiate into neurons in a site-specific manner when grafted to the cortex and hippocampus of adult rats (Onifer et al, 1993, Exp.Neurol. 22:130, Shihabuddin et al, 1995, J. Neurosci. 15:6666). To investigate the fate of RN33B cells in a sub-cortical structure, we grafted RN33B cells into the intact or excitoxically lesioned striatum of adult or neonatal rats. The total number and phenotypic characteristics of the ^{3}H-thy labeled grafted cells were analysed, by combined autoradiography and immunohistochemistry, at different time-points after transplantation. Transplanted RN33B cells were found to survive, integrate and differentiate into both neurons and astrocytes, and a significant proportion of the cells (approx. 10%) were found to differentiate into cells with morphological and phenotypic characteristics of medium-sized striatal projection neurons, as defined by their expression of the striatum-specific proteins DARPP-32 and STEP. Retrograde tracing, using Fluoro-Gold, showed that at least some of the graft-derived neurons were capable of establishing connections with one of the primary striatal targets, the globus pallidus. These findings demonstrate a remarkable capacity of the RN33B cells for site-specific neuronal differentiation in both the adult and developing striatum and suggest that the same differentiating factors that are operating during normal neurogenesis in brain development are retained, at least to some extent, also in the adult CNS. Supported by Swedish MRC (04X-3874), the Göran Gustafsson Foundation, The Swedish Society for Medical Science, the Human Frontiers Science Program, and the National Institutes of Health (NS26887).

28.7

INFLUENCE OF CNS ENVIRONMENT ON C-JUN RESPONSE AND MIGRATION OF SCHWANN CELLS. [1]E.Vaudano* [2]B.Finsen and [1]A.Björklund [1]Wallenberg Neurocenter, S-22362, Lund, Sweden and [2]Dept. Anatomy, Odense University, 5000 Odense C, Denmark.

Schwann cells (SCs) transplanted into the brain promote regeneration of CNS axons, although this regeneration is always limited when compared with PNS regeneration. SCs "activated" following denervation, or grown in vitro, express high levels of the transcription factor c-jun, while c-jun is rapidly downregulated in SCs implanted into the intact brain. This might be due either to the presence in the CNS of an active inhibitor of c-jun expression, or to the lack of factors necessary to maintain the c-jun response. After PNS injury there is a massive invasion of the lesioned nerve by macrophages which secrete interleukin I which induces c-jun expression. The lesion inflicted on the CNS by the transplantation of SCs is followed by a much more limited macrophage reaction, which in itself may be inadequate to maintain c-jun expression. The intensity of the reaction of brain macrophages can be enhanced by lesioning the target brain area prior to grafting. We implanted standard primary SCs, or SCs labelled by retroviral infection with the Lac-Z gene, either into the intact adult rat striatum, or into the striatum previously lesioned either with a) the excitotoxin quinolinic acid or b) with 6OHDA. We found maintenance of c-jun expression in SCs implanted into the excitotoxin lesioned striatum, which also displayed an intense brain macrophage and glial reaction, compared with a very limited c-jun response in the SCs implanted into the intact, or 6OHDA lesioned striatum (where the micro-macroglial responses were much weaker). We also found extensive migration (and possibly proliferation) of SCs transplanted in the excitoxin lesioned striatum, compared to a limited migration in the intact, or 6OHDA lesioned striatum. Thus glial cell reactions, and the brain macrophage reaction in particular, may play a critical role in regulating the c-jun response as well as the migration (and possibly survival and/or proliferation) of SCs implanted into the brain.

This work was supported by the Wellcome Trust.

28.8

INTEGRATION AND DIFFERENTIATION OF EGF-RESPONSIVE NEURAL PROGENITOR CELLS AFTER TRANSPLANTATION INTO THE DEVELOPING RAT BRAIN IS INDEPENDENT OF CELLULAR ORIGIN. C. Winkler*, R.A. Fricker and A. Björklund. Lund University, Wallenberg Neuroscience Center, Sölvegatan 17, S-223 62 Lund, Sweden.

In this study the pattern of migration and differentiation of neural progenitor cells after transplantation into the neonatal or embryonic rat brain was determined. Cells derived from striatum, cortex and ventral mesencephalon (VM) of E14 mice were cultured in serum free medium containing EGF. The arising spheres of progenitor cells were triturated to single cell suspensions and re-seeded every week for 4 weeks. Small spheres derived from striatum, cortex or VM were transplanted into the ventricle of E15 rats. Cells derived from both striatum and VM were transplanted into striatum and midbrain of neonatal rats. Four weeks post-transplantation donor cells were visualized by mouse specific glial and neuronal antibodies and by in situ hybridization to mouse satellite DNA.

Following embryonic transplantation cells from either origin showed a similar pattern of integration and differentiation with many glial cells and neurons in midbrain, hippocampus and thalamus, fewer in cortex, hypothalamus and olfactory bulb and no integration in striatum or septum.

Following neonatal transplantation cells derived from striatum or VM behaved in a similar way. Thus, after intrastriatal transplantation cells migrated in the striatum and differentiated into glial and neuronal cells, partially expressing striatal phenotype and appropriate connections, while after transplantation into the midbrain cells from either origin were almost exclusively observed in the substantia nigra. We conclude that EGF-responsive progenitor cells behave as multipotent neural progenitors after transplantation to the developing brain, expressing features that appear to depend on local environmental cues rather than the subregion of the brain from which the cells are derived.

28.9

EGF-RESPONSIVE NEURAL PRECURSOR CELLS ENGRAFT AND DIFFERENTIATE IN LESIONED ADULT BRAIN. P. Zlomanczuk, L.D. Recht, P.J. Quesenberry, W.J. Schwartz*. Dept. Neurol. & Cancer Center, Univ. Mass. Med. Sch., Worcester, MA 01655

Several laboratories have implanted neural progenitor or stem-like cells into brain, mostly in regions still undergoing neurogenesis. Recently, Svendsen et al. (Exp. Neurol., 137: 376) reported engraftment of EGF-responsive stem-like cells into lesioned adult rat striatum. However, further analysis was confounded by difficulties in labeling the donor cells, and no colocalization data could be obtained.

We have been using EGF - responsive cells derived from striata/subventricular zone of embryonic mice bearing a promoter trap insertion of a β-galactosidase reporter gene (Rosa 26). Cells were propagated in serum-free DMEM/F12 medium with 20ng/ml EGF, and 2μl of single cell suspension (80,000-100,000 cells/μl) were implanted into electrolytic lesions of adult mouse hypothalamus. Brains were processed for X-gal histochemistry and immunocytochemistry 4 or 30 days after implantation. Initially, cells formed grape-like clusters at the injection site. By 30 days transplanted cells had developed into morphologically distinct structures, either as thin layers surrounding the lesion cavity, dense cellular nodules or multicellular filaments bridging the lumen of lesion cavity. Immunoreactivity for S-100 and GFAP was clearly colocalized to X-gal positive cells at 30 days, but no colocalization was observed at 4 days. Control injections of Rosa 26 hematopoietic stem cells or EGF - responsive neural stem cells without β-galactosidase reporter gene did not yield X-gal positivity. Abundant macrophages in the lesion cavity showed no X-gal reaction product in any of the implantations.

Our results show that transplanted cells differentiate in adult host brain, primarily into glia. Rosa 26 transgenic mice are a source for reliably-marked donor cells for neural transplantation. (Supported by UMass Cancer Center, Worcester, MA).

28.10

EXPRESSION OF TH-IMMUNOREACTIVITY IN LONG-TERM NON-PASSAGE EGF-RESPONSIVE NEUROSPHERES Y.H. Chiang* and F.C. Zhou, Program of Medical Neurobiology and Department of Anatomy, Indiana Univ. Sch. Med., Indiana, IN 46202

The epidermal growth factor (EGF)-responsive neurospheres procured from rat fetal brain tissue are able to differentiate into neurons and glia in the absence of EGF. The differentiation fate of these cell can be affected by exogenous factors (Chiang, Silani and Zhou, 1996). However, there is little available data about the differentiation of dopamine neurons. Here, we present the expression of tyrosine hydroxylase immunoreactivity (TH-im) among long-term nonpassage neurosphere cells in vitro and in vivo.

Mesencephalon of gestational day 14 (E-14) rat or mouse fetuses were collected and dissociated into single cells. The single cells were cultured in DMEM/F12 containing N2 supplement and EGF (20 ng/ml). After 3 to 9 months in culture, rat neurospheres were subplated onto 16-well chamber slides with Neurobasal medium and 2% fetal calf serum and were given 14 days to differentiate. Exogenous factors including bFGF (20 ng/ml), GDNF (1 ng/ml & 10 ng/ml), retinol (10 μM), ibotenic acid primed striatal soup and fetal brain tissue extracts were tested to increase TH-im expression, respectively. Another group of neurospheres obtained from either E-14 rat or mouse fetal mesencephalon were collected and transplanted into either the 6-OHDA lesioned striatum of adult rats or normal striatum of adult mouse. Animals were sacrificed 2 months after transplant and the striatum was sectioned. The cultured cells and tissue sections were examined by TH immunocytochemistry.

The frequency of neurospheres that gave rise to TH-im positive neurons was about 6% (72/1200 neurospheres). Of these neurospheres, the TH-im neurons were about 504 cells/mm^2 (24% of total cells) in the area surrounding the neurosphere (about 1 mm wide) which had a higher population of neurons and other cells. However, the exogenous factors failed to increase the frequency of neurospheres giving rise to the TH-im cells. A low population of TH-im cells was identified outside of grafts among neurospheres transplanted into either 6-OHDA adult rat striatum or the normal mouse striatum,. These cells had TH-im soma as well as TH-im neurites. Taken together, these results suggest that neurons derived from long-term nonpassage neurospheres are capable of receiving signals and differentiating into TH-im neurons. (PIRC 22-821-0)

28.11

PROGENY OF LONG-TERM MOUSE AND HUMAN CNS STEM CELL CULTURES SURVIVE TRANSPLANTATION AND DIFFERENTIATE INTO NEURONS AND GLIA. Laurie L. Borg, Angelo L. Vescovi, Karen Blote, Ann L. Kyle, Paula Hettiaratchi, Brent A. Reynolds, Lori A. Mudrick-Donnon*. NeuroSpheres Ltd., 3330 Hospital Dr. N.W., Calgary, AB, T2N 4N1 CANADA.

CNS stem cells maintained *in vitro* will continue to divide indefinitely under appropriate conditions and, therefore, may provide an attractive cell source for transplantation therapy. In the present study CNS stem cells were isolated from embryonic mouse and human subependyma and put into primary culture. Cells proliferated to produce clusters of cells or "neurospheres" that were passaged every 7-10 days, 3-4 times (mouse) or 11-35 times (human) prior to transplantation. BrdU was added to the media 16 hr (mouse) or 10 days (human) prior to transplantation and incorporated into the DNA of cells undergoing cell division. For transplantation, cells were washed, dissociated, and resuspended to a final density of 10-50 X 10^6 cells/ml. Aliquots (1-3 ml) of suspension were injected into various CNS regions of nonlesioned adult mouse or rat hosts or to the striatum of Parkinsonian and Huntingtonian rat hosts. After a survival time of 1 week to 12 months anatomical analyses were undertaken to assess survival, differentiation, and migration of the stem cell progeny. Cell survival was estimated from the total number of BrdU-immunoreactive cells and ranged from 2-10% (mouse) and 10-35% (human). With time, transplanted cells moved away from the injection tract in a radial fashion and extended 0.5-1.5 mm mediolaterally and rostrocaudally. Double immunostaining techniques using antibodies to BrdU and antibodies to NSE or Human Neurofilament (proteins found in neurons), or GFAP (an astrocytic protein) demonstrated that the transplanted stem cell progeny underwent migration and differentiated into both neurons and astrocytes. The specific phenotype of the differentiated neurons is now under investigation.

28.12

ULTRASTRUCTURE OF GRAFT- OR HOST-DERIVED 5-HT INNERVATIONS IN VENTRAL MESENCEPHALIC GRAFTS IMPLANTED INTO THE ADULT OR NEWBORN RAT STRIATUM. H. Moukhles*, M. Geffard and G. Doucet. Département de pathologie and Centre de recherche en sci. neurol., Université de Montréal, Montréal, Qc, Canada.

The majority of serotonin (5-HT) neurons innervating the substantia nigra also send collaterals to the neostriatum (Van der Kooy and Hattori, Brain Res 186: 1, 1980). Nevertheless, the 5-HT innervation is entirely synaptic in the substantia nigra pars reticulata (Moukhles et al, Submitted) while almost asynaptic in the neostriatum (Soghomonian et al, Brain Res 481: 67, 1989), suggesting an influence of the cellular environment on the formation of 5-HT synapses. To test the possibility of an epigenetic control on 5-HT synaptology, we prepared intrastriatal grafts of ventral mesencephalic tissue, either devoid of 5-HT neurons in newborn rats or containing small numbers of 5-HT neurons in adult rats. The former grafts are innervated by 5-HT neurons of the host whereas the latter are innervated only by the co-grafted 5-HT neurons (Mounir et al, Eur J Neurosci 6: 1307, 1994). Ultrastructural analyses were performed 6-8 months later on single ultrathin sections, following pre-embedding immunolabeling using a monoclonal antibody against a 5-HT-glutaraldehyde-protein conjugate. The morphometric data show that 5-HT varicosities are highly comparable in the two types of grafts to those of the host neostriatum and that only few established synaptic specializations. These results demonstrate that the synaptic incidence of the 5-HT innervation of the grafts is much lower (8-30%) than the one determined in the normal substantia nigra and plead against an epigenetic control of 5-HT synaptogenesis. Alternatively, the ventral mesencephalic grafts might not provide the "factors" normally promoting the formation of 5-HT synapses in the substantia nigra. Supported by MRC grant MT10982, an MRC fellowship to H.M. and FRSQ scholarship to G.D.

28.13

LONG TERM CULTURE AND CHARACTERIZATION OF NORMAL HUMAN FETAL SPINAL CORD CELLS. E.A. Zompa*, M.P. Moyer, L. Cain and C.E. Hulsebosch. Anat. & Neurosci., Univ. of Texas Med. Br., Galveston, TX 77555 and Cell & Gene Therapy Inst., UTHSC, San Antonio, TX 78258

The use of cultured fetal cells as a source for replacement neurons, growth factors, and glial cells to restore function after spinal cord injury is promising; however, maintaining normal cells in culture for any length of time is difficult. We report the long term culture and phenotypic analysis of normal human fetal spinal cord cells cultured in M3:5 medium (InCell Corp., San Antonio, TX) for longer than 2 years. No other media we have tested has permitted this long term propagation. The cells have been categorized with respect to morphology, neuronal and glial specific markers, transmitters and receptors. Preliminary results indicate that a population of precursor or stem cells is maintained, with the ability to differentiate into neuronal cells or proliferate for the duration of the period in culture. A minimum of five different morphological types are present. These have continued to replicate after 75 or more passages. Many of the neuronal-like cells extend processes as long as 3cm to target cells and form intricate networks in culture. The cells survive enzymatic treatment with trypsin upon subculture, often reforming aggregates of neuronal-like cells which then extend numerous and long processes. The cells have been characterized for the expression of embryonic markers Vimentin and Chromogranin A, neuronal markers NF68, NF200, NSE, transmitters GABA and acetyl-choline, glutamate receptor subunits GluR 2/3, NMDAR subunits R1 and R2 and glial markers GFAP, and GalC in the cultured cells. Preliminary data indicate that subpopulations of the cells differentiate into these various lineages, but perhaps more importantly a stable population of precursor cells is maintained. (Supported by Kent Waldrep National Paralysis Foundation and NS 11255,)

28.14

THY-1 ALLELIC DIFFERENCES: A POWERFUL TOOL FOR REVEALING RECIPROCAL FIBER PENETRATION AND NEURON MIGRATION IN NEURAL TRANSPLANTATION. K. R. Hendricks*, R. C. Wilson, J. N. Kott and L. E. Westrum, Dept. of Neuro. Surgery, U. of Washington, Seattle, WA 98195.

The functional significance of neural transplants is directly related to their ability to integrate with host brain. Thus, the ability to identify and delineate neurons and processes as to host versus donor origin is essential. The membrane glycoprotein Thy-1 is an ideal marker since it is present at high density on almost all neurons (except olfactory nerve) and it exists in two immunologically distinct forms in mice. In this experiment, neonatal, Thy-1.1 mouse pups have had one olfactory bulb ablated and immediately replaced with a Thy-1.2, fetal, donor olfactory bulb. Results from the present experiment show: 1) inter-strain transplants are viable; 2) host olfactory nerve (via olfactory marker protein labeling) penetrates into donor tissue, entering glomerulus-like structures that are also penetrated by donor processes; 3) donor fibers are able to penetrate appropriate (olfactory peduncle and olfactory cortex) and novel (subependymal cell layer and rostral frontal cortex) areas of brain; 4) host fibers from the forebrain penetrate into the transplant and 5) host neurons can migrate into transplant tissue and remain viable. Our previous reports using traditional tract-tracing methods have demonstrated donor-host fiber integration in rats but only of selected fiber pathways. This experiment shows the robustness of host and donor fiber integration along with demonstrating, for the first time, that host neurons are able to migrate into donor olfactory bulbs.

(Supported by NIH/NINDS grants NS09678 and NS07144)

29.1

CULTURED CELLS FROM THE CLEARNOSE SKATE, RAJA EGLANTERIA, R. M. Gould* and C. A. Luer, N.Y.S. Inst. For Basic Res.,Staten Island, NY and Mote Marine Lab, Sarasota, FL

The nervous systems of all gnathostomes, from cartilaginous fishes to humans have their origins in a common ancestor, all are well myelinated by oligodendrocytes in the CNS and Schwann cells in the PNS. Though highly similar at the structural and ultrastructural level, CNS myelin of fishes is biochemically different from mammals. The major protein in fishes is related to Po, the dominant protein in mammalian PNS myelin, and not proteolipid protein, the major protein in mammalian CNS myelin. Other biochemical differences have been noted. Whereas mammalian oligodendrocytes in culture differentiation from highly motile, proliferating O-2A progenitors (A2B5+) to cells that extend multiple processes and elaborate membrane sheets (they express myelin lipids and proteins), neural cells present in skate and spiny dogfish (Glia (1995) **15**:401) cultures, which grow in standard mammalian medium supplemented to 1000 mOsm with urea, glucose, sodium chloride sucrose and HEPES, remain as A2B5+, HNK-1+ progenitors. Currently we are growing cells from 4, 5 and 6 week skate embryos in three different media supplemented with fetal calf or horse serum (2-10%). To see if these cells will differentiated in culture, we plan to grow these cells in chemically defined medium, to purify O-2A cells using the shake-off procedure of McCarthy and DeVellis (J.Cell Biol (1980) **85**:890), and add gangliosides and forskolin to the medium. Markers that will be used to characterize possible differentiation stages in culture have been characterized using frozen sections of skate embryo spinal cord. These studies will hopefully resolve the issue whether or not the ability to differentiate in culture is another difference between fish and mammalian oligodendrocytes.

29.2

The myelin gene P_0 is expressed in a subgroup of neural crest cells, and is respectively down- and up-regulated during the development of non-myelin and myelin-forming Schwann cells. M.-J. Lee, A. Brennan, A. Tabernero, A. Blanchard, G. Zoidl, J. S. Altman*, K. R. Jessen and R. Mirsky. Department of Anatomy and Developmental Biology , University College London, Gower Street, London WC1E 6BT

The expression of the myelin protein P_0 was examined in the rat Schwann cell lineage using in situ hybridisation and immunohistochemical methods adjusted to higher sensitivity than needed to detect the exceptionally high levels of P_0 in myelinating cells in vivo. This reveals unambiguous P_0 mRNA expression in migrating neural crest cells, Schwann cell precursors from embryo day 14 (E14) nerves, and embryonic Schwann cells. Although 95% of cells in this nerve remain non-myelin-forming in the adult, the Schwann cells in the E18 sympathetic trunk are P_0 positive using this adjusted method, and the level is much the same as most pre-myelinating Schwann cells in E18 sicatic nerves. Transecting the sciatic nerve at birth does not alter this basal P_0 expression in nerves observed two days later (postnatal day 2). In contrast, it abolishes the strikingly high P_0 mRNA and protein seen in cells forming myelin sheaths in the contralateral control nerves. Furthermore, non-myelin-forming cells in adult nerves fail to show the basal P_0 levels, indicating P_0 expression has been suppressed in these cells. In this case, nerve transection results in up-regulation of P_0 mRNA expression in the denervated non-myelin-forming cells.

We conclude that the postnatal diversification of immature Schwann cells to form myelin-forming and non-myelin-forming cells involves axon-dependent amplification and suppression, respectively, of myelin-independent P_0 expression. In the Schwann cell lineage, this basal expression is an early phenotype that is likely to appear as one of the first signals of glial lineage choice in neural crest development.

29.3

Perisynaptic Glia at Frog Neuromuscular Synapses Express Schwann Cell Marker Protein Zero (P_0), but Not Myelin Proteins. J.Georgiou*, and M.P.Charlton. *Department of Physiology, University of Toronto, Toronto, Canada, M5S 1A8.*

Neuromuscular synapses are enveloped by perisynaptic Schwann cells (**PSCs**) which do not make myelin wrappings. These specialized glial cells have properties similar to astrocytes in the CNS. For instance, they express neurotransmitter receptors and respond to nerve-evoked activity with changes in $[Ca^{2+}]_i$. Since the physiology of PSCs differs from myelinating Schwann cells (**MSCs**) surrounding motor nerves, we wondered whether these two cell types have similar origins.

Using immunocytochemistry and monoclonal antibodies to galactocerebroside and myelin-associated glycoprotein, we found that MSCs expressed both of these myelin proteins, but PSCs had neither.

We next used monoclonal antibody 1E8 to ask whether PSCs expressed the glycoprotein P_0 , which is expressed on all Schwann cell types, including non-myelinating Schwann cells, MSCs, and Schwann cell precursors (Bhattacharyya et al., 1991; Neuron *7*, 831). We detected 1E8 immunoreactivity on both MSCs and PSCs.

We conclude that PSCs belong to the Schwann cell lineage, but their lack of myelin protein expression indicates they are biochemically similar to non-myelinating Schwann cells. We speculate that factors released by the nerve terminal may block expression of the myelinating phenotype.

Supported by grants to M.P.C. from NeuroScience Network and MRC of Canada, and a NeuroScience Network scholarship to J.G.

29.4

TOPOLOGY AND RELATIONSHIP BETWEEN CELLULAR LOCALIZATION AND FUNCTION OF THE PERIPHERAL MYELIN PROTEIN 22 D. D'Urso* and H.W. Müller. Molecular Neurobiology Lab., Dept. of Neurology, University of Düsseldorf, 40225, Germany

It has been suggested that the peripheral myelin protein PMP22 plays a role in the formation and maintenance of myelin in the PNS and in the regulation of cell growth. Immunohistochemistry and in situ hybridization studies have shown that PMP22 is largely synthesized by Schwann cells and mainly localized in compact myelin. Based on the amino acid sequence and computer analysis, the predicted disposition of PMP22 with respect to the membrane bilayer postulates the existence of three distinct hydrophilic loops and four transmembrane domains. Different mutations in the coding region of the PMP22 gene are associated with common inherited peripheral neuropathies in mice (Trembler and Trembler J) and humans (CMT1a, HNPP, DS). Whether the mutant proteins, which are probably misfolded, are correctly transported and targeted to the plasmamembrane is unknown, and it remains to be determined what cellular mechanisms affect myelination.We investigated the folding and sorting of wild type and mutant forms of PMP22 as well as its membrane topology. We inserted an octapeptide tag sequence at the N-terminus or the C-terminus of the PMP22 open reading frame and generated different chimeric constructs which were then transfected into HeLa or rat Schwann cells. The expression of the tagged PMP22 proteins was analyzed using antibodies directed against specific PMP22 epitope and the tag sequence. Cellular localization and membrane topology was examined by immunofluorescence and confocal microscopy. PMP22 interactions with other peripheral myelin proteins, using the same experimental paradigm will be also discussed. Supported by DFG (Mu 630 5-3).

29.5

EXPRESSION OF PROTEOLIPID PROTEIN AND A PLP-LacZ TRANSGENE IN MOUSE SCIATIC NERVE. C.S. Duchala[a]*, H. Jiang[b], M. Shy[b], S. Scherer[c] and W.B. Macklin[a]. [a] Dept. Neurosciences, The Cleveland Clinic Foundation, Cleveland, OH 44195. [b] Dept. Neurology, Wayne State Univ., Detroit, MI 48201. [c] Dept. Neurology, Univ. Of Penn., Philadelphia, PA 19104.

Proteolipid protein (PLP) is the major myelin protein in the central nervous system and is also expressed by Schwann cells (SC) in the peripheral nervous system (PNS). PNS PLP is not thought to be incorporated into compact myelin and its expression has not appeared to be regulated like other myelin specific genes. We examined PLP expression in the PNS using a PLP-LacZ transgenic mouse (Wight et al., J. Cell Biol., 1993). X-gal histochemistry of adult sciatic nerves revealed β-gal expression predominantly in myelinating SC. When sciatic nerves were transected, with resultant degeneration of their axons and myelin sheath (Wallerian degeneration) PLP and β-gal decreased dramatically when analyzed by Western blot , X-gal staining and Northern blot. When the nerves were crushed, to permit axonal regeneration following axonal degeneration, regenerating axons re-induced β-gal expression in previously denervated SC. Finally, developing sciatic nerves demonstrated increasing PLP and β-gal expression between postnatal days 7 (the earliest day examined) and 21. Taken together, these results suggest that, contrary to our previous beliefs, PLP expression in the PNS is being regulated by axons as part of a coordinated program of myelin gene expression that includes myelin proteins P0, MBP and PMP-22. Ultrastructural studies to more precisely localize PLP with respect to PNS myelin, are currently underway. Supported by NS25304.

29.6

IN VITRO CHARACTERISATION OF HUMAN SCHWANN CELLS FROM CHARCOT MARIE TOOTH PATIENTS. C. Rosenbaum, S. Kupfer, S. Wosch, C.O. Hanemann*, H.W. Müller. Mol. Neurobiology Lab., Dept. of Neurology, University of Düsseldorf, 40225 Düsseldorf, Germany

Charcot Marie Tooth neuropathy is a frequent (1/2500) hereditary demyelinating neuropathy. The vast majority of patients (CMT1A) shows a PMP22 gene duplication, whereas in a few cases PMP22 point mutations have been described. PMP22 is a myelin protein produced in Schwann cells and is homologous to the growth arrest specific gene gas3. PMP22 expression levels seem to be increased in the disease and might be responsible for abnormal Schwann cell growth in CMT1A. We analysed the Schwann cell phenotype in different stages of CMT1A neuropathy and found a pathological expression of LNGF-R and NCAM in early and late stages (onion bulbs) of the disease. These results suggest that abnormal PMP22 expression alters the differentiation status of Schwann cells.

To elucidate if the altered Schwann cell phenotype is primarily due to PMP22 gene duplication or is secondarily changed during the disease process we established a modified method for culturing human Schwann cells isolated from sural nerve preparations of CMT1A patients and control nerves from multiorgan donors. We are able to grow Schwann cells up to the 7th passage with high proliferation rates and decreased numbers of fibroblasts (around 2%). Schwann cells from CMT1A patients show a significantly lower proliferation rate than control Schwann cells. Currently, we investigate the expression of PMP22 in cultured human Schwann cells from CMT1A and control nerves. In addition, we performed an extensive study on the expression of several Schwann cells markers under different growth conditions in order to compare the CMT1A Schwann cell phenotype to control cells in culture.

Supported by the DFG

29.7

PROCESSING AND RAPID DEGRADATION OF PERIPHERAL MYELIN PROTEIN-22. S. Pareek[1], L. Notterpek[1], G. J. Snipes[1*], R. Naef[#], W. Sossin[1], J. Laliberté[1], S. Iacampo[1], U. Suter[#], E. M. Shooter[1], and R. A. Murphy[1]. [1]Montreal Neurol. Inst., McGill Univ., Montreal, Quebec, Canada, [1]Dept. of Neurobiol., Stanford Univ. School of Medicine, Stanford, California, and [#]Inst. of Cell Biol., Swiss Federal Inst. of Tech., ETH-Hönggerberg, Zürich, Switzerland.

We have studied the biosynthesis of peripheral myelin protein-22 (PMP22) in cultured Schwann cells, myelinating co-cultures of Schwann cells and neurons, and in sciatic nerve explants. Metabolic labelling and pulse chase studies indicate that most of the newly synthesized PMP22 is rapidly turned-over within the cell and does not become translocated to the cell membrane. A small fraction of PMP22 is complex glycosylated and is localized within the Golgi compartment. Similarly, in myelinating co-cultures of Schwann cells and neurons and in sciatic nerve explants *ex-vivo*, most of the newly synthesized PMP22, as measured by short term radiolabelling, is rapidly degraded. Long-term labelling studies show an accumulation of endoglycosidase H resistant PMP22 in Schwann cells alone or when combined with neurons. Furthermore, in long term myelinating co-cultures, PMP22 is localized in the Schwann cell membrane and in myelin. Taken together, the data suggest that most of the PMP22 produced by Schwann cells in culture, in the presence or absence of neurons, and in *ex vivo* sciatic nerve explants is rapidly degraded within the cell. In myelinating co-cultures, only a small fraction of the newly synthesized protein is transferred to the cell membrane where it is incorporated into myelin. These results suggest that during myelination, signals from neurons promote the trafficking, insertion, or stability of PMP22 in myelin, but do not alter the rapid rate of PMP22 turnover within Schwann cells.

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29.8

INSULIN-LIKE GROWTH FACTOR-I (IGF-I) PROMOTES MYELINATION IN NEURONS. J.W. Russell*, H-L. Cheng and E.L. Feldman. Neuroscience Program and Department of Neurology, University of Michigan, Ann Arbor, MI 48109.

IGF-I is an important neuronal mitogen and is able to promote the formation of adult, myelin forming oligodendrocytes in the central nervous system. Although IGF-I is produced by Schwann cells, the effect of IGF-I on peripheral neuronal myelination has not been previously established. E15 dissociated rat dorsal root ganglion neurons (DRG) were cultured in serum free defined medium (SFDM) containing selenium, transferrin, hydrocortisone, ß-Estriadiol, glutamate, ascorbic acid, glucose, and 10 ng/ml 2.5S NGF to promote neuronal growth. DRG neuronal myelination was measured in SFDM alone or with one of the following additions: 5% calf serum, 5-10 nM IGF-I, IGF-I + 1 mM 8-bromo cAMP, forskolin, or dibutyryl cAMP. Differentiating Schwann cells were photographed during development. At 21 d the cultures were fixed and stained with 2% osmium and Sudan black and the number of fully myelinated fibers/μm^2 was measured in each sample by a blinded observer using a random order measuring system. Within 72 h, both cAMP and IGF-I promoted Schwann cell differentiation into mature forms which lined up and then formed a sheath along the growing axon. Neuronal survival at 21 days was increased in the following order IGF-I>serum treated>cAMP>SFDM. IGF-I promoted myelination of the DRG neurons, however, the number of myelinated fibres was reduced by 32% compared to serum treated neurons ($p<0.01$). Myelination did not occur in DRG cultures treated with cAMP or in surviving neurons treated with SFDM. These results imply that 1) cAMP and IGF-I promote differentiation of Schwann cells 2) IGF-I is an important promotor of survival and myelination in sensory neurons, and 3) An additional permissive factor present in serum is required to induce optimum myelin production.

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29.9

INITIAL SHEATH LENGTHS ALONG DEVELOPING RAT PNS AND CNS AXONS C. Bjartmar* and C. Hildebrand. Dept of Cell Biology, Faculty of Health Sciences, University of Linköping, Sweden.

In myelinated axons, nodal spacing, i.e. sheath length, is a parameter of functional importance. One of the determinants of nodal spacing is the initial sheath length at onset of myelination. The present study examines the lengths of newly formed sheaths along prospective myelinated rat PNS and CNS axons. Developing axons in the ventral root L5, the spinal cord ventral funiculus and the corpus callosum were subjected to serial section EM analysis. On the average, uncompacted Schwann cell sheaths were about 50 µm long, while partly compacted sheaths were some 70 µm long. The corresponding adult sheaths are 1250 µm long. Thus, these Schwann cell sheaths exhibit a developmental elongation of 17 times. The roots elongate 11 times only. This necessitates a myelin sheath remodelling. In developing white matter, many uncompacted sheaths were < 10 µm long. In the spinal cord, the average uncompacted and partly compacted sheath was 21 and 102 µm long respectively. The corresponding callosal lengths were 33 and 69 µm. Since the unmyelinated/ensheathed axon length ratio is too low to accomodate the calculated developmental sheath elongation, early white matter sheaths must undergo a remodelling. Finally, we found that the individual early myelinating PNS or CNS axon present is myelinated at some levels but unmyelinated at other levels. These data show that initial sheath length is markedly different in the PNS and the CNS, and that both sheath types undergo an early remodelling.

Supported by SMRC grant 3761.

29.10

POST-MITOTIC OLIGODENDROCYTES ARE INCAPABLE OF REMYELINATION IN VIVO. H.S. Keirstead* and W.F. Blakemore Department of Clinical Veterinary Medicine and MRC Cambridge Centre for Brain Repair, University of Cambridge, Robinson Way, Cambridge U.K., CB2 2PY.

In order to investigate the remyelinating potential of mature, post-mitotic oligodendrocytes *in vivo*, we have developed a model of demyelination in the adult rat spinal cord in which not all myelin bearing oligodendrocytes are killed by the demyelinating process. A single intraspinal injection of serum complement proteins plus antibodies to galactocerebroside (the major sphingolipid in myelin) results in demyelination which is rapidly followed by oligodendrocyte remyelination. This remyelination was not seen when the spinal cord was exposed to 40 Grays of x-irradiation prior to demyelination to prohibit a contribution by dividing cells. Histological, immunohistochemical and electron microscopic examination of the lesion at early and late time points following x-irradiation and intraspinal injection indicate that a proportion of oligodendrocytes in the area of demyelination survive. With time these cells extend processes which engage axons, and on occasions form compact myelin. These studies demonstrate that oligodendrocytes which survive within a region of demyelination in the adult rat spinal cord are not induced to divide by the presence of demyelinated axons. Furthermore, the radiation resistance of such cells indicates that differentiated oligodendrocytes are post mitotic and thus cannot be considered to make a significant contribution to remyelination in the CNS. Supported by the Medical Research Council of Great Britain and Northern Ireland.

29.11

VITRONECTIN IS ASSOCIATED WITH MYELIN SHEATHS IN THE ADULT RAT BRAIN. S. Einheber[1*], T. A. Milner[3], K.T. Preissner[4] and J. L. Salzer[1,2] Depts. of [1]Cell Biology and [2]Neurology, New York Univ. School of Med., New York, NY 10016, [3]Dept. of Neurology and Neuroscience, Cornell Univ. Med. Coll., New York, NY 10021· [4]Haemostasis Research Unit, Kerckhoff-Klinik, Max-Planck-Inst., Bad Nauheim, Germany.

Oligodendrocytes ensheathe and myelinate axons in the absence of a surrounding basal lamina. However, *in vitro* studies suggest that extracellular matrix (ECM) components, such as fibronectin and vitronectin (Vn) promote oligodendrocyte adhesion (Cardwell and Rome, J. Cell Biol. 107:1541-1549, 1988). Recently, integrin receptors for Vn were identified in cultured oligodendrocytes, further implicating this ECM protein in oligodendrocyte function (Milner and ffrench-Constant, Development. 120:3497-3506, 1994). Vn is a multifunctional protein· that promotes cell spreading and migration, modulates pericellular proteolysis and inhibits complement mediated cell lysis. In this study, we demonstrate that Vn is closely associated with myelin-forming oligodendrocytes *in vivo*. Vn immunoreactivity was localized in acrolein-fixed sections from adult rat brain using an affinity-purified polyclonal antibody against mouse Vn. By light microscopy, moderate Vn immunoreactivity was observed in bundles of myelinated axons in several fiber tracts, including the corpus callosum, internal capsule and ventral hippocampal commissure. At the ultrastructural level, Vn immunoreactivity was associated with the outer surfaces of myelinated axons as well as some unmyelinated axons. In addition, intracellular immunoreactivity was commonly observed in glial cells adjacent to immunolabeled myelin sheaths. The identity of these cells and their possible role in secreting Vn in the fiber tracts is currently under investigation. These results provide further evidence that Vn may influence oligodendrocyte function *in vivo* and provide a foundation for future studies focused on potential interactions between myelin-associated Vn and the immune system. Supported by Multiple Sclerosis Grant RG2678 to J.L.S and HL18974 to T.A.M.

29.12

OLIGODENDROCYTES DO NOT MYELINATE AXONS BY DEFAULT. A. Meyer-Franke* and B. A. Barres, Stanford University School of Medicine, Department of Neurobiology, Stanford, CA 94305.

Myelination is a sequential, multistep process in which the process of an oligodendrocyte adheres to an axon, ensheathes and wraps it. The timing, rate and amount of myelination are controlled by signals from neurons, astrocytes, and some endocrine cells. The recent finding that signals from the same cell types promote the survival of oligodendrocytes has raised the following question: Is myelination primarily regulated by permissive signals, which promote oligodendrocyte survival, or does myelination also require instructive signals, which regulate specific steps of the myelination program independently of survival? Because surviving oligodendrocytes synthesize myelin proteins in the absence of other cell types, we hypothesized that myelination is a default program, which progresses inexorably forward in surviving oligodendrocytes.

The recent development of procedures to purify and culture defined types of postnatal CNS neurons and glial cells in serum-free medium (Barres et al., Cell 70:31-46, 1992; Meyer-Franke et al., Neuron 15:805-819, 1995) has allowed us to test this hypothesis. We purified and cultured oligodendrocytes from the rat optic nerve in the presence of saturating concentrations of oligodendrocyte survival signals and asked whether they would myelinate the axons of purified retinal ganglion cells in culture. Although axons freely grew over the oligodendrocytes in culture, the axons did not become ensheathed or myelinated. In contrast, when the co-cultures were treated with conditioned medium from purified optic nerve astrocytes, the oligodendrocytes dramatically altered their morphology, aligning their processes parallel to the axons. Immunostaining with antibodies to myelin proteins and neurofilaments showed that the axons were ensheathed by the oligodendrocyte processes. Electron microscopic studies are in progress. These findings demonstrate that surviving oligodendrocytes do not myelinate by default, but that other cell-cell interactions are required.

This work was funded by the National Eye Institute (BAB) and by the Deutsche Forschungsgemeinschaft (AM).

29.13

SERUM-INDUCED DEMYELINATION IN ORGAN CULTURES OF MATURE MOUSE SCIATIC NERVE. C. Lobato*, J.M. Spies, and J.W. Griffin, Johns Hopkins University School of Medicine, Baltimore, MD 21287

We have established a system in which we can monitor and manipulate the cellular events that take place during demyelination *in vitro*. A number of immune-mediated disorders exist in which peripheral nerves undergo demyelination. In many of these disorders, antibody may play a role, but the cellular mechanisms of antibody-induced demyelination are uncertain. To examine the requirements for antibody-induced demyelination, organ cultures of whole mature C57/BL6/Wld^s mouse sciatic nerves are maintained; the fibers survive for several days before Wallerian-like degeneration ensues. We have used this system to examine the effect of serum from rabbits with experimental allergic neuritis (EAN) induced by immunization with whole myelin. Demyelination of sciatic nerves occurred when nerves were cultured with immunoglobulin purified from EAN serum. Some demyelination took place in the absence of added complement. Circulating macrophages were not required for the initiation of vesicular demyelination. These results indicate that serum factors in EAN are sufficient to induce vesicular demyelination in mature nerves *in vitro*.

(Funding: National Institutes of Health, NS34846)

29.14

EXPRESSION OF PLATELET-DERIVED GROWTH FACTOR α RECEPTOR AND LIGAND DURING CNS REMYELINATION. J.M. Redwine* and R.C. Armstrong, Uniformed Services University of the Health Sciences, Bethesda, MD 20814, USA.

Oligodendrocyte precursors from neonatal rodent CNS express PDGFαR, and PDGF-AA ligand increases *in vitro* proliferation, migration, and survival of these cells. During remyelination following demyelination and oligodendrocyte loss, surviving oligodendrocytes or oligodendrocyte precursors must proliferate, migrate into the lesion area, and form new myelin. To determine the role of PDGF during remyelination, we first compared the expression of PDGFαR and PDGF-AA in normal versus remyelinating spinal cord. We used C57/Bl6 mice experimentally demyelinated by intracranial injection with murine hepatitis virus strain A59, which produced motor dysfunction due to focal areas of demyelination throughout the spinal cord. In this model, widespread remyelination is achieved followed by functional recovery. Immunocytochemical markers demonstrated areas of astrocytic gliosis and myelin loss. Immunoreactivity for PDGFαR was increased in these lesion sites and immunoreactive cells included those with the morphology of oligodendrocytes or precursors. Immunoreactivity for PDGF-AA was present in neurons and axons of both control and lesioned tissues, and in reactive astrocytes in lesioned areas. Supported by National Multiple Sclerosis Society and National Institutes of Health.

29.15

SHAKER-TYPE POTASSIUM CHANNEL (KV1.1) CLUSTERING IN REMYELINATING RAT AXONS. M. N. Rasband, T. L. Schwarz, M. H. Ellisman, M. Schachner, S. D. Novakovic*, and P. Shrager. Dept. of Biophysics, University of Rochester, Rochester NY, 14642.

Kv1.1 has been localized to the juxtaparanode in normal rat axons (J. Neurosci. 15, 3761-3774). We have induced demyelination by intraneural injection of lysolecithin into the sciatic nerve of Lewis rats. The distribution of Kv1.1 has now been investigated during subsequent remyelination using immunofluorescence applied to teased axon preparations. 6 days after lysolecithin injection, demyelinated axons showed broad diffuse labeling with elevated fluorescence at the edges of newly associated, MAG (myelin associated glycoprotein) negative, Schwann cells. 10 days after injection, Schwann cells had extended processes along the axon, had begun to elaborate myelin, and were MAG positive. No Kv1.1 labeling was seen at newly forming nodes. This is in contrast to our earlier report of sodium channel clustering at the edges of Schwann cell bodies as early as 8 days after injection. Kv1.1 perinuclear labeling of Schwann cell bodies, however, was pronounced and persisted in all stages of remyelination. 12 to 16 days following injection, Kv1.1 labeling appeared in the vicinity of nodes. Although it has not been definitively shown, Kv1.1 label appeared to be axonal and extended through the nodal gap between apposing Schwann cells. In comparison, sodium channel clusters are well formed by this stage of remyelination. Our findings indicate Kv1.1 clustering follows MAG expression. At 16 days, Kv1.1 began localizing to paranodes, indicating either active clustering or possibly exclusion from the center of the node. Labeling was clearly paranodal by 32 days, with a gap between adjacent paranodal clusters. The location of Kv1.1 remained paranodal even at periods of remyelination as long as 62 days. The time course of clustering and final distribution of Kv1.1 potassium channels differs from that of sodium channel clustering. This implies the mechanisms of clustering are likely different. The functional significance of sodium channel distribution is well understood, but remains unknown for Kv1.1 potassium channels. Supported by NIH and NMSS.

BLOOD-BRAIN BARRIER I

30.1

UPREGULATORY CIS-ELEMENT WITHIN THE 3'-UNTRANSLATED REGION OF GLUT1 GLUCOSE TRANSPORTER mRNA. H.Tsukamoto, R.J.Boado and W.M. Pardridge*, Dept. of Medicine, UCLA School of Medicine, Los Angeles, CA 90095

The principal glucose transporter at the blood-brain barrier is GLUT1, and this gene expression may be regulated posttranscriptionally by specific interactions of cytosolic trans-acting factors and regulatory cis-elements within the untranslated regions (UTRs) of the GLUT1 mRNA. Our recent studies demonstrate that C6 rat glioma cytosol contains at least two proteins that react with specific sequences within the GLUT1 3'UTR and form two RNA/protein complexes with molecular masses of 88 and 44 kDa in ultraviolet crosslinking (UVCL) studies. The putative role of the GLUT1 3'UTR cis-acting element was studied using the luciferase expression vector pGL2. Transfection of C6 cells with pGL2 containing nucleotides 2,100-2,300 of the bovine GLUT1 3'UTR, encompassing the binding site of C6 proteins that form the 88 kDa complex, inserted at the PflM1 site within the luciferase 3'UTR resulted in a fivefold increase in luciferase gene expression in C6 cells (484±33 pg/35 mm dish), compared to levels with control pGL2 (95±19 pg/dish). Deletion of 10 nucleotides (2,181-2,190) of bovine GLUT1 3'UTR, the binding site of the proteins forming the 88 kDa complex, was performed by site-directed mutagenesis. Luciferase expression in C6 cells transfected with this mutagenized plasmid was markedly reduced to levels obtained with control pGL2. UVCL study showed that the transcript of pGL2 containing the intact fragment of bovine GLUT1 3'UTR formed the 88 kDa complex with C6 proteins. The present study provides evidence suggesting that the interaction of the cis-regulatory sequence, nucleotides 2,181-2,190 of bovine GLUT1 3'UTR, and the cytosolic proteins that form the 88 kDa complex in C6 cells, may serve to upregulate GLUT1 gene expression.

30.2

IMMUNOHISTOCHEMICAL LOCALIZATION OF INTRANEURONAL TRANSFERRIN RECEPTOR IMMUNOREACTIVITY IN THE ADULT MOUSE CENTRAL NERVOUS SYSTEM. T. Moos*. Inst. Med.Anat, sect A, Panum Inst., Univ. Copenhagen, Denmark

Iron is an essential determinant for a variety of intracellular functions. Accordingly, the transfer of iron from blood to brain is vital for normal brain function. In the CNS, the receptor for iron-transferrin is generally accepted to be located in endothelial cells, whereas its occurrence in other cell types is less well established. I have investigated the distribution of the transferrin receptor in the adult mouse central nervous system by immunohistochemistry using a monoclonal antibody raised against the transferrin receptor protein. Immunoreactive cell types comprised brain capillary endothelial cells, excluding those of circumventricular organs, and choroid plexus epithelial cells. Moreover, transferrin receptor immunoreactivity was detected intraneuronally in several brain regions without access to peripheral blood. The immunoreactive cell bodies were mainly confined to the cerebral cortex, hippocampus, habenular nucleus, red nucleus, substantia nigra, pontine nuclei, reticular formation, several cranial nerve nuclei, deep cerebellar nuclei, and cerebellar cortex. Transferrin receptor immunoreactivity was not ascertained in astrocytes, oligodendrocytes or microglial cells. The occurrence of transferrin receptors at brain-barrier sites, i.e. brain endothelium and choroid plexus epithelium, and the presence of the receptors intraneuronally are in accordance with the generally held belief that iron is released from liver transferrin and transported through capillaries and the choroid plexus into the brain interstitium. Subsequently, iron may be linked to brain transferrin synthesized within oligodendrocytes and choroid plexus epithelial cells followed by a concomitant uptake of iron-transferrin in neurons expressing transferrin receptors. Additional analyses of iron-transport within the brain are presented.

30.3

OXOPROLINE REGULATES AMINO ACID TRANSPORT ACROSS THE BLOOD-BRAIN BARRIER

W.-J. Lee[1], J. R. Viña[2], D. R. Peterson[1] and R. A. Hawkins[1]*, [1]Department of Physiology and Biophysics, Finch University of Health Sciences/The Chicago Medical School, North Chicago, IL 60064. [2]Department of Biochemistry and Molecular Biology, University of Valencia, Valencia-46010, SPAIN.

Regulation of neutral amino acid transport was studied using isolated plasma membrane vesicles derived from the bovine blood-brain barrier. Neutral amino acids cross the blood-brain barrier by facilitative transport system L1, which may allow both desirable and undesirable amino acids to enter the brain. The Na-dependent amino acid systems A and $B^{0,+}$ are located exclusively on abluminal membranes, in a position to pump undesirable amino acids out. Gamma-glutamyl transpeptidase, the first enzyme of the gamma-glutamyl cycle, is an integral protein of the luminal membrane of the blood-brain barrier. We demonstrate that oxoproline, an intracellular product of the gamma-glutamyl cycle, stimulates the rates of the Na-dependent systems A and $B^{0,+}$ by 70% and 20% respectively. Study of system A showed that 2 mM oxoproline increased the affinity for its specific substrate methyl-amino isobutyric acid by 50%. This relationship between the activity of the gamma-glutamyl cycle and system A transport may provide a short-term regulatory mechanism by which the entry of potentially deleterious amino acids (i.e. neurotransmitters or their precursors) may be retarded and their removal from brain accelerated.

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30.4

POLYAMINE MODIFICATION INCREASES THE PERMEABILITY OF PROTEINS AT THE BLOOD-NERVE AND BLOOD-BRAIN BARRIERS

Joseph F. Poduslo* and Geoffry L. Curran
Mayo Clinic and Mayo Foundation, Rochester, MN 55905 U.S.A.

The permeability of the BNB and BBB to superoxide dismutase (SOD), insulin, albumin, and IgG in normal adult rats was quantified by measuring the permeability coefficient-surface area product (PS) with the i.v. bolus injection technique before and after covalent protein modification with the naturally occurring polyamines - putrescine (PUT), spermidine (SPD), and spermine (SPM). The PS value of the BNB for PUT-SOD was 21.1-fold greater than the native SOD, and the PS values of the BBB for PUT-SOD ranged from 17.6-fold greater for the thalamus to 23.6-fold greater for the caudate-putamen compared to native SOD. Similarly, polyamine modified insulin showed a 1.7 to 2.0-fold increase in PS of the BNB and BBB compared to the high values of native insulin. Polyamine modified albumin showed a remarkable 54 to 165-fold increase in PS of the BNB and BBB compared to native albumin, while PUT-IgG resulted in an even higher increase in the PS that ranged from 111 to 349-fold for nerve and different brain regions compared to native IgG. Polyamine modification of proteins, therefore, can dramatically increase the permeability at the BNB and BBB of a variety of proteins with widely differing M_r and function. Surprisingly, the PS values of the BNB and BBB decreased with the increasing number of positive charges of the protonated amino groups on the polyamines (PUT > SPD > SPM). While cationic proteins are known to interact with fixed anionic charges on the lumen of the microvascular endothelium, this observation of decreased permeability with increased positive charge distribution along the aliphatic carbon chain of the polyamines implies mechanisms other than simple electrostatic interaction involving charge density. Systemic administration of polyamine modified proteins might prove to be an efficient approach to deliver therapeutic agents into the CNS and PNS for the treatment of a variety of neurological diseases (Mayo Foundation).

30.5

MORPHOLOGICAL PROPERTIES, ELECTRICAL RESISTANCE AND POTASSIUM TRANSPORT ACROSS AN "IN VITRO" BLOOD-BRAIN BARRIER

K.A. Stanness, L.A. Beck, L.E. Westrum, H.R. Winn, L.G. Costa*, D. Janigro, Depts. Neurosurgery & Env. Health, Univ. of Washington, Seattle, WA

We have developed an *in vitro* model of the mammalian blood-brain barrier (DIV-BBB) by co-culturing endothelial cells (EC) with glia in a hollow fiber apparatus. Induction of BBB properties was characterized by a selective barrier to intraluminal potassium and glucose transport across the endothelial monolayer. We further characterized the BBB nature of our culture system by electron microscopic analysis of both intraluminal EC and extraluminal (*ecs*) glia. EC formed numerous tight junctions following co-incubation with glia; the latter did not develop any junctional specialization. During induction of BBB properties we monitored transendothelial electrical resistance (TER) by applying a voltage signal to the *ecs* while recording voltage changes in the lumen. The electrical resistance of the BBB was around 800 Mohms. "Osmotic openings" of the DIV-BBB were induced by intraluminal applications of high [KCl] and were characterized by a sudden and reversible decrease of TER (by approximately 25%). TER was also dramatically reduced by intraluminal perfusion with proteolitic enzymes but was not affected by exposure of the extraluminal glia to low pH, further suggesting the endothelial nature of the BBB. While the DIV-BBB effectively prevented intraluminal potassium movement to the *ecs*, potassium was actively transported from the *ecs* to the lumen in an ouabain-sensitive (100 µM) fashion. Intraluminal furosemide application (2.5 mM) further decreased the potassium movement from the lumen to the *ecs* suggesting that a K/Cl/Na endothelial co-transporter is expressed in the DIV-BBB. Our results suggest that the DIV-BBB may be an useful tool for the study of brain ionic homeostasis and cellular development *in vitro*. Supported in part by Cellco Inc. (Germantown, MD) and NIH 51624 and NIEHS ES 07033.

30.6

AMMONIUM IONS IMPAIR POTASSIUM EFFLUX ACROSS THE BLOOD-BRAIN BARRIER OF THE RAT.

K.A. Smart and M.B. Segal*. *Physiology Dept, UMDS, London, SE1 7EH, UK.*

Ammonium ions (NH_4^+) are believed to be the major toxin involved in the manifestation of the symptoms of Hepatic Encephalopathy (HE) (Schenker et al, 1974). Following acute liver failure, the plasma level of the NH_4^+ ion rises to in excess of three times the normal (39.9 ± 3.7µM), and it has been proposed that the lipid insoluble NH_4^+ ion crosses the blood brain barrier (BBB) by competing with K^+, leading to neuronal damage (Cooper and Plum, 1987). In this study we have loaded the brain with ^{86}Rb using the bilateral in situ brain perfusion technique (Preston et al 1995), then collected jugular vein effluent over a 30 minute period in the presence and absence of NH_4^+. We have taken terminal CSF and brain tissue samples. Using dextran density capillary depletion analysis (Triguero et al 1990), we have been able to show an increase in the levels of ^{86}Rb remaining in the brain parenchyma and capillary endothelial cells, and loss of ^{86}Rb to CSF and blood. These data fit a 2 compartment efflux model as evident from the 2 different half-life ($t_{1/2}$) values. ($t_{1/2}$ is a measure of the time taken for half of the contained activity to be lost). This suggests that there is a fast efflux component from the capillary endothelium, $t_{1/2}$fast= 0.715±0.12 min, and a slower component from the brain parenchyma, $t_{1/2}$slow = 20.125±1.2 min. NH_4^+ had no effect on $t_{1/2}$fast, but increased $t_{1/2}$slow to 28.8±0.1 min. NH_4^+ also decreased loss of ^{86}Rb to the blood and the amount remaining in the cerebral tissue. These results suggest that NH_4^+ inhibits efflux by acting on the brain side of the capillary endothelial cell.

30.7

POTASSIUM TRANSPORT SYSTEMS IN BRAIN CAPILLARY ENDOTHELIAL CELLS: RESPONSE TO ENDOTHELIN-1 AND HYPOXIA.

N. Kawai, R. M. McCarron, and M. Spatz*
Stroke Branch, NINDS, NIH, Bethesda, MD 20892-4128

Endothelin-1 (ET-1), oxygen deprivation, or oligomycin treatment was shown to stimulate Na^+-K^+-Cl^- cotransport in cultured rat brain capillary endothelial cells (RBEC), a constituent of blood-brain barrier (BBB). This report compares the effect of ET-1 and chemically induced hypoxia (by oligomycin, sodium azide, or antimycin A) on RBEC K^+ transport activity. $^{86}Rb^+$ was used as a tracer for K^+; bumetanide-sensitive K^+ uptake was defined as Na^+-K^+-Cl^- cotransport activity. ET-1, phorbol myristate acetate (PMA), or thapsigargin increased Na^+-K^+-Cl^- cotransport activity by 60, 150, and 200%, respectively. A protein kinase C (PKC) inhibitor, bisindolylmaleimide (BIS) inhibited PMA- and ET-1- (but not thapsigargin-) induced Na^+-K^+-Cl^- cotransport activity. In contrast, the basal and induced (by all the above substances) Na^+-K^+-Cl^- cotransport activity was reduced by intracellular Ca^{2+} chelator, BAPTA/AM. Oligomycin, sodium azide, or antimycin A increased Na^+-K^+-Cl^- cotransport activity by 80-200%; BAPTA/AM (but not BIS) reduced oligomycin-induced Na^+-K^+-Cl^- cotransport activity. These data indicate that hypoxia stimulates Na^+-K^+-Cl^- cotransport activity through Ca^{2+}-dependent mechanisms, whereas ET-1 augmentation occurs through both Ca^{2+}- and PKC-dependent pathways. ET-1 or oligomycin also increased K^+ efflux (50-120%) which was abolished by 300 µM quinine, indicating involvement of Ca^{2+}-activated K^+ channels in this response. Thus, the stimulation of K^+ uptake by ET-1 or hypoxia coincided with increased K^+ efflux through Ca^{2+} activated K^+ channels in RBEC. Similar mechanisms may be involved in altering the ion-transport activity at BBB and contribute to disturbances of water-electrolyte homeostasis reported in ischemic brain.

30.8

THE PATTERN OFBLOOD -NERVE BARRIER BREAKDOWN AFTER DORSAL ROOT GANGLIONECTOMY.

J.M. Spies, K.A. Sheikh, P. Kessins, T.W. Brushart, A.K. Asbury*, J.W. Griffin, Johns Hopkins University School of Medicine, Baltimore, MD 21287

Increasing evidence suggests that humoral factors are important in the pathogenesis of a number of immune-mediated neuropathies. The blood-nerve barrier (BNB) has hindered efforts to establish animal models to investigate the role of these humoral factors. Nerves undergoing active Wallerian degeneration are known to have defective BNBs, and evidence suggests that restoration of the BNB depends on axonal regeneration. We investigated the pattern of BNB breakdown in the rat sciatic nerve after dorsal root ganglion (DRG) removal, a model in which axons are not expected to regenerate.

The left 5th lumbar DRG was removed from adult Lewis rats. Permeability of BNB to albumin at various times was assessed by quantitating endoneurial content of Evans blue dye in control and ganglionectomized nerves after intravenous injection. Permeability to immunoglobulin (Ig) M was assessed by immunostaining for rat IgM in the endoneurium.

Sciatic nerves on the side of ganglionectomy showed a significant increase in permeability to both albumin and IgM. This increased permeability was not seen diffusely throughout the nerve fascicles but was most marked in those parts of the fascicles where sensory axons were degenerating. Permeability was maximal at 3-5 weeks and thereafter returned towards baseline. By 6 months only minor increases in permeability were seen. These data suggest that, in the rat, the BNB can be restored in the absence of axonal regeneration.

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30.9

LEUKOCYTE-DEPENDENT BLOOD-BRAIN BARRIER BREAKDOWN FOLLOWING ASPHYXIA IN PIGS. E.R. Gonzales, T.S. Park, J.W. Beetsch, A.R. Shah, R.G. Maceren, Y.-B. Lee, J.M. Gidday*. Department of Neurosurgery, and St. Louis Children's Hospital, Washington University School of Medicine, St. Louis, MO 63110

Microvascular damage following ischemia contributes to brain edema and associated tissue injury, but the mechanisms responsible for disruption of the blood-brain barrier have yet to be conclusively identified. We tested the hypothesis that adherent leukocytes are involved in mediating post-ischemic edema. Newborn pigs were equipped with closed cranial windows for quantification by dual-filter videomicroscopy of the adherence of rhodamine 6G-stained leukocytes to pial venules, and the leakage of sodium fluorescein from these venules 20 min after its intravenous injection (1 ml/kg of a 1% solution). The latter was quantified by measurements of extravascular optical density on the videotape record using image analysis software. Three groups of animals (n=5 each) were studied: Non-asphyxic controls, animals rendered asphyxic for 9 min by discontinuing ventilation (which causes hypoxia, hypercapnia, hypotension, and acidosis), and asphyxic animals pretreated with a mouse anti-human monoclonal antibody against the leukocyte CD18 integrin (R15.7 from Boehringer Ingelheim; 2 mg/kg). Asphyxia induced a progressive and significant increase in adherent leukocytes during the initial 2 h of reperfusion, at which time a 103% increase in fluorescein leakage was measured. However, adherence and fluorescein leakage in the group treated with the CD18 antibody were reduced to levels equivalent to those measured in non-asphyxic controls. These findings indicate that adherent leukocytes are responsible for the increase in cerebrovascular permeability following asphyxia, and therefore may contribute to injury following ischemia. (NIH NINDS 21045 and 32568).

30.10

RELATIONSHIP BETWEEN DEMYELINATION (DM) AND BLOOD BRAIN BARRIER (BBB) DISRUPTION FOLLOWING RAPID CORRECTION OF HYPONATREMIA. S. Adler, J.G. Verbalis, D. Williams and A.J. Martinez*. U. Pittsburgh Sch. Med., and NMR Biomedical Research Center, CMU, Pittsburgh, PA 15261 and Georgetown Univ., Wash. D.C. 20007.

This study examines the hypothesis that the demyelination (DM) which may follow overly-rapid correction of hyponatremia (HN) is due to BBB disruption. Chronic (10d) HN was induced in rats by infusing dDAVP via osmotic minipump. HN was corrected by administering 1 M NaCl IP at 1.0 ml/100 g. At either 3 hours (n=23) or 16-24 hours (n=44) after initiating HN correction, BBB leakage was assessed by NMR imaging using IV gadolinium-DTPA. After 5-6 days, rats were sacrificed and brain DM determined histopathologically by luxol blue. Of the 67 rats studied, 45 showed BBB leakage; the table shows the relationship between leakage and DM.

	Number	Leakage (+)	Leakage (-)
DM (+)	35	31 (89%)	4 (11%)
DM (-)	32	14 (44%)	18 (56%)

The rate of (+) leakage and (-) DM was equal at both times. A separate study performed in 29 HN rats used a V_2 receptor antagonist to more slowly correct HN. BBB leakage and DM occurred in only two rats in this group. Data from all 96 rats reveals that DM occurred in 69% of rats with BBB leakage but in only 5% without a leak. These studies show that BBB disruption is closely associated with subsequent demyelination but that BBB disruption does not inevitably lead to DM. The results support the hypothesis that leakage of blood components, such as complement, into the brain could be in part responsible for the DM observed in this model.

30.11

A REPEATABLE TECHNIQUE FOR HYPEROSMOTIC BLOOD BRAIN BARRIER DIRUPTION IN THE DOG. B. W. Culver, K. R. Dyer , J. Jones, B. Jortner*, R. Kroll Virginia Maryland Regional College of Veterinary Medicine, Blacksburg, VA 24061

Reversible hyperosmotic blood-brain barrier disruption (BBBD) enhances drug delivery across the blood brain barrier. A technique for repeatable BBBD in the canine has not been described. The purpose of this study was to develop a repeatable technique for BBBD in the dog and evaluate the effects of multiple BBBD.

Ten dogs were randomly assigned to treatment or control groups. A Norman catheter was introduced into the femoral artery and advanced under fluoroscopy into the internal carotid artery. Treatment dogs received intracarotid mannitol (2 - 2.3 ml/sec for 30 sec). Control dogs received saline at the same rate. Following BBBD, Iohexal was administered prior to a CT scan. Dogs were allowed to recover after the first procedure and were monitored for clinical signs related to BBBD for 2 wk. A second, non survival procedure was performed as before with the addition of Evan's blue administration 15 min prior to BBBD. The degree of BBBD was estimated using CT densitometry values as well as Evan's blue staining graded (scale of 0-3).

All Treatment dogs had evidence of successful BBBD by CT and/or Evan's blue evaluation. None of the control dogs had CT densitometry values consistent with successful BBBD. Procedural complications consisted of; 1) hematoma of the internal carotid, 2) rupture of the femoral artery, and 3) self resolving, hematoma of the femoral artery catheter site. Side effects included; ipsilateral head tilt and circling, contralateral hemiparesis, and blindness. These side effects resolved in 12-48 hr. and while present in both groups, tended to be most pronounced in treatment dogs. Life threatening side effects were only seen in 2 treatment dogs and consisted of; CNS edema 12 hours post BBBD, and brain herniation immediately following BBBD.

This study describes a technique of repeatable BBBD in the dog using fluoroscopic guidance to place a percutaneous transfemoral catheter system into the internal carotid artery and open the way for further investigations of BBBD using the dog as a model.

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30.12

CHLOROQUINE/BLOOD-BRAIN BARRIER I. CHLOROQUINE ATTENUATES KAINATE-INDUCED IgG EXTRAVASATION IN MICE. **J. Maritz, M.P. Murphy, J.G. Mielke, K.M. Bengualid, and G.O. Ivy.* University of Toronto, Scarborough College, 1265 Military Trail, Division of Life Sciences, Scarborough, Ontario, Canada M1C 1A4.**

Kainic acid (KA) induced seizures damage the blood-brain barrier (BBB), as indicated by the presence of extravasated serum proteins, such as IgG. Since the commonly used anti-malarial drug chloroquine (CHL) has proconvulsant properties in both animals and humans, a BBB effect may exist similar to that of KA. BCF1 and BDF1 male and female mice were treated with either CHL (45 mg/kg, n=18) or vehicle (0.9% physiological saline, n=24), i.p., once/day for 6 consecutive days. On the day following the final dose, the subjects were given an i.p. injection of KA (12 mg/kg) or SAL (half of each group). Animals pretreated with CHL developed seizures an average of 45 minutes before controls [$F(1,25)=3.81$, $p<0.06$]. There were no strain or sex differences. Following 45 minutes of status epilepticus, the mice were sacrificed with an overdose of sodium pentobarbital and perfused transcardially with 4% paraformaldehyde. Coronal sections were taken (30 μm) from the septum through the hippocampus, and every 8th section processed for immunocytochemical localization of IgG. KA caused an increase in the number of IgG incursions in the brain [$F(1,30)=8.56$, $p<0.0065$], and CHL pretreatment attenuated this effect [$F(1,17)=7.41$, $p<0.015$]. These results suggest that CHL may exert its proconvulsant activity indirectly through an interaction with the BBB. Research supported by the Natural Sciences and Engineering Research Council (NSERC) of Canada.

30.13

CHLOROQUINE/BLOOD-BRAIN BARRIER II. CHLOROQUINE MEDIATED DISRUPTION OF THE BLOOD-BRAIN BARRIER IN THE RAT. **M.P. Murphy*, J. Mielke, S.C. Barsoum, and G.O. Ivy. University of Toronto, Scarborough College, 1265 Military Trail, Division of Life Sciences, Scarborough, Ontario, Canada M1C 1A4.**

We have shown that chloroquine (CHL) has proconvulsant properties, possibly mediated by an interaction with the blood-brain barrier (BBB). Male Sprague-Dawley rats were implanted s.c. with an osmotic minipump (Alzet) containing either 0.9% saline (SAL; n=5) or 30 mg/ml CHL (n=13), delivered into the left lateral ventricle. One week later, rats were given kainic acid (KA; 12 mg/kg i.p.: 3 without pump, 3 SAL, 8 CHL) or 0.9% saline. CHL was administered orally (gavage) to another group of animals for 7 days [dosage/day, mg/kg (n=7-8/dose): 0, 7.5, 15, 22.5, 30, 45]; 2 hrs. after the final dose, half of the animals were injected with KA as above. CHL had a proconvulsant effect both i.c.v. and orally (45 mg/kg only). Animals were sacrificed with an overdose of sodium pentobarbital after 30 minutes of status epilepticus. Coronal sections (30 μm) were processed for immunocytochemical localization of extravasated IgG. CHL infusion caused massive IgG immunoreactivity, confined almost entirely to the treated side. Oral CHL caused a marked increase in IgG extravasation around blood vessels; this effect declined with increasing dose, probably reflecting CHL immunosuppression. For both methods, CHL and KA had an additive effect. Hence, CHL may damage the BBB, thereby allowing potentially toxic substances easier access to the CNS. Alternatively, CHL may have additional therapeutic use as a means to open the BBB to allow passage of beneficial compounds. Supported by NSERC, Canada.

30.14

TRAFFICKING OF B AND T LYMPHOCYTES TO THE BRAIN FOLLOWING ANTIGEN INFUSION INTO CAUDATE-PUTAMEN OF PASSIVELY IMMUNIZED LEWIS RATS. J. Szmydynger-Chodobska[1,2]*, S.C. Nolan[1], C.J. Harling-Berg[1] and P.M. Knopf[1]. [1]Div. Biol. Med., Brown Univ., Providence, RI 02906 and [2]Dept. Clin. Neurosci., Brown Univ./ R.I. Hosp., Providence, RI 02903.

We have previously shown that in the absence of central nervous system disease, intrathecal antibody synthesis can occur within the brain following soluble antigen administration into CSF. In order to perform an immunohistochemical study of trafficking and retention of B and T lymphocytes to the site of antigen administration within the brain, we have developed a bilateral cannula model. A stainless steel cannula was implanted into each caudate-putamen of Lewis rats. One week later, these rats were passively immunized via i.v. injection of splenocytes (~10^7 cells) from ovalbumin (OVA)-primed syngeneic donors to increase the circulating pool of activated lymphocytes in the recipient. Sixteen hours prior to this cell transfer, recipient rats received OVA (45 μg/rat) infusion into one cannula and saline or an irrelevant antigen into the contralateral cannula. Brains were then evaluated at 4, 24, and 48 h after cell transfer. B and T cells were detected by immunofluoresence using mouse anti-rat IgG and CD4/CD8, respectively. In addition, OX6 antibody against class II MHC molecule (MHCII) was used. B and T lymphocytes were prominent in OVA-infused brain tissue 4 h following passive immunization, and diminished in quantity at 24 and 48 h. At all times cellular infiltration at the control site was significantly lower. The temporal profile of immunopositive staining for MHCII was similar to that of B and T cells, and may indicate presence of both macrophages/activated microglia and B lymphocytes. In conclusion, there is an early influx of B and T cells into the OVA-infusion site. Future studies will focus on antigen-specific retention of these cells. Supported by NIH Grant NS33070.

30.15

EFFECTS OF UROKINASE & VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) ON BRAIN VESSELS.
M. Kaya, L. Chang, A. Truong, M. Brightman*. LN, N.I.H., Bethesda, Md. 20892-4062

Retinoic acid (RA) and phorbol myristate acetate (PMA), continuously infused into the cerebral cortex of rats for 28 days, create a cyst containing blood vessels. About 20-29 % of the vessels are converted from the continuous, barrier type to the permeable, fenestrated vessel (FV) type. It is now found that the conversion is reversed between 1-2 months after ending the infusion. By 1 month, very little circulating horseradish peroxidase enters brain and only 1-3% of the vessels are FV. At 2 months, no FV are found.

An activity common to both RA and PMA is to stimulate production of a proteinase that degrades extracellular matrix: urokinase. Because of its short half life, urokinase was infused into cortex intermittently: 1.5µg in 10µl for 1 h every 3rd day for 28 days. About 12% of cyst vessels became FV. VEGF, associated with FV of some organs, was also infused continuously at 500ng/ml for 28 days and produced vessels with short, 20nm thick segments, a few FV and, in addition, open endothelial junctions. It is inferred that urokinase, made by endothelial cells, may autocrinely mediate the action of RA and PMA, but not VEGF. (NIH Intramural funding).

30.16

MATRIX METALLOPROTEINASE INHIBITION SELECTIVELY REGULATES BLOOD-BRAIN BARRIER OPENING IN LPS-INJECTED BRAIN.
S. Mun-Bryce, G. A. Rosenberg*. Dept. of Neurology, Univ. of New Mexico School of Medicine, Albuquerque, NM 87131.

The up-regulated production of inflammatory cytokines and matrix metalloproteinases (MMP) has been associated with several neuroinflammatory-related diseases such as ischemia and multiple sclerosis. An intracerebral injection of lipopolysaccharide (LPS) causes a significant rise in gelatinase B production, and increases blood-brain barrier permeability to sucrose (M.W. 340 Da) and dextran (M.W. 50-90 kD) molecules. These studies examined whether hydroxymate compound, BB-1101, regulates MMP production and blood-brain barrier breakdown in the inflammatory mediator-stimulated brain. Four hours after LPS-injection, treated animals received an i.p. injection of BB-1101. The zymogram lysis activity of 92 kD and 84 kD gelatinase B, and the 72 kD gelatinase A dropped significantly in treated animals, as compared to untreated animals, 12 hrs post-LPS injection. The MMP inhibitor markedly decreased blood-brain barrier permeability to ^{14}C-sucrose, but had no effect on the amount of ^{14}C-dextran uptake in brain tissue samples, 12 hr after LPs-injection. This data suggests that BB-1101 selectively inhibits blood-brain barrier opening to smaller sucrose-sized molecules in the LPS-injured brain.

30.17

BRAIN AND SPINAL CORD DISTRIBUTION OF BIPHALIN, CORRELATION WITH OPIOID RECEPTOR DENSITY AND MECHANISM OF CNS ENTRY.
T.J. Abbruscato*, S.A. Williams, V.J. Hruby, T.P. Davis. Departments of Pharmacology and Chemistry, University of Arizona, Tucson, AZ 85724

Biphalin (Tyr-D-Ala-Gly-Phe-NH)$_2$ is a bivalent, opioid peptide containing two pharmacophores linked by a hydrazide bridge. When administered ICV, it has been shown to be more potent than morphine and etorphine at eliciting antinociception. Biphalin has also been shown to cross both the blood-brain and blood-CSF barriers (Abbruscato et al., 1996 JPET 276, 1049-1057). To understand the mechanism for biphalin potency, regional brain and spinal cord distribution studies with[^{125}I-Tyr]biphalin were performed after 5, 20 and 40 minute IV bolus injection. A statistically greater amount of [^{125}I-Tyr^1]biphalin was detected in the nucleus accumbens compared to other brain regions ($P<0.05$). This correlates with the high density of delta and mu opioid receptor mRNA and binding sites shown to be expressed in the nucleus accumbens (Mansour et al., 1995 TINS **18**, 1995). Considerable amounts of [^{125}I-Tyr^1]biphalin were detected in spinal regions (cervical, thoracic, lumbar and cauda equina) in comparison to brain regions including frontal cortex, caudate-putamen, cerebellum and brain stem. Also, a statistically greater amount of [^{125}I-Tyr^1]biphalin was detected in two circumventricular organs, the choroid plexus and pituitary, when compared to other brain regions. These studies provide evidence that biphalin can not only reach brain sites that express mu and delta-opioid receptors, but it also can reach spinal sites to elicit antinociception. Additional *in situ* brain perfusion experiments identified a saturable component that contributes to CNS entry of [^{125}I-Tyr^1]biphalin. This work provides evidence that biphalin is be a promising, potent analgesic that has a unique mechanism for reaching both spinal and supra-spinal opioid receptor sites. (supported by NIDA #DA-06284).

30.18

IN VITRO BLOOD-BRAIN BARRIER PERMEABILITY AND STABILITY OF A SERIES OF [D-ALA^2]DELTORPHIN I AND II ANALOGUES[1].
S. A. Williams, T. J. Abbruscato, V. Hau, T. J. Gillespie, J. Zsigo, V. J. Hruby and T. P. Davis*. Departments of Pharmacology and Chemistry, The University of Arizona, Tucson, AZ 85724 USA.

[D-Ala^2]Deltorphins are enzymatically stable, amphibian heptapeptides that have a higher affinity and selectivity for *delta*-opioid receptors than any other endogenous mammalian compound known. This study investigated the *in vitro* blood-brain barrier (BBB) permeability, using primary cultured bovine brain microvessel endothelial cells, and the resistance to enzymatic degradation, in 15% mouse brain homogenates and 100% plasma, of [D-Ala^2]deltorphin I, [D-Ala^2]deltorphin II and several analogues. Derivatives were designed with the addition of N-terminal neutral and basic amino acids or with alterations of the amino acids present within the deltorphin sequences. The results indicated that the N-terminal sequence and the amino acids in position 4 and 5 are critical to deltorphin analogue BBB permeability and biological stability i.e. T½ brain; 4.8 hr- [D-Ala^2]deltorphin I; > 15 hr- [D-Ala^2,Ser^4,D-Ala^5]deltorphin. Although, no analogue was found to increase the BBB permeability coefficient (PC; x10^{-4} cm/min) of the parent compounds ([D-Ala^2]deltorphin II, PC=23.49±2.42), analogues were identified ([Arg^0,D-Ala^2]deltorphin II, PC=19.06±3.73; [Ala^1,Pro^0,D-Ala^2]deltorphin II, PC=17.38±1.01) which had similar permeability coefficients, even though they had larger molecular weights and, in the case of the cationic pro-drug, a significantly lower lipophilicity. These results provide directions in the development of future pro-drugs for the treatment of pain and this study further clarifies the structure-activity relationship of the deltorphins. This work was supported by NIDA # DA-06284.

30.19

DEXAMETHASONE INCREASES ZO-1 AT THE JUNCTIONS OF BOVINE RETINAL ENDOTHELIAL CELLS.
A.J. Barber*[1], G.E.W. Rutherford[1], S. Khin[1], E. Lieth[2], and T. W. Gardner[1].
[1]Ophthalmology, and [2]Neuroscience and Anatomy, Penn State University College Medicine, Hershey, PA 17033.

The steroid dexamethasone is known to decrease the transcellular permeability of cerebral endothelial cells, but the mechanism by which this occurs is not known. We tested the hypothesis that dexamethasone increases the accumulation of the tight junction protein ZO-1.

Endothelial cells isolated from bovine retina were purified and grown on fibronectin coated glass coverslips to a confluent monolayer. The cells were treated with 1µM or 100µM dexamethasone for 72 hrs. ZO-1 was detected by fluorescent immunocytochemistry using a monoclonal antibody (Chemicon).

ZO-1 immunoreactivity was clearly visible at the junctions between cells. Immunoreactivity was greater at the junctions of cells treated with 100µM dexamethasone, compared to both 1µM dexamethasone and untreated controls.

Dexamethasone increases the accumulation of ZO-1 at inter-endothelial cell junctions. The mechanism by which this occurs is not known but since dexamethasone has also been reported to upregulate enzymes associated with barrier function it may mimic an endogenous signal to cause expression of barrier properties in endothelial cells. We suggest that dexamethasone causes functional reinforcement of the blood-CNS barrier by increasing tight junction ZO-1.

Funded by NIH (K11 EY00331) and The PA Lions Sight Conservation and Eye Research Foundation.

30.20

POSSIBLE REGULATION BY DEXAMETHASONE OF THE HISTAMINERGIC SYSTEM IN CEREBRAL ENDOTHELIAL CELLS.
K. Karlstedt, T. Sallmén, K.S. Eriksson*, M. Lintunen, K. Wasowicz, H. Fukui, F. Joó and P. Panula. Dept. of Biology, Abo Akademi Univ., 20520 Abo/Turku, Finland, Dept. of Pharmacology II, Osaka Univ., Osaka, Japan and Lab. of Mol. Neurobiol., Inst. of Biophysics, Biological Research Center, 6701-Szeged, Hungary.

Histamine has powerful effects on brain endothelial cells through specific receptors. Previous studies have suggested that the endothelial cells can store histamine and transport it in both directions. The purpose of this study was to find out if endothelial cells possess the capacity to synthesize histamine, and if they express histamine receptors. We also studied the effect of dexamethasone *in vitro*. *In situ* hybridization experiments revealed expression of specific L-histidine decarboxylase (HDC) mRNA in tuberomammillary neurons but not in capillary endothelial cells in rat brain. Histamine immunofluorescence was detected in these neurons and thalamic mast cells, but not in capillary endothelium. RT-PCR amplification showed that an immortalized rat capillary endothelial cell line (RBE-4) does not express HDC indicating that these cells do not synthesize histamine. This was further verified by HPLC-measurements. Histamine could be detected neither in the cultured cells nor in the media. RT-PCR amplification results also indicated the presence of both H_1 and H_2 receptor mRNA in RBE-4 cells. They express H_1 and H_2 receptors and thereby possess the capability to react on histamine. Preliminary RT-PCR studies indicated that dexamethasone downregulates the expression of both H_1 and H_2 mRNA in cultured RBE-4 cells. This again suggests that glucocorticoid hormones may regulate the histaminergic system in the brain not only through HDC but also through specific histamine receptors. Supported by the MRC of the Academy of Finland.

31.1

A CHANNEL FOR MONOAMINE EFFLUX IN SYNAPTIC VESICLES? RAPID EFFLUX OF 1-METHYL-4-PHENYL-PYRIDINIUM ION (MPP+). E. Floor* and A. Gaut. Div. of Biological Sciences, University of Kansas, Lawrence KS 66045

Dopamine (DA) storage in brain synaptic vesicles is highly dynamic and has rapid uptake and efflux components. Efflux by the uptake-independent pathway may involve passive diffusion through the membrane. To test this hypothesis, efflux of the dopamine neurotoxin, MPP^+, a quaternary ammonium ion, was examined. MPP^+ is taken up by synaptic vesicles but, unlike DA, can not exist as a neutral, membrane permeant species. [^{3}H]MPP^+ escaped from rat brain synaptic vesicles with a half-time ($t_{1/2}$) of 2.1 min at 30°C after its uptake was blocked by tetrabenazine. Similarly, [^{3}H]DA escaped with a $t_{1/2}$ of 3.7 min. By contrast, efflux of [^{3}H]MPP^+ ($t_{1/2}$ = 51 h) or [^{3}H]DA ($t_{1/2}$ = 13 h) from 150 nm phosphatidylcholine/cholesterol liposomes was much slower. The identity of [^{3}H]MPP^+ or [^{3}H]DA in synaptic vesicles or liposomes was confirmed by chromatography. [^{3}H]MPP^+ did not escape from synaptic vesicles at 0°C and was taken up by the same vesicles as DA as shown in competition experiments. These results suggest that a transmembrane channel mediates efflux of MPP^+ or DA from synaptic vesicles.

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31.2

EFFECT OF AMPHETAMINE ON SULPHYDRYL GROUPS IN MONOAMINERGIC SYNAPTIC VESICLES FROM RAT PINEAL NERVES. A.Pellegrino de Iraldi* and J.Pablo Corazza. Inst. de Biología Celular y Neurociencias Prof.E.de Robertis, Facultad de Medicina, U.B.A., Paraguay 2155, 1121 Buenos Aires, República Argentina.

Previous studies of our laboratory demonstrated that a mixture of zinc iodide-osmium tetroxide (ZIO) reacts intensely with sulphydryl groups giving an electron dense precipitate. It was also shown that this reaction was intensely positive in monoaminergic synaptic vesicles. In this work we studied the effect of d-amphetamine on the ZIO reaction in rat pineal nerves synaptic vesicles because it has been shown that amphetamine significantly reduces non-protein sulphydryl groups in mouse tissues. Male Wistar rats, 200-300 g body weight, were injected intraperitoneally with 10 mg/Kg d-amphetamine and killed 5, 7, 10 and 15 min later. The pineal glands were extracted, trimmed in two blocks, treated with ZIO and studied at the electron microscope. The ZIO reaction was intensely positive in synaptic vesicles from control untreated animals but it decreased significantly in amphetamine injected rats. In some cases the sulphydryl group reaction was completely negative. Those results suggest that amphetamine may regulate dopamine-β hydroxylase activity through sulphydryl compounds which are known to act as endogenous inhibitors of the enzyme.

This work was supported by CONICET and U.B.A., Rep. Argentina.

31.3

THE FUNCTIONAL COUPLING OF THE CELL BODY TO THE NERVE TERMINAL IS GATED BY AN I_A-LIKE K^+ CONDUCTANCE IN THE AXON OF HIPPOCAMPAL PYRAMIDAL CELLS IN VITRO.

D. Debanne*, N.C. Guérineau, B.H. Gähwiler and S.M. Thompson.
Brain Research Institute, University of Zurich, Switzerland

Pairs of monosynaptically coupled pyramidal cells were recorded with microelectrodes or with the whole-cell technique in hippocampal slice cultures. At the resting membrane potential, presynaptic action potentials (pAPs) evoked a postsynaptic response in all trials, i.e. there were no *release* failures. When the induction of the pAP was preceded by a brief hyperpolarization, no EPSP/C was elicited by the pAP. Such failures of *transmission* were associated with faster repolarization of the pAP. Functional coupling was also blocked when the pAP was preceded by an extracellularly evoked IPSP in the presynaptic cell, thus showing that this process is physiologically relevant. We suggest that activation of a voltage-dependent conductance impedes the conduction of the pAP to the axon terminal. Continuous hyperpolarization of the presynaptic cell to -70/-80 mV blocked pAP-EPSP coupling when pAPs were triggered ≤15 ms after the onset of a depolarizing step but not with longer delays, indicating that the conductance has a fast voltage-dependent inactivation. Recovery from inactivation was tested at hyperpolarized potentials with a depolarizing prepulse. Transmission recovered when the prepulse preceded the pAP by ≤15 ms, but not with longer intervals. Latencies of the monosynaptic EPSPs/Cs increased at the failure threshold, suggesting a local reduction of pAP conduction velocity due to shunting of the pAP (Segev 1990). Block of pAP-EPSP coupling was not observed with all postsynaptic partners of a single presynaptic cell, suggesting that pAP propagation fails at particular axonal branch points. We conclude that an axonal I_A-like potassium conductance can shunt pAPs, and prevent their propagation to all axon terminals.

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31.4

TRANSMISSION OF INFORMATION THROUGH EXCITATORY SYNAPSES.

Budelli, R., Saa, R.E. and Segundo, J.P*. Biomatemática, Facultad de Ciencias, Universidad de la República, Montevideo, Uruguay. Dept. of Neurobiology and Brain Research Institute, University of California, Los Angeles, Ca., 90095-1763 U.S.A.

The conceptual background for applying Communication Theory to the relation, or "coding," between spike trains across synapses was explained earlier (Segundo, Perkel and Moore, 1966, Kybernetik 3:67-82). The probability of a particular presynaptic spike triggering a postsynaptic spike depended on a bounded number of the former called "influential" and packed within a recent epoch called "integration period." Reasonable formulae were proposed for the entropies that measure uncertainities involving the individual trains, as well as for how much uncertainity is removed, --i.e., how much information is provided-- by each train about the other. Stressed were the advantages of using the entire timings --i.e., jointly averages and patterns-- of influential spikes rather than exclusively averages. Finally, called attention to was that physiological issues such as discharge statistics, PSP summation rules and refractoriness were strongly influential.

The present work models an excitatory synapse acting upon a spontaneously inactive neuron represented by a leaky integrator, and then measures precisely the uncertainties and informations present in this simple situation. Results illustrate the magnitudes to be expected. The differential information provided by rates and patterns over rates alone was evaluated for different sets of parameters. The dependence of the influential time and the number of influential presynaptic spikes as a function of the model parameters was determined.

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31.5

RELEASE OF A SOLUBLE PROTEIN KINASE FROM BRAIN NEURONS TO THE EXTRACELLULAR SPACE CAN REGULATE INTERCELLULAR INTERACTIONS

H-A. Yang, M.V. Hogan*,and Y.H. Ehrlich, CSI/IBR Ctr. Dev.Neurosc., Coll. Staten Island, Biol. Phd Prog., Grad. Sch. of the City Univ. of NY, NY 10314.

Extracellular protein phosphorylation that occurs on the surface of brain neurons serves as a target for factors that stimulate neurite outgrowth or can cause neurodegeneration, such as Alzheimer's β-amyloid peptides (Hogan et al., J. Neurochem. 65: 2022-2030,1995). When primary neurons cultured from chick telencephalon were assayed for ecto-protein kinase (PK) activity, about 50% of the surface-radiolabeled 12/13kDa protein duplex phosphorylated as endogenous substrate was found to be released to the soluble fraction (100,000xg/ 90min) prepared from the extracellular medium. These released surface phosphoproteins may be related to the intermediates produced during the processing of amyloid precursor protein (APP) to β-amyloid peptides. When the same soluble fraction was prepared first from the medium, a prominent endogenous phosphorylation of a 17kDa protein was in evidence, and phosphorylation of the 12/13kDa substrates was not detected. We have obtained evidence that different protein kinases phosphorylate the 12/13kDa protein-duplex and the 17kDa protein: MBP (myelin basic protein) competes for the phosphorylation of the 12/13kDa substrates but not for the phosphorylation of the 17kDa protein. Antibody against the catalytic domain of PKC-δ inhibits the phosphorylation of the 12/13kDa, while stimulating the phosphorylation of the 17kDa protein (possibly by indirectly inhibiting its phosphatase). The PKC inhibitor bisindoylmaleimide has the same dual effect. We conclude that the 12/13kDa proteins are specific substrates of a membrane-bound ecto-protein kinase, and the 17kDa protein is a specific substrate of a soluble, exo-protein kinase that is released by neurons to the extracellular environment, and regulates interactions among cells and between the cell surface and components of the extracellular matrix. Supported by NIH grant HD28788 to YHE.

31.6

THE PARS INTERMEDIA OF THE AMPHIBIAN *XENOPUS LAEVIS* AS A MODEL FOR SYNAPSE PLASTICITY,

C.A.F.M. Berghs[1], J.J.H. Hens[2], G.W. Eagleson[1]* and E.W. Roubos[1]. [1]Department of Cellular Animal Physiology, Nijmegen Institute for Neurosciences, University of Nijmegen, Toernooiveld, 6525 ED Nijmegen and [2]Department of Medical Pharmacology, Rudolf Magnus Institute for Neurosciences, University of Utrecht, The Netherlands.

The process of background adaptation of the clawed toad *Xenopus laevis* is regulated by the melanotrope cells in the pars intermedia of the pituitary gland, which produce and release the pro-opiomelanocortin (POMC)-peptide αMSH. Suprachiasmatic neurons are responsible for the inhibitory control of the melanotropes. Immunoelectron microscopy, using freeze-substituted material, shows that their synapses in the pars intermedia contain GABA, NPY and dopamine. GABA is present in electron-lucent vesicles, whereas NPY and dopamine coexist in dense vesicles. Morphologically specialised synaptic contacts ("active zones") with melanotrope cells show clustering of exclusively lucent vesicles to the presynaptic membrane, indicating release of GABA. Ultrastructural morphometry reveals a strong plasticity of the synapses, in relation to background adaptation: transferring animals from a black to a white background leads to a significant increase in the number and size of the synapses and their active zones. This morphological plasticity of the synapses is reflected in a plasticity of the neurotransmitter contents: biochemical studies demonstrate effects of background on the storage and release of dopamine, GABA and NPY. These findings show that the pars intermedia is a valuable tool in the study of synapse plasticity. Studies of the underlying mechanisms of neurotransmitter release in *Xenopus* are currently in progress.

Supported by university grant.

31.7

CHARACTERIZATION OF NEURONAL PROPERTIES OF HUMAN POSTMITOTIC NT2N CELLS. D.B. Hawver*, A. Khaldi, A. Rashid, S. Doron, C.C. Chiueh, and W.Z. Potter, Section of Clinical Pharmacology, Experimental Therapeutics Branch, and Laboratory of Clinical Science, NIMH, Bethesda, MD 20892.

Cultures of NT2N cells were studied after 5 weeks of treatment with retinoic acid, 2 weeks with mitotic inhibitors, and 4-7 weeks of outgrowth on Matrigel-coated dishes. NT2N cells were able to transport 3H-GABA>>3H-glutamate>3H-choline>3H-glycine, but not 3H-NE, 3H-DA, or 3H-5HT. Uptake of 3H-GABA, 3H-glutamate, and 3H-choline was substantially reduced in the presence of the specific transporter inhibitors NO-711, L-trans-pyrrollidine-2,4-dicarboxylic acid, and hemicholinium-3, respectively. Stimulation with 100 mM KCl evoked release of transmitter from cells preloaded with 3H-glutamate or 3H-choline, but not 3H-GABA or 3H-glycine. KCl-evoked release of transmitter was dependent on extracellular Ca++ and inhibited by verapamil. Fura-2 studies showed increases in intracellular Ca++ levels in NT2N cells exposed to KCl, glutamate, veratridine, 4-aminopyridine, or BAYK8644. Calcium responses to KCl were blocked by verapamil, nimodipine, and cobalt chloride. The response to veratridine was blocked by tetrodotoxin. Thus, depolarization of NT2N cells with KCl is able to evoke release of neurotransmitters by increasing intracellular calcium through activation of calcium channels with L-type pharmacology. In summary, our results demonstrate that NT2N cells exhibit many properties of CNS neurons, and therefore provide a valuable system for the study of multiple aspects of CNS neuronal function.
(supported by NIGMS PRAT Program and NIMH, IRP, NIH)

31.8

CALCIUM-SECRETION RELATIONSHIPS OBSERVED IN BOVINE CHROMAFFIN CELLS IN RESPONSE TO PULSE TRAINS. N.I. Chernevskaya*, K.L. Engisch, & M.C. Nowycky, Dept. Neurobiol. & Anat., Med. Coll. Penn., Philadelphia PA, 19129.

We have examined Ca^{2+}-secretion coupling in individual bovine adrenal chromaffin cells, using the capacitance detection technique to monitor exocytosis (ΔC_m). Recordings were made in the perforated patch mode and cells were stimulated with trains of 5, 40, or 80 ms duration pulses (200 ms interval, V_H = -90 mV, V_T = +10 mV, 10-40 pulses). ΔC_m was plotted against the cumulative integral of the Ca^{2+} current during a train. At the beginning of a train, the slope of the ΔC_m-Ca^{2+} load relationship was similar for the 3 different pulse durations. However, significant deviations occurred later in the train. Many (57%) of the 5 ms pulse trains exhibited an increased sensitivity to Ca^{2+} entry later in the train, while the majority (90%) of 40 and 80 ms pulse trains showed either a decreased responsiveness or maintained the initial relationship. These findings are strikingly different from results obtained in whole cell recordings (J. Neurosci. 16; 553, 1996). To determine the role of intracellular Ca^{2+} [Ca^{2+}]i on differences in Ca^{2+}-secretion-coupling, we loaded cells with fura-red AM and monitored [Ca^{2+}]i as changes in dye fluorescence with a Gen-3 video camera. During trains, [Ca^{2+}]i quickly reached a plateau (within 1-3 pulses of 40 or 80 ms duration and ~10-15 pulses of 5 ms duration) which did not change for the remainder of the train, again unlike the whole cell configuration. Thus, changes in [Ca^{2+}]i levels alone cannot account for the different Ca^{2+}-secretion relationships. Supported by NS22281 and Muscular Dystrophy Association.

31.9

A MIXED GIANT SYNAPSE BETWEEN THE MEDIAL AND MOTOR GIANT FIBERS IN THE ABDOMINAL NERVE CORD OF *PENAEUS ORIENTALIS*. K .XU and X.Q. Shu*. Shanghai Institute of Physiology, Chinese Academy of Sciences, Shanghai 200031, China.

The preparations of the chemical giant synapse of loligo and the electrical giant synapse of crayfish have been successfully used for analysing the mechanism of the two kinds of synapses accordingly. In our experiments the giant synapse between the medical and motor giant fibers was identified as not only an electrical one by demonstrating the dye (lucifer yellow) coupling in it, but also a chemical one by finding the densely populated transparent synaptic vesicles in diameter of about 50 nm in its presynaptic side. The identification of the transmitter of the mixed giant synapse is going on.

SODIUM CHANNELS: EXPRESSION AND CLONING

32.1

ANALYSIS OF VOLTAGE-SENSITIVE Na^+ CHANNEL β1 SUBUNIT GENE EXPRESSION USING QUANTITATIVE RT-PCR. Youngsuk Oh*. Department of Medicine, Division of Nephrology, and Neurobiology Research Center, University of Alabama, Birmingham, AL 35294.

Two isoforms ($\beta_{1.1}$ and $\beta_{1.2}$) of the Na^+ channel β1 subunit (Naβ1) mRNAs have been found in certain glial cells as well as in neurons. The coding regions of the $\beta_{1.1}$ and the $\beta_{1.2}$ mRNAs are nearly identical, but the $\beta_{1.2}$ mRNA contains an additional 86-nucleotide insert (which is an intron) in 3' noncoding regions. In the present study, I have constructed a competitive Naβ1 DNA template that is cloned into pCR II vector and can be readily discriminated from the $\beta_{1.1}$ and the $\beta_{1.2}$ because of its different nucleotide size. The same primers were used to co-amplify the $\beta_{1.1}$ (974 bp), the $\beta_{1.2}$ (1060 bp), and the competitor (880 bp). For the application of the Naβ1 competitor, two different methods were evaluated by determining basal levels of Naβ1 mRNAs in cultured cortical or spinal cord astrocytes derived from postnatal day 0-5 Sprague-Dawley rats. First, a dilution series of the competitor DNA was added into a constant amount of astrocyte cDNA during PCR reaction. Second, a dilution series of the synthesized competitor cRNA was added into a constant amount of astrocyte RNA during RT reaction. The PCR products were resolved by gel electrophoresis, and quantitated by analyzing a digitalized image of the ethidium bromide stained gel. The results indicate that both DNA and RNA competitors are suitable for analyzing Naβ1 gene expression by quantitative RT-PCR. This quantitative competitive RT-PCR will be useful to study Naβ1 gene expression in response to various stimuli, such as cAMP treatment. [Supported by NS 34877]

32.2

IDENTIFICATION OF SODIUM CHANNELS IN HUMAN DORSAL ROOT GANGLIA. D.K. Rabert*, M.J. Ilnicka and L. Sangameswaran. Institute of Pharmacology, Roche Bioscience, Palo Alto, CA 94303.

RT-PCR was used to amplify voltage-gated sodium channel α-subunits that are expressed in human dorsal root ganglia (DRG). Nested, degenerate primer sets were designed to amplify either one of the major domains (I-IV) or one of the interdomain regions. Two known human α-subunits were detected: 1) human brain HBA, 100% identity for the amino acid sequence between IIIS5 and IVS1, and 2) human neuroendocrine, 100% identity for the amino acid sequence between IVS1 and IVS6. The human neuroendocrine sodium channel was previously detected in the thyroid and adrenal gland (EMBO J., 14:1084, 1995). This study suggests that the neuroendocrine channel is a homologue of the rat PN1 channel based on the expression of the neuroendocrine channel in the DRG and the 93% identity of the amino acid sequence that is shared between the two channels. Tissue distribution of the PN1-like channel will be discussed.

32.3

SPINAL SENSORY NEURONS EXPRESS MULTIPLE SODIUM CHANNEL ALPHA SUBUNIT mRNAs J.A. Black, S. Dib-Hajj, K. McNabola, S. Jeste, M.A. Rizzo, J.D. Kocsis and S.G. Waxman. Dept. of Neurology, Yale Univ. Sch. of Med., New Haven, CT and Neuroscience Res. Ctr, VA Medical Center, West Haven, CT 06516

Spinal sensory (dorsal root ganglion [DRG]) neurons constitute a heterogeneous population of cells, and multiple, distinct voltage-gated Na currents have been described in these cells. Neural Na channels are trimeric complexes composed of α-, β1- and β2-subunits. The α-subunits belong to a multigene family, with at least eight distinct isoforms being described in neural tissue. The molecular correlates underlying Na current diversity in DRG neurons has not been established. In the present study, we use *in situ* hybridization and RT-PCR to examine the expression of Na channel mRNAs in DRG neurons *in vitro* and *in vivo* from adult rats.

Riboprobes specific for Na channel mRNAs I, II/IIA, III, NaG, Na6, hNE-Na, SNS, mNav2.3, β1 and β2 were constructed, and DRG neurons *in vitro* and *in vivo* processed for *in situ* hybridization, as previously described (Black et al. 1994a,b). RT-PCR was performed on total cellular RNA extracted from brain and L4/5 DRG with primers designed against sequences in domains 1 and 4, and also against 3' untranslated sequences as previously described (Dib-Hajj and Waxman 1995)

The results demonstate that multiple Na channel α-subunit mRNAs, in addition to β1- and β2-subunit mRNAs, are expressed in DRG neurons *in vitro*, with high levels of expression for mRNAs I, NaG, Na6, hNE-Na, SNS, β1 and β2. Moreover, the pattern of expression of Na channel mRNAs in DRG neurons *in vitro* at 1 day in culture is similar to that observed in DRG neurons *in vivo*. [Supported by Medical Research Service, VA and NMSS]

32.4

STRUCTURE, FUNCTION AND CHROMOSOME MAPPING OF A NOVEL VOLTAGE-GATED, TETRODOTOXIN-RESISTANT SODIUM CHANNEL SPECIFIC TO SENSORY NEURONS. L. Sangameswaran, S. G. Delgado, L. M. Fish, B. D. Koch, C. Kozak[1], L. B. Jakeman, G. R. Stewart, P. Sze, J. C. Hunter, R. M. Eglen and R. C. Herman. Institute of Pharmacology, Neurobiology Unit, Roche Bioscience, Palo Alto, CA 94304. [1] Laboratory of Molecular Microbiology, NIAID, NIH, Bethesda, MD 20892.

Small neurons of the dorsal root ganglia (DRG) play a significant role in nociception. These neurons express two types of sodium current, which differ in their inactivation kinetics and sensitivity to tetrodotoxin (TTX). We have cloned the α-subunit of a novel voltage-gated sodium channel, PN3 (Peripheral sodium channel 3) from rat DRG. In *Xenopus* oocytes PN3 exhibited slow inactivation kinetics and little or no activation at -10mV, whereas most cloned sodium channels begin to activate between -60 and -30mV in this sytem. PN3 sodium current is resistant to TTX (IC_{50} ≥100μM). PN3 mRNA was expressed at high levels only in DRG and nodose ganglia. It was not detected in brain, spinal cord, superior cervical ganglia, heart and skeletal muscle. *In situ* hybridizaion to rat DRG indicated that PN3 expressed primarily in small sensory neurons of the peripheral nervous system. Using a specific DNA probe for PN3, we have mapped the location of the PN3 gene (*SCN10a*) to mouse chromosome 9, close to the TTX-insensitive cardiac sodium channel, *SCN5a*.

32.5

NA^+ CHANNEL β1-A: AN AUXILIARY SUBUNIT EXPRESSED IN EMBRYONIC BRAIN AND NEUROENDOCRINE TISSUES. L. L. Isom* and K. Kazen-Gillespie. Department of Pharmacology, The University of Michigan Medical School, Ann Arbor, MI. 48109-0632.

Voltage-gated Na^+ channels are composed of a central, pore-forming α subunit and one or two auxiliary β subunits. The β subunits have been shown to be important modulators of channel kinetics and plasma membrane expression levels. Na^+ channel β1 subunits cloned from brain have been shown to be expressed only after birth, whereas β2 subunits have been shown to be expressed early on in embryonic development and have been postulated to play a role in neurogenesis and synaptogenesis. Both β1 and β2 contain immunoglobulin (Ig) -like domains with homology to neural cell adhesion molecules . Thus, β subunits may also function in Na^+ channel localization during brain development. We now describe the cloning of a β1 subunit isoform that is significantly different from that originally reported. This new isoform, β1-A, was isolated from a rat adrenal cDNA library. β1-A contains a central region of identity to β1 and two regions of non-identity, but retaining structural homology, at the 5' and 3' ends. Interestingly, the central region of identity includes most of the putative Ig domain found in β1. The domains of non-identity retain structural homology to the remainder of the Ig domain. Outside of the putative Ig loop, however, the amino acid sequence of β1-A contains little or no homology to β1 or β2. Thus, β1-A is a new member of the Ig superfamily, homologous to members of the superfamily that are termed V-like, including the neural cell adhesion molecules. Investigation of the tissue-specific expression of β1-A mRNA by Northern blot analysis revealed two positive hybridization signals at 1.4 and 4 kb. The mRNA migrating at 4 kb is expressed in embryonic brain, adult adrenal, and PC12 cell line cells. The signal in embryonic brain diminishes as development progresses and is not detected after birth. The lower band is more widely expressed, showing positive signals in embryonic and adult brain, heart, skeletal muscle, and adrenal. *In situ* hybridization analysis indicated that β1-A is expressed in adrenal medulla but not in adrenal cortex, consistent with the hypothesis that β1-A may be an auxiliary subunit of adrenal chromaffin cell Na^+ channels. Supported by the Michigan Diabetes Research and Training Center 5-P60-DK20572-19.

32.6

LOCALIZATION OF SODIUM CHANNEL ALPHA SUB-UNIT NaG mRNA IN THE ADULT RAT. P.A. Felts*, J.A. Black, S. Dib-Haj and S.G. Waxman. Department of Neurology, Yale Univ. School of Medicine, New Haven, CT 06512.

The rat sodium channel alpha subunit NaG was originally cloned from a cultured cortical astrocyte cDNA library. NaG mRNA has been shown by RNase protection assay and Northern blot to be expressed at only very low levels within the adult rat brain, but at substantially greater levels within the dorsal root ganglion (DRG) (Gautron *et al., PNAS*, 89:7272). Previous studies in our laboratory (Waxman and Black, *Neurochem. Res.*, in press), utilizing non-radioactive *in situ* hybridization with an antisense riboprobe against a coding region of NaG (436 bp; GeneBank numbering 872-1308), demonstrated a lack of labeling in the adult CNS, but showed that NaG mRNA was present in adult rat DRG neurons. As DRG neurons are: 1) primary afferents and 2) derived from neural crest, we have used this technique, in some cases in conjunction with RT-PCR, to examine expression of NaG mRNA in several related tissues to determine if the expression of this transcript is correlated with one or both of these properties.

Hybridization signal with the NaG antisense riboprobe was observed in the primary afferents of the trigeminal ganglion, and the mesencephalic nucleus of cranial nerve V (the only primary afferent neuronal somata located within the CNS). However, labeling was absent from neurons within the spiral ganglion, vestibular ganglion, olfactory epithelium and retina. Of the non-afferent, neural crest derived tissues examined, light labeling was present in the chromaffin cells of the adrenal medulla (with scattered cells exhibiting dense label); neurons of the superior cervical ganglion were unlabeled, although satellite cells surrounding these neurons did exhibit moderate labeling. RT-PCR confirmed the lack of NaG mRNA in the retina, and demonstrated the presence of NaG mRNA within the trigeminal ganglion, and, in agreement with the findings by Gautron *et al.*, within the adrenal gland.

Our findings demonstrate that NaG, or a closely related alpha subunit, is expressed in some, but not all, primary afferent neurons, and in some, but not all, neurons derived from the neural crest. [Supported by the VA and NMSS]

32.7

CLONING AND DISTRIBUTION OF THE MECHANOSENSORY- LIKE GENE EXPRESSED IN THE RAT NERVOUS SYSTEM.
Y. Minami[1,2], S. Shimada[1]*, K. Inoue[1], H. Morimura[1], T. Matsunaga[2] and M. Tohyama[1], [1]Dept.of Anatomy & Neurosci., Osaka Univ. Medical School, 565,Japan. [2]Dept.of Otolaryngol., Nara Medical Univ., Nara, 634,Japan.

Mec (mechanosensory) genes are involved in the transduction of touch sensory in the nematode *C. elegans*. Especially mutation of degenerin gene family (mec-4, mec-10, mec-6, deg-1) cause neuronal degeneration. On the other hand, the amiloride-sensitive sodium channel was identified from rat colon, kidney and lung, and the subunit sequences of this channel showed significant identity with degenerin gene family. Recently, peptide-gated sodium channel was identified from *Helix* nervous tissue, it is suggested that these genes constitute a new gene family which is associated with mechanotransduction or the maintenance of the cellular volume or neurotransmission. We isolated a novel gene belong to these gene family from rat brain, and examined the distribution of this mechanosensor-like gene expression by in situ hybridization. Hybridization signal was seen in the almost all neuronal cells in the brain and retina. It is suggested that this gene may be involved in the osmosensor or the unknown peptide-gated ion channel in the rat nernous system.

32.8

CHARACTERIZATION OF VOLTAGE-DEPENDENT ION CHANNELS IN HUMAN BREAST CANCER CELLS. C.E. Davis, T.J. O'Shaughnessy, L.S. Gray, H.R. Brashear*, and Y.I. Kim. Departments of Biomedical Engineering, Neurology, and Pathology, University of Virginia School of Medicine, Charlottesville, VA 22908.

Paraneoplastic neurological syndromes (PNS) are "remote effects" of cancer on nervous structures. They are believed to be autoimmune in nature, with disease symptoms brought about by autoantibodies against tumor antigens cross-reacting with the pathological target. In this study, we tested the possibility that human breast cancer cells may express voltage-dependent ion channels acting as potential antigens for PNS. Using the whole-cell patch-clamp technique, we initially characterized the types of ionic currents present in the tumor cells.

Of six breast cancer cell lines studied, five showed expression of at least one type of voltage-dependent ion channel. Cell line DU4475 was found to express a TTX-sensitive sodium current with a mean peak amplitude of 74 pA (n=88 cells), as well as a 90 pA (n=20) potassium current in 87% of the cells tested. In addition, preliminary data suggest that a calcium current (10-50 pA) may be present in these cells. Cell line MDA-MB-157 expressed I_{Na} (52 pA, n=7) in 35% of the cells and a 28 pA current in 30% of the cells tested (n=6), which had biophysical properties characteristic of I_{Ca}. Cell line MCF-7 was found to contain a 375 pA (n=12) whole-cell I_K in 57% of the cells. In 27% of these cells, only a rapidly inactivating I_K was seen (169 pA, n=7). Cell lines MDA-MB-468 and BT-549 also expressed I_K in the majority of cells tested.

These results provide experimental evidence for the presence of voltage-dependent ion channels in human breast cancer cells. Possible cross-reactivity of autoantibodies in PNS with these ion channels remains to be determined. (Supported by grants from NIH and MDA)

32.9

STRUCTURAL AND FUNCTIONAL FEATURES OF VOLTAGE-GATED SODIUM CHANNELS IN A HYDROZOAN JELLYFISH J.D. Spafford*, N. Grigoriev, A.N. Spencer and W.J. Gallin. Department of Biological Sciences, University of Alberta, Edmonton, Alberta, Canada T6G 2E9.

Whereas most animals' Na^+ currents are blocked by nanomolar concentrations of TTX, all known cnidarians' currents are insensitive to concentrations in excess of 100 µM. In fact, no common Na^+ channel-specific drugs effective on mammalian currents alter the TTX-insensitive Na^+ current in swimming motor neurons ("SMNs") of the hydrozoan jellyfish, *Polyorchis penicillatus*. The Na^+ current in SMNs also differs in its activation and inactivation curves, which are displaced 35 to 45 mV in the depolarizing direction relative to most Na^+ currents. In other regards, the current is typical, with multiple modes of inactivation gating and steeply voltage-dependent rates of activation and steady-state inactivation. The current also exhibits a channel permeability to monovalent cations and sensitivity to block by di-(tri)valent cations in rank orders that are typical of mammalian Na^+ channels. The Na^+ current in SMNs is one of three which have been identified in primary culture of jellyfish cell networks in whole cell patch clamp recording.

We have identified a single 6 kb Na^+ channel transcript in a Northern blot with probes made from fragments of a 13.5 genomic clone. Sequences from coding regions of the genomic clone have an amino-acid similarity of ~70% to cloned mammalian and *Drosophila* Na^+ channels and 85% similarity with a Scyphozoan jellyfish clone. We are currently isolating a full-length cDNA clone of the 6 kb transcript. Data from the full-length expressed clone will help our understanding of the structural and functional features that make the subclass of Na^+ currents in jellyfish unique among those in the voltage-gated Na^+ channel family.

This research was supported by grants from the Natural Sciences and Engineering Research Council.

32.10

DOPAMINE MODULATES SODIUM CURRENT VIA PKA ACTIVATION IN RAT HIPPOCAMPAL NEURONS. A.R. Cantrell*, T. Scheuer, and W.A. Catterall, Department of Pharmacology, University of Washington, Seattle, WA 98195.

Voltage -gated sodium channels play an important role in neuronal excitability in the hippocampus but are infrequently recognized as targets for neuromodulation. As previously reported by our laboratory, phosphorylation of brain sodium channels by protein kinase A (PKA) decreases peak current. D1-like dopamine receptors on hippocampal neurons activate PKA. Therefore, we have tested whether sodium channels can be modulated by activation of D1-like receptors in acutely-isolated adult (3-5 wks postnatal) hippocampal neurons using whole-cell voltage clamp recording.

Application of the D1 agonist, 6ClPB (5µM), reduced peak sodium current an average of 25.3 ± 1.5%. No changes in the voltage dependence of activation or inactivation were detected. These effects were mediated via PKA as they were eliminated when a specific PKA inhibitor peptide ($PKAI_{5-16}$) was included in the pipette solution and mimicked by the extracellular application of the PKA activator, DClcBIMPS.

These results demonstrate that activation of endogenous D1-like dopamine receptors on hippocampal neurons strongly modulates sodium channel activity by activation of PKA. Such modulation is expected to have profound effects on overall neuronal activity. This work was supported by NIH grant NS15751 to W.A.C. and a postdoctoral fellowship from NIDA training grant DA07278 to A.R.C.

32.11

DIFFERENTIAL REGULATION OF SODIUM CHANNEL α - AND β -SUBUNIT mRNAS BY PROTEIN KINASE C ACTIVATION IN ADRENAL CHROMAFFIN CELLS. T. Yanagita, H. Kobayashi, K. Masumoto, R. Yamamoto, T. Yuhi, H. Yokoo and A. Wada*. Dept of Pharmacology, Miyazaki Medical College, Miyazaki 889-16, Japan.

Our previous [^{3}H]saxitoxin binding and $^{22}Na^+$ influx studies have shown that an activation of protein kinase C (PKC) α - and/or ε -isoforms by 12-0-tetradecanoylphorbol-13-acetate (TPA) down-regulates the number of functional Na^+ channels at plasma membranes with IC_{50} 19 nM and $t_{1/2}$ 4.5 h, and it is mediated via the de novo synthesis of protein(s) (J. Neurochem. 66:1249-1253, 1996).

Present northern blot analysis with cDNA probes for human neuroendocrine type Na^+ channel (hNE-Na) α -subunit and rat brain β_1-subunit was undertaken to examine the effects of TPA on the level of Na^+ channel subunit $mRNA_s$. Teatment of cultured bovine adrenal chromaffin cells with 100 nM TPA decreased α -subunit mRNA within 1 h, the reduction being maximal (by 58%) at 6 h, and restoring toward control level within 24-48 h, while TPA raised β -subunit mRNA by 130% at 24 h with an initial small decrease at 6 h. These results suggest that α - and β - subunits of Na^+ channels in adrenlal chromaffin cells are structurally related to those of hNE-Na and rat brain, and that PKC reduces the abundance of transcript of α - (but not β -) subunit, culminating in the down-regulation of cell surface expression of Na^+ channels.

(Supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture, Japan.)

32.12

INDUCTION OF PERSISTENT Na^+ CURRENT BY OVEREXPRESSION OF G-PROTEIN βγ SUBUNITS. J.Y. Ma*, W.A. Catterall and T. Scheuer. Dept. of Pharmacol., Box 357280, Univ. of Washington, Seattle, WA 98195.

We have shown that rat brain Na^+ currents are modulated by activation of G proteins. To determine the effects of particular G-protein subunits on brain Na^+ channels, we transiently overexpressed them in combination with rat brain type IIA Na^+ channel α subunits in tsA201 cells. Overexpression of G-protein subunits modulated the presence of a TTX-sensitive, slowly and incompletely inactivating fraction of Na^+ current in whole cell voltage clamp recordings from these cells. In the absence of exogenous G-protein subunits, a small component of Na^+ current persisted after 2 ms of depolarization to +20 mV (8%). Overexpression of Gβ2γ3 increased the component of current not inactivated at 2 ms 2.5-fold to 30%, with individual cells having >50% non-inactivated current. Conversely, overexpression of Gαo or Gαi resulted in a rapidly inactivating current (13% not inactivated at 2 ms), even in the presence of overexpressed Gβ2γ3. These results suggest that Gβ2γ3 subunits induce the slowly inactivating component of Na^+ current, and Gα subunits inhibit the effect by binding and sequestering Gβγ subunits. The slowly inactivating current inactivated at more positive potentials than the rapidly inactivating current. Inactivation curves in cells expressing Gβ2γ3 showed two well-defined Boltzmann components with the positive component corresponding to the slowly inactivating current and shifted >30 mV positive to the negative component. We used prepulses to -25 mV to isolate the slowly inactivating component of current. Its voltage-dependence of activation was similar to that of the rapidly inactivating current but inactivation was slowed and incomplete at all test pulse potentials. Such slowly inactivating and persistent Na^+ currents are characteristic of a variety of central neuronal cell types and are thought to be important modulators of cell function. These persistent Na^+ currents may result from modulation of a single Na^+ channel subtype by G protein βγ subunits.

Supported by NIH training grant NS09797 to J.Y.M.

32.13

TRANSCRIPTIONAL REGULATION OF HUMAN BRAIN SODIUM CHANNEL GENES: CLONING AND CHARACTERIZATION OF THE β1 SUBUNIT 5'-FLANKING REGION. M.L. Beckman* and G.B. Brown. Psychiatry and Behavioral Neurobiology and The Department of Biochemistry and Molecular Genetics, Univ. of Alabama at Birmingham, Birmingham, AL 35294

Voltage-dependent sodium channels from central neurons are composed of a heterotrimeric complex of α, β1 and β2 subunits in a 1:1:1 stoichiometry. Several alpha subunit cDNAs as well as cDNAs for the β1 and β2 subunits have been obtained in rat, and with the exception of β2, in human as well. Coexpression of the β1 subunit with α modifies the electrophysiologic properties of the expressed α subunit by accelerating inactivation, shifting the voltage-dependence of inactivation to more negative membrane potentials and increasing the peak amplitude of sodium current (W.A. Catterall, Annu.Rev.Biochem. 64:493-531, 1995).

Toward gaining an understanding of the transcriptional regulation of the β1 subunit we have undertaken to clone and characterize the 5'-flanking region. A digoxigenin-labeled 416 base pair probe was generated by the polymerase chain reaction and used to screen a human genomic library in λ phage MG14 (Rotwein, et al. JBC. 261:4828-4832, 1986). Initial first round positive clones were taken through two additional rounds of screening and phage were then purified from a liquid lysate. A clone was obtained and the restriction map determined for a number of enzymes. Subsequently, the clone was confirmed to be a β1 subunit by the polymerase chain reaction using β1 specific primers.

Further characterization will include sequencing, subcloning into luciferase reporter vectors and functional studies of the promoter activity of segments of the 5'-flanking region. These studies will make use of the human neuroblastoma cell line LA-N-5 which has been shown to express the β1 subunit (Beckman and Brown, Soc.Neurosci.Abs. 21(3) 714.6). (Supported by USPHS grant DA07237)

32.14

TRANSCRIPTIONAL REGULATION OF HUMAN BRAIN SODIUM CHANNEL GENES: IDENTIFYING THE TRANSCRIPTIONAL START SITE OF THE SUBTYPE II GENE. S.D. Schade*, C.-M. Lu, J.S. Eichelberger, M.L. Beckman, and G.B. Brown. Dept. of Psychiatry and Behavioral Neurobiology. Univ. of Alabama at Birmingham. Birmingham, AL 35294

Voltage-gated sodium channels are responsible for the rising phase of the action potential in excitable cells. Human neuronal sodium channel subtype-specific mRNAs are differentially distributed regionally (Lu et al., FEBS Lett. 303(1):53-58, 1992), suggesting differential transcriptional regulation. Previously we described cloning, sequencing, and putative transcriptional start sites by primer extension and 5' RACE (rapid amplification of cDNA ends) for the subtype II gene (Eichelberger et al., Soc. Neurosci. Abs. 20(1), 33.14, 1994). Here we describe additional studies to identify the transcriptional start site and promoter region of this gene.

Two genomic subclones upstream of the translational start site identified by Ahmed et al. (PNAS 89: 8220-8224, 1992) were constructed to investigate potential promoter and regulatory elements in the immediate 5'-flanking region. The 2.7kb subclone overlaps and extends 5' to the translational start site. The 1.9kb subclone abuts the 2.7kb subclone further upstream. The 1.9kb subclone has been cloned into the promoterless and basic luciferase vectors for transient transfection into the human neuroblastoma cell line, SH-SY5Y. Luciferase assays show the 1.9kb subclone does not drive luciferase expression and thus does not contain the promoter region. Concordantly, ribonuclease protection assays (RPA) showed the 1.9kb clone does not contain the transcriptional start site. Within the 2.7kb subclone, primer extension and 5' RACE products map potential transcriptional start sites to 261 and 318 nucleotides upstream of the 5' end of a 1.7kb intron within the 2.7kb subclone, but preliminary RPA experiments suggest the transcriptional start site is 70 nucleotides further upstream.

Supported by USPHS grant DA 07237.

32.15

THE REGULATION OF SODIUM CHANNEL SUBUNITS IN THE HEK293 MAMMALIAN CELL LINE. S.L. Stewart*, S.J. Wieland, and J.E. Fletcher. Dept. of Anesthesiology, Med. Col. of PA and Hahnemann Univ., Phila., PA 19102

The human skeletal muscle Na^+ channel consists of one of two α subunits (adult and embryonic forms), which are the functional subunits of the channel, and a modulatory β_1 subunit. In *Xenopus* oocytes injected with mRNA for the adult α subunit alone, Na^+ channels are expressed which exhibit slow inactivation kinetics, however, co-injection with mRNA for the β_1 subunit restores normal rapid inactivation kinetics. In contrast, plasmids containing α subunits alone expressed in mammalian cells produce channels which exhibit normal rapid inactivation kinetics. In the present study, human embryonic kidney (HEK293) cells, with very low or no expression of Na^+ current, were transfected with a plasmid containing either the adult skeletal muscle sodium channel α subunit (SkM1), or the embryonic skeletal muscle α subunit (SkM2). Poly A^+ mRNA was reverse transcribed and the cDNA was amplified by PCR using primers to the Na^+ channel subunits. Transfection with either SkM1 or SkM2 induced transcription of the β_1 subunit. These findings suggest that α subunit expression induces β_1 subunit transcription, and that the normal rapid inactivation kinetics exhibited by mammalian cultures transfected with Na^+ channel α subunits may reflect the interaction between the α and β_1 subunits.

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32.16

Temporal regulation of changes in the expression of specific Na channel α subunits in adult rat DRG neurons following axotomy. S. D. Dib-Hajj*, P. Felts, A. Kenney, J. A. Black and S. G. Waxman. Department of Neurology, Yale University School of Medicine, New Haven, CT. and Neuroscience & Regeneration Research Center, VA Medical Center, West Haven, CT. 06516.

Our group has previously demonstrated that, following axotomy, there is an increase in the amplitude of Na currents and the expression of the previously silent α III subunit mRNA in adult rat DRG neurons. More recently, we also demonstrated that there is an enhancement of the fast, TTX-sensitive current and a decline in the slow, TTX-resistant current in identified medium size cutaneous afferent DRG neurons. We used RT-PCR to investigate the changes in the α subunit expression profile in adult rat DRG neurons following axotomy at 1, 3, 5, 7, 14, 21 and 210 days post ligation (dpl).

Preliminary results show that the previously silent αIII subunit is expressed, as previously reported, and the expression level of the slow, TTX-resistant αSNS is reduced in the DRG neurons of the Ipsilateral (lesioned) side compared to the contralateral (control) side. Transcripts of αIII were detectable at 5 dpl but were undetectable by 21 dpl. Restriction enzyme polymorphism analysis shows only the adult form of αIII. Therefore, the expression of this subunit may not be a recapitulation of a developmental program, or axotomy does not restore factor(s) responsible for the embryonic-specific alternative splicing of this transcript. Transcripts of αSNS appear to first increase at 1 and 3 dpl, then decrease between 5-14 dpl and partially recover by 21 dpl. No detectable difference from control is seen at 210 dpl.

This work is supported in part by the Medical Research Service, VA and NMSS.

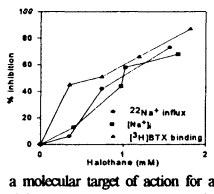
SODIUM CHANNELS: PHARMACOLOGY

33.1

INHIBITION OF VOLTAGE-DEPENDENT SODIUM CHANNELS BY HALOTHANE IN RAT CORTICAL SYNAPTOSOMES.

L.Ratnakumari and H.C.Hemmings, Jr.* Departments of Anesthesiology and Pharmacology, Cornell University Medical College, New York, NY 10021, USA

Although voltage-gated ion channels have been claimed to be insensitive to the action of general anesthetics, recent studies have shown that the volatile anesthetic halothane reduces Na^+ currents through Na^+ channels.[1] Volatile anesthetics inhibit synaptosomal glutamate release, probably through an effect on Na^+ channels.[2] In the present study, the effects of halothane on Na^+ channel function and ligand binding were examined by measuring changes in $^{22}Na^+$ influx, free intracellular sodium levels ($[Na^+]_i$) and binding of [^{3}H]saxitoxin ([^{3}H]STX), [^{3}H]batrachotoxin-A 20-α-benzoate([^{3}H]BTX) and [^{3}H]brevetoxin-3 ([^{3}H]Pbtx-3) in rat cortical synaptosomes. Synaptosomes were isolated from cerebral cortex of adult Sprague-Dawley rats by the Percoll gradient method. $[Na^+]_i$ was measured by ion-specific spectrofluorimetry using SBFI-AM as Na^+-sensitive fluorophore. Halothane inhibited veratridine-evoked $^{22}Na^+$ influx and changes in $[Na^+]_i$ dose-dependently with IC_{50} values of 1.1 and 0.96 mM (MAC=0.3 mM), respectively, without affecting basal levels. [^{3}H]BTX binding to receptor site 2 of the Na^+ channel was also significantly reduced in the presence of halothane with 50% inhibition at 0.5 mM; [^{3}H]STX binding to receptor site 1 was unaffected. [^{3}H]Pbtx-3 binding at site 5 of Na^+ channel was increased marginally (10%, $p<0.05$) by halothane. These results provide biochemical evidence that halothane inhibits Na^+ channels at clinically relevant concentrations and support a role for Na^+ channels as a molecular target of action for anesthetics. ([1]Anesthesiology, 75:716, 1991; [2]Anesthesiology, 82:1406, 1995; Supported by NIH grant # GM 52441and Cornell Scholar Award).



33.2

THE NMDA RECEPTOR ANTAGONISTS IFENPRODIL AND ELIPRODIL BLOCKED VOLTAGE-GATED NA^+ CHANNELS S. J. Stoehr* G. W. Campbell and D. M. Rock. Neuroscience Therapeutics, Parke-Davis Pharmaceutical Research, Division of Warner-Lambert Co., 2800 Plymouth Rd., Ann Arbor, MI 48105.

The in vivo anticonvulsant and neuroprotective actions of ifenprodil and eliprodil have generally been associated with blockade of NMDA receptor channels. While both compounds have been shown to block voltage-gated Ca^{2+} channels, contributions from Ca^{2+} channel blockade to anticonvulsant properties or neuroprotection are not clear. There is a more direct link between blockade of voltage-gated Na^+ channels and the neuroprotective action of anticonvulsant drugs such as phenytoin and lamotrigine.

We investigated the effects of ifenprodil and eliprodil on voltage-gated Na^+ channels in acutely isolated rat superior cervical ganglion (SCG) neurons using whole-cell voltage-clamp techniques. Both ifenprodil and eliprodil blocked voltage-gated Na^+ channels in SCG neurons in a concentration- and voltage-dependent manner. The IC_{50} for block of Na^+ channel currents with ifenprodil was approximately 30 µM when neurons were voltage-clamped at a holding potential of -90 mV. However, the same concentration of ifenprodil (10µM) blocked 70% of the Na^+ currents when neurons were held at -60 mV, a holding potential at which approximately 50% of the Na^+ channels were inactivated. Eliprodil also blocked Na^+ channel currents in SCG neurons with similar potency.

This potent, voltage-dependent blockade of Na^+ channels may be involved in the neuroprotective and anticonvulsant actions of ifenprodil and eliprodil. Mechanisms that may account for the voltage-dependent block of Na^+ channels by ifenprodil and eliprodil will be discussed.

[Supported by Warner-Lambert Co.]

33.3

VOLTAGE-DEPENDENT INHIBITION OF TETRODOTOXIN-RESISTANT NA^+ CURRENTS IN RAT SENSORY NEURONS BY LAMOTRIGINE

X.M. Xie*, T.J. Dale and D.J. Trezise Neurosciences Unit, GlaxoWellcome Medicines Research Centre, Stevenage, Hertfordshire SG1 2NY, U.K.

The antiepileptic, Na^+ channel blocking drug, lamotrigine (LTG, Lamictal), exhibits analgesic effects both in a variety of animal models and in man (*Pain* 63:33, 1995; *Br. J. Clin Pharmacol.* 39:549P, 1995). However, the cellular and molecular mechanisms underlying these effects are poorly understood. In the present study, we have used whole cell voltage-clamp recording techniques to examine the actions of LTG on voltage-gated Na^+ currents in rat dorsal root ganglion (DRG) neurons. We have focused on tetrodotoxin-resistant Na^+ currents (TTX-R) in these cells, which are believed to be sensory neuron specific (*Nature* 379: 257, 1996).

Na^+ currents were recorded from acutely dissociated, neonatal rat (1-3 days old) DRG neurons (15-25µm) in the presence of 500nM TTX. Pilot studies demonstrated that 100nM TTX was sufficient to abolish fast TTX-sensitive Na^+ currents. TTX-R currents were activated by membrane depolarisation from a holding potential (V_h) of -90mV (voltage for 50% activation, $V_{0.5}$, was -17mV) and reached maximum at 0 to +10mV. The $V_{0.5}$ for fast inactivation was -18mV. At the peak current, the mean time constants for activation and inactivation (decay) were 0.6ms and 3.9ms, respectively. Bath application of LTG caused a reversible inhibition of TTX-R, in a concentration-dependent and voltage-dependent manner. The mean IC_{50} obtained at a V_h of -90 mV was 550µM, whereas an IC_{50} of 260µM was obtained at a V_h of -60 mV. No pronounced effects of LTG (50 µM) on either activation/ fast inactivation kinetics, or the voltage dependence of these parameters were observed.

Our preliminary data demonstrate that LTG is capable of inhibiting TTX-R Na^+ currents in sensory neurones in a voltage-dependent manner. Further mechanistic studies on this drug action are underway. This inhibitory effect of LTG on TTX-R Na^+ channels may, in part, underly the analgesic effects of this compound *in vivo*.

33.4

EFFECTS OF HALOPERIDOL ON TRANSIENT SODIUM CURRENT IN THE RETINAL GANGLION CELL OF YOUNG RATS K. ITO[1,2], Y. NISHIMURA[1], Y. UJI[2], K. NAKANO*[3], T. YAMAMOTO[1] Dept. of Physiology[1], Ophthalmology[2] and Anatomy[3], Mie University, School of Medicine, Tsu, 514, Japan

Some neuroleptic compounds are reported to suppress the transient sodium current(INa,t) in central neurons and peripheral nerves. We have examined the effects of one of them, haloperidol on INa,t of retinal ganglion cells in young rats. We used a whole-cell patchclamp technique in the acutely dissociated cells by enzymatic treatment and gentle trituration. Ganglion cells were identified by retrograde labeling of Fast-Blue injected into the superior colliculus in advance. INa,t was recorded in the perfusate containing tetraethylammonium, CsCl and $CoCl_2$ with a pipette filled with CsF- and EGTA- containing solution. INa,t was voltage-dependent and reversibly blocked by tetrodotoxin(1 μM). Application of haloperidol in the perfusate reversibly reduced the peak amplitude of INa,t in a dose-dependent manner. The dose of half inhibition was $3\text{-}4\times10^{-5}$M and was intermediate between the doses reported in central neurons and peripheral nerves. Furthermore, we investigated the changes of the threshold, the reversal potential and the kinetics of INa,t. We discuss the ionic mechanism underlying the pharmacological effects of the neuroleptic agent on the retina. This study was supported by Ministry of Education, Science and Culture of Japan.

33.5

μ-CONOTOXIN PIIIA: A NEW LIGAND FOR SODIUM CHANNELS. D. Yoshikami[1], K. Shon[3], B. M. Olivera[1], J. S. Imperial[1]*, B. Harris[1], M. Marsh[2], D. Hillyard[2], H. Terlau[4] and W. R. Gray[1]. Depts. of Biology[1] and Pathology[2], Univ. of Utah, Salt Lake City, UT, 84112; Dept. of Physiology and Biophysics[3], Case Western Reserve Univ., Cleveland, OH, 44106; Molekulare Biologie Neuronaler Signale[4], Max-Planck-Inst. für Exp. Medizin, D-37075, Göttingen, Germany.

The μ-conotoxins are a family of peptides that target Na channels; a well studied example is μ-CTx GIIIA. We have recently identified a new peptide (μ-CTx PIIIA) from *Conus purpurascens* venom which belongs to this family. PIIIA irreversibly blocks action potentials in frog skeletal muscle without blocking synaptic potentials. Biochemical assays reveal that PIIIA blocks ^{3}H-saxitoxin binding to electroplax membrane. In these respects PIIIA behaves like other μ-CTx's, such as GIIIA, except that it acts essentially irreversibly in frog preparations. Thus, this new μ-conotoxin provides a simple and permanent means to pharmacologically 'isolate' synaptic transmission from interfering action potentials and consequent muscle contractions.

PIIIA was also tested on voltage-gated I_{Na} in neurons and *Xenopus* oocytes injected with cloned mRNA for Na channels. PIIIA is of exceptional interest because, together with GIIIA, it resolves TTX-sensitive sodium channels in mammalian CNS neurons into 3 classes: sensitive to both toxins, sensitive to PIIIA but not to GIIIA, and sensitive to neither conotoxin. Thus, μ-CTx PIIIA provides a promising tool to discriminate among different Na channel subtypes in the CNS. (Supported by GM PO1 48677.)

33.6

INTERACTION OF THE LOCAL ANESTHETICS LIDOCAINE AND PRILOCAINE. J. M. Sidie* and A. J. Mishizen, Ursinus College, Collegeville, PA 19426.

Lidocaine and prilocaine are amide-linked local anesthetics both with a pKa of 7.9. Utilizing the weakly electric fish *Eigenmannia* as a bioassay, we investigated the effects of these compounds on the electric organ discharge (EOD) frequency. The EOD frequency is driven by the medullary pacemaker nucleus and is very stable. At a concentration of 5x10-5M/pH9, lidocaine alone depresses the EOD frequency 25-30%, while under the same conditions prilocaine gives 10-15% depression. At this pH (9.0) these compounds are 93% in the uncharged (lipid-soluble) form. When the two drugs are given sequentially (first prilocaine then lidocaine or vice versa) the final EOD depression (15% vs 30%) is a function of the second drug delivered. The two drugs given simultaneously produce an intermediate effect. When the drug is removed, EOD frequency recovery is rapid and nearly complete (90-95%). The data support the view that there is one local anesthetic binding site, the two compounds exert different potencies at this site and under conditions investigated one drug displaces the other equally well regardless of which drug is bound first. The recovery data indicate that both compounds are rapidly metabolized.
Supported in part by HHMI.

33.7

EFFECTS OF RILUZOLE ON TETRODOTOXIN-SENSITIVE AND TETRODOTOXIN-RESISTANT SODIUM CHANNELS IN RAT DORSAL ROOT GANGLION NEURONS. J.-H. Song*, K. Nagata, J. Z. Yeh and T. Narahashi. Dept. of Mol. Pharmacol. & Biol. Chem., Northwestern Univ. Med. Sch., Chicago, IL 60611.

The effects of riluzole, a neuroprotective drug, on tetrodotoxin-sensitive (TTX-S) and tetrodotoxin-resistant (TTX-R) Na channels in rat dorsal root ganglion neurons were studied using the patch clamp technique. When the membrane potential was held near the resting level, riluzole preferentially blocked the TTX-S Na channels, whereas at more negative potentials, it blocked both types of Na channels equally. The apparent dissociation constants for riluzole to block TTX-S and TTX-R Na channels in their resting state were estimated to be 90 and 143 μM, respectively. Riluzole accelerated the time course of the inactivation of TTX-R Na currents, whereas it had little or no effect on that of TTX-S Na currents. Riluzole shifted the voltage dependence of activation in the depolarizing direction more efficaciously in TTX-R than in TTX-S Na channels. The voltage dependence of the inactivation was shifted in the hyperpolarizing direction in a dose-dependent manner, indicating that riluzole has a high affinity for the inactivated channels. From the concentration-dependent shift in the inactivation curve, the dissociation constants for riluzole to bind to the inactivated channels were estimated to be 2 and 3 μM, respectively, for the TTX-S and TTX-R Na channels. Thus, riluzole has a similar affinity for both types of Na channels at either resting or inactivated state. Since the TTX-S Na channel has its mid-point of inactivation at a more negative potential than the TTX-R channel, more channels are inactivated at the resting membrane potential to which riluzole can avidly bind. Therefore, riluzole appears more potent to block the TTX-S than TTX-R Na channel. Supported by NIH grant NS14144.

33.8

EFFECTS OF QX-314 ON THE TETRODOTOXIN-RESISTANT SODIUM CURRENT IN RAT DORSAL ROOT GANGLION NEURONS. J.G. McGivern*, Roche Bioscience, 3401 Hillview Avenue, Palo Alto, CA 94304,

The permanently charged quaternary amine derivative of lidocaine, QX-314, binds to the local anesthetic site and rapidly blocks rat neuronal tetrodotoxin-sensitive (TTX_S) sodium channels only when applied to the intracellular side of the membrane (Strichartz, 1973, *J. Gen. Physiol.*, **62**, 37-57). In contrast, QX-314 rapidly blocks TTX-insensitive (TTX_I) sodium channels in rat heart when applied to either side of the membrane (Alpert *et al.*, 1989, *Am. J. Physiol.*, **257**, H79-H84). The present experiments were designed to investigate whether the TTX-resistant (TTX_R) sodium current in small (<30 μm diameter) acutely isolated adult rat (200 g) dorsal root ganglion (DRG) neurons is susceptible to inhibition by extracellular QX-314. The whole-cell patch-clamp technique was used to record sodium currents from single enzymatically dissociated DRG neurons in the presence of 1 μM TTX. Extracellular application of QX-314 (30 μM to 1 mM for 10 minutes) failed to rapidly inhibit the TTX_R sodium current in 12 neurons. Increasing the rate of depolarization from 0.1 to 3.3 Hz caused the TTX_R sodium current to rundown by 15% in the absence of drug. In the presence of 1 mM extracellular QX-314, the rundown was similar to control. In contrast, intracellular application of QX-314 (10 to 300 μM) caused a reduction in the amplitude of the TTX_R sodium current in 12 neurons, with an IC_{50} of approximately 100 μM at a depolarization rate of 0.1 Hz. Increasing the rate of depolarization from 0.1 to 3.3 Hz induced additional reversible block of the current. The present results provide evidence that, in contrast to rat heart, QX-314 cannot directly access the local anesthetic site on TTX_R sodium channels in rat DRG neurons from the extracellular side of the membrane.

33.9

MODULATION OF SODIUM CHANNELS IN THE NEURONAL CELL LINE B50 BY BREVETOXIN-3 AND ITS DERIVATIVES. S.L. Purkerson*, L.A. Fieber, K. S. Rein, and D.G. Baden. NIEHS Marine and Freshwater Biomedical Sciences Center, RSMAS, University of Miami, Miami, FL 33149.

Among neurotoxins that affect Na^+ channel activity are those associated with Neurotoxic Shellfish Poisoning, called brevetoxins. These are multi-ring polyether toxins produced by marine dinoflagellates. The effects on voltage-gated sodium channels of brevetoxin-3 (PbTx-3) and PbTx-3 derivatives synthesized in our laboratory were studied in the stable cell line B50. The B50 cell line was derived from the rat central nervous system and expresses type I /II sodium channel mRNA (Schubert et al., Nature, 1974, 239:224-227; Baines et al., Mol Br Res, 1992, 16:330-338). Unitary Na^+ currents were studied using the cell-attached patch configuration of the patch clamp technique in a bath solution that contained (in mM) 140 NaCl, 3 KCl, 1 $CaCl_2$, 0.6 $MgCl_2$, 7.7 glucose, and 10 HEPES, pH 7.4. K^+ and Ca^{2+} currents were blocked with the inclusion of TEA, Ba^{2+} and Cd^{2+}in the pipette. Na^+channels activated with depolarizing steps to approximately -50 to -30 mV. 75% of membrane patches contained active sodium channels. When Na^+ channel modifier was present, the channels inactivated within ~20 ms during a sustained (50 ms) depolarization. When cells were bathed in 30 nM PbTx-3 or certain derivatives of PbTx-3 prior to an experiment, the open time was increased and the activation curve was shifted to more hyperpolarized potentials. When 100 nM TTX was present in the pipette, Na^+ currents were not observed (n=10). B50 cells provide a good model for the study of Na^+ channels before and after modification of the channels by brevetoxins. Supported by R01 ES05853, P30 ES05705.

33.10

LIDOCAINE EFFECTS ON A MUTANT NA^+ CHANNEL LACKING FAST INACTIVATION. K.J. Gingrich* and L.Wagner II. Anesthesiology, U. of Rochester, School of Medicine, Rochester, NY 14642

Local anesthetics (LA) block voltage dependent Na^+ channels. Fast inactivation is eliminated when hydrophobic amino acids on the intracellular linker between domains III and IV are mutated to polar residues. Bennett *et al* (1995) reported that such mutated cardiac channels do not exhibit long-term LA block and concluded that fast inactivation is required for use-dependence. We became interested in confirming these observations in skeletal muscle. Therefore, we studied the effects of lidocaine (LDCN) on a mutant form of the rat skeletal muscle α-subunit (μ1) Na^+ channel (rskm1) that lacks fast inactivation. Rskm1 was mutated to the QQQ genotype (Q1303,Q1304,Q1305). *Xenopus* oocytes were injected with RNA and Na^+ currents were measured using a two-electrode voltage clamp 2-3 days after injection.

QQQ manifested slow biexponential inactivation (τ_{SLOW1} = 0.33s, τ_{SLOW2} = 1.2s, -10mV) with biexponential recovery (τ_{REC1}= 0.12s, τ_{REC2}=5.7s, -100mV). LDCN (0.5mM) induced a new rapid component (τ_{FAST}= .004s) in apparent inactivation that likely represents open channel blockade but also accelerated slow inactivation by nearly 2-fold (τ_{SLOW1}= 0.12s, τ_{SLOW2}=0.7s). Slow inactivation enhancement was confirmed by a doubling of channels in the faster (τ_{SLOW1}) inactivated state.

These data indicate that LAs enhance slow inactivation. Further, these states are sufficiently long-lived (tenths of seconds) to provide for use-dependent block. Therefore fast inactivation may not be essential for use-dependent block in muscle, in contradistinction to heart. These results suggest genuine differences between skeletal and cardiac Na^+ channel isoforms that may be exploited to reveal the mechanistic details underlying long-lived LA blockade.

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33.11

EFFECT OF VERATRIDINE ON CNaIIA-1 CELLS. L.D. Margolin*, F.R. Wason, & S. Connaughton Cambridge Neuroscience, Inc., Cambridge, MA 02139.

Veratridine-induced [^{14}C]-guanidine flux in a Chinese hamster ovary cell line that stably expresses the alpha-1 subunit of the mammalian type IIA neuronal sodium channel (CNaIIA-1) is a useful high throughput screen for compounds with putative Na^+ channel blocking activity (Connaughton *et al.*, 1995). To better define the role of veratridine in the [^{14}C]-guanidine flux assay, electrophysiological studies were performed in order to characterize its effect on the activity of known Na^+ channel blockers and on the resting membrane potential (RMP) of CNaIIA-1 cells.

Experiments were conducted by either voltage clamping cells in the whole cell configuration (Hamill *et al.*, 1981) for the Na^+ channel blockers or by using whole cell current clamp for the resting membrane potential studies. Cells used for voltage-clamp experiments were held at -90mV and stepped to test potentials that evoked maximal inward Na^+ currents. Cells used for current-clamp experiments were clamped at their RMP. All drugs were applied by bath perfusion (2ml/min).

When corrected for veratridine's ability to reduce transient inward Na^+ current, blockade of Na^+ channels by the local anaesthetic lidocaine and the anti-convulsant lamotrigine was not affected by treatment with 200μM veratridine. The resting membrane potential of CNaIIA-1 cells ranged from -10mV to +20mV. Current-clamp experiments were inconclusive; application of 200μM veratridine did not produce the expected depolarization of the RMP. In contrast similar experiments using N1E-115 cells (which contain K^+ and Ca^+, as well as Na^+ channels) exhibited a rapid depolarization of RMP to +20mV. The voltage-clamp results suggest that veratridine activity does not interfere with the action of Na^+ channel blockers. Additional current-clamp experiments are planned.

33.12

WHOLE-CELL PATCH-CLAMP ASSESSMENT OF VOLTAGE-DEPENDENT SODIUM CURRENT IN ACUTELY DISSOCIATED RAT HIPPOCAMPAL CA1 NEURONS DURING CHRONIC COCAINE INTOXICATION. J. Zhai*, S.J. Wieland and F.M. Sessler. Dept. Of Neurobiology & Anatomy, Med. Col. Of Pennsylvania & Hahnemann University, Philadelphia, PA 19102.

The development of increased susceptibility to cocaine-induced seizures is not well characterized. High doses of cocaine reaching local anesthetic levels are known to produce these effects. These high doses become pharmacologically relevant when drug users increase their cocaine dose to recapture peak euphoric effects. In this study we began an assessment of potential cocaine-induced changes in excitability in the hippocampus. For this we used CA1 neurons from acutely dissociated adult rat brain slices and studied voltage-dependent sodium currents. A hypothesis tested here is that chronic cocaine produces changes in sodium channel functional characteristics which may participate in the development of sensitization to seizure. Rats were given saline or cocaine 40 mg/kg (i.p.) once every day for more than 4 consecutive days. CA1 neurons from control and drug-treated rats were isolated from hippocampal slices using enzyme and trituration methods. Voltage-dependent sodium channels were activated by a series of depolarizing pulses under whole-cell patch-clamp recording. Steady-state inactivation was characterized with 100 ms prepulses in the range of -140 mV to -45 mV. Preliminary data show that the steady-state inactivation of sodium channel in cocaine-treated rats was substantially shifted in depolarizing direction. This could make a larger number of sodium channels available for activation and increase excitability. Considering the low threshold for seizure activities in the hippocampal formation, these changes may participate in the development of seizure sensitization and/or may be related to compensatory mechanisms in response to drug treatment. (Supported by NIDA DA08405 and Allegheny-Singer Research Institute Award to FMS)

33.13

COUMAROYL SPERMIDINE, A PHENOLIC POLYAMINE, INDUCES HYPEREXCITABLILITY IN CRAYFISH AXONS. S. R. Kelly, S. Jenkins, J. K. Atkinson and A. J. Mercier*, Dept. of Biol. Sci. and Dept. of Chemistry, Brock University, St. Catharines, Ont., L2S 3A1.

Recent work investigating pest resistance in plants has led to the identification of several phenolic polyamines, which are structurally similar to acylpolyamines of spider and wasp venoms. This study investigated the effects of coumaroyl spermidine (CS), a synthetic polyamine, on giant axons in the ventral nerve cord of the crayfish *Procambarus clarkii.*

CS, at concentrations of 100-500 μM, caused the appearance of multiple action potentials in response to brief (0.2 ms), single electrical stimuli. The action potentials typically occurred at frequencies of up to 400 Hz. The amplitude and time course of the first action potential in each train were not substantially different from those of impulses recorded in the absence of CS. CS did not alter the resting potential of the axon, and its effects on excitability reversed within 1 minute of washing with physiological saline. The effects CS were neither mimicked by 500 μM spermidine (which had no effect) nor by reducing extracellular calcium concentration. Application of tetrodotoxin initially reduced the number of multiple action potentials induced in the presence of CS and eventually blocked impulses altogether. This suggests that CS may modulate gating of sodium channels.

Supported by NSERC Canada.

33.14

ALTERED BIOPHYSICAL AND PHARMACOLOGICAL PROFILES OF SODIUM CHANNELS IN PYRETHROID-RESISTANT INSECTS. D. Lee*[1], T. M. Brown[2], and M.E. Adams[1]. [1]Dept. of Entomology, University of California, Riverside, CA 92521 and [2]Dept. of Entomology, Clemson University, Clemson, SC 29634.

Heavy selection pressure on insect populations brought about by chronic use of neurotoxic insecticides has resulted in many forms of genetic resistance. A significant factor contributing to pyrethroid insecticide resistance is nervous system insensitivity. Since pyrethroids target voltage-dependent sodium channels, we compared the biophysical and pharmacological characteristics of sodium channels in central neurons of susceptible and pyrethroid-resistant tobacco budworm, *Heliothis virescens* using the whole-cell patch clamp technique. The synthetic pyrethroid permethrin causes a dramatic prolongation of tail currents following test depolarizations due to an extreme slowing of sodium channel deactivation. Steady-state [s-s] tail currents were plotted as a function of permethrin concentration to obtain dose-response curves. Low nanomolar concentrations of permethrin were sufficient to prolong tail currents in neurons from susceptibe insects whereas neurons from resistant insects were ~20-fold less sensitive. Resistant currents showed a ~15 mV shift in the voltage-dependent activation curve to more positive potentials as compared to susceptible currents. Resistant currents also differed in their steady-state inactivation, with the h_∞ curve shifted about 7 mV more positive. Interestingly, resistant currents were ~2 times more sensitive to the scorpion toxin LqhαIT, but showed no change in sensitivity to TTX. We conclude that neuronal sodium channels in resistant insects have altered gating properties, decreased sensitivity to pyrethroids, and enhanced sensitivity to LqhαIT, all of which are consistent with the hypothesis that an alteration in channel structure contributes to neuronal insensivity to pyrethroids.

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33.15

STRUCTURE-ACTIVITY RELATIONSHIP OF DELTAMETHRIN ANALOGS ON SODIUM CHANNELS EXPRESSED IN *XENOPUS* OOCYTES. Timothy J. Smith* and David M. Soderlund. Field of Environmental Toxicology and Dept. of Entomology, New York State Agric. Expt. Station, Cornell Univ., Geneva, NY 14456.

Pyrethroids make up a large class of potent neurotoxic insecticides whose principal target is the voltage-gated sodium channel. In this series of insecticides the R configuration at chiral carbon 1 or 3 of the cyclopropanecarboxylate moiety and the S configuration at the α-cyano carbon of the phenoxybenzyl alcohol moiety are essential for toxicity. To further explore the stereospecific basis of pyrethroid action on sodium channels we expressed the rat brain IIa sodium channel α subunit plus the rat β_1 subunit in *Xenopus* oocytes and determined the effects of pyrethroids on sodium currents measured by two electrode voltage clamp. Deltamethrin, (S)-α-cyano-3-phenoxybenzyl-(1R)-*cis*-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropanecarboxylate, prolonged the time course of sodium channel inactivation and produced tail currents following repolarization that were qualitatively similar to the effects produced on sodium channels in intact nerves. The only other structurally related compound to produce tail currents was NRDC 158B, the 1R,*trans*,αS isomer of deltamethrin, which varies from deltamethrin in the absolute configuration only at carbon 3 of the cyclopropane ring. Other stereoisomers of deltamethrin that lack the preferred configuration for high neurotoxicity did not modify sodium currents. None of the compounds significantly modified the voltage dependence of activation or inactivation. The stereospecific action of pyrethroids on sodium channels are consistent with the high degree of stereospecificity observed in the neurotoxic actions of these compounds.

34.1

MODELS OF ACTIVITY-DEPENDENT CHANGES IN THE BACK-PROPAGATED ACTION POTENTIALS OF CA1 NEURONS.
M. Migliore*, Inst. for Interdisciplinary Applications of Physics - Natl. Res. Council, 90123 Palermo, Italy.

Recent experimental advances using simultaneous somatic and dendritic recordings from hippocampal CA1 neurons (Spruston et al., *Science* 268:297-300,1995) have shown that the active properties of dendrites can change the amplitude of the action potentials in the dendrites in an activity-dependent manner. Experiments, however, have not been paralleled by the development of a clear model of the mechanisms that could be involved with this effect. I modeled two different mechanisms, a shunting conductance and a slow sodium inactivation, to test if they could modulate the active propagation of a train of action potentials in a dendritic tree. Computer simulations, using a compartmental model of a pyramidal neuron, suggest that each of these two mechanisms could account for the activity-dependent attenuation and failure of the action potentials in the dendrites during the train. Each mechanism is shown to be in good qualitative agreement with experimental findings on somatic or dendritic stimulation and on the effects of hyperpolarization. The conditions under which branch point failures can be observed, and a few experimentally testable predictions, are presented and discussed. (Supported by C.N.R.)

34.2

PROPERTIES OF THE PERSISTENT Na CURRENT GENERATING SUBTHRESHOLD OSCILLATIONS IN ENTORHINAL CORTEX (EC) LAYER II NEURONS A. Galue* and A. Alonso Dept. Neurol. & Neurosurg, MNI and McGill University, Montreal, Canada.

Neurons in EC layer II display subthreshold membrane potential oscillations that according to current-clamp and SEVC data in the slice are driven by a persistent TTX-sensitive Na current (I_{NaP}). It is possible that I_{NaP} represents a window current resulting from the overlap of the m_∞ and h_∞ curves of the fast transient Na current (I_{Na}) or that it is generated by a distinct Na conductance. To adress this issue, the properties of different Na-current components were studied in acutely isolated neurons from EC layer II with the use of the whole cell configuration of the patch-clamp technique. A TTX-sensitive (100nM), persistent Na current but no transient current was seen in response to depolarizations of up to 20 mV from a H.P. of -80mV. Depolarizations positive to -50 mV elicited both a persistant and a transient component. The steady state I/V relation of I_{NaP} could be studied in isolation either by applying long depolarizing voltage steps or slow (<0.1mV/ms) ramp depolarizations. I_{Nap} reached a peak amplitude of -50 to -500pA at -35 mV, reversed at +40mV and the potential for half-maximum conductance was -55 to -60mV. In EC layer II neurons the transient I_{Na} displays a TTX sensitive and a TTX resistant component whose voltage dependent and kinetic properties are indistinguishable (White et al., Neuron, 1993). This allowed us to estimate the m_∞ curve for I_{Nap} and, following block of I_{Nap} with 100nM TTX, the m_∞ and h_∞ curves of I_{Na} which displayed minimal overlap. The data demonstrated that in EC layer II neurons the persistent Na current activated negative to -50mV and which provides the drive for the generation of subthreshold oscillations is mainly due to a distinct persistent Na current and not to a "window" current. Funded by the MRC and FRSQ.

34.3

DIFFERENCES IN CHACTERISTICS OF IONIC CURRENTS IN BAG CELLS FROM SEXUALLY IMMATURE ADULT *APLYSIA*.
L. A. Fieber*. Division of Marine Biology and Fisheries, Univ. Miami Rosenstiel School, Miami, FL 33149.

Experiments to assess whole cell ionic currents were executed with the whole cell variation of the patch clamp technique on bag cells 10-30 μm in diameter from sexually immature adult *Aplysia* maintained in short term tissue culture. Na^+ and Ca^{2+} currents were recorded in an intracellular solution of (mM): 450 CsCl, 2.9 $CaCl_2$, 2.5 $MgCl_2$, 10 EGTA, 5 Na_2ATP, 300 μM GTP, and 40 HEPES-CsOH, pH 7.4. For recording K^+ and Na^+ currents, the bath contained (mM): 417 NaCl, 55 $MgCl_2$, 10 $CaCl_2$, 10 KCl, 10 HEPES-KOH, pH 7.6. For recording Ca^{2+} currents the bath contained (mM): 460 tetraethylammonium Cl (TEACl), 10.4 CsCl, and 11 Ba^{2+}, 55 $MgCl_2$, and 10 HEPES-CsOH, pH 7.6. In addition to K currents, less than 50% of cells also had inward currents for Na^+, Ca^{2+} or both. Na^{2+} currents were recorded in 10 of 33 cells and Ca^{2+} currents in 14 of 33. Na^+ currents in immature bag cells were similar in many respects to those in mature bag cells (Fieber 1995, J. exp. Biol., 198:2237-47), with one important difference that might influence excitability. Time constants for recovery from inactivation using a double pulse protocol were 2.7 ms at V_h=-70 mV, similar to the adult value of 2.9 ms, and 13 ms at V_h=-30 mV, which is twice the adult value 0f 6.8 ms. Ca^{2+} currents were short-lived (<10 min) and were smaller than Ca^{2+} currents in mature bag cells. These data suggest that immature bag cell Na^+ and Ca^{2+} currents have some characteristics that make them less likely than currents from mature animals to contribute to repetitive action potential firing. Supported by NSF IBN-9508705 and NIH RR-10294.

34.4

POST-NATAL DEVELOPMENTAL INCREASE IN THE DENSITY OF SODIUM CURRENTS IN MURINE MOTONEURONS, K.D. García[a], L.K. Sprunger[a], M. Meisler[a] and K.G. Beam[a]. Dept. Anat. Neurobiol. Colo. St. Univ. Ft. Collins Co 80525 and Dept. Human Genetics Univ. Mich. Ann Arbor MII 48109

At birth, mice display weak and uncoordinated motor activity, but during the first postnatal week acquire substantial motor skills. Because propagated action potentials in motor axons are required for coordinated motor activity, we have investigated whether changes in expression of motoneuronal sodium currents accompany the acquisition of motor ability. The whole-cell variant of the patch clamp technique was applied to motoneurons from neonatal mice ages P0-P8. Motoneuronal cell bodies, labelled by injection of dil into fore- and hind-limbs of anesthetized mice, were isolated by enzymatic dissociation and cultured 12-24 hours. Cultures were made from either whole spinal cords or spinal cords divided into cranial and caudal portions. Labelled cells were held at a potential of -80 mV and sodium currents (35 mM extracellular sodium) were elicited by test potentials ranging from -50 to +50 mV. In motoneurons obtained from whole spinal cords, the sodium current density (peak current divided by linear cell capacitance) was 46.8±8.3 pA/pF (mean±sem) for P0 mice and 154±31.7 for P8 mice. Sodium current densities for cranial and caudal cells, respectively, were 48.4±14.0 and 48.7±9.2 pA/pF at P0, 77.0±26.0 and 49.8±20.4 pA/pF at P1, 100.6±6.7 and 70.9±16.5 pA/pF at P2, 127.0±16.3 and 90.3±21.6 at P4. These results suggest that significant up-regulation of motoneuronal sodium currents occurs postnatally, proceeds rostrally to caudally, and may contribute to maturation of motor function. Ongoing studies are examining sodium current expression in motoneurons from mice with motor endplate disease (*med*), which have a null mutation in the sodium channel gene *Scn8a*. Supported by NIH grants NS26416, NS24444 and NS34509.

34.5

PRESENCE OF CARDIAC-LIKE ZN^{2+}-SENSITIVE NA^+ CHANNELS IN LIMBIC REGIONS OF RAT BRAIN. H.A. Hartmann[1]*, L.V. Colom[2], M.L. Biophysics[1] and Neurology[2], Baylor College of Medicine, Houston, TX 77030.

Zn^{2+}-sensitivity is a property of the Na^+ currents expressed from the cardiac tissue isoform, however classical brain Na^+ channels are Zn^{2+}-resistant. Recently Zn^{2+}-sensitive Na^+ currents have been described in neurons from the rat medial entorhinal cortex (White *et al.*, Neuron: 11, 1037-47, 1993). We used *in situ* hybridization of mRNA to localize the expression of the cardiac Zn^{2+}-sensitive isoform in the rat brain. Two antisense oligonucleotide probes (P1 and P2) were designed to the rat heart Na^+ channel cDNA nucleotides 1914 to 1958 for P1, and 6301 to 6350 for P2. The 45-mer for P1 corresponded to amino acids 638 to 652 of the intracellular linker between repeats I and II, and the 50-mer for P2 corresponded to a 3'-untranslated region. The 3' end-labelled oligos with [35S]*dATP were hybridized to adult rat brain slices. Two sets of control probes were used: antisense probes were hybridized in the presence of 50-fold excess of unlabelled oligo, and sense probes were also used. Both P1 and P2 gave an identical, highly specific localization, labeling the pyriform cortex, septal area, dorsal and medial hypothalamus, mamillary nuclei, stria terminalis, and amygdaloid complex. Whole-cell patch recordings from enzymatically-isolated rat septal neurons demonstrated the presence of a Zn^{2+}-sensitive Na^+ current. In 50% of neurons tested, 1, 10, and 100 μM Zn^{2+} blocked 10, 19, and 21% of the Na^+ current, respectively. These results demonstrate the mRNA expression of a heart-like Na^+ channel in limbic system structures and the functional expression of this channel in septal neurons of the rat brain. Since Na^+ currents contribute to pacemaker and subthreshold potentials, this Zn^{2+}-sensitive Na^+ current may play a critical role modulating excitability in these networks. Supported by Hankamer Foundation (HAH), Methodist Foundation (LVC), and NS29709 (JLN).

34.6

PROPERTIES OF HODGKIN-HUXLEY TYPE POTASSIUM AND SODIUM CHANNELS IN SUBTRESHOLD SIGNAL BOOSTING. M. Kauranen, K. Djupsund*, and M. Weckström Department of Physiology, University of Oulu, Finland

Many neurons especially in peripheral sensory systems do not generate action potentials. However, they do express voltage-activated ion channels. Recent experimental evidence shows that many non-spiking cells show boosting, or resonance-like behaviour. How could nonspiking graded potentials be boosted in some frequency band in nerve membrane? The boosting could occur when membrane ion-channels are suitably activated in this frequency band so that electrochemical gradients of these ions could be used in signal boosting. We tested simulated models of sodium and potassium ion-channels with nonlinear differential equations of Hodgkin and Huxley type. Single channel frequency response under voltage clamp either with sinusoidal or white noise stimulus was simulated in several holding potentials. In addition, a membrane model with sodium or potassium channels with "co-expressed" leak channels and membrane capacitance was simulated under current clamp conditions. The results show that the Na^+ channels are able to amplify the graded changes in membrane potential with holding potentials between -60 to -30 mV, while the resonance is between 500-1000 Hz. The +3 dB level is reach already near 100 Hz with sinusoidal stimulation. The amplification is most prominent when r.p. is between -50 to -35 mV. The amplification takes place also with very small stimulus amplitudes. With white noise stimulation the resonance is generally smaller, but the small-signal sensitivity is better. In a cell model the membrane capacitance shifts the resonance to smaller frequencies. The low-passing properties of the K^+-channels cause an increase in membrane impedance at high (50-500 Hz) frequencies. The voltage-gated ion channels are thus able to produce the required boosting effect, although the exact matching of real neuronal membranes' responses with simulation needs adjustment of the kinetic parameters of the channels.

34.7

OPTIMAL WAVEFORM POLARITY AND SEQUENCE FOR NEUROMAGNETIC EXCITATION OF PERIPHERAL NERVE: IN-VITRO AND SIMULATION STUDY. P.J. Maccabee*, S.S. Nagarajan, V.E. Amassian, D.M. Durand, L.P. Eberle, R.Q. Cracco and K.S. Lai, Department of Neurology, SUNY Health Science Center at Brooklyn, Brooklyn, NY 11203.

Neuromagnetic excitation of mammalian phrenic nerve in a trough filled with saline was studied at the negative-going first spatial derivative of the induced electric field along a straight nerve, and at low threshold sites such as at a bend and at a cut nerve ending. With identical first phases, polyphasic (70 μs, then 140 μs, etc. durations) and predominantly monophasic (70 μs duration) stimuli were induced by the MC. Experiments were also simulated using the identical waveforms.

In-vitro, at all excitation sites, largest amplitude responses were elicited by an induced polyphasic hyperpolarizing-depolarizing pulse; smaller responses by a monophasic depolarizing pulse; smallest responses by a polyphasic depolarizing-hyperpolarizing pulse. The simulation studies confirmed the in-vitro findings and further delineated the mechanism. 7% of the increased efficacy of the hyperpolarizing-depolarizing pulse sequence reflects the greater integrated depolarizing charge transfer afforded by the 140 μs vs 70 μs cycle. The remaining 3% of the increased efficacy of the hyperpolarizing-depolarizing pulse sequence represents reversal of Na inactivation by the initial hyperpolarization resulting in an increased fraction of open Na channels available to the subsequent depolarization.

34.8

ACTION POTENTIAL-LIKE RESPONSES IN B104 CELLS WITH LOW Na^+ AND K^+ CHANNEL DENSITIES. S.G.Waxman and X.Q. Gu*,Department of Neurology, Yale School of Medicine, New Haven, CT 06510; and PVA/EPVA Neuroscience Research Center, VA Hospital, West Haven, CT 06516.

In order to study the generation of action potentials in cells with low densities of Na^+ channels, whole-cell patch-clamp methods were used to record Na^+ and K^+ currents, and responses to depolarization and hyperpolarization in B104 neuroblastoma cells in vitro. Action potential-like responses were elicited in 38 of 42 cells, with a threshold close to -55.4 mV for depolarizing stimuli, and -56.7 mV for anode-break stimuli. These responses were voltage-dependent, and were larger when cells were held at more negative prepulse potentials. Response amplitudes, plotted against prepulse potentials, were well fit by a Boltzmann distribution with a midpoint of ~ -75 mV, close to the $V_{1/2}$ for Na^+ current steady-state inactivation in these cells. Cells displaying action potential-like responses exhibited a peak Na^+ current density of 133.0±0.1 pA/pF (range, 10.2-196.2 pA/pF). Cells which did not support action potential-like responses displayed a peak Na^+ current density of 30.3±20.1 pA/pF. The $g_K:g_{Na}$ ratio was 0.0067±0.0023 for cells which displayed action potential-like responses, and was 0.1027±0.0296 for cells which did not generate responses. Exposure to 0.1 mM Cd^{2+} did not block the generation of action potential-like responses in B104 cells, while 1 μM TTX abolished the responses in cells exposed to Cd^{2+}. We conclude that low densities of Na^+ channels (<3/μm^2, and <1/μm^2 in some cells) can support the generation of action potential-like responses in B104 cells. The low K^+ conductance of these cells may contribute to their ability to generate action potential-like responses. (Supported in part by the NMSS and Medical Research Service, Veterans Administration).

34.9

CURRENT CLAMP SIMULATIONS OF A MODEL CELL USING A MICROSOFT EXCEL™ SPREADSHEET. A.M. Brown*. Department of Pharmacology, Neurosciences Institute, University of Dundee, Ninewells Hospital, Dundee, DD1 9SY, U.K.

A method has been devised whereby a model cell containing multiple voltage gated ion channels can be modelled under current clamp conditions using a Microsoft Excel™ spreadsheet. This method does not require any programming or macro language knowledge. The features of spreadsheets which allow this are firstly, 'drag and drop to fill a range', secondly, the ability to insert formulae into cells allowing new columns of data to be generated based on existing columns, and thirdly, the instant charting of data.

In the example described the squid giant axon with I_{Na}, I_K and I_{leak} was modelled. Ionic currents calculations were based on the Hodgkin & Huxley (HH) series of equations where the currents are described in the fashion:

$$I_{Na} = m^3hg_{Na}(E-E_{Na})$$

In these types of simulation an initial potential is set and the voltage- and time-dependent parameters are calculated for this potential. The spreadsheet is set up so that each voltage dependent parameter (αm, βm, αh, βh, αn, βn, g_{Na}, g_K, g_{leak}, I_{Na}, I_K, I_{inj}, I_{leak}) occupies a separate column. δt is set at 20 μs and each row increments the value of that particular variable and its change in value according to the change in voltage. The HH parameters are calculated by Euler integration and the membrane potential is calculated by integrating the total ionic current transfer (I_{Na} + I_K + I_{inj} + I_{leak}) across the membrane for the time interval δt. The specific membrane capacitance is assumed to be 1 μF cm^{-2}. Resting membrane potential, action potential generation and repetitive firing are intrinsic properties of the model.

34.10

USING μ-CONOTOXIN TO TEST A MODEL OF THE STRUCTURE OF THE VOLTAGE-GATED SODIUM CHANNEL OUTER VESTIBULE, N. S. Chang*, S. Dudley, Jr., G. Lipkind, R. J. French, and H. Fozzard. Dept. of Pharmacological and Physiological Sciences, Univ. of Chicago, Chicago, IL 60637.

Data on sites critical for tetrodotoxin and saxitoxin binding to the voltage-gated sodium channel allowed our laboratory to develop a model of the pore outer vestibule. With this model, we predicted a binding configuration for μ-conotoxin (μ-CTX). We have demonstrated a specific interaction, as predicted, between E758 of the adult rat skeletal muscle Na^+ channel (μ1) and R13 of μ-CTX. We sought to examine binding of the toxin mutant R13D to the μ1 mutant E758K. Wild-type and mutant μ1 channels were heterologously expressed in Xenopus oocytes. IC50's of mutant and wild-type μ-CTX blockade of peak sodium current (shown below in μM ± SEM) were determined under two-electrode voltage clamp. Although E758K has a 260-fold lower affinity for μ-CTX than does wild-type μ1, R13D binds to E758K with slightly greater affinity than to wild-type μ1. This partial recovery of wild-type binding supports our hypothesis that there is at least one other critical interaction site for R13 which is most likely at the putative selectivity filter

We also examined interaction of the μ1 mutant E403Q with R13A, R13D and R13Q. The calculated coupling coefficients (Ω) are 7, 5, and 18, respectively. This implies some contribution of E403 to the binding of R13. However, consistent with our model, this contribution appears to be much smaller than that of E758, where coupling coefficients were 111, 54, and 56 for E758Q vs. R13A, R13D and R13Q.

		μ-CTX	R13Q	R13D	R13A
Work supported by NIH	**μ-1**	0.017 ± 0.005	1.37± 0.13	9.2 ± 1.1	2.6 ± 0.8
NHLBI HL20592-20 and	**E758Q**	0.822 ± 0.105	1.11± 0.15	8.3 ± 1.2	1.1 ± 0.2
MRC (Canada)	**E758K**	4.53 ± 0.69	10.3 ± 1.0	5.3 ± 0.9	----------
MT-10053	**E403Q**	0.098 ± 0.009	0.43± 0.10	10.7± 2.6	2.0 ± 0.3

34.11

S(357)F confers tetrodotoxin sensitivity on the sensory neuron specific sodium channel SNS.

K. Okuse, L. Sivilotti*, S. Moss, A.N. Akopian and J.N. Wood Departments of Anatomy and Developmental Biology, and Pharmacology, University College, London WC1E 6BT, UK.

Small diameter sensory neurons, many of which are nociceptors, express an unusual voltage-gated sodium channel (SNS or PN3) that is resistant to tetrodotoxin (IC_{50}=60μM). SNS is 65% homologous to the tetrodotoxin-insensitive cardiac channel. A number of residues that line the channel atrium have been implicated in tetrodotoxin binding. SNS exhibits sequence identity to other tetrodotoxin-sensitive sodium channels in 7 out of 9 such residues (Urenjak J. and Obrenovitch T.P. Pharm Revs. 1996, **48**, 21-67). One difference is a conservative substitution at D(905)E. Interestingly, a single residue (C-357) has been shown to play a critical role in tetrodotoxin binding to the cardiac channel. In SNS, a hydrophilic serine is found at this position. Using site-directed mutagenesis we have substituted phenylalanine for serine at position 357. The mutated SNS channel, when expressed in Xenopus oocytes produces voltage-gated sodium currents similar in amplitude and time-course to the native channel. However, sensitivity to tetrodotoxin is restored to give an IC_{50} of 2.5 nM (+/-0.4 n=5), similar to other voltage-gated sodium channels that have aromatic residues at the equivalent position. These data are consistent with current models of tetrodotoxin binding to sodium channels, and provide further clues to the rational design of selective blockers of SNS.

34.12

STRUCTURE-FUNCTION SURVEY OF THE S4-5 LINKER OF THE SODIUM CHANNEL. G.N. Filatov, S.D. Kraner and R.L. Barchi*. Depts. of Neurology and Neuroscience and the Mahoney Inst.of Neurological Sciences, Univ. of Penn Sch. of Med.,Philadelphia, PA 19104.

The S4-5 linker has been proposed to play a major role in sodium channel inactivation. Schiffer-Edmondson analysis of the S4-5 interhelical sequence suggests an amphipatic helical structure, in which a conserved region of small hydrophobic residues occupies one contiguous surface. This surface may interact with the other channel helices, while the opposite face which contains multiple charged and polar residues may help to form the cytoplasmic mouth of the pore. We hypothesize that the polar face of S4-5 forms a part of the docking surface for the ID3-4 inactivation gate after conformational changes during activation. We replaced 16 residues on the helix with substitutions which differed in charge, polarity, size and the presence of an aromatic ring. A total of 62 single amino acid substitutions were analyzed. The introduction of any charge into the hydrophobic surface resulted in failure of functional channel expression. All other mutations in this face produced channels with activation, inactivation, recovery from inactivation and steady-state inactivation kinetics similar to wild type. In the polar surface, charged mutations caused a pronounced slowing of inactivation, shifted voltage dependence of steady-state inactivation toward more depolarized potentials and decreased recovery from inactivation time constants. Noncharged substitutions usually showed the opposite effect. All mutations at P1473 (rat SkM1 numbering) resulted in nonfunctional channels and the effects of mutations beyond this proline differed from those at preceeding residues. Charged mutations at hydrophobic residues caused a slowing in inactivation and produced different effects on recovery. An interpretation of our results in terms of channel function will be presented. **Supported by NIH NS 18013.**

34.13

EPITOPE-TAGGING OF THE RAT SkM1 SODIUM CHANNEL CORROBORATES AN EXTRACELLULAR LOCATION FOR THE S1-S2 INTERHELICAL LOOP. S.D. Kraner*, W.-J. Sun, G.N. Filatov, J. Lindstrom, R.L. Barchi, Depts. of Neuroscience and Neurology and Mahoney Inst. of Neuroscience, Univ. of Penn., Philadelphia, PA 19104.

The SkM 1 channel was epitope-tagged at positions within and between the S1, S2, and S3 helices of domain 2 (584, 595, 599, 605, 619, 630, 651, and 654) by insertion of a sequence from the Torpedo AChR alpha subunit. The epitope is found only in the Torpedo sequence and not in the mammalian AChR or sodium channel sequences, and a high-affinity monoclonal antibody (mAb 142) to it is available for our use. All insertional mutations were first screened for function by expression of the cRNA in Xenopus oocytes followed by two-electrode voltage clamp. Insertional mutants at positions 595, 599, 630, 651, and 654 all gave rise to large (>2 μA) currents in oocytes, whereas those at positions 584, 605 and 619 failed to function. Functional mutants were transfected into primary muscle cells and analyzed for surface membrane staining following immunofluorescent labeling by confocal microscopy. Intracellular as opposed to extracellular availability of the epitope was determined by permeabilizing the cells with the detergent saponin. The mutant at positon 599 gave rise to a clear pattern of membrane staining along the surface of the myotube, both before and after membrane permeabilization with saponin. In addition, internal staining, presumably of nascent proteins, is seen in saponin-treated cells. The mutants at postions 595, 630, 651, and 654 failed to yield a signal in the absence of saponin and gave rise only to internal staining in the presence of saponin. These results provide corroboration that the S1-S2 interhelical segment has an extracellular location, as predicted by models of tertiary structure. Supported by NIH NS 18083.

34.14

MOLECULAR MOTION IN THE PORE OF THE SODIUM CHANNEL: EVIDENCE FOR FLEXIBILITY OF THE P LOOPS

Jean-Pierre Benitah, Maria Janecki, Ravi Ranjan, Gordon F. Tomaselli, Eduardo Marban*. The Johns Hopkins University School of Medicine, Baltimore MD

Permeation through ion channels is generally conceptualized as ion passage through rigid structures that discriminate particles of varying size and charge. In contrast, molecular dynamics simulations reveal that proteins can undergo sizable thermal motions on a submillisecond time scale. We investigated the extent of motion in the pore-lining (P) loops of the rat skeletal muscle Na^+ channel by constructing double-cysteine mutants and quantifying their propensity to form disulfide bonds. Whole-cell Na^+ currents were measured in oocytes before, during and after exposure to the redox catalyst Cu(II)(1.10-phenanthroline)$_3$ (CuP, 0.1 mM). Disulfide bond formation was inferred by redox-sensitive occlusion of ion flux, and by concomitant changes in sensitivity to Cd^{2+} and Zn^{2+}. Only one of 14 double mutants (Y401C-E758C, bridging domains I & II) spontaneously formed a disulfide bond. Nevertheless, three other sites (W1239C in domain III, and G1530C & D1532C in domain IV) could form disulfide bonds with Y401C when the reaction was catalyzed by CuP. These reactions occurred over 1-5 minutes at 22^o C and were partially reversed by dithiothreitol. The selectivity filter position K1237 (domain III) formed disulfides catalyzed by CuP with two P loop residues in domain IV (G1530 and W1531), but interestingly not with the other classical "selectivity filter" sites. We will present a structural model of the pore which depicts the minimal motions required to account for the salient results. We conclude that the pore of the Na^+ channel, rather than being rigid, exhibits considerable internal flexibility.

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34.15

CELLULAR EXPRESSION AND FUNCTIONAL ANALYSIS OF WILD TYPE AND MUTANT FORMS OF EPITOPE-TAGGED NA CHANNEL β1 SUBUNITS R.M. Shah[1], L.P. Henderson[1,2], J.R. Jones[1], G.J. Huang[1], and R.A. Maue[1,2]* Depts. of [1]Biochem. and [2]Physiol., Dartmouth Medical School, Hanover, NH 03755.

During assembly and expression of voltage-dependent Na channels, the noncovalent interaction between the β1 and α subunits has important functional implications. For example, in Xenopus oocytes co-expression of β1 increases Na channel expression, speeds activation and inactivation kinetics, and causes a hyperpolarizing shift in the voltage-dependence of activation and inactivation. As an approach to understanding the molecular and cellular basis of β1 actions in neurons and muscle cells, a β1 cDNA was isolated from rat brain mRNA using RT-PCR, sequences encoding an epitope of the hemaglutinin protein (HA) were added by PCR, and then the cDNA encoding the HA-tagged β1 subunit inserted into expression vectors. HA-tagged β1 subunits were then expressed in Xenopus oocytes, Cos cells, and primary cultures of embryonic neurons. The tagged β1 subunit could be detected in Western blots, with a commercially available HA antibody binding to a protein of the predicted molecular weight in lysates from transfected, but not mock transfected, cells. Immunofluorescent labelling and confocal microscopy of transiently transfected Cos cells and embryonic neurons suggested that the HA-tagged β1 will facilitate analysis of Na channel expression and distribution in muscle cells and neurons, as the expression of the tagged β1 subunit on the cell surface was nonuniform, and in primary neurons was detected in both cell bodies and neurites. Xenopus oocyte expression studies confirmed that the HA-tagged β1 modified the functional properties of the SKM1 (μ1) α subunit as expected, increasing Na current amplitude and speed of inactivation, and causing a hyperpolarizing shift in the voltage-dependence of steady state inactivation. Deletions and point mutations of the HA-tagged β1 subunit had differential effects on Na channel function, and highlight the utility of this approach in determining structural elements necessary for the interaction with α subunits and effects on Na channel function. Supported by the Hitchcock Foundation. (RAM), Waterhouse Foundation. (JRJ), Richter Memorial Scholarship Trust (JRJ, GJH), Dartmouth Presidential Scholars Fund (JRJ, GJH), Dartmouth Biology (JRJ) and Chemistry (GJH) Depts., Dartmouth Class of '39 (JRJ), and NIH grant NS28767 (RAM).

34.16

A TALE OF TWO VALINES: Na CHANNEL MUTATIONS IN NEUROMUSCULAR DISEASE. D.S. Green[1], L.J. Hayward[1], A.L. George[2], and S. C. Cannon[1]* (1) Dept. of Neurobiology Harvard Med. Sch. Boston, MA 02114; (2) Dept. of Pharmacology Vanderbilt Univ. Med. Center Nashville TN 37232

Mutations of the skeletal muscle α subunit (hSkM1) cause hereditary forms of myotonia and periodic paralysis. We have tested the functional consequences of two previously uncharacterized mutations: V781I - at the extracellular end of IIS6 and associated with HyperPP + cardiac dysrhythmia; V1293I - at the -NH end of the III-IV linker and associated with a variant of paramyotonia congenita (PMC) without paralysis on exposure to cold. Na currents were recorded from HEK cells transfected with cDNAs coding the hβ_1 subunit and WT hSkM1 or either mutant α subunit.

V1293I disrupted fast inactivation as evidenced by a 3-fold increase in the persistent Na current at 50 msec and a 2-fold increase in the rate of recovery from inactivation. The voltage-dependence of steady-state inactivation (h_∞) was not significantly altered, and the decay of I_{Na} (τ_h) was similar for WT and V1293I. The voltage-dependence of activation was identical for WT and V1293I. In contrast, the only feature of fast inactivation that was altered for V781I was a small shift, -3.5 mV, in the midpoint of h_∞(V). Furthermore, this shift was in the wrong direction to cause paralysis. We conclude that V781I is a benign polymorphism and not a disease-causing mutation. V781I does not occur in the general population, and has been found in only one individual with a HyperPP-like phenotype. The proband was adopted so molecular confirmation was not possible in affected relatives.

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EXCITATORY AMINO ACID RECEPTORS: PHYSIOLOGY, PHARMACOLOGY, AND MODULATION—NMDA I

35.1

BOTH *d*- and *l*-METHADONE BIND TO THE NMDA RECEPTOR. A.L. Gorman*, K.J.Elliott, C.E.Inturrisi. Dept. of Pharmacology, Cornell University Medical College, NY, NY 10021.

Recently, Ebert et al. (1995), reported that racemic methadone (MET) binds to and inhibits the NMDA receptor. To characterize further this effect, competition binding studies were conducted to determine (1) stereoselectivity of MET's affinity for the NMDA receptor and (2) whether other opioids exhibit a similar binding profile. Well-washed rat forebrain synaptic membranes were incubated with 5 nM [^{3}H]MK-801, vehicle or 200 μM MK-801 (non-specific binding),1 μM Glycine, 30 μM *l*-Glutamate, and 0 - 100 μM concentrations of the competing drugs. The opioid agonists, *l*-morphine (MOR) and hydromorphone (HM), and the opioid antagonist naltrexone (NTX) exhibited negligible affinity for the NMDA receptor. However, both isomers of MET exhibited moderate affinity for the NMDA receptor (Ki values: *l*-MET = 2.9 μM; *d*-MET = 5.4 μM), and produced inhibition curves similar to that of the NMDA receptor antagonist, dextromethorphan (DM: Ki = 3.3 μM). Therefore, MET, unlike the other opioids (MOR, HM, or NTX) has affinity for the NMDA receptor. Since previous studies (Elliott et al., 1994) have shown that NMDA receptor antagonists attenuate the development of MOR tolerance, NMDA receptor antagonism by the analgesically inactive *d*-MET and/or the active *l*-MET isomers may contribute to the incomplete analgesic cross-tolerance observed between MET and MOR *in vivo*. Supported by NIDA grants DA 01457, DA 07274, DA 00198 and DA 00255.

35.2

SYNTHETIC CONANTOKIN ANALOGS: NOVEL POLYAMINE ANTAGONISTS OF NMDA RECEPTORS. L.-M. Zhou*, G. I. Szendrei1[1], M.-L. Maccecchini[2], L. Otvos, Jr.[1] and P. Skolnick. Lab. of Neuroscience, NIDDK/NIH, Bethesda, MD 20892, [1]The Wistar Institute, Philadelphia, PA 19104, [2]Symphony Pharmaceuticals, Malvern, PA 19355.

Conantokin G (con-G) is a 17-amino acid peptide originally isolated from marine snails of the genus Conus. This peptide is a NMDA antagonist as evidenced by its ability to reduce glutamate-induced neurotoxicity and inhibit NMDA-stimulated cGMP formation in primary neuron cultures. The NMDA antagonist properties of con-G have been attributed to a specific, non-competitive inhibition of polyamine actions. Thus, con-G potently inhibits spermine-enhanced [^{3}H]MK-801 binding to NMDA receptors from rat brains at concentrations without significant effect on radioligands binding to glutamate or glycine sites. Structure-activity studies indicate that the γ-carboxyglutamate (Gla) residues at positions 7, 10, and 14 could be replaced individually without altering the NMDA antagonist properties of con-G. By contrast, Gla^3 and Gla^4 appear to be required for NMDA antagonism. Ala^{7}con-G was found to be the most potent NMDA antagonist among con-G analogs with an IC_{50} of 0.045 μM in inhibiting spermine-enhanced [^{3}H]MK-801 binding, 4-fold more potent than the parent peptide. We report here newly synthesized con-G analogs and their biological activities at NMDA receptors. Ala^{7,10,14}con-G exhibited an IC_{50} value of ~0.075 μM, comparable to Ala^{7}con-G. By contrast, the potency of Glu^{7,10,14}con-G, was dramatically reduced (IC_{50} ~4 μM) while Glu^{4}con-G was inactive at concentrations of up to 20 μM. Several Tyr-substituted con G analogs have been prepared in order to develop potential radioligands for the putative polyamine-sensitive con-G sites. Among these compounds, Ala^{7}Tyr^{10}con-G was as potent as con-G (IC_{50} ~0.17 μM). The present data are in agreement with and provided additional evidence to support previous SAR findings with derivatives of con-G.

35.3

REGIONAL DISTRIBUTION AND PROPERTIES OF [^{3}H]MDL 105,519-LABELED NMDA GLYCINE SITES ASSESSED BY QUANTITATIVE RECEPTOR AUTORADIOGRAPHY, P.A. Chmielewski, B.W. Siegel*, and B.M. Baron. Hoechst Marion Roussel, Cincinnati, OH 45215.

MDL 105,519 is a potent and selective competitive antagonist of glycine at the NMDA receptor. In membrane binding studies, the pharmacology of the site labeled by [^{3}H]MDL 105,519 closely correlated with that of the recognition site labeled by [^{3}H]glycine. These results suggested that [^{3}H]MDL 105,519 could provide an improved tool to characterize anatomical variations in NMDA receptor pharmacology using quantitative autoradiographic techniques (QAR).

[^{3}H]MDL 105,519 binding parameters were optimized for QAR using sagittal rat brain sections. Both the density (B_{max}) and affinity (K_D) of [^{3}H]MDL 105,519 binding sites was heterogeneous in the seven brain regions examined. The B_{max} was highest in the hippocampal formation and cerebral cortex (20-25 fmol/mm^2) and lowest in the cerebellum (6 fmol/mm^2) an observation consistent with results obtained in membrane preparations. QAR K_D values ranged from 7 nM (striate cortex) to 21 nM (caudate). Competition binding experiments were used to determine K_i values for 4 unlabeled ligands. In the cerebral cortex, the rank order of potency was L-701,324 > ACEA1021 > glycine > D-serine. Regional variations in K_i were observed for these glycine site ligands in other areas of the rat brain.

35.4

ZINC INHIBITION OF [^{3}H]MK-801 BINDING IS DIFFERENT IN MOUSE BRAIN AND SPINAL CORD: EFFECT OF GLYCINE AND GLUTAMATE. K.J. Kovács and A.A. Larson*. Dept. of Veterinary PathoBiology, University of Minnesota, St. Paul, MN 55108.

Zinc (Zn^{2+}) inhibits *N*-methyl-D-aspartate (NMDA)-type excitatory amino acid activity by a non-competitive action. Based on regional differences in the CNS in binding characteristics of [^{3}H](+)-5-methyl-10,11-dihydro-5*H*-dibenzo[a,d]cyclohepten-5,10-imine maleate ([^{3}H]-MK-801) and other compounds used to label open channels in the receptor complex, we compared the inhibitory influence of Zn^{2+} on [^{3}H]MK-801 binding in whole mouse brain to that in spinal cord membranes. Radioligand binding techniques were used in the presence and absence of maximally effective concentrations of glycine and/or glutamate. Using extensively washed membranes without exogenous glycine and glutamate, Zn^{2+} was found to be a weaker inhibitor of [^{3}H]MK-801 labeled sites in the spinal cord (IC_{50} > 50 μM) than in the whole brain (IC_{50} = 9.8 μM). In contrast, addition of exogenous glycine and glutamate decreased the inhibitory effect of Zn^{2+} in the brain (IC_{50} = 42.6 μM) but dramatically increased the inhibitory effect of Zn^{2+} in the spinal cord (IC_{50} = 4.1 μM). Thus, Zn^{2+} can inhibit NMDA activity over a wider range of concentrations in the spinal cord than in the brain. In addition, increases in NMDA activity appear to potentiate the inhibitory effect of zinc in the spinal cord but attenuate the inhibitory effect of zinc in brain. The different actions of Zn^{2+} may be attributable to a differential distribution of NMDA NR2 receptor subunits in mouse brain and spinal cord. Zinc may play a role in sensory processing at the spinal cord level as the density of zinc is highest in the superficial layers of the dorsal horn. [NIDA04090 to AAL]

35.5

ETHANOL INHIBITION OF NMDA CURRENTS IN ACUTELY DISSOCIATED RAT MEDIAL SEPTUM/N. DIAGONAL BAND (MS/DB) CELLS. C.A. Grover*, A.S. Fincher, and G.D. Frye. Dept. of Med. Pharmacol. & Toxicol., Texas A&M Univ., College Station, TX 77843.

Ethanol inhibits NMDA currents in cultured hippocampal cells (Lovinger et al., *Sci.* 243:1721-1724; 1989), and acute tolerance to ethanol inhibition of NMDA-mediated EPSPs occurs in hippocampal slices (Grover & Frye, *Brain Res.* 642: 70-76; 1994). The present study examined the time dependency of ethanol (60 mM) inhibition of NMDA (100 μM) -induced whole-cell currents in acutely isolated MS/DB cells from adult male Sprague-Dawley rats. Three baseline 3 s NMDA currents were obtained 30 s apart, followed by 3 NMDA responses 30 s apart either with ethanol applied only during 3 s applications of NMDA (Brief), or with ethanol applied continuously for 90 s (Continuous). Ethanol reversibly inhibited NMDA currents by 38, 31, and 39 % (n=7) with the 3 consecutive Brief tests. NMDA currents in each of these cells was inhibited by more than 20 % with the first Brief test, and none of these cells showed reduced inhibition by the third NMDA-ethanol application. Continuous ethanol inhibited NMDA currents by 34, 26, and 26 % (n=31) with the 3 consecutive Continuous tests. However, inhibition was less than 20% in 9 of the 31 cells, and while 17 of the remaining 22 cells showed consistent inhibition (40, 37, 40 %), 5 cells showed at least a 20% reduction of inhibition by the third response during continuous exposure to ethanol (68, 30, 27 %). This suggests that acute tolerance to continuous ethanol inhibition of NMDA currents occurs in a subpopulation of MS/DB neurons. Supported by AA10067-02 (GDF).

35.6

EFFECT OF CHRONIC ETHANOL EXPOSURE ON GLYCINE SITE OF THE NMDA RECEPTOR COMPLEX IN MAMMALIAN CORTICAL NEURONS Xian-Jue Hu* and M.K. Ticku. Department of Pharmacology, University of Texas Health Science Center, San Antonio, TX 78284-7764

We have previously demonstrated that chronic ethanol (50 mM, 5 days) treatment upregulated the NMDA receptor function and binding in cultured cortical neurons, as measured by $[Ca^{2+}]_i$ with Fura-2/AM and [^{3}H]MK-801 binding (Hu and Ticku, Mol. Br. Res. 270:485, 1994). Furthermore, we have shown that it also selectively increased the NMDA receptor R2B subunit mRNA gene expression (Hu et al. Mol. Br. Res. 36:211, 1996). In the present study we further investigated the effect of chronic ethanol treatment on [^{3}H]DCK (a glycine site ligand) binding and the potentiation of [^{3}H]MK-801 binding by glycine in cortical neuronal membrane preparation. The results demonstrate that chronic ethanol treatment increased the specific [^{3}H]DCK binding by ~60%, and the potentiation of [^{3}H]MK-801 binding by glycine was also increased by ~90%. The increased binding and the potentiation were reversible following ethanol withdrawal for 72 hrs. These results suggest that glycine site may also play a role in the upregulation of the NMDA receptor function following chronic ethanol exposure. The potential mechanisms involved in the upregulation of the binding to the glycine site is under investigation.
Supported by NIH-NIAAA grant #AA100552

35.7

CHRONIC ETHANOL INCREASES NMDA RECEPTOR SUBUNIT EXPRESSION IN CORTICAL NEURONS GROWN IN LOW BUT NOT HIGH GLUTAMINE. **L. J. Chandler* and D. Norwood. Department of Pharmacology, Louisiana State University Medical Center, Shreveport, LA 71130.**

The high concentration (2 mM) of glutamine commonly present in culture media has been reported to inhibit upregulation of NMDA receptors in response to chronic exposure to antagonist. We have previously shown that chronic ethanol can enhance NMDA receptor function in cortical neurons grown in high glutamine without an apparent increase in NMDA receptor density. The present study examined the effect of chronic ethanol exposure on the expression of NMDA receptor subunit proteins in cortical neurons grown in high (2 mM) versus low (100 μM) glutamine. Since L-glutamine is relatively unstable in media, cells were grown in media containing dipeptide L-alanyl-L-glutamine, a stabilized form of L-glutamine. Exposure of low glutamine cultures to 100 mM ethanol for 4 days (starting at culture day 9) significantly enhanced expression of NR1, NR2A and NR2B subunit polypeptides as determined by immunoblot analysis using subunit selective antibodies. In contrast, and in agreement with our previous observations, chronic ethanol did not alter NR1, NR2A or NR2B subunit expression in the presence of high glutamine. Reducing the glutamine concentration did not alter the developmental expression of any of the receptor subunit proteins. These results 1) demonstrate the use of a stabilized form of L-glutamine in neuronal cultures; 2) confirm and extend previous observations that upregulation of NMDA receptor expression in response to chronic ethanol is enhance in low glutamine media; and 3) suggest that other mechanisms in addition to increases in NMDA receptor density may contribute to upregulation of NMDA receptor function in response to chronic ethanol exposure. **Supported by a grant from the Alcoholic Beverage Medical Research Foundation.**

35.8

USE OF TRYPSIN IN NEURONAL TISSUE DISSOCIATION ALTERS THE EFFECT OF ETHANOL ON NMDA-ACTIVATED WHOLE-CELL CURRENTS IN VITRO. D.W. Frederick and J.M. Wright*. Howard Univ., Washington, D.C. 20059, and Lab. of Molecular & Cellular Neurobiology, NIAAA, NIH, Bethesda, MD 20892-8205.

One of the problems in the study of alcohol effects on ligand-gated ion-channels has been the variation in response among and within various laboratories. We developed a non-enzymatic dissociation protocol with cell yield and % viable cells similar to that of enzymatic protocols. Neurons from E-16 mouse (NCI C57/BL6) hippocampus or cortex were plated on laminin-coated 35 mm dishes and cultured for 11 to 20 d. Cells dissociated by a non-enzymatic process exhibited a potentiation of the peak NMDA-activated current (254 ± 54%; N = 15) when 1 mM ethanol was preapplied for 10 s. This potentiation was not observed in parallel cultures of neurons dissociated by treatment with trypsin (N = 4) and had not previously been observed over a 3 year period in cultures produced using trypsin in the dissociation protocol. The potentiation was transient with no effect seen after 3 min continuous exposure to 1 mM ethanol. Potentiation occurred both in whole-cell (N = 11) and permeabilized patch (N = 4) configurations. The results suggest that attention should be paid to the enzymes used in the tissue preparation protocol. This work was supported by NIMH grant #5T32MH19547 and NIAAA.

35.9

BLOCK OF NMDA RECEPTOR BY A MILNACIPRAN ANALOG.
K. Yoshii, T. Noguchi, Y. Ohtubo, H. Imoto, and E. Tanaka*, Kyushu Inst. of Tech., Iizuka, 820 Japan, Dept. of Physiology, Kurume Univ. Sch. Med. Kurume, 830 Japan.

A milnacipran analog synthesized by Shuto and Matsuda (Hokkaido Univ. Sapporo, Japan) blocked NMDA receptors of cultured mouse hippocampus neurons and those produced by Xenopus oocytes injected with rat brain mRNA under voltage clamp conditions. The analog blocked NMDA responses to 30 μM NMDA (+ 1 or 10 μM Gly) with IC50 of 1.7 μM and decreased the maximum responses of concentration-response curves for NMDA (+ 10 μM Gly) and those for Gly (+ 30 μM NMDA) in uncompetitive manner. The blocking effect was use-dependent and potentiated on hyperpolarization. The time constants of block and recovery from the block were much shorter than those for MK-801. The analog hardly blocked the responses of non-NMDA, metabotropic Glu, $5HT_{1C}$, $GABA_A$, and ACh_{M1} receptors produced by the oocytes. These results indicate that this analog acts as a faster open channel blocker than MK-801.

This study was supported by The Ministry of Education, Japan.

35.10

WAGLERIN-1 MODULATES GLYCINE AFFINITY FOR NMDA RECEPTORS OF MURINE CNS NEURONS. J.H. Ye* and J.J. McArdle. Depts Pharm & Phys and Anesthesiology, NJ Med Sch (UMDNJ) Newark, NJ 07103-2714

In this study we explored the effect of Waglerin-1 (WTX), a lethal peptide purified from the venom of *Wagler's* pit viper (*Trimeresurus wagleri*), on the NMDA-activated current of freshly isolated murine hypothalamic neurons. Whole cell currents were recorded with the nystatin perforated patch technique. Current in response to NMDA and glycine increased when WTX was co-applied. This effect of WTX was not observed either in the absence of added glycine or when saturating glycine concentrations were co-applied. Evaluation of the dependence of NMDA current on glycine concentration revealed that WTX increased the affinity for glycine. For rat cortical membrane preparations, 10 μM WTX caused a 20 fold enhancement of the affinity of the strychnine-resistant glycine binding site. WTX did not potentiate the response of hypothalamic neurons to kainate. These data suggest that WTX selectively potentiates the NMDA type of glutamate receptor through an action on the glycine modulatory site. (Funds supporting this work were derived from NIH grant NS31040 as well as the Department of Anesthesiology.)

35.11

EFFECT OF NMDA ON HIPPOCAMPAL NEURONS FROM TS16 FETAL MICE.
R.J. Siarey, E. Coan, S.I. Rapoport, and Z. Galdzicki*. LNS/NIA, NIH, Bethesda, MD 20892, USA.

The trisomy 16 mouse (Ts16) is regarded as a model of Down's syndrome. Voltage-dependent ion channels from fetal Ts16 hippocampal neurons have been shown to be abnormal (Galdzicki et al., Brain Res. 604, 69-78, 1993). The NMDA receptor ion current is voltage-dependent (Nowak et al., Nature 307, 462-465, 1984). In order to determine if there were similar defects in ligand-gated channels we examined NMDA responses on Ts16 neurons.

Hippocampal cultures were prepared from diploid and Ts16 mouse fetuses (E15-17) as previously described (Galdzicki, et al., 1993). The initial plating medium consisted of MEM, 1% glutamine, 10% fetal bovine and 5% horse serum (all v/v), after 24h the fetal bovine serum was omitted. Whole-cell patch clamp recordings were performed at culture days 16-29. Neurons were superfused with extracellular medium consisting of (in mM) NaCl, 140; KCl, 5; $MgCl_2$, 2: $CaCl_2$, 1; NaOH HEPES, 10 and dextrose, 15 at pH 7.4, plus 1μM glycine, 1μM TTX and 50μM picrotoxin. The pipette solution contained (in mM) CsF 130; $CaCl_2$, 1; BAPTA, 11; CsOH HEPES, 10 and Mg-ATP, 2. Passive membrane properties of the neurons were not significantly different, resistance, τ and capacitance values were 228±50MΩ, 6.0±0.8ms and 35.3±4.8pF (n=17) for diploid and 181±28MΩ, 7.0±1.3ms and 36.3±3.6pF (n=11) for Ts16 neurons. Whole-cell currents were evoked from an holding potential of -60 mV by four 30 mV steps, in the absence and presence of NMDA (10-80μM). I-V relationships for the NMDA-induced current were plotted and used to estimate the reversal potential and conductance, which was then normalized. The presence of NMDA evoked an inward current in both diploid and Ts16 neurons. There was no significant difference in the conductance at the concentrations applied or reversal potentials, -6.6±3.6mV (n=20) and -9.9±4.4mV (n=12) for diploid and Ts16 respectively.

Therefore, in contrast to our observations on voltage-dependent ion channels, these data suggest that the trisomic condition does not produce abnormal NMDA evoked ionic current in fetal hippocampal neurons.

35.12

PRE- AND POSTSYNAPTIC ACTIONS OF HALLUCINOGENS ON NMDA-NEUROTRANSMISSION IN THE RAT MEDIAL PREFRONTAL CORTICAL (mPFC) SLICES. V.I. Arvanov*, P.G.Magro and R.Y. Wang. Dept. Psychiatry, SUNY/SB, Stony Brook, NY 11794.

Serotonin$_{2A}$ ($5\text{-}HT_{2A}$) receptors are implicated in the action of hallucinogens and antipsychotic drugs. The aim of the present study was to examine the modulatory action of the hallucinogens DOB and LSD, which are $5\text{-}HT_{2A}$ receptor agonists, on NMDA-induced effect in pyramidal cells. Application of NMDA evoked EPSPs and depolarizations. DOB exerted a concentration-dependent modulation of NMDA's responses: at low (0.01-1 μM) and high (10-40 μM) concentrations, DOB facilitated and inhibited, respectively, NMDA-evoked depolarization and EPSPs. LSD also produced a biphasic effect in the presence of the $5\text{-}HT_{1A}$ antagonist WAY 100635: at 0.01-0.1 μM, LSD facilitated and at 1-10 μM, it inhibited NMDA response. In contrast, lisuride (a non-hallucinogenic congener of LSD) potentiated NMDA's response in a monophasic fashion. Without WAY 100635, LSD inhibited NMDA's response. These modulatory actions of DOB, LSD and lisuride were also observed in voltage-clamp mode, in the presence of TTX, glycine and bicuculline. The biphasic modulatory action of hallucinogens (but not the effect of lisuride) was blocked by the selective $5\text{-}HT_{2A}$ receptor antagonists MDL 100907 and ketanserin but not by bicuculline or WAY 100635. Interestingly, a non-NMDA receptor antagonist CNQX (20 μM) decreased NMDA's response. Moreover, it totally eliminated the potentiating action and unmasked the inhibitory action of hallucinogens at 100 nM on NMDA's response. Riluzole (30 μM), an inhibitor of glutamate release from presynaptic terminals, produced similar results to those of CNQX. Combined, these results suggest that the hallucinogens DOB and LSD interact with $5\text{-}HT_{2A}$ receptors on presynaptic glutamate terminals and post-synaptic pyramidal cells, respectively, to produce the potentiating and inhibitory effects. In addition, they suggest that the hallucinogenic effect of DOB and LSD is mediated by their postsynaptic action (Supported by MH-41440, DA-07193).

35.13

SIGNAL TRANSDUCING MECHANISMS IN THE MODULATION OF NMDA - INDUCED INWARD CURRENT BY THE $5\text{-}HT_{2A/2C}$ RECEPTOR AGONIST DOB IN RAT CORTICAL CELLS.
X.F. Liang, V.L. Arvanov, R.Roberts, J.Y.Zhang* and R.Y. Wang. Dept. Psychiatry, SUNY/SB, Stony Brook, NY 11794-8790.

We have previously demonstrated that at low (0.01-1 μM) and high (10-40 μM) concentrations, DOB facilitated and inhibited, respectively, NMDA-evoked inward current in pyramidal cells of the medial prefrontal cortex slice preparations. The aim of the present study was to determine the cellular mechanisms by which DOB modulates NMDA-induced current. Using the techniques of intracellular recording and single electrode voltage clamp, we examined the effects of agents modifying cytosolic [Ca^{2+}] as well as inhibitors of Ca^{2+}-related protein kinases. We found that inclusion of Ca^{2+}-chelator BAPTA (200 mM) in the recording micropipette had no effect on the facilitating effect but it eliminated the inhibitory action of DOB on NMDA's response, whereas bath application of membrane permeable BAPTA-AM (30 μM) prevented both effects of DOB. Bath application of thapsigargin (1 μM), a Ca^{2+}-mobilizing agent, produced a time-dependent action: it facilitated NMDA-induced current 10 min after superfusion, mimicking the action of low concentrations of DOB, which disappeared after 30-40 min. Additionally, thapsigargin blocked both the facilitating and inhibitory action of DOB on NMDA's response. Chelerythrine (2 μM), a selective PKC inhibitor, completely prevented the facilitating effect but did not affect the inhibitory action of DOB. In contrast, the calmodulin antagonist W-7 (10 μM) had no effect on DOB's potentiating action, but prevented DOB-induced inhibition of NMDA's response. In conclusion, these results support our previous findings that the potentiating and inhibitory effects of DOB on NMDA-induced inward current are the results of its action on presynaptic glutamate terminals and postsynaptic pyramidal cells, respectively. Our results further suggest that the pre- and postsynaptic actions of DOB were mediated via the PKC and Ca^{2+}-calmodulin transducing mechanisms, respectively (Supported by USPHS grants MH-41440 and DA-07193).

35.14

THE POTENTIATION OF NMDA-INDUCED INWARD CURRENT BY ANTI-PSYCHOTICS IN CORTICAL CELLS.
R.Y. Wang* and V.L. Arvanov. Dept. Psychiatry, Sch. of Med., SUNY/SB, Stony Brook, NY 11794-8790

The glutamate hypothesis of schizophrenia proposes that N-methyl-D-aspartate (NMDA) receptor hypofunction may cause the behavioral deficits associated with schizophrenia. Accordingly, NMDA receptor agonists or compounds that facilitate NMDA-mediated neurotransmission might be potential antipsychotic drugs (APDs). Using the techniques of intracellular recording and single electrode voltage clamp, we examined the effects of typical and atypical APDs on NMDA-induced inward current and the role of $5\text{-}HT_{2A}$ receptors and corresponding second messengers in the action of APDs in pyramidal cells of the medial prefrontal cortex in *in vitro* brain slice preparations. The following drugs (μM) were included in the superfusion solution: 1 glycine, 0.5 TTX and 5 bicuculline methiodide. Our preliminary results show that neither haloperidol nor clozapine altered membrane current and input resistance of pyramidal cells. However, both haloperidol (50 - 100 nM) and clozapine (50 - 100 nM), but not fluoxetine (50 - 100 nM), produce a marked 3-4 fold potentiation of NMDA-induced inward current. By comparison, responses evoked by AMPA in the same neuron were not affected by haloperidol but depressed 59-78% by clozapine. MDL 100907, a selective $5\text{-}HT_{2A}$ receptor antagonist (K_i = 0.5 nM) and a putative atypical APD, but not its less active enantiomer MDL 100009, also produced a striking potentiation of NMDA-induced inward current. As clozapine (K_i = 10 nM) and haloperidol (K_i = 50 nM) have a moderate affinity to $5\text{-}HT_{2A}$ receptors *in vitro*, it is likely that the potentiating effect of putative APDs on NMDA-induced inward current is the result of their interactions with $5\text{-}HT_{2A}$ receptors. (Supported by USPHS grants MH-41440 and DA-07193).

35.15

CP-101,606 ANTAGONIZES NMDA RECEPTORS BY INTERACTING WITH THE POLYAMINE MODULATORY SITE. David D. Mott*, Sunan Zhang, Mark S. Washburn, Morris Fendley and Ray Dingledine. Dept. of Pharmacology, Emory Univ. Sch. of Med., Atlanta, GA 30322.

CP-101,606, an ifenprodil analogue, is a potent and selective antagonist of NMDA receptors that protects cultured neurons from glutamate neurotoxicity. The mechanism by which CP-101,606 antagonizes NMDA receptors has not been clearly defined. To better understand its mechanism of action we examined the effect of CP-101,606 on heteromeric NMDA receptors expressed in *Xenopus* oocytes.

NR1a/2B receptors were reversibly inhibited by CP-101,606 in a non-competitive and voltage independent manner. Maximum inhibition was 78% at a concentration of 3 μM. Interestingly, at drug concentrations below 100 nM, inhibition was initially large but then faded over 20-30 sec to a steady state value. Concentration response curves suggest that CP-101,606 inhibits NR1a/2B receptors through both high affinity (transient; *IC50* <10 nM) and low affinity (steady state; *IC50* =*200* nM) mechanisms.

We initially examined the site of action of CP-101,606 by testing its effect on NMDA receptors with different subunit composition. CP-101,606 produced steady state (*IC50*=250 nM), but no transient inhibition of NR1b/2B receptors, suggesting that the transient inhibition was suppressed by the presence of exon 5 in the NR1b subunit. In contrast, CP-101,606 had a markedly reduced effect on NR1a/2A receptors (*IC50* = 540 nM) with a maximum inhibition of only 30%, suggesting that the NR2B subunit contributed to the development of the steady state inhibition. Since the NR1b and NR2B subunits are both involved in the actions of spermine on the NMDA receptor, we examined the effect of spermine on the inhibition of NR1a/2B receptors produced by CP-101,606. Spermine (100 μM) significantly inhibited both the peak and steady state inhibition produced by CP-101,606. These results suggest that CP-101,606 produces both a high and low affinity inhibition of NMDA receptors by interacting with the polyamine modulatory site. Supported by the Epilepsy Foundation and NS-27452.

35.16

S-3,5-Dihydroxyphenylglycine Potentiates the NMDA Response of Cortical Neurons H.J. Flavin* and N.W. Daw Dept. Ophthalmology and Visual Science, Yale University School of Medicine, New Haven, CT. 06520-2089.

Recent work demonstrated that the metabotropic glutamate receptor (mGluR) agonist (1S,3R)-1-aminocyclopentane-1,3-dicarboxylic acid (ACPD) potentiates the NMDA response in Layer V/VI neurons and in some Layer II/III visual cortical neurons (Wang, X.F. and Daw N.W., Investigative Ophthalmology & Visual Science 37(3); S486, 1996). In order to elucidate the specific mGluR subtype(s) responsible for this potentiation, the current study compared the effect of the Type I (mGluR 1,5) agonist S-3,5-dihydroxyphenylglycine (DHPG) and the Type II (mGluR 2,3) agonist (2S,3S,4S)-alpha-(carboxycyclopropyl)glycine (LCCGI) on the NMDA response of rat visual cortical neurons. Whole cell recordings were obtained for each cell with iontophoretic drug application. In Layers II/III and Layers V/VI, DHPG always potentiated the NMDA response. The mean ratio of the cell's response to NMDA + DHPG to the control NMDA response was 3.93 for Layer II/III (8 cells) and 1.97 for Layer V/VI cells (11 cells). Some cells in Layers II/III (observed in 4 of 8 cells) and Layers V/VI (4/11 cells) responded to LCCGI by potentiating the NMDA response while other cells responded by attenuating the NMDA response (4/8 cells in Layer II/III and 7/11 cells in Layers V/VI). These data suggest that mGluR 1 and/or 5 stimulation always enhances the NMDA response whereas mGluR 2 and/or 3 stimulation has at least two effects. Further work will establish whether these multiple effects are due to presynaptic versus postsynaptic localization of mGluR's.
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35.17

MODULATION OF [^{3}H]MDL 105,519 BINDING TO THE GLYCINE SITE OF THE NMDA RECEPTOR BY POLYAMINES. T.A. Constantine* and L.J. Reynolds. Dept. of Pharmacology, University of Pittsburgh School of Medicine, Pittsburgh, PA 15261.

Several studies have suggested that polyamines modulate the interaction of glycine with the NMDA receptor. We have further investigated the effects of polyamines using the glycine site antagonist [^{3}H]MDL 105,519. [^{3}H]MDL 105,519 binding assays were performed using well washed membranes prepared from frozen rat brains. Non-specific binding was determined by the addition of 1mM glycine. The polyamines spermine, spermidine, and 1,5-(diethylamino)piperidine (DEAP) increased the fraction of non-specific binding in the [^{3}H]MDL 105,519 binding assay from 40% to 60% when spermidine or spermine concentration was increased from 1 to 100μM.

We investigated the characteristics of the polyamine induced increase in [^{3}H]MDL 105,519 non-specific binding. We performed a 5,7-dichlorokynurenic acid (DCK) dose response curve in the presence of 300μM spermine and 1mM glycine. High concentrations of DCK (10-100μM) were necessary to displace the polyamine induced binding suggesting [^{3}H]MDL 105,519 is binding to a site that is unrelated to the high-affinity glycine antagonist site. An arcaine dose response curve was performed in the presence of 300μM spermine and 1mM glycine and a decrease in [^{3}H]MDL 105,519 binding was observed only at high arcaine concentrations (≥100μM). This suggests that polyamines are working on a different site than the "arcaine-sensitive" site to cause the increase in non-specific [^{3}H]MDL 105,519 binding.

Spermine, spermidine, and DEAP (30μM) do not appear to have a significant effect on the displacement of [^{3}H]MDL 105,519 binding by glycine. Further studies of the effects of polyamines on [^{3}H]MDL 105,519 binding are currently in progress in order to resolve the ambiguity of whether polyamines alter the affinity of both glycine agonists and glycine antagonists.
Supported by NIH grant DA 07409

35.18

FREE INTRACELLULAR Mg^{2+} CONCENTRATION AND BLOCK OF WHOLE-CELL NMDA-ACTIVATED CURRENT. Y. Li-Smerin*, E.S. Levitan and J.W. Johnson. Depts. of Neuroscience and Pharmacology, University of Pittsburgh, Pittsburgh, PA 15260.

Free intracellular Mg^{2+} (Mg^{2+}_i) was found previously to inhibit NMDA-activated current flow in cell-free patches more effectively than in whole cells. To test whether this difference is due to cellular regulation of Mg^{2+}_i, we measured $[Mg^{2+}]_i$ in cultured cortical neurons using the fluorescent dye mag-indo-1. 50 μM mag-indo-1 and various buffered Mg^{2+} solutions were dialyzed into neurons from whole-cell pipettes. The ratio of the dye emission at 405 to that at 495 nM was converted to $[Mg^{2+}]_i$ using a calibration curve constructed in cells (K_{app} of the dye = 10.6 mM). We then measured $[Mg^{2+}]_i$ in cells dialyzed with pipette solutions which were previously used to test Mg^{2+} block of NMDA-activated currents. Pipette solutions contained 10 mM EGTA with theoretically calculated free Mg^{2+} of 1, 3, and 10 mM. Measured steady-state $[Mg^{2+}]_i$ in these cells was (in mM) 1.3 ± 0.2 (mean ± sem) (n = 3), 2.2 ± 0.08 (n = 3), and 6.2 ± 1.6 (n = 5), respectively. Based on an independent calibration constructed in vitro (K_{app} = 7.5 mM), the free $[Mg^{2+}]$ in the same three pipette solutions was (in mM) 0.8 ± 0.05 (n = 5), 2.1± 0.1 (n = 5), and 6.4 ± 0.2 (n = 7), respectively. The cellular $[Mg^{2+}]$ is not significantly different from the pipette $[Mg^{2+}]$ for the intermediate and the high Mg^{2+} solutions. Thus, cellular regulation of $[Mg^{2+}]_i$ cannot explain the less effective inhibition by Mg^{2+}_i of whole-cell NMDA-activated current. Based on these measurements of $[Mg^{2+}]$ and previous measurements of fractional block, we determined that the K_D of inhibition by Mg^{2+}_i at +60 mV in cell-free patches is 1.8 ± 0.1 mM, whereas the K_D in whole cells is 5.0 ± 0.9 mM. The two values of K_D are significantly different ($P < 0.05$). Thus, the difference observed in the inhibition by Mg^{2+}_i of NMDA-induced current flow through patches and whole cells appears to be due to a difference in the sensitivity of NMDA receptors to Mg^{2+}_i. Supported by NIH.

35.19

MODULATION OF NMDA RESPONSE BY LEUKOTRIENE B4 IN RAT HIPPOCAMPAL NEURONS. N. Horimoto*, M. Abe, J. Nabekura and T. Ogawa. Dept. of Physiology, Akita Univ. Sch. of Med., Akita 010, Japan.

Leukotriene B4 (LTB4), one of the arachidonic acid metabolites, is produced in various tissues including brain. However, the influence of LTB4 on neurons is not well understood. In the present study, we investigated the effect of LTB4 on NMDA-receptor mediated currents in acutely dissociated rat hippocampal neurons using a patch recording under the voltage clamp. At a holding potential of -40 mV, 10^{-4}M NMDA induced initial peak followed by a rapid decrease of inward current. Extracellular application of LTB4 (300 nM - 3 μM) attenuated the peak NMDA-induced current in 10 of 25 neurons. The intracellular perfusion with LTB4 dissolved in a pipette solution prolonged the time constant of the decreasing phase from the peak to the steady state. These observations show that LTB4 could affect the NMDA response both extracellularly and intracellularly, suggesting that LTB4 has not only an effect related to inflammation but also plays a modulatory role in neuronal excitation in the hippocampus.

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35.20

CHRONIC TREATMENT WITH SIGMA LIGANDS PRODUCES FUNCTIONAL CHANGES IN NMDA RECEPTOR ION-CHANNEL: *in vivo* AND *in vitro* STUDIES. T. Yamamoto*[1], S. Tanaka[1] and H. Yamamoto[2]. [1]Lab. of Mol. Recognition, Grad. Sch. of Integrated Sci., Yokohama City Univ., Yokohama 236; [2]Dept. of Psychopharmacol., Tokyo Inst. of Psychiatry, Tokyo 156, Japan.

Sigma binding sites have been implicated to be involved in the psychotomimetic effects for the certain benzomorphans and in the action for the antipsychotic drugs. Despite the accumulation of data, the function of the sigma sites remains somewhat unclear. Our previous studies demonstrated that sigma sites modulate the NMDA receptor ion-channel complex by using intact cell binding for primary culture cells from rat cerebrum. In the present study, we further characterized the interaction between sigma sites and NMDA receptor ion channel complex. The effects of chronic treatments of haloperidol or (+)pentazocine on sigma sites and NMDA receptor in brain membrane were assessed using [^{3}H]NE-100 and [^{3}H]TCP (or [^{3}H]MK-801) binding. Administration of haloperidol (2 mg/kg, i.p.) or (+)pentazocine (5 and 10 mg/kg, i.p.) to rat for 7 days (twice a day) resulted in 37-48 % decreases or no significance in various regions of brain in the specific binding of [^{3}H]NE-100 (for sigma-1 sites), respectively. Moreover, haloperidol or (+)pentazocine produced 17 % increase or 13 % decrease the specific binding of [^{3}H]MK-801 in the frontal cortex, respectively. These findings suggest that chronic haloperidol and (+)pentazocine treatment inversely modulates [3H]MK-801 binding in frontal cortex.

35.21

NOVEL ANTAGONISTS OF THE GLYCINE SITE OF THE NMDA RECEPTOR: IN VITRO CHARACTERIZATION AND REVERSIBILITY WITH GLYCINE L. J. Robichaud*, L. L. Coughenour, S. Borosky, G. W. Campbell, D. M. Rock, S.J. Hays, D.M. Retz, P-W. Yuen, C. F. Bigge, and P. A. Boxer. Neuroscience Therapeutics and Chemistry, Parke-Davis Pharm. Research, Div. Warner-Lambert Co., Ann Arbor, MI 48105.

Spontaneous epileptiform discharge (SED) frequency in the Mg^{2+}-free rat cortical wedge model is inhibited by various modulators of the NMDA-ion channel receptor complex (e.g. competitive and non-competitive antagonists, glycine-site antagonists) and ion channel blockers. Glycine, a co-agonist of the NMDA receptor, is present in excess in cortical wedges, since addition of glycine at concentrations up to 1 mM has no effect on SED frequency. Furthermore, inhibition of SEDs by known glycine antagonists (e.g., 7-chlorokynurenic acid) is reversed by additional glycine, in contrast to SED inhibition by other functional antagonists. Known and novel compounds representative of diverse chemical series (kynurenates, quinoxalinediones, 2-hydroxyquinoxaline-3-carboxylic acids, indole-2-carboxylic acids, and ester or sulfonamide derivatives) were evaluated in the following assays: [^{3}H]glycine binding, inhibition of SEDs and reversal of this inhibition by addition of glycine, and glutamate-induced LDH release from cultured cortical neurons. There was a good correlation between the IC_{50}s in these assays across all classes and potencies of compounds. Reversal of SED inhibition was a good predictor of glycine receptor affinity and inhibition of LDH release, whereas compounds without glycine reversibility of SED inhibition (CPP, MK-801, ifenprodil, spermine, toxins) frequency did not show glycine receptor affinity although they could inhibit glutamate-induced neurotoxicity.

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35.22

POTENTIATION OF NMDA RECEPTOR-MEDIATED RESPONSES BY DYNORPHIN AT LOW GLYCINE. U. Brauneis*, R. W. Peoples, M.Oz, F. F. Weight, and Li Zhang. Laboratory of Molecular and Cellular Neurobiology, NIAAA, NIH, Bethesda, MD 20892-8205

The effect of dynorphin on NMDA-activated currents in the presence of low glycine was investigated in oocytes expressing heteromeric NMDA receptors. While at 10 μM glycine dyn 1-13 inhibited NMDA-activated currents, lowering glycine to 100 nM caused a dramatic potentiation of NMDA-activated currents by 10 μM dyn 1-13: ε1/ζ1: 3377 ± 1416 % (n = 13); ε2/ζ1: 1897 ± 893 % (n = 5); ε3/ζ1: 4356 ± 846 % (n = 12); ε4/ζ1: 1783 ± 503 % (n = 14). This potentiation was inhibited by the glycine antagonist kynureic acid. Glycine concentration-response curves showed the shift of potentiation to inhibition to be between 1 μM and 10 μM glycine, depending on subunit combination of the NMDA receptor. Potentiation, like inhibition, of NMDA-activated currents by dyn 1-13 was not mediated by an opioid receptor. Dyn 2-13 and dyn 3-13, both of which have a glycine as the first amino acid, potentiated NMDA-activated current more strongly at low glycine than dyn 1-13. No potentiation was observed for dyn 4-13. In hippocampal neurons, dyn 1-13 and dyn 2-13 also potentiated NMDA-activated currents in the nominal absence of glycine. We investigated possible glycine contamination and concluded that the small amount of free glycine present in solutions, equipment and peptide preparations was insufficient to account for the observed potentiation of NMDA-activated current. This work was supported by NIAAA/NIH. U.B. was an NRC Fellow.

EXCITATORY AMINO ACID RECEPTORS: PHYSIOLOGY, PHARMACOLOGY, AND MODULATION—NMDA II

36.1

DISSOCIATION BETWEEN NMDA RECEPTOR MEDIATED INTRACELLULAR CALCIUM SIGNALLING AND ION FLUX. KEG Richter*, MJ Pagnozzi, JT Lazzaro, FS Menniti. Pfizer, Inc., Central Research Division, Groton, CT 06340.

NMDA receptors (NMDAR) are glutamate-gated cation channels that mediate depolarization, increases in cytoplasmic calcium concentration ([Ca2+]i), and calcium accumulation in the cell. We examined the agonist properties of NMDA at its receptor in rat cortical neurons in culture. The EC50 of NMDA measured in whole cell recordings was 60 uM, measured by 45Ca2+ uptake was 100 uM, but measured by changes in [Ca2+]i (using Fura-2) was only 5 uM. This suggests that maximal changes in [Ca2+]i occur at levels of NMDAR activation well below those which mediate depolarizing transmembrane ion flux. CP-101,606, a noncompetitive NMDAR antagonist, potently (IC50= 200 nM) and efficaciously (extent of block 75%) inhibited NMDA-induced ion flux (measured in whole cell recordings) but very weakly inhibited the increase in [Ca2+]i (20% block @ 10 uM). However, CP-101,606 potently (EC50 30 nM) potentiated the ability of Mg++ to block NMDA-induced increases in [Ca2+]i. Thus, by blocking depolarization, CP-101,606 potentiated the inhibitory effect of Mg2+. In summary, these results suggest that the NMDAR regulates [Ca2+]i at a level of activation far below that required to cause depolarization and calcium accumulation. This has implications for our understanding of NMDAR mediated signalling and neurotoxicity. Supported by Pfizer Central Research, Groton, CT.

36.2

ALKALINIZATION PROLONGS RECOVERY FROM GLUTAMATE-INDUCED INCREASES IN $[Ca^{2+}]_i$. Kari R. Hoyt* and Ian J. Reynolds. Dept. Pharmacol. Univ. Pittsburgh, Pittsburgh, PA 15261.

Using indo-1-loaded cultured rat forebrain neurons, we found that increasing extracellular pH from 7.4 to 8.5 caused a dramatic increase in the time required to recover from a glutamate-induced (3μM, in the presence of 1 μM glycine, for 15 s.) increase in $[Ca^{2+}]_i$. Recovery time to 3-fold basal $[Ca^{2+}]_i$ levels, in pH 7.4 Hepes-buffered saline solution (HBSS) was *62.9 ± 23.6 s. (n = 9)*, while recovery in pH 8.5 HBSS required *173.4 ± 18.6 s. (n=22)*. We tested whether this effect of alkalinization resulted from an increased Ca^{2+} influx via a Ca^{2+}/H^+ exchange function of the plasma membrane Ca^{2+} ATPase. This pH 8.5 effect was not due to increased influx of extracellular Ca^{2+} as removal of extracellular Ca^{2+} did not inhibit the prolongation caused by increasing pH *(144.1 ± 25.6 s. (n = 15))*. Cadmium (200 μM) which inhibits Ca^{2+} influx through calcium channels and/or the Na^+/Ca^{2+} exchanger, also did not completely block the effect of pH 8.5 HBSS *(128.13 ± 16.4 s. (n = 8))*. The addition of ketamine (100μM) to the pH 8.5 HBSS did not block the prolongation *(167.1 ± 32.9 s. (n = 13))*, excluding a role for continued NMDA receptor activation as a mechanism for the effect of pH 8.5 HBSS. Exposure of BCECF-loaded neurons to pH 8.5 HBSS resulted in a rapid intracellular alkalinization, thereby raising the possibility that the pH 8.5 HBSS may be exerting its effects on Ca^{2+} recovery by affecting intraneuronal Ca^{2+} buffering mechanisms, including mitochondrial Ca^{2+} uptake. The effect of pH 8.5 HBSS on Ca^{2+} recovery was similar to the mitochondrial uncoupler, FCCP (750 nM) in pH 7.4 HBSS *(201.5 ± 49.2 s. (n = 6))*, and combining pH 8.5 HBSS and FCCP did not have a substantially greater effect than either treatment alone *(252.9 ± 61.2 s. (n = 7))*. Removal of extracellular Na^+ along with pH 8.5 HBSS resulted in a longer recovery time *(555.7 ± 75.9 s. (n = 7))*, that was also not potentiated by FCCP *(591.8 ± 48.2 s. (n = 6))*. For comparison, Na^+ free-pH 7.4 HBSS resulted in a recovery time of *315.5 ± 68.0 s. (n=4)*, while addition of FCCP to this Na^+ free pH 7.4 HBSS resulted in a substantial additional prolongation of recovery time *(777.4 ± 164.7 s. (n = 5))*. In summary, these results suggest that the prolongation of $[Ca^{2+}]_i$ recovery time by alkalinization is not caused by an increase in Ca^{2+} influx (by a Ca^{2+}/H^+ exchange function of the plasma membrane Ca^{2+} ATPase), but instead may reflect an effect on intracellular Ca^{2+} buffering mechanisms, with inhibition of mitochondrial Ca^{2+} uptake representing a possible target. Support NIH grant NS 34138.

36.3

HOW IMPORTANT ARE ENDOPLASMIC RETICULUM CALCIUM STORES TO GLUTAMATE-INDUCED CHANGES IN $[Ca^{2+}]_i$? L.J. Reynolds*. *Dept. of Pharmacology, Univ. of Pittsburgh School of Medicine, Pittsburgh, PA 15261.*

Glutamate receptor activation results in increases in intracellular free Ca^{2+} ($[Ca^{2+}]_i$) due to Ca^{2+} entry. Endoplasmic reticulum (ER) Ca^{2+} stores may influence the effects of glutamate on $[Ca^{2+}]_i$, either by contributing to the buffering of cytoplasmic Ca^{2+} or by amplifying the response subsequent to Ca^{2+}-induced Ca^{2+}-release. Previous studies have suggested that the latter effect makes a substantial contribution to the NMDA component of the glutamate response. The present study has investigated the contribution of ER stores to the effects of glutamate on $[Ca^{2+}]_i$.

Experiments were performed on cultured forebrain neurons from e17 rats maintained for 14-18 days *in vitro*. $[Ca^{2+}]_i$ was usually measured with indo-1. Typically, two responses were obtained to glutamate in each cell, and the second, drug modified, response was compared to the first, as previously described (J. Neurosci. 15:1318, 1995). Drug effects were monitored in neurons stimulated with 0.3, 3.0 or 30μM glutamate.

Inhibition of the ER Ca^{2+} ATPase with thapsigargin (0.05-1.25μM) or cyclopiazonic acid (0.25-12.5μM) alone produced small increases in $[Ca^{2+}]_i$ (<50nM) in <20% of neurons tested. These drugs decreased subsequent glutamate responses by ≤40%. They did not measurably alter the rate of recovery of $[Ca^{2+}]_i$ to baseline. Inhibition of Ca^{2+} release with dantrolene (30μM) inhibited responses to a similar extent. Caffeine (10mM, $[Ca^{2+}]_i$ measured using fura-2) infrequently produced small increases in $[Ca^{2+}]_i$, and also decreased glutamate-induced responses by about 40%.

Thus, in these studies the release of Ca^{2+} from ER stores appear to make a smaller contribution to glutamate-induced $[Ca^{2+}]_i$ changes than has previously been reported. Also, there is no evidence for a substantial contribution of ER Ca^{2+} ATPase to Ca^{2+} buffering following glutamate receptor activation.

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36.4

MITOCHONDRIA ACCUMULATE Ca^{2+} STORES FOLLOWING INTENSE GLUTAMATE STIMULATION IN CULTURED RAT FOREBRAIN NEURONS

R. J. White* and I. J. Reynolds. *Center for Neuroscience and Dept. of Pharmacology, Univ. of Pittsburgh School of Medicine, Pittsburgh, PA 15261.*

In cultures of rat forebrain neurons, mitochondria buffer the Ca^{2+} influx resulting from brief NMDA receptor activation by glutamate. Here, we have independently varied both the glutamate concentration and the duration of exposure to investigate the $[Ca^{2+}]_i$ transients and cellular mechanisms recruited to buffer Ca^{2+} within different stimulation paradigms. For a 15 s. stimulus, the recovery time doubled as the glutamate concentration was raised from 3 to 300 μM. Changing the duration of exposure from 15 s. to five minutes increased the recovery time tenfold even when the glutamate concentration was held at 3 μM.

We then used a selective inhibitor of the mitochondrial Na^+/Ca^{2+}-exchange, CGP-37157, to explore the Ca^{2+} buffering role of mitochondria under these more intense stimulation paradigms. When applied immediately after a 15 s., 100 μM glutamate challenge, CGP-37157 consistently caused a rapid fall in $[Ca^{2+}]_i$ followed by a slow rise after the drug was washed out. A similar pattern was seen with the 5 min., 3 μM glutamate stimulus. Since this pattern was not seen following a 15 s., 3 μM glutamate stimulation, we propose that the slow rise following CGP-37157 washout reflects the release of mitochondrial Ca^{2+} stores which would have otherwise been expelled while the mitochondrial Na^+/Ca^{2+}-exchanger was inhibited.

These studies suggest that mitochondria become progressively more important for buffering glutamate-induced Ca^{2+} loads as the stimulus intensity increases. Moreover, the recovery of $[Ca^{2+}]_i$ to baseline following glutamate removal is critically regulated by the release of Ca^{2+} from mitochondrial stores via mitochondrial Na^+/Ca^{2+}-exchange; this highlights a previously under-appreciated role for $[Na^+]_i$ in the regulation of $[Ca^{2+}]_i$.

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36.5

GLUTAMATE-INDUCED $[Mg^{2+}]_i$ CHANGES IN NUCLEAR AND NON-NUCLEAR AREAS IN CULTURED FOREBRAIN NEURONS USING CONFOCAL MICROSCOPY. C. Cheng* and L.J. Reynolds. Dept. of Pharmacology, University of Pittsburgh School of Medicine, Pittsburgh, PA 15261.

Previously in our laboratory, it has been shown that a glutamate stimulus that causes excitotoxicity in cultured rat forebrain neurons also causes millimolar increases of intracellular free magnesium ($[Mg^{2+}]_i$) (Brocard *et al.*, Neuron 11: 751-757, 1992). This suggests that $[Mg^{2+}]_i$ may be important in the mechanism(s) underlying glutamate's excitotoxic effect. Although magnesium is an abundant intracellular cation, and is essential for many cellular functions, little is known about how it is regulated within neurons. In order to gain a better understanding of magnesium buffering within cells and how glutamate perturbs this balance, we sought to identify the possible intracellular pools of magnesium affected by glutamate exposure. Cultured forebrain neurons were loaded with the fluorescent magnesium indicator, Mag-indo-1, and confocal microscopy of single cells was used to visualize a 1μm section across the neuron. $[Mg^{2+}]_i$ was monitored by measuring the ratio of fluorescence emission at 405 and 490nm. The nucleus was identified in the same samples using the fluorescent indicator, Hoechst 33342. Neurons loaded with Mag-indo-1 showed significantly higher levels of fluorescence in the nucleus compared to the cytoplasm; however, the 405/490 ratio was not different across the cell suggesting that the $[Mg^{2+}]_i$ does not vary between the nucleus and cytoplasm. The addition of glutamate, in the presence of calcium or in Ca^{2+}-free buffer supplemented with 9.0mM Mg^{2+}, resulted in an increase in $[Mg^{2+}]_i$ in both cytoplasm and nucleus of similar magnitude and duration. Thus, the impact of glutamate-induced changes in $[Mg^{2+}]_i$ on neuronal function may not be limited to cytosolic effects.

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36.6

SIMULTANEOUS MEASUREMENT OF INTRACELLULAR FREE CALCIUM AND INTRACELLULAR FREE MAGNESIUM CONCENTRATIONS IN NEURONS. A.K. Stout* and I.J. Reynolds. Department of Pharmacology, University of Pittsburgh, Pittsburgh, PA 15261.

Previous fluorescence microscopy experiments using the magnesium-sensitive dye mag-fura-2 have demonstrated that glutamate can cause millimolar increases in intracellular free magnesium concentrations ($[Mg^{2+}]_i$) in cultured rat forebrain neurons (*Neuron*, 11: 751-757, 1993). Since mag-fura-2 is also sensitive to micromolar increases in intracellular free calcium concentrations ($[Ca^{2+}]_i$) and is frequently used as a low-affinity calcium probe, it is therefore important to demonstrate that mag-fura-2 is measuring increases in $[Mg^{2+}]_i$ rather than very large increases in $[Ca^{2+}]_i$ in our experimental paradigm. Since previous results from this lab have shown that the increases in $[Ca^{2+}]_i$ stimulated by glutamate (100 μM) plateau or decline over the course of a 5-minute stimulation while $[Mg^{2+}]_i$ continue to increase over the course of the same stimulation, and since previous studies have also shown that glutamate-induced $[Ca^{2+}]_i$ increases are not of a magnitude to result in significant binding to mag-fura-2 (*Neurosci. Letters*, 162: 149-152, 1993), these results suggest that mag-fura-2 is indeed measuring increases in $[Mg^{2+}]_i$. Using a monochromator-based imaging system, we are currently measuring glutamate-induced changes in $[Mg^{2+}]_i$ and $[Ca^{2+}]_i$ simultaneously in neurons loaded with both mag-fura-2 and either a high- or a low-affinity calcium-sensitive dye such as fluo-3 or calcium green-5N. The results of these studies will help to differentiate whether we are indeed measuring $[Mg^{2+}]_i$ changes with mag-fura-2 or whether the excitotoxic glutamate stimulations employed in these studies are causing large (*i.e.*, tens of micromolar) increases in $[Ca^{2+}]_i$. Supported by the American Heart Association and NIH grants NS 09998 and NS 34138.

36.7

TRH ATTENUATES GLUTAMATE-STIMULATED INCREASES IN INTRANEURONAL Ca^{2+} BY REDUCING THE INFLUX OF Ca^{2+} THOUGH NMDA, AMPA, AND VOLTAGE-GATED CHANNELS. M.L. Koenig*, D.L. Yourick, and J.L. Meyerhoff. Div. of Neurosciences, Walter Reed Army Institute of Research, Washington, DC, 20307-5100.

TRH, a tripeptide with anticonvulsant properties which is widely distributed in the mammalian central nervous system, has been reported to reduce glutamate-induced excitation of cerebral cortical neurons (Science 205:1275, 1979). Further, we have found that the reduction in excitation can be attributed, at least in part, to an attenuation of glutamate-stimulated increases in intraneuronal Ca^{2+} ($[Ca]_i$) (in primary neuronal cultures - Soc. Neurosci. Abstr. 20:278, 1994). To determine the mechanism by which TRH reduces glutamate-stimulated elevations in $[Ca]_i$, we loaded neurons (in primary culture) with the ratiometric Ca^{2+} indicator dye indo-1, and quantified changes in $[Ca]_i$ induced by exposure to ionotropic and metabotropic glutamate receptor agonists and depolarizing concentrations of KCl in the absence and presence of the tripeptide. We have found that a 2 min pre-exposure of neurons to TRH significantly reduces Ca^{2+} influx through channels opened by AMPA and NMDA (by 49% and 28% respectively), but has no effect on influx mediated by kainate. Prior exposure of cultured neurons to the tripeptide also has no effect on increases in $[Ca]_i$ generated by exposure to the Class I metabotropic receptor agonist *trans*-ACPD. Interestingly, TRH does seem to have an inhibitory effect on Ca^{2+} influx through voltage-gated Ca^{2+} channels. This is significant, because these channels are opened indirectly by glutamate, as the increased $[Ca]_i$ effects a depolarization.

36.8

HETEROMERIC NMDA RECEPTOR CALCIUM RESPONSES ARE SUBUNIT DEPENDENT IN TRANSFECTED HEK-293 CELLS. E.R. Grant*, B.J. Bacskai, L. Kricka, D.E. Pleasure, and D.R. Lynch. Dept. of Pharmacology, Neurology, and Pathology, U. Pennsylvania and Children's Hosp. of Philadelphia, PA 19104

The NMDA receptor mediates increases in intracellular calcium levels. Previously we have shown that application of 100 μM glutamate, 100 μM glycine, and 2 mM Ca^{++} to cells transfected with various NMDA receptors results in a subunit specific rise in intracellular calcium. Recently, using both the calcium sensitive bioluminescent protein aequorin, and the fluorescent calcium indicator fluo-3, we have quantitated peak rises for the NR 1a/2A combination as 1.36±0.09 μM and 1.14±0.19 μM measured with aequorin and fluo-3 respectively. The NR 1a/2B combination also elicited a significant intracellular calcium response (0.61±0.03 μM) to agonists, as did the $5HT_{2A}$ receptor to 10 μM serotonin (0.78±0.06 μM). However, no intracellular calcium responses to NMDA receptor agonists in cells transfected with the NR 1a/2C or NR 1a/2D subunit combinations were observed, even though differences in expression levels from NR 1A/2A were not found with ^{3}H-glutamate binding, ^{3}H-MK-801 binding, or western blotting. To determine the calcium source which mediates the rise in calcium for the NR 1a/2A combination, we found that substitution of the divalent cation barium for calcium in the extracellular buffer eliminated the NR 1a/2A calcium response to agonists. Transfection of the mutant NR1 (N598Q) with the 2A subunit decreased, but did not eliminate, intracellular calcium responses to agonists compared to wildtype NR 1a/2A. Ca^{45} influx studies revealed a 2.0±0.2 fold increase in Ca^{45} uptake upon agonist application compared to vehicle control in NR 1a/2A transfected cells, but not in NR 1a/2C transfected cells. NMDA receptor mediated changes in intracellular calcium are partly dependent on the receptor subunit composition and require extracellular calcium. (NIH-DA07130, NS01789)

36.9

ANGIOTENSIN II TYPE-2 RECEPTOR (AT_2)-MEDIATED INHIBITION OF NMDA RECEPTOR SIGNALING IN NEURONAL CELLS. **W.R. Schelman, J.L. Kurth, S.-W. Norby, B.C. Wheeler* and J.A. Weyhenmeyer. Department of Cell and Structural Biology, University of Illinois, Urbana, Illinois, 61801.**

N-methyl-D-aspartate (NMDA) type-1 receptor subunit (NR1) has a critical role in normal brain function and in neurologic disease. Here, we present immunochemical evidence for the expression of the NR1 receptor subunit in undifferentiated PC12W cells, which selectively expresses only the AT_2 receptor subtype, and in undifferentiated NG108-15 (NG108) cells, a murine neuroblastoma/glioma hybrid. We have recently reported that NMDA stimulated PC12W cells produce nitric oxide (NO) as measured by *in vitro* spin-trapping combined with electron paramagnetic resonance (EPR) spectroscopy (Norby et al., 1996). In the present studies, we show that the addition of angiotensin II (AngII) to the PC12W cells resulted in a 27.8% ± 5.1 decrease in NMDA-induced NO formation. Pretreatment with the isoform specific AT_2 antagonist, PD 123319, attenuated the inhibitory effect of AngII on NO production. Incubation with NMDA did not have an effect on cGMP accumulation in PC12W cells. Similar experiments were performed using undifferentiated NG108 cells since the binding and functional properties of the NMDA receptors expressed by these cells have been previously reported (Ohkuma et al., 1994). NMDA induced a dose-dependent increase in the levels of cGMP, which was decreased in the presence of AngII. The effect of AngII on NMDA-mediated changes in cGMP levels was partially blocked by PD 123319, but was not significantly altered by the AT_1 isoform specific antagonist, losartan. Further, AngII action in NG108 cells was completely inhibited by the addition of both the AT_1 and AT_2 antagonists. Taken together, these results suggest that AngII inhibits NMDA-mediated NO and cGMP production through a mechanism involving the AT_2 receptor subtype. (Supported by NSF IBN-9320158, W.R.S. is supported by AHA SS-06/CS-02).

36.10

NITRIC OXIDE MODULATES RESPONSIVENESS TO NMDA IN PARAVENTRICULAR NUCLEUS. 1) TYPE I NEURONS. J.S. Bains* and A.V. Ferguson. *Department of Physiology, Queen's University, Kingston, Ontario, Canada, K7L 3N6.*

Electrophysiological and anatomical evidence suggests that glutamate is the primary transmitter at fast, excitatory synapses in the paraventricular nucleus of the hypothalamus (PVN). Activation of glutamate receptors, and more specifically, the NMDA subtype, in other brain areas results in the subsequent synthesis and release of the diffusible messenger, nitric oxide (NO). The localization of NO synthase within PVN neurons led us to investigate the putative interactions between NO and NMDA receptor activation within this nucleus.

Whole cell recordings, using K-gluconate filled electrodes were obtained from PVN neurons in coronal brain slices (400 μm thickness) of adult, male Sprague-Dawley rats. Cells were classified as type I (putative oxytocinergic or vasopressinergic) if they displayed prominent outward rectification when depolarized from negative potentials (Tasker and Dudek, J. Physiol. 434: 273-294). Bath application of NMDA (10 nM - 1 μM) elicited robust depolarizations in all cells tested (n=48). At hyperpolarized potentials, NMDA application elicited profound oscillations of the membrane potential resulting in the emergence of burst like activity. Following bath application of the NO synthase inhibitor, N_ω-Nitro L-arginine methyl ester (L-NAME), subsequent depolarizing responses to NMDA were potentiated by 52 ± 27 % (n=7). NMDA application was also accompanied by inhibitory postsynaptic potentials (IPSPs) in 35 % of the cells. These IPSPs were inhibited by bath application of the $GABA_A$ receptor antagonist, bicuculline methiodide (n=4). IPSPs were also elicited by bath application of the NO donor, N-Acetyl-S-nitroso-D-penicillamine (SNAP) (n=4). IPSPs activated by NMDA were completely abolished in the presence of 1 mM L-NAME (n=6). These results support the hypothesis that NO regulates the excitability of Type I neurons in PVN by increasing GABA'ergic input to these cells.

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36.11

NITRIC OXIDE MODULATES RESPONSIVENESS TO NMDA IN PARAVENTRICULAR NUCLEUS. 2) TYPE II NEURONS. A.V. Ferguson* and J.S. Bains. *Department of Physiology, Queen's University, Kingston, Ontario, Canada, K7L 3N6.*

Electrophysiological and anatomical evidence suggests that glutamate is the primary transmitter released at fast, excitatory synapses in the paraventricular nucleus of the hypothalamus (PVN). Activation of glutamate receptors, and more specifically, the NMDA subtype, in other brain areas results in the subsequent synthesis and release of the diffusible messenger, nitric oxide (NO). The localization of NO synthase within PVN neurons led us to investigate the putative interactions between NO and NMDA receptor activation. We have previously reported that NO depolarizes the majority of type II cells in PVN (Bains and Ferguson, Soc. For Neurosci., 1995. 450.16)

Whole cell recordings, using K-gluconate filled electrodes were obtained from PVN neurons in coronal brain slices (400 μm thickness) of adult, male Sprague-Dawley rats. Cells were classified as type II if they displayed inward rectification at hyperpolarized potentials and a prominent low-threshold calcium spike. Bath application of NMDA (10 nM - 1 μm) elicited robust depolarizations in all cells tested (n=43). At hyperpolarized potentials, NMDA application elicited profound oscillations of the membrane potential resulting in the emergence of burst like activity. Following bath application of the NO synthase inhibitor, N_ω-Nitro L-arginine methyl ester (L-NAME), subsequent depolarizing responses to NMDA were inhibited by 39 ± 18 % (n=4). Inhibitory postsynaptic potentials (IPSPs) were also inhibited by application of NO (n=4). The IPSPs were also blocked by 1 mM bicuculline methiodide (BMI), confirming their identity as $GABA_A$ mediated events. These results support the hypothesis that NO regulates NMDA receptor activity on Type II neurons by decreasing GABA'ergic input to these cells.

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36.12

INTERACTION BETWEEN PERMEANT CATIONS AND CHANNEL BLOCKERS WITHIN THE PORE OF THE NMDA RECEPTOR. S.M. Antonov*, V.E.Gmiro† and J.W. Johnson. Dept. of Neuroscience, Univ. of Pittsburgh, Pittsburgh, PA 15260; †Institute of Experimental Medicine RAMS, St.Petersburg, A-22, Russia.

Single-channel currents through native NMDA receptors were recorded in outside-out patches excised from cultured rat cortical neurons. The mechanism of open-channel block of the NMDA receptor pore by IEM-1857 and IEM-1754 applied in the external solution revealed considerable deviations from conventional models of channel block. (i) The voltage dependence of the rate constant of binding (k_+) for either blocker was not single-exponential: the voltage dependence was over two times weaker at membrane voltages (V_ms) from -90 to -140 mV than at V_ms from -20 to -90 mV. (ii) External and internal permeant cations affected the k_+ of the IEM drugs. The effect of a reduction of $[Cs^+]_{in}$ was voltage-dependent. When $[Cs^+]_{in}$ was reduced from 130 to 20 mM the values of k_+ were increased, but only at voltages more positive than -90 mV. The magnitude of the increase of k_+ became progressively greater as V_m was depolarized. A reduction of $[Na^+]_{out}$ from 140 to 70 mM caused an increase of approximately three-fold of the values of k_+ over the entire V_m range tested. (iii) The reduction of $[Cs^+]_{in}$ decreased the values of the rate constant of unbinding (k_-) of IEM-1754 at V_ms from -60 to -140 mV. (iv) Finally, the reduction of $[Na^+]_{out}$ caused a voltage-independent increase in the values of k_- of IEM-1857 and IEM-1754.

The data show that experimentally measurable rate constants of channel block by the IEM drugs applied externally are influenced by permeant cations. A model which is consistent with our data suggests the existence of permeant cation binding sites in the external and internal vestibules of the NMDA receptor pore. These binding sites appear to be located outside the region of the pore where the drop of the membrane electric field occurs. Supported by NIMH.

36.13

MODULATION OF NMDAR CHANNELS BY Ca^{2+}-CALMODULIN IN RAT CEREBELLAR GRANULE CELLS. N. Filippova,† K. Hatta* & P. Bregestovski
Koltzov Inst. Develop. Biology, Moscow†, INSERM, U-29 & U-261, Paris.

Transient inactivation of NMDA receptor (NMDAR) channels by cytoplasmic calcium ($[Ca^{2+}]i$) was described in neurons and transfected HEK-293 cells. According to Medina et al. (J. Physiol., in press) the ability of $[Ca^{2+}]i$ to inhibit NMDAR channels decreases with time during whole-cell recording and disappears shortly after excision of inside-out patches. This suggests that Ca^{2+} actions on NMDAR may be mediated by a putative diffusible factor. From the study of Ehlers et al. (Cell, **84**:745-755, 1996), the Ca^{2+}-dependent protein, calmodulin (CaM), is a likely candidate. These authors demonstrated that NMDAR function can be regulated by direct binding of CaM to the NR1 subunit.

Using patch-clamp techniques we studied the effect of CaM on NMDAR channels in rat cerebellar granule neurons in culture. During whole-cell recording, to increase $[Ca^{2+}]i$, long (5 s) conditioning pulses of 100 μM NMDA (2 mM $[Ca^{2+}]o$) were applied to neurons. For a quantitative estimation of Ca^{2+}-induced inactivation, the amplitude of brief (100 ms) NMDA test-currents was analyzed before and after conditioning applications. With 4 mM Mg-ATP in the pipette solution, in cells that did not show rundown, the Ca^{2+}-induced inactivation decreased continuously during the recording: 28±7 % and 6±5 % on 3-rd and 22-nd min respectively (n=15). Addition of 200 nM CaM to the internal solution did not prevent this continuous decrease of $[Ca^{2+}]i$ action: inactivation of NMDA test-currents was 47±14% on 3-rd and 8±4% (n=6) on 23-rd min of recording.

In inside-out patches (5 μM NMDA, 10 μM glycine in the pipette), direct application of 10 μM Ca^{2+} to the cytoplasmic side of the membrane resulted in a reversible decrease in the open probability (Po) of single NMDAR channels (50±20%, n=5). However, 3-5 min after obtaining the inside-out patches, NMDAR channels became Ca^{2+} insensitive (0.1-1 mM Ca^{2+} was ineffective). Application at this time of 10 μM Ca^{2+} with 200 nM CaM induced 71±19% reversible decreases in Po (n=5). Our data indicate that in cerebellar granule neurons direct application of Ca^{2+} + CaM to excised inside-out patches causes inhibition of NMDAR channels, however, during whole-cell recording intracellular dialysis leads to the loss of NMDAR channel sensitivity to $[Ca^{2+}]i$ independently of CaM.

36.14

NMDA RECEPTOR SUBUNIT COMPOSITION EFFECTS THE KINETICS OF DESENSITIZATION AND RECOVERY FROM DESENSITIZATION. T.Priestley, P.Laughton and R.G. Hill*. Merck Sharp & Dohme Research Laboratories, Terlings Park, Harlow, Essex. CM20 2QR. UK.

A characteristic feature of NMDA receptors is that they desensitize during prolonged agonist exposure and the desensitization has both glycine- and calcium-dependent components. Molecular cloning has revealed that these ligand-gated ion channels are hetero-oligomeric assemblies of subunit proteins. We have examined the macroscopic kinetics of glycine-independent desensitization and recovery from desensitization in L(tk-) cells permanently transfected with human NR1a/NR2A and NR1a/NR2B subunit combinations.

Whole-cell currents were recorded from cells following rapid steps into glutamate (30μM) in the presence of a saturating concentration of glycine and 2mM external calcium. NR1a/NR2B receptor desensitization proceeded with a monophasic exponential time course and relatively slow kinetics (τ_d = 5.5s, n = 16); recovery from desensitization was slow (τ_{rec} = 21s) and monophasic. Recombinant NR1a/NR2A receptors showed biphasic desensitization kinetics comprising fast (τ_{fast} = 0.4 ± 0.04) and slow (τ_{slow} = 3.5 ± 0.7s, n = 5) components together with biphasic, time-dependent recovery (τ_{rec} = 1.6s and 49s). The influence of the slow component of desensitization of NR1a/NR2A receptors declined with time during prolonged whole-cell recordings. This had two consequences: NR1a/NR2A receptors desensitized more quickly but also recovered from the desensitized state more quickly at the end of a 30min recording period compared to time zero. Thus, the macroscopic kinetics of desensitization and recovery from desensitization are also effected by changes in receptor state brought about by protracted whole-cell recording. These time-dependent changes appear to be due to an altered phophorylation state of receptor proteins which also appears to be dependent upon subunit composition.

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36.15

1-AMINOCYCLOPROPANECARBOXYLIC ACID (ACPC) PROTECTS AGAINST NMDA RECEPTOR-MEDIATED INJURY TO CULTURED SPINAL CORD NEURONS BOTH ACUTELY AND THROUGH DESENSITIZATION. Y. Lin* and J.B. Long. Div. of Neurosciences, Walter Reed Army Inst. of Res., Wash., D.C. 20307.

ACPC is a high affinity partial agonist for the glycine binding site within the NMDA receptor complex. This compound has been shown to protect against ischemic brain and spinal cord injury in vivo and to block NMDA receptor-mediated neurotoxicity in vitro. Chronic treatment with ACPC in vivo also appears to reversibly desensitize the NMDA receptor complex, prompting suggestions that it might provide an effective means of ameliorating degenerative mechanisms mediated through this ligand-gated ion channel. In these experiments, cultured rat spinal cord neurons were used to further examine the effects of acute and sustained ACPC exposures on NMDA-induced neurotoxicity and changes in $[Ca^{2+}]_i$. Cell damage was quantitatively assessed using a tetrazolium salt colorimetric assay, and changes in $[Ca^{2+}]_i$ were measured in single identified neurons preloaded with the Ca^{2+}-sensitive dye Fluo-3. With coincubation, 1mM ACPC significantly reduced the neuronal cell damage caused by 30 min exposure to 25 or 50 μM concentrations of NMDA, but failed to alter the cell injury induced by 100 μM NMDA. The protective effects of ACPC were competitively abolished by co-addition of glycine (1-1000 μM), verifying that they were mediated through glycine binding sites. Sustained 20 hr exposure to 1 mM ACPC (which was removed 30 min before addition of 25 μM NMDA) also caused cells to be significantly less responsive to the neurotoxic effects of NMDA than were vehicle-pretreated control cells. Pre-exposure to ACPC for shorter intervals (< 1 hr) failed to alter subsequent NMDA toxicity. Acute or sustained exposures to ACPC alone did not affect cell viability or $[Ca^{2+}]_i$. Also, paradoxically, ACPC did not alter the NMDA-triggered elevations in $[Ca^{2+}]_i$. These results support earlier indications that: 1) ACPC provides an effective means of antagonizing excitotoxic phenomena, and 2) sustained exposure to ACPC desensitizes the NMDA receptor complex.

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36.16

FREQUENCY-DEPENDENT MODULATION OF NMDA RECEPTOR DESENSITIZATION IS MEDIATED BY RECEPTOR MECHANO-SENSITIVITY AND GLYCINE. L.A. Raymond*[#] and R.L. Huganir[&]. [#]Div. Neurosci., Dept. Psych., U. of British Columbia, Vancouver, B.C. and [&]HHMI, Dept. Neurosci., Johns Hopkins Med. Inst., Baltimore, MD.

N-methyl-D-aspartate (NMDA) receptors play a critical role in synaptic plasticity, and studies have shown that synaptic stimulation of these receptors can result in their desensitization. In whole-cell patch clamp recordings from human embryonic kidney 293 cells transfected with NR1/NR2A, we have found that the extent of desensitization of agonist-evoked current can be modulated by changes in hydrostatic pressure and glycine concentration. Desensitization of current evoked by 0.5-1 s applications of glutamate or NMDA is <20% under conditions of constant hydrostatic pressure, independent of glycine concentration. However, when transfected cells are subjected to intermittent pulses of increased hydrostatic pressure, the extent of desensitization is inversely related to the fraction of time exposed to higher pressures. Therefore, high frequency pressure pulses result in high steady-state to peak current ratios (I_{ss}/I_p) while low I_{ss}/I_p result from low frequency pressure pulses. Switches between low and high I_{ss}/I_p states begin after a delay of a few pressure pulses at the new frequency, and occur gradually over several pulses, thus exhibiting "memory" for the previous pulse frequency protocol. Modulation of I_{ss}/I_p by pressure pulses is due to a change in receptor affinity for glycine and can be overcome by >10 μM glycine. Pressure-induced modulation of NMDA receptor desensitization may represent a novel mechanism for short-term NMDA receptor-mediated synaptic plasticity. (Supported by MRC of Canada (LAR); HHMI (RLH)).

36.17

PROTONS INCREASE DESENSITIZATION OF *N*-METHYL-D-ASPARTATE RECEPTORS EXPRESSED IN *XENOPUS* OOCYTES. Y. Liu*, R. H. Hill, G. von Euler and P. Århem. Dept. of Neuroscience, Karolinska Institute, S-171 77 Stockholm, Sweden.

It has been reported that protons inhibit *N*-methyl-D-aspartate (NMDA)-activated currents in cultured hippocampal and cerebellar neurons. The results indicated the presence of a proton binding site, which is located at the extracellular surface of the membrane.

In the present study we have investigated the effects of pH on NMDA receptors expressed in *Xenopus* oocytes. Oocytes were injected with *in vitro*-synthesized mRNA of the NMDA receptor subunits NR1 and NR2A. The results showed that the inward current induced by 100 μM NMDA and 100 μM glycine was reduced by decreasing the pH levels stepwise from 9.0 to 6.0, and that the current reduction is voltage-independent. The ratio of steady state (corresponding to a desensitized state) current to peak current varied at different pH levels. At pH 8.5, NMDA and glycine induced a large current with little or no desensitization. However, at pH 7.4, NMDA and glycine induced a rapidly rising current followed by a pronounced desensitization. At pH 6.5, agonist application lead to a small steady state current without a clear peak current. When the agonists were applied at the same time as pH was changed from 7.4 to 6.5, a clear but small peak current was observed, followed by a strong desensitization. This can be explained by an onset of desensitization when the pH was quickly lowered.

In conclusion, our results suggest that protons inhibit the inward current induced by glutamate and glycine, at least partly, by enhancing the desensitization of NMDA receptors. The mechanism of the proton-dependent desensitization appears to be different from the glycine-sensitive and the calcium-dependent desensitization. This work was supported by the Swedish MRC (04X-06552 and 14X-10377).

36.18

GLYCINE: ITS ROLE IN MODULATING THE NMDA RECEPTOR RESPONSE AND REGULATING DESENSITIZATION. G. A. Worrell, B. N. Christensen and M. Phillips* Department of Physiology and Biophysics and Department of Pharmacology, University of Texas Medical Branch, Galveston, TX, 77555.

The response from acutely dissociated catfish (*Ictalurus punctatus*) horizontal cells (CFHC) to N-methyl-D-aspartic acid (NMDA) and glycine were recorded using the whole-cell recording technique. A system that provided rapid concentration clamp for the agonist, of the order ~20 msec, over the entire neuron surface was used to study the modulation of the NMDA response by glycine. The glycine concentration was determined for each experiment using HPLC. Nominal glycine was routinely < 10 nM. The best fit of the Hill equation to the glycine concentration-response data yields an apparent dissociation constant (K_d) of 195.0 nM and a Hill coefficient (N) of 1.1. The current response in the complete absence of glycine (I_0) was also used as a fitting parameter and was found to be 74% of the response measured at a saturating glycine concentration. This result indicates that most of the response to NMDA can be produced in the complete absence of glycine.

Cells that were pretreated with 5.0 μM glycine and then concentration clamped with NMDA and a nominal glycine concentration, demonstrated rapid desensitization. Desensitization was eliminated if the agonist solution contained a saturating concentration of glycine. The rate constant of the rapid phase of desensitization is approximately a linear function of the NMDA concentration with slope of 15.1 $(mSec\text{-}M)^{-1}$ and an intercept of 0.85 sec^{-1}. The intercept provides an estimate of the dissociation rate of glycine from the receptor in the absence of NMDA. This result indicates that desensitization in these cells is a result of unbinding of glycine from its receptor site. The rate of unbinding increases as the concentration of NMDA is increased. Supported by grant NEI-01897 from the NIH.

OPIOID RECEPTORS I

37.1

LOCUS COERULEUS-LIKE CATH.a CELLS EXPRESS DELTA OPIOID RECEPTOR WHICH ARE FUNCTIONALLY COUPLED TO INHIBITION OF ADENYLYL CYCLASE ACTIVITY. M. Leid*, V.J. Peterson, B.D. Hettinger-Smith, K. Soderstrom, T.F. Murray and C. Bouvier. College of Pharmacy, Oregon State University, Corvallis, OR 97331.

CATH.a cells are a locus coeruleus (LC)-like cell line which exhibit a strongly catecholaminergic phenotype. We have carried out a pharmacological characterization of CATH.a cells to define the opioid receptor complement expressed by these cells. Three lines of evidence strongly suggest that CATH.a cells express predominantly δ opioid receptor (DOR): (1) the rank order of potency with which a series of prototype, subtype-selective opioid receptor agonists inhibited [^{3}H]Diprenorphine binding in CATH.a cell membranes was identical to that carried out using membranes derived from CHO cells stably expressing recombinant DOR:

(DPDPE >>> DAMGO > U50488),

(2) CATH.a cell membrane preparations were directly labeled with high affinity using the DOR-selective radioligand [^{3}H]DPDPE and the number of receptors labeled with this agonist radioligand corresponded to fraction of [^{3}H]Diprenorphine binding sites exhibiting high affinity for agonists in competition experiments, and (3) DPDPE potently (IC_{50} = 1.86 nM) and robustly (~ 40% inhibition) inhibited forskolin-stimulated, CATH.a cell adenylyl cyclase activity in a naloxone-reversible manner. These findings demonstrate that CATH.a cells express DOR and that at least one of the opioid signaling pathways (inhibition of adenylyl cyclase) is operant in this cell line. This cell line may be of use in the characterization of DOR-mediated events without the confounding variable of expression of other opioid receptor subtypes. We thank D. Chikaraishi and D. Grandy for CATH.a cells and CHO cells stably transfected with recombinant DOR, respectively.

Supported by a grant from the PMA Foundation

37.2

EFFECTS OF ESTRADIOL ON cAMP IN OPIOID-RESPONSIVE SK-N-SH CELLS AFTER CHRONIC MORPHINE TREATMENT. Anna Ratka* and Val McKinley, Department of Pharmaceutical Sciences, College of Pharmacy, Idaho State University, Pocatello, ID 83209.

The SK-N-SH human neuroblastoma cells express opioid receptors. Cyclic AMP responses to morphine (M), attributed to the activation of opioid receptors, are well defined in these cells. The inhibitory effect of an acute M on cAMP is significantly reduced after chronic pretreatment with M. Differentiated and undifferentiated SK-N-SH cells were exposed to M (10μM) for 6 days. Treatment with 5 nM estradiol (E) was for 1 hr, 1 day and 6 days. Changes in cAMP level, measured by radioimmunoassay, induced by an acute 10 μM dose of M, served as a marker of opioid receptor function. E had a time-dependent attenuating effect on M-induced inhibition of prostaglandin E_1(PGE_1)-stimulated accumulation of cAMP in differentiated SK-N-SH cells. The degree of M inhibition declined from 63, 42, 28 to 26 % in M-naive cells, and from 34, 31, 20 to 15% in M-treated cells, after no E, 1hr, 1 day and 6 days of E treatment, respectively. Moreover, 1 and 6 day treatment with E significantly potentiated the PGE_1-induced cAMP accumulation in M-treated cells, from 616.9+/-18.0 to 1405.9+/-62.0 and 1142.2+/-250.0 pMol/mg protein, respectively. We conclude that in opioid-responsive differentiated SK-N-SH neuroblastoma cells, E has a profound attenuating effect on the function of both M-naive and M-treated opioid receptors.

[Supported by Faculty Reserch Committee (grant #750) and NSF-EPSCoR (REU)]

37.3

μ RECEPTOR ACTIVATION IN SH-SY5Y HUMAN NEUROBLASTOMA AND CHO-MOR CELLS IS BLOCKED BY G-PROTEIN α SUBUNIT ANTISENSE DNA. K.M. Standifer*[1], G.P. Brown[2] and G.W. Pasternak[2], [1]Dept. Pharmacological and Pharmaceutical Sciences, University of Houston, Houston, TX and [2]Cotzias Laboratory of Neuro-Oncology, Memorial Sloan-Kettering Cancer Center, NY, NY.

A cultured cell line stably expressing the cloned μ receptor allows for examination of interactions between second messenger systems and a molecularly defined receptor, an advantage over a natively expressing cell line such as SH-SY5Y. One disadvantage is that the receptor is expressed in an environment which may not accurately reflect native conditions. Thus, when utilizing these different model systems, it is important to understand differences in receptor activation and signalling between the systems. One approach is to determine which G-proteins are responsible for transducing agonist-mediated effects in each cell line by down-regulating α subunit expression with administration of antisense DNA. SH-SY5Y and CHO-MOR cells were treated for 42-48 hr with antisense DNA (1.2 μM) directed against $G_{i}\alpha1$, $G_{i}\alpha2$, $G_{i}\alpha3$ or $G_{o}\alpha$, and the ability of DAMGO to inhibit forskolin-stimulated cAMP accumulation was determined. Treatment with $G_{o}\alpha$ antisense significantly reduced the EC_{50} value in both cell lines, while $G_{i}\alpha2$ and $G_{i}\alpha3$ antisense treatment was effective in SH-SY5Y and CHO-MOR, respectively. $G_{i}\alpha1$ antisense was without effect. This suggests that different G-proteins are responsible for μ receptor-mediated cAMP inhibition in the two cell lines. This work was supported by NIDA and the Wendy Will Case Cancer Fund.

37.4

EFFECT OF KINASE SITE MUTATION ON MU OPIOID RECEPTOR FUNCTION. L. S. Bye and L. Yu*. Dept. of Med. and Mol. Genetics, Indiana Univ. Sch. of Med., Indianapolis, IN 46202

The mu opioid receptor is the site of a number of clinically used opioids for analgesia and anesthesia. Prolonged use of mu receptor agonists, however, often results in tolerance to these drugs. The cellular mechanism of the development of tolerance is unknown.

Using the cloned mu opioid receptor we established a model to study desensitization at the cellular level. Using *Xenopus laevis* oocytes as an expression system, the mu opioid receptor was coexpressed with an inwardly rectifying K^+ channel (GIRK1). Activation of the mu opioid receptor results in an increase in the K^+ current. We previously showed that desensitization of this receptor-mediated K^+ current is modulated by cAMP-dependent protein kinase (PKA). To further study the molecular mechanism of PKA-mediated modulation, a mutant was generated using oligonucleotide-mediated, site-directed mutagenesis in which the putative PKA phosphorylation site threonine was changed to alanine. The mutant receptor, when expressed in *Xenopus* oocytes, can be activated to elicit the K^+ current. Compared with the wildtype mu receptor-mediated K^+ current, however, the mutant receptor-mediated current displayed altered kinetics upon agonist washout, with a significantly larger decay time constant. This suggests that the capability to be phosphorylated by PKA plays an important role in mu opioid receptor function. (Supported in part by NIDA and NINDS.)

37.5

INHIBITION OF cAMP ACCUMULATION AND STIMULATION OF GTPγS BINDING CORRESPOND CLOSELY TO RECEPTOR NUMBER IN μ RECEPTOR-CONTAINING CHO CELLS. Lawrence Toll* and Willma E. Polgar. SRI International 333 Ravenswood Ave. Menlo Park, CA 94025.

μ-Receptor-containing CHO cells were subcloned and individual clones tested for binding parameters using [^{3}H]bremazocine, inhibition of forskolin-stimulated cAMP accumulation, and opiate-stimulated [^{35}S]GTPγS binding. In four individual clones, receptor levels varied from 0.1 to 1.5 pmol/mg protein, without any changes in affinity of bremazocine, approximately 0.15 nM in each clone. For both inhibition of cAMP accumulation and [^{35}S]GTPγS binding, maximal activity correlated closely with receptor number. The clone with the lowest receptor number was essentially devoid of activity in either functional assay. The clones with intermediate receptor number produced intermediate levels of opiate-induced inhibition of cAMP accumulation and stimulation of [^{35}S]GTPγS binding. In the clone with the highest receptor number DAMGO produced 75% inhibition of cAMP accumulation, and 250% stimulation of [^{35}S]GTPγS binding. The potency of the selective μ agonist DAMGO was also tested in the [^{35}S]GTPγS binding assay, and found to be independent of receptor number in this assay. In each clone the EC_{50} for DAMGO was found to be approximately 5 nM. This indicates that there is no receptor reserve with respect to the [^{35}S]GTPγS binding assay, and activity is directly related to the receptor number and efficacy of the agonist tested.

Supported by NIDA grant DA06682.

37.6

μ-SPECIFIC OPIOID COMPOUNDS AFFECT SECRETION FROM PEPTIDERGIC NERVE TERMINALS WITHOUT ALTERING THEIR ELECTROPHYSIOLOGICAL PROPERTIES. V.J. Coccia, G. Wang, G. Dayanithi†, and J.R. Lemos* ,Worcester Foundation for Biomedical Research, Shrewsbury, MA 01545 and †CNRS, University of Montpellier II, France 34095

Peptidergic nerve terminals in the neurohypophysis (NH) secrete either oxytocin (OT) or vasopressin (AVP) in response to depolarization. We investigated the effects of μ-specific opioids on both the electrophysiological and secretory responses of these terminals to depolarization. μ-specific opioid compounds have profound effects on the secretion of both OT and AVP from isolated NH terminals. We treated NH terminals with either DAMGO (μ-agonist) or naloxonazine (μ-antagonist) and examined the change in the amount of either OT or AVP secreted by high K^+. Increasing the dose of DAMGO decreased the amount of both AVP and OT secreted; OT secretion was reduced 76% by 100 nM DAMGO. 100 nM DAMGO reduced AVP secretion by 20%. Treatment with naloxonazine potentiated release of OT and AVP from isolated terminals; OT secretion was increased 52% after treatment with 1 μM naloxonazine. AVP secretion was increased 10% by the identical treatment. The mechanism of the μ-opioid compound's effect on peptide secretion in the NH is not clear, since DAMGO has been shown to have no effect on calcium currents in isolated terminals (Rusin et al., 1995, *Neurosci. Abs.* **21**: 1092). We, therefore, examined action potentials and their underlying sodium-, A- and Ca^{2+}-activated K^+-currents from isolated NH terminals using the perforated, "whole-cell" patch-clamp configuration. Treatment with up to 13 μM DAMGO or naloxonazine did not alter either the amplitude or duration of the action potential of electrically stimulated terminals. Consistent with this, alterations in the underlying currents were not statistically significant from control currents. These results challenge the notion that modulation of the ion channels in the terminal are solely responsible for changes in peptide secretion from the terminal. (Supported by NIH grants NS07366 & NS29470).

37.7

EXPRESSION OF THE G PROTEIN-COUPLED RECEPTOR KINASES 2 AND 3 DOES NOT AFFECT THE COUPLING OF THE MU OPIOID RECEPTOR TO THE G PROTEIN-GATED INWARDLY RECTIFYING POTASSIUM CHANNEL COMPLEX IN *XENOPUS* OOCYTES A. Kovoor, D. Henry*, C. Chavkin, Dept. of Pharmacology, Univ. of Washington, Seattle, WA

Chronic morphine exposure leads to an uncoupling of the mu opioid receptor from downstream G proteins. Previously, agonist-dependent phosphorylation of the mu opioid receptor has been reported in cell lines and homologous desensitization of mu opioid receptor-effector coupling has been reported in the brain. We used the *Xenopus* oocyte system to test the hypothesis that the above events were a result of phosphorylation of the mu opioid receptor by G protein-coupled receptor kinases (GRKs) leading to a functional uncoupling to the effector. The application of mu opioid agonists evoke an increase in K^+ conductance in *Xenopus* oocytes expressing the rat mu receptor and the G protein-gated, inwardly rectifying K^+ channel subunits, GIRK and CIR. GRK2 and GRK3 were separately coexpressed with the mu receptor and the inwardly rectifying channel complex (Kir) and overexpression of either kinase failed to alter the coupling of the receptor to the channel. Neither the peak response nor the rate of desensitization of the response to the mu opioid agonist DAMGO was altered. Subsequent coexpression of either arrestin 2 or arrestin 3 with GRKs 2 and 3 also failed to alter the coupling of the mu receptor to the Kir. Functional expression of the GRK and arrestin proteins was tested by verifying their ability to inhibit the β2-adrenergic receptor stimulation of CFTR mediated Cl^- currents in oocytes exogenously expressing these proteins. Supported by DA04213.

37.8

THE PHOSPHORYLATION STATE OF THE KAPPA OPIOID RECEPTOR IS INCREASED IN GUINEA PIG BRAINS FOLLOWING AN ACUTE INJECTION OF U50,488H, BUT DECREASED IN GUINEA PIGS TOLERANT TO U50,488H. S.M. Appleyard, W. Jin, T.A. Patterson and C. Chavkin* Dept. of Pharmacology, University of Washington, Seattle, WA 98195.

Phosphorylation has been shown to modulate opioid receptor function. In this study we used antibodies generated against the kappa opioid receptor to examine whether the phosphorylation state of the kappa receptor is altered in the brains of guinea pigs following acute or chronic exposure to U50,488H. Guinea pigs were given a single injection of U50,488H (25 mg/kg, sc), sacrificed an hour later, and then brain membranes were solubilized in the presence of phosphatase inhibitors. The kappa opioid receptor was immunoprecipitated, then phosphorylated *in vitro* with ^{32}PγATP and PKC. The amount of protein immunoprecipitated was quantified by immunoblot and the ^{32}P incorporation was measured by phosphoimage analysis. The ^{32}P incorporated into the kappa receptor was decreased by 23±7.5% (n=3) following acute U50,488H compared to saline injected controls. In contrast, for guinea pigs made tolerant to the effects of U50,488H by repeated injections of increasing doses of U50,488H, the ^{32}P incorporation was increased 187±37% (n=3) compared to saline treated controls (=100%). These results suggest that an acute injection of U50,488H leads to a small increase in the phosphorylation of the kappa opioid receptor, but that following chronic exposure to U50,488H the phosphorylation state of the receptor is reduced. Supported by DA04123.

37.9

OPIOID-INDUCED CURRENTS IN ACUTELY DISSOCIATED LOCUS COERULEUS NEURONS. S. L. Ingram* and J. T. Williams. Vollum Institute, Oregon Health Sciences University, Portland OR 97201.

Whole-cell recordings from acutely dissociated locus coeruleus neurons were made to further study activation of the G-protein coupled inward rectifying potassium channels by opioids. Opioid-induced inward currents were recorded in a high potassium external solution (30 mM K+). Agonists were applied with a fast flow system (exchange < 100 ms). One second applications of saturating concentrations of [Met]^5 enkephalin (ME) elicited inward currents ranging from 100 pA to 1 nA at -60 mV. The time course of decay ranged from 1.3 to 4.1 s. One second applications of a saturating concentration of the selective μ-receptor agonist DAMGO produced inward currents ranging from 100 pA to 500 pA at -60 mV and time to decay ranged from 0.88 to 1.5 s. Therefore, DAMGO currents seem to recover to baseline faster than ME currents. The latency of response (36 to 306 ms) and time to peak (1.2 to 1.5 s) were similar for both agonists. These results show that the opioid responses were substantially slower than solution exchange. There was no evidence for desensitization for applications of agonists up to 20 s. Interestingly, saturating concentrations of morphine did not produce inward currents but reversed the DAMGO current in all cells tested. Naloxone (1 μM) also reversed the inward current produced by ME and DAMGO. Under these conditions, morphine appears to act as an antagonist, suggesting that the efficiency of coupling may be compromised during the dissociation procedure. Supported by NIDA grant DA08163.

37.10

DIFFERENTIAL EFFECTS OF ω-CONOTOXIN, NIMODIPINE, CALMIDAZOLIUM, AND KN-62 INJECTED SPINALLY ON ANALGESIA INDUCED BY ß-ENDORPHIN AND MORPHINE ADMINISTERED SUPRASPINALLY. H. W. Suh, D. K. Song*, S. R. Choi, and Y. H. Kim, Department of Pharmacology, Institute of Natural Medicine, College of Medicine, Hallym University, Chunchon, Kangwon-Do, 200-702, S. Korea.

Several lines of evidence have demonstrated that ß-endorphin and morphine administered supraspinally produce the antinociception by activating different descending pain inhibitory systems. In the present study, the effects of nimodipine (a L-type calcium channel blocker), ω-conotoxin (a N-type calcium channel blocker), calmidazolium (a calmodulin antagonist), or KN-62 (a calcium/calmodulin-dependent protein kinase II inhibitor) injected intrathecally (i.t.) on the antinociception induced by morphine, DAMGO, or ß-endorphin administered intracerebroventricularly (i.c.v.) were examined in the present study. Antinociception was assessed by the tail-flick test. The i.t. injection of nimodipine, ω-conotoxin, calmidazolium, or KN-62 at the doses from 0.01 to 1 ng alone did not affect the basal tail-flick latencies. The pretreatment of mice i.t. with nimodipine, ω-conotoxin, calmidazolium, or KN-62 dose dependently attenuated the inhibition of the tail-flick response induced by ß-endorphin administered i.c.v. However, the inhibition of the tail-flick response induced by morphine or DAMGO administered i.c.v. was not changed by i.t. pretreatment with nimodipine, ω-conotoxin, calmidazolium, or KN-62. The results suggest that spinally located L-, and N-type calcium channels, calmodulin, and calcium/calmodulin-dependent protein kinase II may be involved in the modulation of antinociceptive action induced by ß-endorphin, but not morphine and DAMGO, administered supraspinally (supported by grant 941-0700-009-2 from KOSEF).

37.11

ACUTE AND CHRONIC DRUG EFFECTS ON OPIOID AND CANNABINOID RECEPTOR ACTIVATED G-PROTEINS IN RAT BRAIN. S.R. Childers*, L. Sim, R. Xiao, & D. Selley, Dept. Physiol./ Pharmacol., Bowman Gray Sch. Med., Winston-Salem, NC 27157.

Receptor-activated G-proteins can be measured by agonist stimulation of [^{35}S]GTPγS binding in membranes or brain sections in the presence of excess GDP. In membranes from thalamus or CHO cells transfected with mu opioid receptors, different mu agonists stimulated [^{35}S]GTPγS binding with different efficacies; these differences were determined by the concentration of GDP, with higher [GDP] increasing efficacy differences between agonists. Chronic morphine treatment of rats (7 days) produced specific decreases in mu-stimulated [^{35}S]GTPγS binding in certain brainstem nuclei, including parabrachial nucleus, locus coeruleus, and dorsal raphe nucleus, but no changes in other brainstem nuclei or in any region in the forebrain. In these same sections, no changes were observed in stimulation of [^{35}S]GTPγS binding by either delta agonists or ORL1 peptide, thus suggesting a homologous-type desensitization in these areas after chronic morphine treatment. In contrast, chronic THC treatment of rats (20 days) produced widespread desensitization of cannabinoid (WIN-55212-2)-stimulated [^{35}S]GTPγS binding (decreasing agonist activation by 50-70%) in every region that contained appreciable WIN-stimulated [^{35}S]GTPγS binding. These data suggest that different mechanisms of desensitization predominate for these two drugs of abuse.

Supported by NIDA (DA02904, DA06784, DA06634 and DA07246).

37.12

CLONED μ- AND δ-OPIOID RECEPTORS INHIBIT PROLACTIN SECRETION FROM TRANSFECTED GH$_3$ CELLS.

E. T. Piros*; C. E. Marounian, T. G. Hales, & C. J. Evans, Departments of Anesthesiology & Psychiatry, UCLA, Los Angeles, CA, 90095.

Endogenous opioids modulate pain pathways and endocrine functions by inhibiting neurotransmitter and hormone release. Vesicular release can be inhibited by hyperpolarization or reduced Ca^{2+} entry into the cell, as well as by direct interference with the secretory mechanism (e.g. phosphorylation-dephosphorylation of proteins involved in release). Opioid receptors activate K^+ channels and inhibit Ca^{2+} channel and adenylyl cyclase activity. The contribution of these actions to the inhibition of vesicular release is unclear. We have used the prolactin (PRL) secreting GH$_3$ cell line, stably transfected with μ- (GH$_3$MOR), and both μ-and δ-receptors (GH$_3$MORDOR), to investigate the mechanisms by which these receptors modulate PRL release. Previously we have shown that μ-receptors inhibit L-type Ca^{2+} channels and cAMP accumulation in GH$_3$MOR cells (Piros *et al.*, Mol. Pharmacol. **47**:1041, 1995), while δ-receptors have similar inhibitory actions only in GH$_3$MORDOR cells.

The μ-selective ligand DAMGO (1 μM) inhibits PRL release, measured by a competitive ELISA, by 32.2 ± 4.7% and 49.1 ± 6.8% in GH$_3$MOR and GH$_3$MORDOR cells, respectively. In GH$_3$MOR cells, expressing μ-receptors only, the δ-agonist DPDPE was without an effect. However, DPDPE inhibited PRL release dose-dependently (IC_{50} = 3.8 nM) in GH$_3$MORDOR cells. The inhibitory actions of DAMGO and DPDPE were attenuated by pertussis toxin pretreatment. We are currently investigating whether μ- and δ-opioids inhibit PRL release through modulation of adenylyl cyclase, K^+ or Ca^{2+} channels in GH$_3$ cells.

Supported by a NIDA Center Grant (DA05010), and an NRSA Fellowship (DA05627-01).

37.13

IN VIVO ETHANOL CONSUMPTION INCREASES OPIOID INHIBITION OF CAMP PRODUCTION IN MOUSE STRIATUM WITHOUT AFFECTING δ-OPIOID RECEPTOR DENSITY OR MRNA. K. Chan, F. Sehba, A. Duttaroy, S. Shah, J. Philippe, T. Monderson, B. Chen, J. Carroll and B.C. Yoburn*. College of Pharmacy, St. John's University, Queens, NY 11439.

Interactions between systems that mediate the effects of opioids and ethanol have been suggested. *In vivo* ethanol consumption decreases the analgesic potency of μ and δ opioid agonists in mice without affecting the density or affinity of brain or spinal cord opioid receptors (*FASEB J.*, 1996, **10**, A451). This effect was unrelated to changes in access of opioids to the CNS. In this study, the effect of *in vivo* ethanol consumption on cAMP in striatum and opioid inhibition of forskolin stimulated cAMP production was determined. Effects on striatal δ-opioid receptor (DOR) density, affinity, and mRNA were also examined. Mice had unlimited access to 7% ethanol alone or water for 7 days and were then sacrificed, and striata removed for analysis. There was no difference in basal cAMP between controls and ethanol-treated striata, respectively (98, 106 pmole/min/mg protein). Forskolin (10μM) stimulated cAMP production equally in both groups(3.4-3.6-fold). However, there was a significant effect of ethanol treatment on the inhibitory effect of DADLE on forskolin-stimulated cAMP formation ($p<.001$). The mean DADLE IC_{50} for controls (0.61±0.36 μM) did not differ ($p>.05$) from the ethanol treated IC_{50} (0.66±0.50μM); whereas maximal inhibition was significantly less ($p<.02$) for controls (13.7%±0.65) compared to ethanol treated (17.6%±1.36). Saturation binding studies ([^{3}H] deltorphin II) indicated no differences in B_{max} or K_d between controls and ethanol treated mice, respectively (B_{max}:257, 274 fmoles/mg protein; K_d: 0.53, 0.54 nM). No difference between control and ethanol-treated striata was observed in DOR mRNA using a 550 NT riboprobe in a solution hybridization assay, respectively (14.4±1.0; 13.6±1.8pg DOR transcripts/μg RNA). These data indicate that ethanol consumption can alter the effect of DADLE on striatal mechanisms that regulate cAMP. However, this effect is not related to changes in any δ-opioid receptor parameters that were examined.

(supported by DA 04185)

37.14

ULTRASTRUCTURAL LOCALIZATION OF μ OPIOID RECEPTORS IN DENDRITIC TARGETS OF DOPAMINE TERMINALS IN RAT NEOSTRIATUM. H. Wang*, A. Moriwaki°, J.B. Wang°, G.R. Uhl°, and V.M. Pickel. Dept. of Neurol. & Neurosci., Cornell Univ. Medical College, New York, NY 10021. °Mol. Neurobiol. Branch, IRP, NIDA, NIH & Johns Hopkins Univ. School of Medicine, Baltimore, MD 21224.

Many motor effects of opiates acting at μ opioid receptors (MOR) are thought to reflect functional interactions with dopamine released from nigrostriatal afferents to the neostriatum. Since dopamine is the major catecholamine identified by the presence of the tyrosine hydroxylase (TH) in this region, we examined the cellular and subcellular basis for this interaction in the striatal patch region by dual immunocytochemical labeling for MOR and TH. MOR-like immunoreactivity (MOR-IR), labeled by immunogold particles, was mainly seen in medium-sized spiny neurons, while immunoperoxidase labeling for TH was exclusively located in axon and axon terminals devoid of detectable MOR-IR. Many MOR and TH immunolabeled profiles were in close apposition to each other, although most lacked well-defined synaptic junctions. From 639 noted appositions, over 90% were between MOR-labeled dendrites and/or dendritic spines and TH-containing terminals. The dendritic spines also often received the asymmetric synapses characteristic of excitatory inputs from unlabeled terminals. Endogenous opioids acting at MOR in the striatal patch region are well positioned to preferentially modulate the output of spiny neurons at sites having most access to released dopamine.

(Supported by NIDA DA 04600 and NIDA Intramural Research Program)

37.15

THE MU-OPIOID RECEPTOR (μOR) IS LOCATED ON THE PLASMA MEMBRANE OF DENDRITES WHICH RECEIVE ASYMMETRIC SYNAPSES FROM AXON TERMINALS CONTAINING LEUCINE-ENKEPHALIN (L-ENK) IN THE RAT LOCUS COERULEUS (LC) E.E.O. Colago*, E.J. Van Bockstaele, A. Moriwaki#, G. Uhl#. Dept. of Neurol. and Neurosci., Cornell Univ. Med. Coll., NY, NY, 10021; #NIDA Intramural Research Program, Baltimore, MD, 21224.

We have recently shown using immunoelectron microscopy that the μOR is prominently distributed within noradrenergic perikarya and dendrites many of which receive excitatory-type (i.e. asymmetric) synapses from unlabeled axon terminals in the LC (Van Bockstaele et al., J. Neurosci, in press). To further characterize the neurotransmitter present in these terminals, we examined the ultrastructural localization of μOR in sections which were also dually labeled for the opioid peptide, L-ENK. Gold-silver labeling for μOR was localized to para- and extra-synaptic portions of the plasma membranes of perikarya and dendrites. The μOR-labeled dendrites were usually postsynaptic to axon terminals containing heterogeneous types of vesicles and forming asymmetric specializations characteristic of excitatory-type synapses. The majority of these were immunolabeled for L-ENK. Some μOR-labeled dendrites received synaptic contacts from unlabeled terminals in fields containing L-ENK. In such cases, the μOR-labeled dendrites were in proximity to L-ENK terminals which contained intense peroxidase labeling within large dense core vesicles along the perimeter of the axoplasm suggesting that L-ENK may be released by exocytosis from dense core vesicles and diffuse within the extracellular space to reach μOR sites on the postsynaptic dendrite or dendrites of other neighboring neurons. Unlabeled terminals apposed to μOR-labeled dendrites may also contain other opioid peptides such as methionine-ENK. These data demonstrate several sites whereby opioid peptides may interact with μOR receptive sites in the LC and may provide an anatomical susbtrate for the LC's involvement in mechanisms of opiate withdrawal. Supp. by R29DA09082, NARSAD to EJVB, MH18974, DA04600, NIDA Intramural Research Program.

37.16

ULTRASTRUCTURAL LOCALIZATION OF MU OPIOID RECEPTOR IMMUNOREACTIVITY TO GABAERGIC NEURONS IN RAT DENTATE GYRUS. C.T. Drake* and T.A. Milner, Dept. Neurology and Neuroscience, Cornell University Medical College, New York, NY 10021

Physiological studies have determined that mu opioid receptor agonists (e.g. morphine, enkephalin) exert their effects in the dentate gyrus by inhibiting GABAergic neurotransmission. The objective of the present study was to determine the morphological correlate of these effects by analyzing the light and electron microscopic distribution of mu opioid receptor immunoreactivity (MOR-I) in the dentate gyrus, using an antiserum to the predicted C-terminus of the MOR (Incstar). Some sections were dually labeled for MOR- and GABA-immunoreactivity. By light microscopy, MOR-I was present in scattered perikarya and processes in all lamina of the dentate gyrus. Labeled neurons were most common in the granule cell layer and the subgranular region of the hilus. In sections dually-labeled for MOR- and GABA-I, MOR-I was present in a subset of GABA-I neurons. By electron microscopy, perikarya with MOR-I exhibited the infolded nuclei characteristic of interneurons, and most MOR-I perikarya also contained GABA-I. Additionally, in all subfields MOR-I was present in dendrites, axons, and axon terminals, most of which also contained GABA-I. Most dendrites with MOR-I were aspiny, and received numerous asymmetric synapses on their shafts. Most axons with MOR-I were unmyelinated. MOR-labeled terminals formed exclusively symmetric synapses with dendrites and somata of granule cells, as well as with unidentified dendrites. In experiments where gold particles were used to visualize MOR-I, labeling in perikarya, dendrites, and terminals was predominantly associated with the plasmalemma and to a lesser extent with the membranes of cytoplasmic organelles. We conclude that the MOR in dentate gyrus is positioned to effect both pre-synaptic modulation of GABA release and post-synaptic modulation of GABAergic neurons. Supported by DA08259 and an Aaron Diamond Postdoctoral Fellowship.

37.17

OPIATE-PICROTOXIN INTERACTIONS: EFFECT OF NATRINDOLE ON PICROTOXIN-INDUCED SEIZURES. J.Thomas* and W.L. Nores. Dept. of Psychology, University of New Orleans, New Orleans, LA 70148.

The effects of natrindole (NTI), an opiate antagonist selective for delta receptors, on picrotoxin-induced seizures and sensitivity to morphine was investigated. For seizure testing, female rats were injected IP with NTI (.5 mg/kg) or saline 30 min prior to seizure testing with picrotoxin (1.5 mg/kg, SC). On the first day they received this treatment, animals were observed for 45 minutes for the latencies to myoclonic jerks and focal seizures. To induce morphine sensitivity, animals received the respective pretreatments followed by an injection of picrotoxin on two additional occasions at one week intervals. Four days after the last treatment, tailflick latencies were tested after pretreatment with morphine. NTI increased the latencies to myoclonic and fully developed focal seizures during the acute phase of the experiment. Pretreatment with NTI also enhanced the development of the sensitivity to morphine that results from repeated seizure episodes. The findings support the thesis of opiate receptor involvement in picrotoxin seizures. However, the mechanisms involved in seizure-induced alter-ation of opiate receptor sensitivity are still not known.

37.18

KAPPA OPIOID RECEPTOR EXPRESSION ON CELLS OF THE IMMUNE SYSTEM. Tracey A. Ignatowski* and Jean M. Bidlack. Department of Pharmacology and Physiology, University of Rochester, Rochester, NY 14642-8711.

A sensitive method for receptor detection has recently been implemented to determine the presence of κ opioid receptors on cells of the immune system. Indirect immunofluorescence using a fluorescein-conjugated arylacetamide (FITC-AA), a κ-selective opioid, in conjunction with an amplification procedure and flow cytometric analysis was employed. Thymocytes, isolated from 6-8 week old C57BL/6ByJ mice, were incubated with FITC-AA, followed by biotinylated anti-fluorescein IgG and extravidin-R-phycoerythrin, in order to demonstrate labeling of the receptor. Specific labeling was greater than 50% above background as determined by inclusion of the κ-selective antagonist nor-binaltorphimine. Phenotypic characterization using monoclonal antibodies directed against the cell surface markers CD3, CD4, and CD8, combined with labeling of the κ opioid receptor, revealed that the majority of thymocytes express the receptor, and 82% were double-positive ($CD4^+/CD8^+$). Similarly, nylon-wool purified splenocytes demonstrated greater than 50% specific labeling of the κ opioid receptor. Additional data will be presented identifying specific immune cell subpopulations which express this receptor. Thus, these studies characterize distinct populations of immune cells involved in opioid responsiveness. (Supported by USPHS grants DA04355 and DA09676).

NEUROTRANSMITTER INTERACTIONS I

38.1

SIGMA$_1$ RECEPTOR REGULATION OF N-METHYL-D-ASPARTATE (NMDA) STIMULATED [^{3}H]DOPAMINE RELEASE IN GUINEA PIG STRIATUM. G. M. Gonzalez-Alvear* and L. L. Werling. Dept. of Pharmacology, The George Washington University Medical Ctr., Washington, D.C. 20037.

We have previously reported inhibition of NMDA-stimulated [^{3}H]dopamine release from rat striatal slices by sigma receptor agonists (+)pentazocine and BD737. We have now investigated the effects of these sigma agonists on NMDA-stimulated [^{3}H]dopamine release from guinea pig striatal slices.

Striata were dissected, chopped, and washed in Mg^+-free modified Krebs-HEPES buffer, then incubated with 15 nM [^{3}H]dopamine for 30 min. Slices were loaded into a superfusion apparatus and superfused with buffer to establish a low, stable baseline release. Tissue was stimulated to release [^{3}H]dopamine by a 2 min exposure to 50 μM NMDA. Inflow was returned to non-stimulating buffer, with or without potential inhibitor of release for 10 min. Tissue was then stimulated a second time (S2) with NMDA for 2 min in the presence or absence of an inhibitor.

(+)Pentazocine inhibited NMDA-stimulated [^{3}H]dopamine release in a concentration dependent, monophasic manner. Additionally, the sigma$_1$ agonist BD737 was also found to inhibit NMDA-stimulated [^{3}H]dopamine release from guinea pig striatal slices. Both (+)pentazocine and BD737 inhibited NMDA-stimulated [^{3}H]dopamine release in a naloxone-insensitive manner. Furthermore, the sigma$_1$ antagonist DuP 734 and BD1008, a non-selective sigma antagonist, completely reversed the inhibition of release. Neither DuP 734 nor BD1008 had any effect on NMDA-stimulated [^{3}H]dopamine release in the absence of a sigma agonist. These data suggest that sigma$_1$ receptors are involved in the regulation of dopamine release in guinea pig striatum. (Supported by a grant from NIDA to LLW and by NIGMS predoctoral fellowship to GMG)

38.2

CHRONIC TREATMENT WITH L-DOPA DECREASES LIGAND BINDING TO STRIATAL NMDA AND NON-NMDA GLUTAMATE RECEPTORS IN RATS WITH NIGROSTRIATAL INJURY. S.J. O'Dell* and J.F. Marshall. Department of Psychobiology; University of California, Irvine; Irvine, CA 92717.

Changes in the activity of CNS dopamine (DA) systems modulate corticostriatal neurotransmission, suggesting that DA-influenced changes in striatal GLU receptors may play an important role in neurodegenerative disorders such as Parkinson's disease. To investigate the influence of dopaminergic modulation on striatal GLU receptors, rats were given unilateral nigrostriatal damage via intracerebral 6-OHDA injection. One month later, these lesioned rats and unlesioned control rats were given 2 daily injections of Sinemet (100 mg L-dopa + 25 mg carbidopa/kg, ip) or vehicle for 21 days. Three days after the last injection, their brains were processed for in vitro autoradiography, quantifying DA uptake sites and the AMPA, kainate (KA) and NMDA subtypes of GLU receptor in the caudate-putamen (CPu), nucleus accumbens (NAc) and cingulate cortex (Cing). Nigrostriatal 6-OHDA injections alone reduced ipsilateral striatal DA uptake sites by 83-99%, but had little effect on radioligand binding to striatal AMPA, KA, or NMDA receptors. In contrast, chronic Sinemet treatment of 6-OHDA-injected rats produced significant decreases in AMPA, KA, and NMDA receptors in the striatum and cortex. In 6-OHDA-injected rats, Sinemet produced the greatest reductions in KA and NMDA receptors (30-40%) in the denervated CPu, with moderate declines (18-25%) observed in the CPu, NAc and Cing of the intact hemisphere. In contrast, Sinemet produced moderate (15%), uniform decreases in AMPA receptors in the CPu, NAc and Cing of both the intact and lesioned hemispheres. Additionally, Sinemet administration produced prolonged, vigorous rotation in rats with unilateral nigrostriatal damage, but not in neurologically intact rats. Taken together, these results suggest that after nigrostriatal injury prolonged treatment with Sinemet differentially influences corticostriatal glutamatergic transmission mediated by AMPA, KA and NMDA receptors. *Funded by NS 22698.*

38.3

DIFFERENTIAL REGULATION OF NON-NMDA SUBUNIT mRNA BY ANTIPSYCHOTICS IN RAT. D.J. Healy*, R.E. King, K.A. Bovenkerk, S.P. Damask, J.H. Meador-Woodruff. Mental Health Research Institute and Department of Psychiatry, University of Michigan, Ann Arbor MI 48109.

Dopamine is the neurotransmitter most often implicated in the pathogenesis of schizophrenia. However, glutamatergic antagonists cause both negative and positive psychotic symptoms in otherwise normal humans, and exacerbate these symptoms in schizophrenics. These findings have led investigators to attempt to synthesize these data, resulting in a model of dopamine-glutamate interactions in limbic cortex and striatum as a potential substrate for symptom production in schizophrenia. We are interested in exploring this model, and in this study have examined the effects of chronic antipsychotic treatment on the expression of some glutamate receptors within discrete neuroanatomical regions in this model. Glutamate receptors are divided into four subtypes, two of which are ionotropic receptors recognized by their relative affinty for AMPA and kainate, respectively. These receptors are composed of five subunits with potentially varying stoichiometries. Some of the variability in subunit stoichiometry is due to the fact that these subunits are encoded by separate genes, referred to as gluR1-gluR7, and KA1-KA2. We examined the regulation of gene expression of the AMPA (gluR1-gluR4), low affinity kainate(gluR5-gluR7), and high affinity kainate (KA1-KA2) receptor subunits by clozapine (20 mg/kg/day) and haloperidol (2 mg/kg/day) treatment for two weeks. Both clozapine and haloperidol caused region-specific alterations in the mRNA levels of these subunits, but in dissimilar patterns. This dissimilarity may represent an interesting mechanism for some of the differential therapeutic or toxic effects of clozapine and haloperidol, and also may be relevant to our understanding of dopamine-glutamate interactions as a potential substrate of schizophrenia. (Supported by MH53327 (JHMW) and MH15794 (DJH)).

38.4

STRESS INCREASES EXTRACELLULAR GLUTAMATE AND DOPAMINE IN THE VENTRAL TEGMENTAL AREA. Z.L. Rossetti* and R.A. Wise, CSBN, Concordia University, Montreal, Quebec, Canada H3G 1M8 and Dept. of Neuroscience, University of Cagliari, 09124 Cagliari, Italy.

Stress is known to activate DA neurons and to increases neurotransmitter release from dopaminergic terminals. Stress also activates glutamatergic neurons causing increased extracellular glutamate (GLU) in cortical and subcortical structures. Since VTA DA neurons receive major excitatory innervation and express GLU receptors, we determined whether stress was associated with increased GLU input to the VTA by measuring GLU and DA levels in microdialysis samples. DA, released in the VTA by dendrites as a correlate of impulse flow, was measured as means of estimating dopaminergic activation by GLU. DA and GLU were measured by HPLC in split 20-min samples (0.6 μl/min), starting 24 h after probe implantation, with fluorescence and electrochemical detection, respectively. Baseline GLU and DA concentrations were 7.65±0.71 μM and 0.43±0.06 nM, respectively. Tail pinch tress was delivered for 10 min. In the first sample after stress, extracellular GLU increased to 4-51±58 % of baseline, remaining elevated for at least 1 h. An increase in extracellular DA (to 176±23 % of baseline) was time-locked to GLU elevation. Thus glutamatergic input to the VTA is increased by stress and this effect is associated with neuronal activation of DA neurons. Given the increasing evidence for the regulation of subcortical DA function by glutamatergic afferents to DA cell bodies, we suggest that GLU release in VTA may be a primary mechanism for the response of mesolimbic DA neurons to stress.

38.5

GLUTAMATERGIC MODULATION OF FEEDING EVOKED DOPAMINE RELEASE IN THE RAT NUCLEUS ACCUMBENS.

M.T. Taber*, L. Crookall, and H.C. Fibiger. Division of Neurological Sciences, Dept. of Psychiatry, University of British Columbia, Vancouver, BC, V6T 1Z3, Canada.

Glutamatergic projections synapse in both the cell body region (ventral tegmental area: VTA) and the terminal region (nucleus accumbens: NAc) of the mesolimbic dopamine (DA) system. Glutamatergic mechanisms in both areas have been proposed to modulate DA neurotransmission in the NAc. The present study used *in vivo* microdialysis to determine the contribution of glutamatergic receptors in these two areas to the modulation of DA release in the NAc that has been shown to occur during feeding. Feeding in 18 hour food-deprived rats increased DA release by approximately 50 percent, an effect which lasted for about 90 minutes, while food was still present. Local application in the NAc of the ionotropic glutamate receptor antagonist kynurenic acid (1 mM) through the microdialysis probe potentiated the effect of feeding on DA release by about 80%. To investigate the possibility that ionotropic glutamate receptors in the ventral tegmental area mediate the effect of feeding, a combination of antagonists, CNQX (50 μM) and AP5 (200 μM), was applied in this region while recording accumbal DA concentrations during feeding. The antagonists produced a small (20%) decrease in basal DA release and reduced the effect of feeding by 70%. Neither of these treatments affected the amount of food eaten over the two hour feeding period or the latency to begin eating. These results demonstrate that glutamate receptors in the ventral tegmental area mediate feeding-induced increases in DA release whereas glutamate receptors in the NAc have an inhibitory influence.

Supported by the Medical Research Council of Canada. MTT is supported by the Scottish Rite Schizophrenia Research Program.

38.6

IMPAIRED LEARNING OF A SHORT-TERM MEMORY TASK AND DECREASED NMDA-RECEPTOR-MEDIATED REGULATION OF IN VIVO PREFRONTAL CORTEX DOPAMINE RELEASE IN ADULT RATS NEONATALLY EXPOSED TO THE NMDA-ANTAGONIST MK-801

M.Feenstra*, M.McLean, L.Ogle, M.Botterblom, R.Joosten, J.de Bruin, Neth Inst Brain Res, Grad Sch Neurosci A'dam, 1105AZ Amsterdam ZO, The Netherlands.

We recently showed that neonatal blockade of NMDA-receptors affects adult spatial learning and DA metabolism (Gorter et al: Brain Res 580(1992)176, Neurosci Lett 137(1992)97). As the mesocortical dopaminergic (DA) projection and NMDA-receptors are both important for the involvement of the prefrontal cortex (PFC) in short-term learning and memory, we studied these parameters in adult male rats that were treated as pups with the noncompetitive NMDA-antagonist (+) MK-801 (0.05 mg/kg, twice daily, postnatal day 5-15).

Acquisition of a Delayed-Matching-To-Position task was assessed in Skinner-boxes and was delay-dependently impaired in the MK-801-exposed rats. For 15 and 30 sec delays the level of controls was reached only after 14 days of testing. In vivo DA overflow was measured in the PFC using microdialysis. Basal levels and novelty-induced increases were not altered, but the increase in DA overflow induced by 0.3 mM D-AP-5 was significantly less in neonatally MK-801-exposed rats compared with saline controls.

Neonatal blockade of NMDA-receptors led to a severe deficit in adult short-term learning and memory. The decreased response to D-AP-5 indicates a reduction of tonic NMDA-receptor dependent inhibition of DA release. The increased DA metabolism in the ventromedial PFC, the most densely DA-innervated PFC area, found in tissue measurements, points in the same direction.

(no external funding).

38.7

THE ROLE OF GLUTAMATE IN DOPAMINE-MEDIATED GENE EXPRESSION IN THE RAT STRIATUM.

J.C. Leveque and C. Konradi*. Neuroscience Center, Mass. General Hospital East, Charlestown, MA 02129.

Dopamine activates the expression of target genes in the striatum in a D_1 as well as D_2 receptor-dependent manner. Treating rats with amphetamine induces the immediate early gene *c-fos* and the *prodynorphin* gene, through the stimulation of D_1 receptors. The neuroleptic haloperidol stimulates *c-fos* as well as *proenkephalin* gene expression in a D_2-dependent manner. Interestingly, the NMDA antagonist MK-801 is able to inhibit this dopamine-mediated gene expression. Experiments in rat primary striatal culture demonstrate that this inhibition is due to an intra- rather than inter-neuronal mechanism. Results in primary striatal culture indicate that the influx of calcium through NMDA receptors, rather than calcium channels, is necessary for dopamine-mediated gene expression. Moreover, low levels of glutamate lead to a superinduction of dopamine-mediated gene expression. While dopamine-induced cAMP levels are unperturbed by MK-801, the dopamine-mediated phosphorylation of the transcription factor CREB is blocked by MK-801. We will present data that further elucidates the interaction between dopamine and glutamate in the expression of the proenkephalin gene and the immediate early gene c-fos and characterizes the second messenger pathways of both transmitters.

38.8

MODULATION OF EXTRACELLULAR DOPAMINE LEVELS IN THE STRIATUM BY A METABOTROPIC GLUTAMATE RECEPTOR AGONIST: A MICRODIALYSIS STUDY,

Anita Verma* and Bita Moghaddam. Department of Psychiatry, Yale University School of Medicine, VA Medical Center 116A/2, West Haven, CT 06511

In vivo microdialysis was used to assess the effect of the metabotropic agonist, trans-(±)-1-amino-1,3-cyclopentanedicarboxylic acid (ACPD) on the extracellular levels of dopamine in the rat striatum. ACPD increased dopamine release in a concentration range of 50 to 500 μM. The lower dose of ACPD (50 μM) failed to elicit a significant effect on extracellular dopamine levels. The higher doses of 100 μM and 500 μM produced a significant increase in extracellular levels of dopamine. The ACPD-induced increases in dopamine levels were decreased by local perfusion of tetrodotoxin, indicating that activation of dopamine release by ACPD is impulse flow dependent. Further, ACPD attenuated the increased dopamine release in response to 30mM KCl. These data suggest that during basal conditions metabotropic glutamate receptors activate striatal dopamine release; however, under stimulated conditions, they attenuate these levels. Thus, during conditions of hyperstimulation, activation of metabotropic receptors, in contrast to ionotropic receptors, may act to reduce the excess dopamine release.

This work was supported in part by USPHS awards, MH-48404, MH-44866 and The Scottish Rite Foundation.

38.9

GLUTAMATERGIC REGULATION OF GABA RELEASE IN THE PREFRONTAL CORTEX DURING BASAL AND STRESS-ACTIVATED CONDITIONS,

Bita Moghaddam*, James Bagley, and Herman Bruins, Department of Psychiatry, Yale University School of Medicine, VA Medical Center 116A/2, West Haven, CT 06516

Intracerebral microdialysis in awake rats was used to study the glutamatergic regulation of GABA release in the prefrontal cortex. Glutamatergic neurotransmission was increased by local application of exogenous ionotropic agonists, and by stress, which has been shown to increase glutamate neurotransmission in the prefrontal cortex. Local perfusion of NMDA at 100 μM and 500 μM, through the microdialysis probe, produced a dose-dependent increase in GABA efflux. Kainate did not affect these levels at 10 μM, but produced a small increase at the relatively high concentration of 50 μM. AMPA, at 20 μM and 100 μM, produced a small increase of about 25% in GABA levels. Exposure to stress (20 min tail pinch) increased GABA efflux by about 50% in the PFC. To examine whether an increase in endogenous glutamate in response to stress alters GABA release in the prefrontal cortex, the effect of locally applied AMPA/kainate receptor antagonist, CNQX, and NMDA receptor antagonist, AP5, during the stress procedure was examined. Infusion of CNQX did not alter the basal or stress-activated GABA levels. In contrast, AP5 produced a trend toward a decrease in basal GABA efflux and it blocked stress-induced GABA release. These studies demonstrate that glutamatergic regulation of GABA release in the prefrontal cortex occurs primarily via the NMDA receptors. Supported in part by USPHS awards MH-48404 and MH-44866, the Scottish Rite Foundation, and West Haven VA Center for Schizophrenia.

38.10

ROLE OF GLUTAMATE RECEPTOR SUBTYPES IN REGULATING AMPHETAMINE-INDUCED ALTERATIONS OF STRIATAL ACETYLCHOLINE EFFLUX

Elizabeth D. Abercrombie* & Michael J. Bickerdike, Center for Molecular & Behavioral Neuroscience, Rutgers University, Newark, NJ 07102.

Striatal ACh efflux is modulated by opposing dopaminergic influences: striatal D2 receptors directly inhibit ACh release, whereas extrastriatal D1 receptors indirectly potentiate ACh efflux. Previous studies have demonstrated that D1-mediated increases in ACh efflux are dependent on glutamate.[1] The current study employed microdialysis to investigate the effect of locally administered NMDA antagonists (MK801 and AP-5) and an AMPA antagonist (CNQX) on the response of striatal ACh to a systemic amphetamine (AMPH) challenge. Male rats received local CNQX (10μM), MK801 (10μM), AP-5 (100μM), or CSF, then were given 4mg/kg AMPH i.p 105 min later. Local administration of the antagonists had varying effects on striatal ACh: the AMPA antagonist CNQX produced a brief 23% increase in striatal ACh; MK801 likewise produced a transient increase in ACh (17%) which then settled 15% below baseline; and AP-5 produced a significant reduction in basal ACh (35%). By itself, AMPH produced a mild, slowly developing increase in striatal ACh efflux (30% after 2 hr). However, in the presence of CNQX, AMPH challenge induced a significant decrease in ACh efflux (40%). In the presence of MK801, AMPH produced a significant, prolonged, increase in ACh (79%), while AP-5 did not significantly affect the ACh response to AMPH (17% increase). These results suggest a clear differentiation of function between AMPA and NMDA receptors in their influence on striatal ACh release. The decrease in ACh produced by AMPH in rats given CNQX likely results from indirect blockade of the positive D1-mediated effects produced by AMPH. Striatal perfusion with the NMDA receptor antagonists MK801 and AP-5 did not result in a similar decrease. It therefore follows that D1 receptor-mediated increases in striatal ACh are ultimately driven by AMPA rather than NMDA receptor activation[1]. Moreover, the increase in ACh observed after AMPH in MK801-treated rats suggests that NMDA receptors mediate a strong inhibitory effect on striatal ACh perhaps via activation of striatal GABAergic processes. (Supported by NIDA 08086)

1. DeBoer P. and Abercrombie E.D. (1994) Soc. Neurosci. Abstr. **20**:126.17

38.11

REGULATIONS OF NMDA-EVOKED RELEASE OF ACETYLCHOLINE IN STRIOSOME- AND MATRIX-ENRICHED AREAS OF THE RAT STRIATUM. M.L. Kemel*, F. Blanchet, S. Perez, M. Desban, J. Glowinski and C. Gauchy. INSERM U114, Collège De France, 11pl M. Berthelot, 75005 Paris France.

Using local microsuperfusions of saggital striatal slices, in the absence of Mg^{2+}, NMDA (50µM) was shown to enhance the release of ^{3}H-ACh previously formed from ^{3}H-choline in striosome- and matrix-enriched areas. D-serine (10µM) potentiated the reponse induced by 10µM NMDA but surprisingly it reduced those evoked by 50µM or 1mM NMDA suggesting, in these cases, the intervention of released inhibitory transmitters such as dopamine (DA) or GABA.

..Confirming the inhibitory role of released DA, the NMDA (1mM+D-serine)-evoked release of ^{3}H-ACh was enhanced under the complete blockade of DA transmission with (-)sulpiride (1µM) and SCH23390 (1µM) (D1 and D2 antagonists respectively), this effect being more important in striosomes (X4) than in the matrix (X1,6). In contrast, the blockade of GABA transmission with the GABA A antagonist (bicuculline, 5µM) was without effect on the NMDA response in the striosomes but reduced this response in the matrix. Moreover, the presence of the GABA A antagonist did not prevent the desinhibitory effects of the combined application of the D1 and D2 antagonists on ^{3}H-ACh release in both compartments suggesting that the reduced NMDA response induced by bicuculline alone in the matrix results from a blockade of the inhibitory effect of GABA on the release of DA. Additional experiments performed with the D2 or D1 antagonist alone in the presence of bicuculline indicated that D2 receptors play a prominent role in the inhibitory effect of DA on NMDA-evoked responses in both compartments and that D1 receptors are involved in an excitatory effect of DA on NMDA-evoked ACh release in the matrix compartment.

38.12

MUSCARINIC MODULATION OF NMDA RESPONSES REQUIRES A G-PROTEIN MEDIATED EVENT. V. B. Aramakis*, A. E. Bandrowski, M. F. Bashir and J. H. Ashe. Departments of Neuroscience and Psychology, University of California, Riverside, CA 92521.

The muscarinic receptor agonist, acetyl-β-methylcholine (MCh) facilitates both the NMDA-mediated late-EPSP and NMDA-induced membrane depolarizations (NMDA-DPs) in the *in vitro* rat auditory cortex (Aramakis et al, submitted). Here we present evidence that the facilitating actions of MCh are G-protein mediated. Whole-cell recordings (WCR) were obtained from layer II/III pyramidal neurons and iontophoretic application of either MCh (1M, 3-100 nA, 1-4 min) or NMDA (50 mM, 13-130 nA, 5 sec) was also applied to layer II/III.

Intracellular infusion of the GTP activator, GTPγS (100 µM) mimicked the facilitating actions of MCh. Facilitation of both the late-EPSP, generated by deep gray/white matter stimulation (n=6), and NMDA DPs (n=5) developed gradually with infusion of GTPγS into the cell, following establishment of the WCR configuration. Facilitation of the late-EPSP, elicited in isolation by blockade of AMPA/kainate receptors with CNQX, did not develop gradually, but could be induced by low current ejection of MCh (3-25 nA, n=6). In contrast, infusion of the competitive G-protein inhibitor, GDPβS (200 µM), prevented the MCh-mediated facilitation of either the pharmacologically isolated late-EPSP (n=5) or the NMDA-DPs (n=7).

Our results suggest that ACh, acting at muscarinic receptors, produces a long-lasting enhancement of NMDA-mediated neurotransmission in auditory cortex, and that this modulatory effect is dependent upon a G-protein mediated event. Supported by NSF (IBN 9310582).

38.13

ACUTE MK-801 ADMINISTRATION INDUCES EXPLOSIVE JUMPING BEHAVIOR AND AN INCREASE IN CENTRAL NICOTINIC RECEPTORS IN MICE. Y. Tizabi*, C. H. Park and S. I. Deutsch. Dept. of Pharmacology, Col. of Med., Howard Univ. Washington, DC 20059, and Psychiatry Service, V. A. Medical Center, Washington, DC 20422.

Administration of MK-801(dizolcipine) to rodents causes a complex series of behaviors including "popping," characterized by involuntary explosive jumping episodes, in an outbred strain of NIH Swiss mice. MK-801, an NMDA receptor antagonist also inhibits nicotinic cholinergic receptor channels. To investigate possible involvement of nicotinic receptors in MK-801-induced popping behavior, mice were injected with MK-801(1.8 mg/kg, i.p.) and following a 30 min test period, low and high poppers were selected for measurements of nicotinic receptor binding. Mice were sacrificed either immediately following the test session or 24 h later. Nicotinic receptor densities were measured by [^{3}H]cytisine binding in homogenates of specific brain regions. There was an increase in nicotinic receptor concentrations in the cortex, striatum and midbrain of both high and low poppers 30 min following the MK-801 injection. There were no changes in hippocampal and cerebellar nicotinic receptor concentrations. At 24 hour following MK-801 injection all nicotinic receptor changes were back to normal. The lack of correlation between nicotinic receptor densities and intensities of "popping" episodes does not support a direct involvement of nicotinic receptors in MK-801- induced popping. However, MK-801 induced increases in nicotinic receptor bindings in discrete brain regions suggests a possible involvement of nicotinic receptors in some aspects of MK-801 actions. Supported in part by a grant from the Department of Veterans Affairs.

38.14

MK-801-INDUCED EXPLOSIVE JUMPING BEHAVIOR IN MICE IS INHIBITED BY NICOTINE PRETREATMENT. S. I. Deutsch*, J. Mastropaolo, R. L. Riggs and Y. Tizabi. Psychiatry Service, V. A. Medical Center, Washington, DC 20422, and Dept. of Pharmacology, Col. of Med. Howard Univ. Washington, DC 20059.

MK-801 is a noncompetitive antagonist of the N-methyl-D-aspartate subclass of glutamate receptor that elicits hyperactivity, stereotypic behaviors, and "popping," an explosive jumping behavior, in mice. The mechanism(s) responsible for MK-801-induced popping is not known. MK-801 also inhibits nicotinic receptor channels. Previously we have observed an increase in central nicotinic receptors following acute administration of MK-801(1.8 mg/kg i.p.). Since nicotine itself may interfere with MK-801 binding, this study was designed to determine whether pretreatment with nicotine may block MK-801-induced popping behavior. Nicotine administration by itself (0.56 - 1.8 mg/kg, i.p.) had no effect on popping behavior. However, nicotine pretreatment dose-dependently inhibited MK-801-induced popping. The mechanism responsible for this effect of nicotine is currently not known. These findings suggest important interactions between nicotine, MK-801 and each other's binding sites. Further elucidations of these interactions are necessary in pharmacotherapeutic considerations of these agents.

Supported in part by a grant from the Dept. of Veterans Affairs and Office of the Vice President for the Health Affairs, Howard Univ.

38.15

ON-LINE MEASUREMENT OF SYNAPTICALLY RELEASED GLUTAMATE IN CULTURED RAT CORTICAL NEURONS USING ELECTROCHEMICAL DETECTION. K. Torimitsu*, A. Kawana and O. Niwa, NTT Basic Research Laboratories, 3-1, Morinosato-Wakamiya, Atsugi-shi, Kanagawa, 243-01, Japan.

The measurement of synaptically released neurotransmitters is essential for understanding the synaptic transmission mechanism and functions of neural networks in the brain. Various methods have been used to measure the neurotransmitters released during synaptic transmission. Most researchers have used microdialysis-sampled liquid chromatography for *in vivo* experiments or labelled neurotransmitters for *in vitro* experiments, in order to estimate the transmitter concentration and movement. However, these methods are problematic as regards long term and real time measurements under stable conditions. Here we have developed a novel method of measuring the glutamate concentration in a medium on-line. E18 rat cortical neurons were cultured for 2 weeks in an MEM-based culture medium. Real time measurements of synaptically released glutamate were carried under serum-free conditions. The glutamate was measured by a glutamate oxidase immobilized glassy carbon detector connected to either a glass capillary or microdialysis tube to provide continuous suctioning of the medium at a rate of 4 µl/min. Spontaneously released glutamate at a submicromolar level was observed. The addition of potassium induced a transient glutamate release. The mechanism which modulates glutamate release will be discussed at the meeting.

38.16

IN MICROCULTURE SINGLE MESENCEPHALIC DOPAMINE NEURONS MAKE TWO CHEMICALLY DISTINCT TYPES OF SYNAPSES. S. Rayport, L. Lin, T. Hattori*. Depts. Psychiatry, Anatomy & Cell Biology, and Ctr. Neurobiology & Behavior, Columbia Univ.; Dept. Neuroscience, N.Y.S. Psychiatric Institute, NY 10032; Dept. Anatomy & Cell Biology, Univ. of Toronto, Ontario M5S 1A8.

In the intact brain, single nigrostriatal dopamine (DA) neurons appear to make DAergic and non-DAergic synapses (Hattori, Neurosci. Res. 1993). Previously we reported (Sulzer *et al.*, Soc. Neurosci. Abstr. 1994; Rayport *et al.*, 1995) that DA neurons grown in microculture form excitatory autapses and stain for glutamate (GLU). We have now double stained mesencephalic DA neurons in microcultures for the DA-synthetic enzyme tyrosine hydroxylase (TH) and for GLU. This reveals that single TH$^+$ neurons have TH$^+$, GLU$^+$, and TH$^+$/GLU$^+$ varicosities. At the ultrastructural level, TH staining, which we visualized with DAB, shows a patchy somatic distribution; both TH$^-$ and intensely TH$^+$ processes arise from the soma and intermingle in the neuritic field. Within individual processes staining sometimes abruptly starts and stops. Synaptic specializations are mainly found near the cell body. Most presynaptic terminals are TH$^-$ and make asymmetric synapses. Rarely TH$^+$ presynaptic terminals make symmetric synapses. Thus single mesencephalic DA neurons can form symmetric DAergic and asymmetric GLUergic terminals; the functional roles of these two types of synapses and their potential impact on DA-dependent processes such as psychostimulant sensitization remain to be determined.

Supported by NIDA DA08675, the Burroughs Wellcome Fund, and the Medical Research Council of Canada MT11770.

38.17

MODULATORY EFFECTS OF SCOPOLAMINE ON PAIRED PULSE DEPRESSION OF DENTATE GRANULE CELL FIELD POTENTIALS. L.J. Burdette*, Dept. Neurology, Graduate Hospital, Philadelphia, PA 19146

We previously have found that the NMDA antagonist, MK-801, potentiates late paired pulse depression of dentate granule cell field potentials. Evidence of cholinergic modulation of GABA-mediated inhibition suggested that the muscarinic antagonist, scopolamine (5 mg/kg, ip), may mimic the enhancement of late paired pulse depression seen with MK-801. Male Long Evans rats were prepared with chronic electrodes in the perforant path and dentate gyrus. Following post-operative recovery, stimulus intensity functions (0.1 ms, 50-1500 μAmps) were obtained using single pulses and paired pulses delivered at 0.05 and 1 Hz under vehicle and drug conditions. In contrast to the effects of MK-801, scopolamine did not significantly alter late paired pulse depression (200 ms interpulse interval) or the 1 Hz depression of the population spike. However, scopolamine produced a significant increase (17.8%, p=0.016) in early paired pulse depression (20 ms interpulse interval) at all stimulus intensities, resulting in a lower threshold for evoking early paired pulse depression (20% v. 40% of maximum stimulus intensity). No drug effects were detected in the stimulus intensity function for the population spike or the slope of the EPSP. The enhancement of early paired pulse depression by scopolamine is consistent with a block of the cholinergically-mediated depression of the fast IPSP observed in hippocampal pyramidal cells, and suggests a similar cholinergic modulation of $GABA_A$-mediated inhibition in the dentate gyrus. (Supported by MH45961).

NEUROTRANSMITTER INTERACTIONS II

39.1

MICRODIALYSIS STUDIES WITH INTRASTRIATAL NEUROTENSIN 1-13 AND NEUROTENSIN 8-13 ON STRIATAL GABA AND DOPAMINE RELEASE IN THE AWAKE RAT. EVIDENCE FOR NEUROTENSIN RECEPTOR HETEROGENEITY IN THE NEOSTRIATUM. W.T. O'Connor[1]*, L. Ferraro[2], T. Antonelli[2], U. Ungerstedt[3], K. Fuxe[4] and S. Tanganelli[2]. [1]Dept. of Physiology, University College, Dublin, Ireland; [2]Inst. of Pharmacology, University of Ferrara and [3]Dept. of Neuroscience, University of Cagliari, Italy; Dept. of [3]Pharmacology and [4]Neuroscience, Karolinska Inst., Stockholm, Sweden.

In the present study we investigated (a) the effects of intrastriatal perfusion with the tridecapeptide neurotensin NT(1-13), and its active fragment NT(8-13) on striatal GABA and dopamine (DA) release and (b) the ability of intrastriatal NT(1-13) to influence D_2 receptor mediated inhibition of striatal GABA release.

Basal striatal GABA and DA levels were and 20.3 ± 1.6 nM and 4.11 ± 0.34 nM respectively. Both NT(1-13) (100 nM) and NT(8-13) (100 nM), increased striatal GABA release (+37 ± 9% and +29±3% respectively) and these effects were antagonised by intrastriatal perfusion with the non peptidergic NT receptor antagonist SR48692 (100 nM). However, while NT(1-13) (100 nM) was associated with a prolonged increase (+55 ± 8%) in striatal DA release, NT(8-13) displayed only a slight and shortlasting effect even at the 1μM concentration.

Intrastriatal NT(1-13) (10 nM) counteracted the pergolide (500 and 1500 nM) induced inhibition of striatal GABA release and the inhibitory action of the D_2 agonist was restored when SR48692 (100 nM) was added to the perfusion medium.

The finding that NT(1-13) (100nM) increases striatal GABA and DA release while its active fragment NT(8-13) (100nM) increases GABA but not DA release indicates that NT receptors located on GABA neurons differ from those on DA terminals and provides *in vivo* evidence for functional NT receptor subtypes in the CNS. Furthermore, the ability of NT (1-13) to counteract the pergolide induced inhibition of striatal GABA release strengthens the evidence for an antagonistic receptor-receptor interaction between striatal NT and D_2 receptors located on GABA neurons.

39.2

PRELIMINARY EVIDENCE OF SUBSTANCE P-INDUCED INHIBITION OF STRIATAL DOPAMINE EFFLUX IN THE AWAKE RAT. R.N. Iyer*, A.B. Giordani and M.D. Davis. Departments of Chemistry and Neuropharmacology, Parke-Davis Pharmaceutical Research, Division of Warner Lambert Co., Ann Arbor, MI 48105.

The observation of a striatonigral pathway containing the undecapeptide substance P (SP) has elicited substantial interest in potential interactions between this neurotransmitter and dopamine (DA). The elucidation of this potential interaction has, however, been complicated by reports of the ability of SP and various SP fragments to both inhibit and facilitate DAergic function in vitro and in vivo (in anaesthetised animals). In light of the potential importance of such an interaction and due to the contradictory observations reported in the literature, we have examined the effect of the peptide SP, when locally infused through a microdialysis probe for 20 minutes, on striatal extracellular DA levels in conscious, freely-moving Sprague-Dawley rats.

Basal dialysate levels of DA were found to be 3.9 +/- 1.0 fmol/μL, n=9. Preliminary data indicate that while the inclusion of 10 μM SP in the perfusate appears to have no significant impact (n=3), infusion of 10 nM SP produces an approximately two-fold attenuation of basal extracellular DA (n=3), consistent with the increase in striatal DA release observed in vivo by Gygi et al. (1993) following local application of the NK_1 receptor blocker CP-96345. Lower perfusate concentrations of the peptide (1 nM) were not found to affect basal DA efflux (n=3).

The data suggest that the neuroactive peptide SP may function, in part, to inhibit striatal DA release in vivo.

Acknowledgment. The support of Parke-Davis Pharmaceutical Research, Division of Warner Lambert Co. is gratefully acknowledged.

39.3

EFFECTS OF INTRACEREBROVENTRICULAR ADMINISTRATION OF ETHYLCHOLINE AZIRIDIUM ON DOPAMINERGIC NERVOUS SYSTEMS. D.K. Lim*, Y. Ma and E.Y. Yi College of Pharmacy, Chonnam National University, Kwang-Ju, 500-757, Korea

Changes in dopaminergic activities were investigated after the intracerebroventricular (icv) administration of ethylcholine aziridium (AF64A) in rats. The levels of dopamine (DA) and metabolites, the activities of tyrosine hydroxylase (TH) and monoamine oxidase (MAO), and the specific bindings of dopamine receptors in striata, hippocampus, and frontal cortex were assessed 6 days after the AF64A treatment with 3 nmol/each ventricle. The levels of DA and metabolites, TH and MAO activities, the specific binding sites of dopamine receptors were measured using HPLC-ECD, enzyme assays, and ligand binding assay, respectively. In frontal cortex, the levels of DA and metabolites were significantly decreased in the AF64A-treated group. However, the ratios of metabolites and DA were significantly decreased in striatum and hippocampus in the AF64A-treatment. The activity of TH in frontal cortex was significantly decreased without changes in the other areas. Although the activity of MAO-A was not changed in the brain regions studied, the activity of MAO-B in striatum was significantly increased. The specific bindings of dopamine D1 and D2 receptors were increased in frontal cortex after AF64A-treatment. However, those were not changed in striatum and hippocampus except the small decrease of dopamine D1 receptor in striatum. These results indicate that the dopaminergic activity was altered in AF64A treatment. Furthermore, it suggests that the dopaminergic activities in each brain regions may be differently affected by AF64A treatment.

(This work was supported by the research grant (1995) from the Ministry of Health and Social affairs, Korea)

39.4

MUSCARINIC ENHANCEMENT OF NMDA-INDUCED ADENOSINE RELEASE IN RAT CORTICAL SLICES IN VITRO. K. Semba* and T.D. White. Depts. of Anat. & Neurobiol. and Pharmacol., Dalhousie Univ., Halifax, N.S. B3H 4H7 Canada.

Acetylcholine (ACh) facilitates neuronal responses to sensory stimulation in the cortex, and is thought to be important in cortical EEG activation. Adenosine is a neuromodulator released in response to increased metabolism and may have sleep-promoting functions by providing inhibitory tone to neurons with a role in behavioural state regulation. In this study we examined the effects of carbachol on NMDA-induced adenosine release in adult rat cortical slices in vitro. Carbachol (up to 300μM) alone did not affect basal release of adenosine. Carbachol (100μM), however, induced a 253±47% increase in NMDA (20μM)-induced adenosine release in the presence of Mg^{2+}. In the absence of Mg^{2+} carbachol induced less of an increase (60±18%). Atropine (1.5μM) reduced, by 59±4%, the facilitatory effects of carbachol on NMDA-evoked adenosine release. Similarly, 4-DAMP (1μM) and pirenzepine (1μM) reduced the carbachol effects by 69±7% and 47±7%, respectively. An M1-selective dose of pirenzepine (50 nM) did not appear to block carbachol's action on NMDA-evoked adenosine release. Carbachol did not affect AMPA-induced adenosine release. These results suggest that ACh does not affect basal adenosine release but enhances NMDA receptor-mediated evoked adenosine release through M3 and, possibly, M1 receptors in the cortex. The facilitatory effects of ACh on evoked adenosine release may have a role in regulating the response of cortical neurons to stimulation. Supported by the MRC of Canada and The Scottish Rite Charitable Foundation.

39.5

DIFFERENTIAL EFFECT OF HALOPERIDOL ON RELEASE OF NEUROTENSIN IN EXTRAPYRAMIDAL AND LIMBIC SYSTEM. W. Huang, G. R. Hanson*. Department of Pharmacology and Toxicology, University of Utah, Salt Lake City, UT 84112.

The effect of the antipsychotic drug haloperidol on extracellular neurotensin-like immunoreactivity (NTLI) was investigated by microdialysis and compared with the time-dependent response of tissue NTLI content in brain structures containing dopamine nerve cell bodies and terminals. Acute administration of haloperidol (1 mg / Kg) increased the extracellular NTLI levels in nucleus accumbens, but decreased it in the striatum. The same treatment increased the tissue content of NTLI in both the nucleus accumbens core and striatum within 24 hours after the injection. Interestingly, although the NTLI concentration in the substantia nigra was not altered by the haloperidol treatment, NTLI levels did decrease significantly in ventral tegmental area. These findings suggest that neurotensin systems associated with nigrostriatal and mesolimbic dopamine pathways do not respond homogeneously to haloperidol. In addition, changes in the release of neurotensin may contribute to altered striatal and accumbens NTLI contents induced by haloperidol treatment, but other factors, such as variation in synthesis also likely influence these changes. Differential effects of haloperidol on neurotensin release might be due to regional differences in dopamine receptor subtypes expressed by the neurotensin-containing neurons. (Supported by NIDA grant # DA 09407).

39.6

EFFECT OF DOPAMINERGIC AGONISTS ON LOCOMOTOR ACTIVITY AND STRIATAL GABA RELEASE: IN VIVO MICRODIALYSIS IN FREELY MOVING RATS. I.Shim', D.Wirtshafter and J.I.Javaid. Department of Psychiatry and Psychology, University of Illinois at Chicago, Chicago, IL 60612.

The majority of neurons in the striatum use gamma-aminobutyric acid (GABA), the major inhibitory transmitter in the mammalian brain. Several studies indicate that dopamine containing terminals within the striatum contact GABAergic neurons, and thus striatal GABA release is likely to be under dopaminergic control. These dopamine-GABA interactions seem to be important for the control of striatal-related motor behaviors. In the present studies, we examined the effect of dopaminergic drugs on locomotor activity and extracellular concentrations of GABA using in vivo microdialysis in the striatum of freely moving rats. When high potassium (100 mM) was included in the perfusion CSF, extracellular GABA levels were increased 300% over basal GABA levels. Injecting rats with D_2 agonists, quinprole and bromocriptine, decreased striatal GABA release and increased activity. Pretreatment with raclopride blocked quinprole-induced locomotor activity and decreases in striatal GABA output. These results suggest that stimulation of D_2 receptors inhibits GABAergic transmission in the striatum, and appears to be important in mediating locomotor activity.

39.7

IN VIVO MODULATION OF STRIATAL AND NIGRAL GABA EFFLUX BY D1 AND GLUTAMATE RECEPTOR LIGANDS. A.M. Reilly*, L.M. Byrnes, & J.P. Bruno. Dept. of Psychology and Neuroscience Program, Ohio State University, Columbus, OH 43210

The modulation of striatonigral GABA efflux via D1 and glutamate receptor ligands was examined using in vivo microdialysis in awake rats. Intrastriatal administration of the glutamate agonists AMPA (100 μM) and kainate (100 μM) both increased striatal GABA efflux (60-90% and 200-250%, respectively); however, only intrastriatal kainate administration led to a concomitant increase in GABA efflux in the substantia nigra. Intrastriatal admininstration of the full D1 agonist SKF 81297 yielded a dose-dependent bidirectional effect; 10 μM decreased striatal GABA efflux (25-50%), while 100 μM increased striatal GABA levels (100-275%). Both of these effects were blocked by local perfusion of the D1 antagonist SCH 23390 (10 μM). Like AMPA, intrastriatal administration of either dose of SKF 81297 had no systematic effect on GABA efflux in nigra. On the other hand, intranigral administration of either kainate (100 μM) or SKF 81297 (100 μM) increased GABA efflux in nigra. These results illustrate the complex modulation of striatonigral GABA release in vivo, and suggest a possible dissociation between the regulation of GABA release in the striatum and in the substantia nigra.

We will also present data on the D1- and glutamate-receptor modulation of striatal and nigral GABA efflux in animals depleted of striatal DA at various stages of development.

(Funding: Whitehall Foundation to J.P.B.)

39.8

SHORT-TERM AND LONG-TERM EFFECTS OF 6-HYDROXYDOPAMINE ON DOPAMINERGIC INHIBITION OF GABA RELEASE IN NEOSTRIATUM. L. G. Harsing, Jr.* and M. J. Zigmond. Department of Neuroscience, University of Pittsburgh, Pittsburgh, PA 15260.

Previous studies have indicated that severe loss of striatal dopamine (DA) is needed to detect deficits in neostriatal functions and that restoration of impaired dopaminergic influences develops even after large lesions. Since GABAergic neurons are known to be the major targets for DA in neostriatum, we examined the changes in dopaminergic inhibition of striatal GABA release after lesions produced by 6-hydroxydopamine (6-OHDA). Rats were injected with 6-OHDA (25-100 µg, icv.) and striatal slices were prepared 3 days or 3 weeks later. The slices were loaded with [^{3}H]GABA and superfused with Krebs buffer containing aminooxyacetic acid and nipecotic acid. [^{3}H]GABA overflow was then measured during electrical field stimulation (8 hz, 3 min). Tissue levels of DA, assessed by HPLC-EC, provided a measure of dopaminergic innervation. In nonlesioned striatum, a 2-fold increase was observed in [^{3}H]GABA overflow in response to the D2 receptor antagonist sulpiride (10 µM), an index of the inhibitory influence of endogenous DA. Three days after 6-OHDA, this effect of sulpiride remained at control levels as long as at the loss of DA terminals did not exceed 60%. Three weeks after 6-OHDA injection, the effect of sulpiride on [^{3}H]GABA overflow had returned to control even in slices with a 90% loss of DA terminals. These results suggest the existence of compensatory mechanisms which maintain dopaminergic control over GABA transmission even after extensive destruction of DA terminals. (Supported by USPHS grant NS19608 and a Fogarty International Center fellowship NS05270).

39.9

GABA AUGMENTS BASAL AND ELECTRICALLY-STIMULATED ^{3}H-NOREPINEPHRINE RELEASE IN HYPOTHALAMIC, PREOPTIC AND CORTICAL SLICES OF FEMALE RATS. J.M. Fiber* and A.M. Etgen. Dept. Neurosci., Albert Einstein Coll. Med., Bronx, NY 10461.

The anatomical delineations of the preoptic area (POA) and hypothalamus (HYP) have been well established; however, the neurochemical circuitry of these regions has been less studied. Using superfusion of 350 μm brain slices of ovariectomized female rats, we have shown that 100 μM GABA enhances the basal release of ^{3}H-norepinephrine (NE) in the POA, HYP and frontal cortex ($n = 9$-10, $p < .01$). This effect is partially Ca^{++} dependent. Females treated with estrogen and progesterone in doses known to induce sexual receptivity show greater GABA-enhanced NE release in the HYP than vehicle-treated controls ($n = 5$, $p < .05$).

GABA also augments evoked ^{3}H-NE release when HYP, MPOA and cortex slices are stimulated with 72 pulses of 3Hz, 18mA. Slices were stimulated once (S1), then superfused with vehicle or 100 μM GABA and simultaneously stimulated a second time (S2). S2/S1 ratios were greater in slices superfused with GABA ($n = 7$-14, $p < .001$). The duration of ^{3}H-NE release during S2 was also greater in GABA-treated slices ($p < .01$). GABA augmentation of electrically-stimulated ^{3}H-NE release in the POA and HYP is mediated through $GABA_A$ receptors as evidenced by its blockade by 10 μM bicuculline, but not by 200 μM 2-OH-saclofen ($n = 5$-10, $p < .05$). GABAergic influence on noradrenergic transmission within the POA and HYP may play a role in the regulation of gonadotropin release and reproductive behavior.

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39.10

CHOLECYSTOKININ REDUCES EVOKED DOPAMINE OVERFLOW IN THE STRIATUM: AN IN VIVO VOLTAMMETRIC STUDY

T. Reum*, R. Morgenstern, A. Marburger and D. Schönfuß, Inst. of Pharmacology and Toxicology, Medical Faculty (Charité), Humboldt-University at Berlin, D-10098 Berlin, Germany

Cholecystokinin (CCK) is capable of modulating the dopaminergic transmission in the striatum. It has been shown that CCK decreases the electrically evoked striatal release of dopamine (DA) *in vitro* (Rakovska, Neurosci. Lett. 195: 151-154). In order to investigate the influence of CCK under *in vivo* conditions an electrical stimulus (2 mA, 200 µsec, 100 pulses, 100 Hz) was applied to the medial forebrain bundle (MFB) every five minutes and 100 nl CCK-8s solution (10^{-3} M-10^{-6} M) was ejected once into the striatum 250 µm away from a calibrated carbon fibre microelectrode. Using amperometry (AMU 130, Tacussel) a potential of +400 mV was applied vs. Ag/AgCl and the oxidation current was recorded continuously. The evoked responses after MFB stimulation, reflecting changes of extracellular DA, were recorded before and up to 120 min after CCK ejection and were analyzed for the maximum overflow and for the rate of DA elimination.

CCK diminished the maximum DA overflow dose dependently with a 50% decrease after 10^{-3} M and a 15% reduction after 10^{-6} M vs. the vehicle-treated control group. This effect was observed immediately after CCK application and remained stable for the whole time of recording. The rate of extracellular DA elimination was not influenced by either concentration of CCK.

These findings indicate that CCK modulates the DA release in the striatum, which is triggered by electrical stimulation of DAergic fibres. Due to the unchanged elimination rate an altered function of the presynaptic DA transporter by CCK seems to be improbable.

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39.11

DISTINCT PHARMACOLOGICAL PROFILES OF TWO CCK-B AGONISTS : FURTHER EVIDENCE OF RAT CCK-B RECEPTOR SUBSITES. I. Léna, V. Daugé, B.P. Roques and C. Durieux *. Département de Pharmacochimie Moléculaire et Structurale, U266 INSERM - URA D1500 CNRS, UFR des Sciences Pharmaceutiques et Biologiques, 75270 Paris Cedex 06.

Previous binding studies have suggested the existence of two affinity states for rat CCK-B receptor. One study, using BC 197 and BC 264, two highly selective CCK-B agonists, has shown that BC 197 is selective for one subsite, B_1 and that BC 264 has the same affinity for the two subsites, B_1 and B_2. Therefore, the possible involvement of CCK-B subsites in the modulation of endogenous dopamine (DA) release from slices of the anterior part of nucleus accumbens was investigated with these two agonists in order to associate a functional response with activation of each subsite. The selective B_1 agonist BC 197 produced a dose-dependent increase (48%) of 35 mM K^+-stimulated DA release. In contrast, at a low concentration (20 nM), BC 264 inhibited the potassium-evoked DA release (29%), whereas at a higher concentration (1 µM), it stimulated the DA release (43%). These two opposing effects were suppressed by the CCK-B antagonist PD-134,308 but not by the CCK-A antagonist L-364,718 and were not prevented by tetrodotoxin, a sodium channel blocker. Moreover, BC 264 at 20 nM, in the presence of PD-134,308 at a concentration which would block the B_2 subsites (0.1 nM), increased the evoked DA release (53%). Furthermore, after stereotaxic injection in the anterior nucleus accumbens, BC 264 and BC 197 induced distinct behavioral responses in the Y maze test. All together, these results support further the existence of distinct CCK-B subsites and suggest that, in the anterior nucleus accumbens, their stimulation mediates different effects.

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39.12

ELECTRICAL STIMULATION OF THE AMYGDALA EVOKES FREQUENCY-DEPENDENT CHANGES IN DOPAMINE EFFLUX IN THE RAT NUCLEUS ACCUMBENS. C.D.Blaha*, S.B. Floresco, C.R.Yang and A.G. Phillips. Departments of Psychology and Psychiatry, University of British Columbia, Vancouver, B.C., V6T 1Z4.

Interactions between the basolateral nucleus of the amygdala (BLA) and ventral striatal dopamine (DA) neurons have been implicated in both rewarding and fear-mediated behaviors. The present study assessed the role of BLA modulation of DA efflux in the nucleus accumbens (NAc) of urethane-anesthetized rats using *in vivo* chronoamperometry (30s sample rate) with stearate-graphite paste electrodes. A concentric bipolar stimulating electrode positioned in the BLA was used to deliver monphasic square-pulse electrical stimulation (800 µA, 300 single pulses). Low frequency stimulation of the BLA (3Hz, 100 s duration), simulating firing patterns observed during fear conditioning paradigms, increased the DA basal signal in the NAc by 25% (0.5 s after stim.), followed by a decrease of 30% (3 min after stim.), with recovery to baseline levels within 5 min. Higher frequency single-pulse stimulation, simulating reward sensitive BLA firing patterns (e.g. 20 Hz, 15 s duration), resulted in an initial increase (35%, 0.5 s after stim.), then a decrease (30%, 3 min after stim.), followed by a prolonged increase (>100%, 15 min after stim.) in the DA signal. Burst stimulation (100 Hz burst, 5 pulses/burst, 1 s inter-burst interval, 60 s duration) resulted in a pattern of DA efflux similar to 20 Hz stimulation but without exhibiting the prolonged increase component. Co-infusions of the AMPA and NMDA glutamate receptor antagonists DNQX (1µg) and APV (1µg), respectively, into the NAc, but not the VTA, abolished the increase in DA efflux following burst-stimulation of the BLA. Additional experiments examining the role that metabotropic glutamate receptors play in mediating these stimulation-evoked effects in NAc DA efflux will be presented.. *Supported by MRC, Canada.*

39.13

SYNERGISTIC INTERACTION BETWEEN AN ADENOSINE ANTAGONIST AND A D1 DOPAMINE AGONIST ON ROTATIONAL BEHAVIOR AND STRIATAL FOS INDUCTION IN 6-HYDROXYDOPAMINE LESIONED RATS. A.E. Pollack* and J.S. Fink Molecular Neurobiology Laboratory, Mass. General Hospital and Dept. of Neurology, Harvard Medical School, Boston, MA 02114; *Dept. of Psychology, Brown University, Providence, RI 02912

In rats depleted of forebrain dopamine coadministration of D1 and D2 dopamine (DA) agonists synergistically increases motor behavior and induces Fos-like immunoreactivity (Fos-LI) in the dorsolateral striatum. Motor activation produced by a D1 DA agonist in DA-depleted rats is also enhanced by adenosine antagonists, but the anatomical basis of adenosine-D1 DA interaction has not been clearly demonstrated. In this study, we sought to (1) determine whether administration of an adenosine antagonist, like a D2 DA agonist, would enhance Fos-LI activation following treatment with a D1 DA agonist, and (2) use the regional pattern of striatal Fos-LI activation to gain insight into the anatomical basis of adenosine antagonist-D1 DA agonist interactions. In rats with unilateral 6-hydroxydopamine (6-OHDA) lesions coadministration of the adenosine antagonist 3,7-dimethyl-1-propargylxanthine (DMPX) (10 mg/kg) and the D1 DA agonist SKF38393 (0.5 mg/kg) synergistically enhanced contralateral rotation and induced Fos-LI expression in the ipsilateral striatum compared to 6-OHDA treated with either drug alone. However, the pattern of striatal Fos-LI activation following treatment of 6-OHDA rats with SKF38393 + DMPX was different from that produced following coadministration of D1 and D2 DA agonists (SFK38393: 0.5 mg/kg + quinpirole: 0.05 mg/kg). These data are consistent with a functional interaction between D1 dopaminergic and adenosinergic receptors in the striatum. However, these data suggest that activation of different subsets of striatal neurons appears to underlie the behavioral synergy observed following adenosine antagonist-D1 DA agonist and D1-D2 DA agonist treatment. Support: NIH: DA07496, NS31579, & T32-DA07282.

39.14

NALTREXONE ATTENUATES ACUTE AMPHETAMINE-INDUCED REARING AND BLOCKS ITS SENSITIZATION BY REPEATED AMPHETAMINE. M. Balcells-Olivero[1,*] and P. Vezina[2]. Department of Pharmacological/Physiological Sciences[1] and Department of Psychiatry[2], The University of Chicago, Chicago, IL 60637.

The effect of opiate receptor blockade on the horizontal locomotion and rearing produced by acute and repeated injections of amphetamine was investigated. Rats in different groups were preexposed to seven injections (one injection every other day) of amphetamine (1.5 mg/kg, i.p.) or saline each preceded by an injection of naltrexone (0, 1 or 5 mg/kg, i.p.). Soon (early withdrawal) and again long (late withdrawal) after the last preexposure injection, all animals were injected with amphetamine (0.75 mg/kg, i.p.) in the absence of naltrexone (test for sensitization). Naltrexone, at doses producing no behavioral effects of their own, acutely reduced the increased rearing produced by amphetamine but had no effect on the increased horizontal locomotion produced by this drug. Animals that received naltrexone prior to amphetamine during preexposure showed no evidence of sensitized rearing on either the early or late withdrawal tests, indicating that naltrexone blocked sensitization of this response to amphetamine. These animals showed sensitized horizontal locomotor responding on the early withdrawal test but less so on the late withdrawal test. These results suggest an important role for dopamine-opioid interactions not only in the production of acute locomotor responding to psychomotor stimulants but also in the sensitization of these responses when these drugs are administered repeatedly. Supported by a grant to P.V. from The Brain Research Foundation.

39.15

D-AMPHETAMINE AND RESERPINE MODULATE KYNURENIC ACID LEVELS IN THE RAT BRAIN IN VIVO. A. Rassoulpour, H.-Q. Wu, B. Poeggeler* and R. Schwarcz. Maryland Psychiatric Research Center. Baltimore, MD 21228.

Interactions between excitatory amino acids and monoamines play important roles in brain physiology and pathology. We have therefore explored the effects of two classic monoaminergic agents, D-amphetamine (Amph) and reserpine (Res) on the levels of the cerebral metabolite and broad spectrum excitatory amino acid receptor antagonist kynurenic acid (KYNA) in the rat brain. The first set of studies was designed to examine Amph and Res effects on extracellular KYNA levels using hippocampal microdialysis in unanesthetized rats. I.p. Amph caused a dose-dependent *decrease* in KYNA levels, reaching a nadir of 70% of control values 1 h after application of 5 mg/kg of the drug. In contrast, Res (10 mg/kg, i.p.) caused a substantial *increase* in extracellular KYNA levels, reaching approximately 300% of baseline values by 6 h and remaining elevated for at least another 12 h. Ex vivo experiments confirmed the results from the microdialysis studies. Thus, 5 mg/kg Amph (i.p.) caused a 27.0 ± 3.9% (SEM) decrease in hippocampal KYNA levels after 1 h (N=14; $P < 0.05$) whereas 10 mg/kg Res (i.p.) resulted in a 87.4 ± 8.6% KYNA increase by 6 h (N=10; $P < 0.05$). No changes in the levels of KYNA's bioprecursor kynurenine were found in these tissue specimens. Quantitatively very similar drug-induced changes were observed in several brain regions but not in the liver. These data suggest that endogenous monoamines modulate KYNA production in the brain.

Supported by USPHS grant NS 28236.

39.16

DIFFERENTIAL REGULATION OF GLUTAMATE AND NORADRENALINE RELEASE IN THE BED NUCLEUS OF STRIA TERMINALIS: IN VIVO MICRODIALYSIS STUDIES. K. Gysling*, M.I. Forray and G. Bustos. Lab. Biochemical Pharmacology, Dept of Cell and Molecular Biology, Faculty of Biological Sciences, Catholic University of Chile, Santiago, CHILE.

The purpose of this work was to study the regulation of noradrenaline (NA) and glutamate (L-GLU) release in the ventral bed nucleus of stria terminalis (vBNST), the area of the brain with the highest concentration of NA nerve terminals. There is evidence indicating that L-GLU afferent projections to the vBNST originate in part from the subiculum. Microdialysis probes were stereotaxically implanted in the vBNST of anesthetized rats and perfused at a rate of 2 µl/min with artificial CSF. NA and L-GLU levels in each sample were measured by HPLC-EC and HPLC coupled to fluorometric detection respectively.

Basal and K^+ evoked release of NA and L-GLU were studied, under the same conditions, and several differences were observed. First, NA was released with lower K^+ concentration than L-GLU. Second, NA levels increased rapidly with K^+ stimulation, but decreased to basal levels even though high K^+ was still present. Instead, L-GLU release is maintained throughout the stimulation period. Third, NA but not L-GLU basal levels were decreased with perfusion with TTX (10-50 µM).

These results indicate that NA but not L-GLU release in the vBNST is under tonic control. They also support a differential regulation of NA and L-GLU extracellular levels in vBNST. (Supported by grants FONDECYT N° 2950010 to M.I.F., N° 1930686 and N° 1960482 to K.G).

39.17

SYNAPTIC INTERACTION BETWEEN CRF-IMMUNOREACTIVE NEURONS AND FIBER TERMINALS WITH VIP AND PACAP IMMUNOREACTIVITY IN THE LATERAL DIVISION OF THE BED NUCLEUS OF STRIA TERMINALS. T. KOZICZ[1], A. ARIMURA[1], A. GERALL[2]*. [1]U.S.-Japan Biomed. Res. Labs., Tulane University Hebert Center, Belle Chasse, LA 70037; [2] Psychology Dept., Tulane Univ., New Orleans, LA, 70118.

The limbic-hypothalamic-pituitary-adrenal system plays an important role in regulation of stress responses. The BNSTL was suggested as a relay nucleus between amygdala (sensory input to BNSTL) and paraventricular, parabrachial, and dorsal vagal nuclei. There are many corticotropin releasing factor (CRF) immunoreactive (ir) neurons in the BNSTL. These cells might be involved in a central response to stress which affects hypothalamic and brain stem neuronal activities. The BNSTL also receives a dense vasoactive intestinal polypeptide (VIP) and pituitary-adenylate-cyclase-activating-polypeptide (PACAP) innervation, probably from amygdala through stria terminalis.

Based on our light microscopic findings, we hypothesized synaptic connections between CRF structures and the fiber terminals of VIP and PACAP neurons. To prove the hypothesis, we performed double-label immunocytochemical study with an electron-microscope. We found synaptic connections between CRF ir cell bodies and VIP and PACAP ir terminals. We also found synapses between VIP or PACAP ir fibers and CRF ir dendrites.

The VIP and PACAP afferents onto CRF neurons in BNSTL could modulate the activity of CRF neurons during stress in animals depending on different sensory stimuli. Further electrophysiological studies will be needed to clearly understand the functional aspects of these synaptic interactions.

(Supported in part by NIH grant DK09094)

NEUROTRANSMITTER INTERACTIONS III

40.1

MODULATION OF EXCITATORY SYNAPSES BY ENDOGENOUS ADENOSINE RELEASED FROM SINGLE HIPPOCAMPAL PYRAMIDAL NEURONS. J.M. Brundege* and T.V. Dunwiddie. Dept. of Pharmacology, Univ of Colorado Health Sci Center, and Veterans Admin Medical Research Service, Denver, CO 80262.

Adenosine is a potent neuromodulator in the central nervous system, but the mechanisms that regulate the concentration of extracellular adenosine remain unclear. To determine whether neurons can modulate their synaptic inputs through the release of adenosine, we increased the intracellular concentration of adenosine in individual CA1 pyramidal neurons in rat hippocampal slices. We recorded excitatory postsynaptic currents (EPSCs) from pyramidal neurons using the whole-cell voltage-clamp technique, and "loaded" the neurons with adenosine by including adenosine in the electrode filling solution. Loading cells with adenosine inhibited EPSCs by increasing the extracellular adenosine concentration at presynaptic A1 receptors on the excitatory nerve terminals. In order to determine whether a nucleoside transporter was responsible for the adenosine release, we included both adenosine and uridine in the electrode filling solution. Uridine blocked the inhibition of the EPSCs, suggesting that adenosine is released by a saturable nucleoside transport system for which uridine is a competitive substrate. In order to determine whether the cell could produce enough adenosine on its own to modulate synaptic activity, we loaded neurons with the adenosine kinase inhibitor 5'-iodotubercidin. 5'-iodotubercidin increased the adenosine-mediated inhibition of EPSCs in a manner similar to adenosine. **These results suggest that individual CA1 pyramidal neurons are capable of forming and releasing enough adenosine to modulate their own excitatory inputs.** Suppported by NS29173 and the VA Medical Research Service.

40.2

GLYCINE AND GABA AS INHIBITORY NEUROTRANSMITTERS IN RAT TRIGEMINAL MOTOR NUCLEUS. H.W. Yang, M.Y. Min, K. Appenteng and T.F.C. Batten (SPON: European Neuroscience Association). Dept. of Physiology and Cardiovascular Research, University of Leeds, Leeds LS2 9NQ, U.K.

The immunogold labelling and whole cell patch recording techniques were employed to investigate the different roles and possible interaction between GABA and glycine in rat trigeminal motor nucleus. In the morphological study, serial untrathin sections were cut, and the alternate sections incubated with antibodies to GABA, glycine and glutamate. Boutons immunoreactive (ir) for GABA, glycine and glutamate formed axo-somatic and axo-dendrites contacts onto motoneurones identified by retrograde transport of HRP following intramusucular injection into the masseter muscles. For 19 out of 34 GABA-ir boutons, their alternate serial sections incubated with anti-glycine also showed glycine-ir. Similarly 22 out of 32 glycine-ir boutons were also GABA-ir. In contrast boutons immunopositive for glutamate were not immunopositive for GABA nor glycine. Some glutamate-ir boutons received axo-axonic contacts with the presynaptic element being found to be GABA-ir but not glycine immunopositive.

In the electrophysiological study, bath application of CGP35348 or strychnine both resulted in an increase of mean amplitude of EPSPs (abolished by CNQX) evoked by stimulation of interneurones caudal to the motor nucleus. The increase in mean EPSP amplitude with CGP35348 was accompanied by an increase in the ratio of square of mean amplitude and its variance (M^2/σ^2). No change of M^2/σ^2 was seen, though there was an increase of the mean amplitude of EPSPs after application of strychnine. Furthermore, CGP35348 or baclofen, but not strychnine, could alter the failure rate of synaptic transmission. Taken together the results suggest 1) GABA and glycine act as inhibitory transmitters and they may colocalize within same boutons, 2) a presynaptic inhibition that is mediated by GABA via $GABA_B$ receptors but not glycine, in this system. (supported by the MRC)

40.3

Protein kinase A-mediated enhancement of glycine response by alpha2-adrenoceptor activation in rat sacral dorsal commissural neuron. J. Nabekura*, T. Xu and N. Akaike. Dept. Physiol., Kyushu Univ. Fac. Med., Fukuoka 812-82, Japan

The effect of noradrenaline (NA) on glycine response was investigated in neurons acutely dissociated from rat sacral dorsal commissural nucleus (SDCN) using nystatin perforated patch recording under voltage-clamp. NA potentiated the glycine-induced Cl^- current (I_{Gly}) in a concentration-dependent manner (>10^{-9}M) without changing the reversal potential of I_{Gly} nor affected the affinity of Gly to its receptor. Clonidine mimicked and yohimbine blocked NA action on I_{Gly}, indicating the α2-adrenoceptor mediated enhancement. Forskolin and IBMX + dibutyl cAMP suppressed the I_{Gly}. H-89 mimicked the effect of NA on I_{Gly}. However, NA failed to affect the I_{Gly} in the presence of these cAMP and PKA modulators, suggesting that cAMP and PKA were involved in the pathway. Phorbol potentiated I_{Gly}. NA further enhanced the I_{Gly} in the presence of the phorbol and chelerythrine, indicating that PKC is not involved in NA facilitatory action. Pertussis toxin (IAP) treatment for 8 hr blocked the NA facilitatory effect on I_{Gly}. In conclusion, the activation of α2 adrenoceptors coupled with IAP-sensitive G protein inhibits adenyl cyclase activity. The reduction of cAMP production decreases PKA activity, resulting in the potentiation of I_{Gly} in the SDCN neurons.

40.4

MODULATION OF ACETYLCHOLINE RELEASE IN THALAMUS AND PONTINE RETICULAR FORMATION BY SEROTONIN IN THE RAT. D.D. Rasmusson* and K. Semba, Departments of Physiology & Biophysics and Anatomy & Neurobiology, Dalhousie U., Halifax, N.S. Canada, B3H 4H7

Presynaptic inhibition of acetylcholine (ACh) release by serotonin (5-HT) could account for the activation of neurons in the pontine reticular formation (PRF) during REM sleep but not during wakefulness. To test this, ACh was collected simultaneously from the thalamus and the PRF using microdialysis probes. After 60 min stabilization period, ten 20 minute samples were collected and analyzed for ACh content. 5-HT (10 µM) was delivered to either the thalamus or PRF by adding it to the perfusion solution beginning with the 5th sample.

Delivery of 5-HT to the PRF produced only a 28% increase in ACh release from the PRF (n=8). Delivery of 5-HT to the thalamus, on the other hand, produced an immediate large (150%) increase in ACh release from the thalamus (n=9). This increase was blocked when TTX (1µM) was applied with 5-HT (n=4). Administration of 5-HT to the thalamus also produced a delayed increase in high frequency cortical EEG activity.

These data suggest that, contrary to expectations, 5-HT does not block ACh release in either the PRF or thalamus and in fact facilitates ACh release in the thalamus. The receptor specificity responsible for these effects is yet to be determined.

Supported by MRC and the Scottish Rite Charitable Foundation of Canada.

40.5

NEUROPEPTIDE Y POTENTIATES α_2 - ADRENERGIC RECEPTOR-MEDIATED INHIBITION OF cAMP LEVELS IN A CATECHOLAMINERGIC CELL LINE. A. Lee, A. E. Wissekerke, and K. R. Lynch.* Neuroscience Graduate Program and Dept. of Pharmacology, University of Virginia, Charlottesville, VA 22908.

Neuropeptide Y (NPY) is colocalized with norepinephrine in a subpopulation of central noradrenergic neurons. Although the functional significance of this colocalization remains unclear, NPY has been shown to modulate noradrenergic signaling mediated by α_2 - adrenergic receptors (α_2 - ARs). In this study, we determined whether such interactions between NPY- and α_2 - ARs occurred in CATH.a cells, a catecholaminergic cell line. CATH.a cells were found to express high levels of NPY and its mRNA by immunocytochemistry and northern blot. α_2 - AR expression was confirmed by saturable, high affinity binding of the α_2 - AR-selective radioligand [^{3}H]RX821002 (B_{max} =35 ± 2.9 fmol/mg, K_d = 0.46 ± 0.06 nM, n=4). In addition, mRNAs encoding the A and C-subtypes of α_2 - AR were detected in CATH.a cell extracts by northern blot. The α_2 - AR agonist UK14304 inhibited forskolin-stimulated cAMP production, an effect prevented by the α_2 - AR antagonist yohimbine. NPY (100 nM) alone had no effect on cAMP levels, but enhanced significantly the inhibition of cAMP levels mediated by α_2 - ARs ($p<0.05$, n=5). NPY (100 nM) administered with UK14304 (1 µM) produced a 50±4.3% inhibition of forskolin-stimulated cAMP production as compared to 29.4±2.7% inhibition by UK14304 alone. NPY may act, in part, through the Y1 receptor subtype since the Y1 but not the Y2 receptor transcript was detected in northern blots of CATH.a cell mRNA. These results suggest that CATH.a cells may provide a useful system in which to study the neuromodulatory functions of NPY in relation to α_2 - AR signaling. (Supported by NIH grant F31 MH11074-01 and AHA grant 95011200).

40.6

TWO DIFFERENT SOMATOSTATIN RECEPTOR SUBTYPES MEDIATE OPPOSITE MODULATION OF AMPA-KA RESPONSES IN HYPOTHALAMIC NEURONS : PHARMACOLOGICAL AND MOLECULAR IDENTIFICATION. C. Lanneau, A. Faivre-Bauman, C. Loudes, J. Rivier', C. Kordon, J. Epelbaum, and R. Gardette*. INSERM U159. 75014 Paris and 'Salk Institute. San Diego

AMPA-KA mediated glutamate (Glu) responses in mouse fetal hypothalamic neurons in culture can either be increased or decreased by somatostatin (Gardette et al., Dev. Brain Res., 1995). In order to investigate whether this differential modulation depends upon activation of different somatostatin receptor subtypes, we have compared the effects of octreotide (OCT), a selective sst2 agonist, 24127515 compound (241), a selective sst1 agonist, and SRIF14 (SRIF), an agonist common to all subtypes, on AMPA-KA mediated Glu sensitivity, and characterized, by single cell RT-PCR, somatostatin receptors borne by these neurons.

Glu peak response was either decreased (64% of cells (18/28), -37 ± 4%) or increased (25% of cells (7/28), +31 ± 6 %) by SRIF. It was constantly decreased by OCT (48% of cells (12/25), -19 ± 3 %) and increased by 241 (35% of cells (6/17), +47.8 ± 17%). No significant difference was found between inhibitory responses to SRIF and OCT or between stimulatory responses to SRIF and 241, both in terms of cell percentage or Glu peak amplitude variations. Preliminary results obtained from 5 neurons processed for single cell RT-PCR analysis after whole cell clamp recordings show that both subtypes were expressed in 3 cells whereas one neuron expressed only sst1 and the last one only sst2.

These data strongly suggest that inhibitory and excitatory effects of SRIF on glutamate responses are respectively mediated by sst2 and sst1 receptor subtypes. Moreover, RT-PCR results support the hypothesis that a differential distribution of somatostatin receptor subtypes within hypothalamic neurons could account for the differential modulation by the neuropeptide.

40.7

FUNCTIONAL UNCOUPLING OF LINKED NEUROTRANSMITTER EFFECTS BY COMBINATORIAL CONVERGENCE. V. Brezina, I.V. Orekhova and K.R. Weiss*. Dept. Physiol. & Biophys., Mt. Sinai School of Medicine, New York, NY.

In this work we consider some basic consequences of the structure of physiological signaling pathways. These pathways characteristically both diverge and converge: a single neurotransmitter has multiple effects in the same target cell, and multiple transmitters have the same effects. Divergence couples the effects of a transmitter so that they are produced together in a relatively fixed ratio. Different physiological circumstances may require a different ratio, however: the coupling must be made modifiable. How may this be achieved? We suggest that an elegant, general mechanism is provided by convergence. If two transmitters couple the effects in a different ratio, then combinations of the transmitters give the effects in all possible intermediate ratios. The coupled effects become functionally uncoupled. Moreover, each transmitter may become tuned to produce mainly just one of the effects.

We have analyzed how this mechanism operates in a well-understood simple invertebrate neuromuscular circuit. The ARC muscle of *Aplysia* is innervated by two motorneurons, B15 and B16, which release ACh to contract the muscle, but also different peptide cotransmitters that modulate the ACh-induced contractions. B15 releases the small cardioactive peptides (SCPs) and B16 the myomodulins (MMs). Both SCPs and MMs have three distinct effects on the muscle: they potentiate contractions by enhancing the Ca current, they depress contractions by activating a K current (these two effects together determine net contraction amplitude), and they accelerate relaxation of the contractions. The SCPs and MMs act equally in all these respects except on the K current: the MMs activate much larger K currents, and so are much stronger depressors, than the SCPs. By virtue of this single inequality in their convergence, the MMs and SCPs produce very different ratios of the ultimate functional effects on contraction amplitude and relaxation rate. However, these are still fixed ratios. Through modeling as well as experimentally, we show how SCP-MM combinations give all otherwise unobtainable intermediate ratios. Furthermore, in the presence of a low, fixed concentration of one modulator, one of the effects of the other modulator becomes particularly emphasized. These phenomena are likely to have direct behavioral significance in this system, as B15 and B16 fire differentially in different behaviors, exposing the muscle to different, and apparently appropriate, SCP-MM combinations. Supported by NIMH and the Whitehall Foundation.

40.8

EFFECT OF SULPIRIDE ON AMPHETAMINE-INDUCED CHANGES IN STRIATAL DOPAMINE AND BEHAVIOR. J.N. Jaworski*, J.S. Randall, D. Crippens, R.A. Gonzales, and P.K. Randall. Division of Pharmacology, University of Texas, Austin, TX. 78712.

Amphetamine (AMPH) administered to rats induces a complex range of stereotypical behaviors. AMPH also produces increases in extracellular dopamine (DA). While these stereotypic behaviors have been shown to be associated with the increased DAergic activity, the precise relationship between the two is unclear. Classical DA antagonists, e.g. haloperidol, block the behavioral response to AMPH, while other antagonists are weakly effective or enhance stereotypy. This study examined the effect of D2 antagonist sulpiride (SULP)(50 mg/kg sc) pretreatment on the biochemical and behavioral response to AMPH (0.5, 2.0, or 8.0 mg/kg ip) in male Sprague-Dawley rats. Extracellular DA in the striatum was monitored using in vivo microdialysis and HPLC-EC. Cage crossing and rearing were measured from videotapes, and stereotypic behavior was quantified using a rating scale technique. DAergic and behavioral time curves were analyzed using non-linear regression and residual F testing. AMPH enhanced extracellular DA and stereotypy scores in a dose dependent manner. SULP augmented the increase in DA induced by 0.5 and 2 mg/kg mainly by decreasing the rate of DA concentration fall off in the extracellular space ($p<.05$). Sulpiride also potentiated the amount of DA increased by 8 mg/kg AMPH, but did so by affecting the maximum concentration achieved (22 pg/µl vs. 8.5 µl for vehicle, $p<.05$), not the onset or offset rates. In contrast, sulpiride enhanced the behavioral effect of AMPH at doses up to and including 2.0 mg/kg AMPH, but had no effect at higher doses. This work was supported by NIDA grant DA06201 and NIAAA grant AA08484.

RECEPTOR MODULATION: UP- AND DOWN-REGULATION I

41.1

CHRONIC COCAINE ADMINISTRATION INCREASES MELANOCORTIN 4-RECEPTOR mRNA EXPRESSION IN RAT STRIATUM. J.D. Alvaro*[1], M.L. Entwistle[2], J.B. Tatro[2], and R.S. Duman[1]. [1]Laboratory of Molecular Psychiatry, Yale Univ. School of Med., New Haven, CT 06508, [2]Dept of Medicine, NEMC and Tufts Univ. School of Med., Boston, MA 02111.

Following our demonstration that exogenous opiates regulate central melanocortin 4-receptor (MC4-R) gene expression, we have examined the regulatory effects of cocaine. Whereas chronic morphine administration in rats down-regulates MC4-R mRNA expression in the neostriatum, chronic cocaine administration has the opposite effect. Rats were treated with either saline or 15 mg/kg cocaine (i.p) twice daily for 14 days, and levels of MC4-R mRNA were determined by RNase protection assay 3 hours following a final injection on day 15. We found that chronic cocaine administration increased levels of MC4-R mRNA in striatum more than 150% above control. This up-regulation did not persist 18 hrs following the final cocaine injection, nor was the regulation seen after 1, 3, or 7 days of drug administration. Preliminary results indicate that chronic cocaine significantly decreases hypothalamic levels of POMC, the precursor to the ACTH and MSH peptides that bind to and activate MC4-R. Examination of the effects of cocaine on MC4-R mRNA expression in other brain regions as well as on levels of melanocortin receptor binding are in progress. Altered MC4-R activation in the striatum may be one neuronal adaptation which contributes to the development of cocaine addiction. (Supported by DA08227 and MH44694.)

41.2

EFFECTS OF LONG-TERM METHAMPHETAMINE AND COCAINE TREATMENT ON NEUROKININ I (SUBSTANCE P) RECEPTORS AND PREPROTACHYKININ mRNA LEVELS IN THE STRIATUM AND NUCLEUS ACCUMBENS OF THE RAT BRAIN. M. Kraft*, Y. Zhang, H. Mueller and J. A. Angulo. Hunter College and the Graduate School of the City University of New York, Department of Biological Sciences, NY NY 10021.

We have assessed the effect of chronic treatment with methamphetamine (METH) or cocaine on ^{3}H-substance P (SP) binding to neurokinin I (NKI) receptors. We also assessed preprotachykinin (PPT) mRNA expression from the same animals. Rats received either 4 mg/kg of methamphetamine or 15 mg/kg cocaine twice daily for 1, 3, 6 and 14 days. ^{3}H-SP binding levels decreased in the dorsalmedial (10%) and ventralmedial (15%) aspects of the striatum at day 14. PPT mRNA levels increased gradually from days 1-14 in all areas of the neostriatum, with increases of up to two-fold at day 14. In contrast, ^{3}H-SP levels showed no changes in cocaine- treated animals although PPT mRNA levels increased significantly in the neostriatum (20-50%). The NMDA non-competitive receptor antagonist MK-801 attenuated the psychostimulant- induced increases in PPT mRNA levels but had no consistent effect on METH-induced decreases in NK1 receptor binding levels. Both methamphetamine and cocaine increase preprotachykinin mRNA levels but only methamphetamine modulates substance P receptor levels.

41.3

NEUROPEPTIDE Y Y1 AND Y2 RECEPTOR BINDING IN THE RAT BRAIN AFTER ANTISENSE OR MISSENSE OLIGODEOXYNUCLEOTIDE TREATMENT. R. T. F. Cheung*, A. J. Stoessl, T. J. McDonald and D. F. Cechetto. Robarts Research Institute, University of Western Ontario, London Ontario, N6A 5K8 Canada.

The regional distribution of neuropeptide Y (NPY) Y1 and Y2 receptor binding in the brain was studied in untreated adult male Wistar rats and rats following treatment with an antisense oligodeoxynucleotide (D-oligo) specific for the NPY Y1 receptor gene or a missense D-oligo. The D-oligos were given into the left lateral ventricle (50 μg in 10 μl of saline every 12 h, 4 times) via an implanted 23-G guide cannula. The treated rats were decapitated 1-2 h after the last injection. Coronal brain sections (20 μm) at 0 and 3 mm Bregma levels were processed for *in situ* receptor binding autoradiography, using porcine [^{125}I]-peptide YY (100 pM; 2200 Ci/mmol). Non-specific binding was determined in the presence of porcine NPY (1 μM), and Y2 sites were defined by occluding Y1 sites with porcine [Leu^{31},Pro^{34}]-NPY (50 nM). A computer-assisted image analysis system was used to quantify the binding density in the frontoparietal cortex (FPC), caudate putamen, lateral septum, amygdaloid region, and dorsal hippocampus. The autoradiographic distribution of the binding in the untreated rats revealed predominantly Y1 binding in the FPC. The other areas had equal Y1 and Y2 binding or mainly Y2 binding. The antisense treatment significantly reduced the Y1 binding in the FPC but did not change the Y2 binding in any area. Missense treatment did not significantly affect Y1 or Y2 binding in any area. We conclude that the antisense D-oligo can selectively reduce receptors in areas of high regional density.
(Supported by the Heart and Stroke Foundation of Ontario)

41.4

NEONATAL EXPOSURE TO ARGININE VASOPRESSIN ALTERS ADULT VASOPRESSIN V1a AND OXYTOCIN RECEPTOR mRNA EXPRESSION IN RAT BRAIN. C.S. Vaccari, Carter, C.S. and N.L. Ostrowski*. NIMH, Bethesda, MD, 20892, and Dept. of Zool., Univ. MD, College Park, MD, 20742.

The possibility that neonatal exposure to vasopressin may play a role in regulating the expression of vasopressin (AVP) and/or oxytocin receptor (OTR) genes was investigated. Neonatal rats were treated for 7 days (1/day) with saline, arginine vasopressin, a selective V1a , or V2 receptor antagonist, or AVP in combination with the antagonists. AVP decreased rate of growth and neurological development during the seven days of treatment. Treated rats demonstrated perturbations in body weight indices as adults, confirming previous work (Boer et al., 1988). *In situ* hybridization experiments on brains of adult males demonstrated that neonatal treatments produced permanent changes in V1a and OTR messenger ribonucleic acid (mRNA) densities. In the lateral septum, the V1a receptor antagonist significantly increased V1a mRNA labeling, whereas AVP, alone, produced little change. Moreover, neonatal treatment with AVP decreased expression of OTR mRNA in the paraventricular nucleus (PVN) where both OT and AVP expressing neurons are located. These data demonstrate that 1) neonatal treatments that affect AVP receptors produce long-term changes in AVP and OT receptor gene expression; 2) AVP may play a determining role in the regulation of V1a receptor mRNA levels in brain; and, 3) the PVN may be a possible site for interactions between the AVP and OT neuropeptide systems. Compounds were donated by Dr. M. Manning; plasmid DNA was prepared by Dr. S. Lolait; brains were sectioned by S. Leonard.
Supported by NSF and NIMH.

41.5

ETORPHINE-INDUCED CHANGES IN μ OPIOID RECEPTOR-IR IN THE CEREBRAL CORTEX. N.C. Brecha§^*, M. Melone°, A. Minelli°, C. Stemini§^, B. Anton#, C. Evans#, and F. Conti°. Inst. Hum. Physiol°, Univ of Ancona, Ancona, Italy; Depts. of Neurobiol.§ and Psych.#; UCLA, and CURE§^, Los Angeles, CA, USA.

μ opioid receptors (MOR) are activated by alkaloids used for pain control and are involved in addiction and tolerance. We have studied MOR immunoreactivity (IR) in the first somatic sensory area (SI) of the rat neocortex and the effects of MOR agonists/antagonists using an antibody directed to the C-terminus of rat MOR. Adult rats received etorphine (0.1 mg/kg i.p), an opioid receptor agonist, and euthanized 15, 30, and 60 min following drug injection. Other rats were injected with etorphine and naloxone, an opioid receptor antagonist, and sacrified 30 min later.

In the SI cortex of non-treated animals, MOR-IR consisted of few lightly stained, nonpyramidal neurons in layers VI and II-III, and strong neuropilar staining in layers II-III and VI. In the SI cortex of etorphine-treated animals: i) at 15 min, MOR- immunoreactivity (IR) appeared slightly denser than in controls; ii) at 30 min, there was an increase in MOR-IR, both at cellular and neuropilar levels. MOR+ neurons were more numerous and more intensely stained than those from control animals. Their processes were shorter and were characterized by a pronounced "granular" appearance. MOR+ cells were all layers II-III and VI nonpyramidal neurons. At 60 min, changes were more pronounced than at 30 min, and the number of MOR+ cells was further increased. Cells were counted in the neocortex of the 4 groups of animals, and statistical analysis was performed using ANOVA and multiple comparisons test. The results show that the number of MOR+ neurons increases significantly as a function of time ($p < 0.001$); and iii) naloxone abolished the effects of etorphine.

The present results demonstrate a rapid, agonist-induced, increase of the number and staining intensity of detectable MOR+ neurons and, given the rapid onset of the changes, they suggest that these effects may be due to MOR internalization. (Funded by EY, MURST, VA, DK, DA, and NATO)

41.6

INTRAVESICAL XYLENE CAUSES SUBSTANCE P RELEASE, UPREGULATION OF NK1 RECEPTORS AND INDUCTION OF NK1R mRNA IN RAT BLADDER. L. Kushner*, M. Sherman, T. Singh, P.Y. Chiu, S. Tillem, S. Press, Y. Chen and R. Moldwin. Long Island Jewish Med. Cent., New Hyde Park, NY 11040

In order to explore the role of substance (SP) in bladder inflammation, we induced inflammation by intravesical instillation of xylene. Bladders of anesthetized adult female Sprague-Dawley rats were either catheterized (controls), instilled with 0.3 ml silicone oil (vehicle) or 0.3 ml 30% xylene in silicone oil (xylene) for 15 min. Evans Blue perfusion to determine plasma protein extravasation demonstrated significant differences for xylene-instilled (72.63 ± 12.37 ng Evans Blue/wet wt. tissue) compared to vehicle-instilled (27.39 ± 6.85, $p<0.01$) or control (14.43 ± 2.43, $p<0.001$) bladders. The SP content of xylene-treated bladders (1.57 ± 0.12 pg/mg protein) was reduced compared to control bladders (4.28 ± 0.89, $p<0.01$) as determined by RIA. Depletion of neuronal SP was confirmed immunohistochemically. High affinity, saturable, and monophasic specific binding of the NK1R selective agonist (9-sar,11-met(O_2),[prolyl-3,4-^{3}H]-SP to bladder membranes indicated an apparent single class of sites with a binding affinity (K_d) of 0.20±0.05, 0.31±0.22 and 0.56±0.17 nM and a binding capacity (B_{max}) of 29.55 ± 2.0, 23.5 ± 1.2 and 94.6 ± 3.9 fmol/mg protein for control, vehicle- and xylene-instilled bladders, respectively. Thus, NK1 receptors were three-fold higher in xylene-treated bladders. Transcripts encoding NK1R were transiently elevated by three-fold between 5 and 7 hrs following xylene instillation as determined by solution hybridization/nuclease protection normalized to the constituitive 1B15 transcript. Intravesical xylene results in SP release from sensory neurons, plasma protein extravasation and upregulation of NK1 receptors as well as induction of NK1 receptor gene transcription, indicating a response to SP depletion consistent with neurogenic inflammation. (Supported by the Interstitial Cystitis Association and an LIJMC Faculty Research Award).

41.7

RAPID BRADYKININ RECEPTOR UPREGULATION IN SKIN FIBROBLASTS IN ALZHEIMER'S DISEASE PROMPTED BY PROTEIN KINASE C (PKC) ACTIVATION. N.L. Baenziger* and Y.-J.I. Jong
Department of Anatomy and Neurobiology, Washington University, St. Louis, MO 63110.

Hyperactivity of bradykinin (BK) receptor signal transduction pathways in Alzheimer's disease (AD) skin fibroblasts interfaces with a complex cast of molecular characters with primary pathophysiology in brain but aberrant behavior reflected at a systemic level. τ protein Ser/Thr hyperphosphorylation lies downstream of BK receptor signal transduction; presenilin 1 and 2 (PS-1,2) cellular functions are still under study. In AD skin fibroblasts with PS-1 mutations A246E and L286V, PKC activation by phorbol ester (PMA) plus phosphoprotein phosphatase 2a inhibitor Calyculin A augmented, within 1 min, BK B_2 subtype receptors with K_d's 5 nM (I,intermediate) and 44 nM (L,low) affinity, implicating Ser/Thr phosphorylation as the augmenting mechanism. Control fibroblasts did not show rapid augmentation. By immunoblotting after nonionic detergent solubilization and SDS-PAGE, AD B_2 receptors were a well-resolved doublet of M, 76-78 kDa and 80-82 kDa, also seen in human lung fibroblasts vulnerable to PKC-mediated upregulation but rarely observed in normal fibroblasts where an M, 76-78 kDa singlet was prevalent. Selective PKC-induced quantitative and qualitative changes in AD fibroblast BK B_2 receptors suggest the cellular role of presenilins may bear on not only intracellular trafficking functions postulated for these species but phosphorylation/dephosphorylation pathways which govern expression and activity of G-protein-coupled receptors over a very short time frame.
Supported by the Alzheimer's Association.

41.8

RAPID DESENSITIZATION OF SOMATOSTATIN INHIBITION OF CALCIUM CHANNEL CURRENT IN NG108-15 CELLS. V. Beaumont, M. Hepworth, E.P. Kelly, J.L. Benovic & G. Henderson*
Dept of Pharmacology, University of Bristol, Bristol BS8 1TD, U.K. and Dept. of Pharmacology, Thomas Jefferson University, Philadelphia, PA 19107 USA.

Somatostatin inhibited the N-type calcium channel current in NG108-15 cells recorded using the whole cell or amphotericin-permeablized patch techniques. However the inhibition of the calcium channel current was not sustained but decayed rapidly during continued exposure to the drug. For a supramaximal concentration of somatostatin (300nM) the inhibition desensitized by 53 ± 2% and was complete within 2.5min of drug application. In contrast the desensitization of the inhibition of the current by the δ-opioid agonist DPDPE (300nM) was much less (17 ± 7%) over the same time period. Both the rate of somatostatin desensitization and the degree of desensitization were concentration-dependent over the range 10 - 1000nM. The time course for recovery from desensitization was exponential with a $t_{1/2}$ of 2min.

The desensitization to somatostatin (300nM) was reduced by intracellular application of Zn (3-30μM) in a concentration-dependent manner. Zn has been proposed as a G-protein receptor kinase (GRK) inhibitor. In cells stably transfected with a dominant negative mutant of GRK2 the rate and degree of the rapid desensitization induced by somatostatin (300nM) was not reduced from that seen in wild type cells or in cells transfected with the plasmid alone.
Supported by the MRC of the UK.

41.9

MOLECULAR MECHANISMS OF THE DESENSITIZATION OF THE SOMATOSTATIN RECEPTOR SSTR2. G. Singh and T. Reisine. Department of Pharmacology, University of Pennsylvania, Philadelphia, PA 19104.

The somatostatin (SRIF) receptor SSTR2 mediates a number of the physiological actions of SRIF. A result of continuous stimulation of SSTR2 with agonist is desensitization. To investigate the mechanisms of SSTR2 desensitization, the splice variants of SSTR2, SSTR2A and SSTR2B, which differ only in their C-terminal amino acid sequences, and mutants of these receptors were expressed in CHO-K1 cells and tested for their coupling to adenylyl cyclase. Both SSTR2A and SSTR2B effectively mediated the inhibition of cAMP accumulation by SRIF. Pretreatment of CHO-K1 cells expressing the SSTR2 splice variants with SRIF (1 μM) for 3 hr diminished the potency and effectiveness of SRIF to inhibit cAMP accumulation. A mutant of SSTR2B (SSTR2BT) truncated at amino acid 331, the point at which the splice variants diverge in amino acid sequence, coupled to adenylyl cyclase and desensitized following prolonged agonist pretreatment, indicating the region required for the desensitization of the receptor does not reside in the C-terminus of the receptor. Two serine residues in the third intracellular loop of SSTR2B were mutated individually to alanines to create the mutants ($SSTR2B_{S244A}$ and $SSTR2B_{S245A}$). Both mutants coupled to adenylyl cyclase. Pretreatment of $SSTR2B_{S244A}$ with SRIF desensitized the receptor. In contrast, $SSTR2B_{S245A}$ was more resistant to desensitization. These findings suggest that phosphorylation of single amino acid in the third intracellular loop of SSTR2B may be critical for desensitization. Supported by MH45533 and MH48518.

41.10

DESENSITIZATION OF CANNABINOID-MEDIATED INHIBITION OF GLUTAMATERGIC SYNAPTIC TRANSMISSION BETWEEN CULTURED RAT HIPPOCAMPAL NEURONS. M. Shen*, S.A. Thayer. Dept. of Pharmacology, Univ. of Minnesota, Minneapolis, MN 55455.

We have shown previously that activation of cannabinoid receptors inhibits the presynaptic release of glutamate from cultured rat hippocampal neurons. Here we describe the effects of prolonged exposure to cannabimimetics on excitatory neurotransmission. Reducing the extracellular Mg^{2+} concentration to 0.1 mM elicited repetitive increases in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$ spikes) that were recorded with indo-1-based microfluorimetry. $[Ca^{2+}]_i$ spikes required glutamatergic synaptic transmission as indicated by pharmacological characterization. Win55212-2 (100 nM), a potent cannabinoid receptor agonist, completely blocked $[Ca^{2+}]_i$ spiking. In the continued presence of Win55212-2, $[Ca^{2+}]_i$ spiking resumed after approximately 1 hr, suggesting that the response had desensitized. The desensitization could be overcome by subsequent application of a high concentration (1 μM) of the agonist. The selective cannabinoid receptor antagonist, SR141716 (300 nM), completely reversed the inhibition of $[Ca^{2+}]_i$ spiking produced by 100 nM Win55212-2. In the neurons desensitized from a 2 hr exposure to 100 nM Win55212-2, 300 nM SR141716 precipitated a rebound increase in the frequency of $[Ca^{2+}]_i$ spiking that exceeded control by 34%. The desensitization process should be considered in evaluating the abuse potential and the clinical utility of cannabimimetics. (Supported by the NIH and the NSF)

41.11

TESTOSTERONE INCREASES AMPA GluR 2/3 PROTEIN- AND GluR 2 mRNA LEVELS IN THE CA1 REGION OF THE HIPPOCAMPUS AND DECREASES NEURONAL DAMAGE DURING ISCHEMIA. S. Diano, F. Naftolin* and T.L. Horvath. Department of Ob/Gyn, Yale University, School of Medicine, New Haven CT, 06520.

We tested the hypothesis that the gonadal steroid-induced increase in GluR 2/3 protein levels in the hippocampus is induced by *local* action of testosterone and involves the regulation of GluR 2 mRNA. Furthermore, since glutamate-induced neuronal death of CA1 pyramidal cells during ischemia was associated with low levels of GluR 2 AMPA receptors, we hypothesized that testosterone upregulating GluR 2 in the CA1 region may increase the survival rate of pyramidal cells. **Experimental:** To verify the local effect of testosterone, male rats received testosterone-implants in the CA1 region of the hippocampus, and were sham-operated on the contralateral side. Seven days later, a group of animals were killed and the hippocampi were used for either immunocytochemical labeling of GluR 2/3 or Northern blot analysis for GluR 2 mRNA. Ischemia was induced in implanted animals by occlusions of the vertebral and carotid arteries. Immunocytochemical studies showed an increased intensity (120% as revealed by image analyzer) of GluR2/3 labeling in the CA1 pyramidal cells in particularly in their dendrites in the vicinity of the testosterone implant but not around the sham implant. Northern blot analysis demonstrated that the increased receptor content corresponded with an increase (100%) in GluR2 mRNA levels in the CA1 region. Seven days after inducing ischemia, animals were killed, and, the hippocampi were counterstained with cresyl violet. Ipsilateral to the testosterone implants, the number of surviving CA1 neurons were higher than that of the control, contralateral side. **Conclusions:** These results suggest that testosterone, acting locally, may decrease the vulnerability of CA1 pyramidal cells during ischemia, at least in part, by increasing the expression of the GluR 2 subunit of the AMPA receptors. (Supported by NIH grant HD 13587)

41.12

NMDA receptor subtype 1a mRNA in the lumbar spinal cord of adult spinal rats in response to spinal transection and locomotor training N.J.K.Tillakaratne*, W.M.Walwyn, R.J. Monti, A.J.Tobin and V.R.Edgerton. Department of Physiological Science, UCLA, Los Angeles, CA 90095.

NMDA receptor activation induces alternating locomotor patterns in the spinal transected animal and plays an important role in spinal cord excitability following repeated conditioning stimuli. The NMDA receptor consists of several subunits, one of which is the NMDAR1, and this subunit exists in several isoforms. Little is known of the effect of spinal transection on the NMDAR1a isoform mRNA and how repeated locomotor patterns may alter the localization and level of this receptor RNA. We have evaluated the effect of transection of the spinal cord and of repeating specific motor patterns on the localization of the mRNA of NMDAR1a in the spinal cord. Adult female rats were transected at T6-8, allowed 7 days to recover and were then trained to step using a combination of modes of sensory stimulation to induce bipedal locomotion. The training protocol included alternating placement of the rear feet in plantarflexion, and light stimulation of the skin over the knee and hip joints, and was performed once daily for 10 min, 5 days a week for 10 weeks. Using non-radioactive in situ hybridization histochemistry we examined the changes in NMDAR1a mRNA in the lumbar spinal cord. Increased levels of mRNA were found in all lamina of the L4 segment of the lumbar spinal cord following spinal cord transection. This was further elevated following 10 weeks of step-training. Training did not alter NMDAR1a mRNA in age-matched intact rats. These results indicate that there is an activity dependent component of regulation of NMDAR1a mRNA in the lumbar spinal cord. Supported by grants to NKJT (NRSA, HDO7416), AJT (NS 22256) and VRE(NS16333).

41.13

REGULATION OF PITUITARY CORTICOTROPIN RELEASING HORMONE RECEPTOR mRNA BY GLUCOCORTICOIDS. C. Rabadan-Diehl, A. Kiss and G. Aguilera*. DEB, National Institute of Child Health and Human Development, NIH, Bethesda, MD 20892

The regulation of pituitary corticotropin releasing hormone receptors (CRH-R) by glucocorticoids was studied in the rat by CRH binding autoradiography and Northern blot and in situ hybridization of CRH-R mRNA after adrenalectomy (ADX) or glucocorticoid administration. CRH-R mRNA levels decreased by 48% of controls 18 hr after ADX but returned to basal levels by 4 and 6 days. Dexamethasone (DEX), 100 μg, s.c. prevented the transient effect of ADX. In sham operated or intact rats, DEX increased CRH-R mRNA only after 6-day repeated injection, while decreasing POMC mRNA, POMC hnRNA and CRH binding. Daily injection of corticosterone (B), 2 mg, sc, to mimic stress levels, had no effect on POMC mRNA, POMC hnRNA, CRH-R mRNA or CRH binding. On the other hand, supraphysiological B doses (10 mg) for 6 days decreased POMC mRNA by 67%, POMC hnRNA by 58% and CRH binding by 35%, but had no effect on CRH-R mRNA levels. However, 2 to 4 hr after injection, DEX (10 to 100 μg) or B (2 to 5 mg) decreased CRH-R mRNA by about 50%, indicating biphasic regulatory actions of glucocorticoids on CRH-R mRNA. The data indicates that regulation of pituitary CRH-R mRNA is complex, probably involving direct effects of glucocorticoids on mRNA transcription and stability, as well as indirect actions mediated by glucocorticoid dependent changes of hypothalamic regulators.

41.14

GLUCOCORTICOIDS DOWN-REGULATE LEVELS OF CRF-R1 mRNA IN AtT-20 CELLS. P.A. Iredale* and R.S. Duman. Lab. of Molecular Psychiatry and Pharmacology, Yale University School of Medicine, New Haven, CT 06508.

Glucocorticoids are known to be involved in a plethora of physiological responses via activation of discrete receptors which are translocated into the nucleus where they act as positive and negative transcriptional regulators. CRF receptors in the anterior pituitary represent one of the most extensively studied sites for negative feedback by adrenocortical glucocorticoid hormones, however the molecular mechanisms have yet to be elucidated.

The present study examines the mechanism by which glucocorticoids regulate CRF-R1 expression in the pituitary cell line, AtT-20. Treatment of these cells with dexamethasone resulted in a concentration- and time-dependent inhibition of CRF-R1 mRNA that was significant by 1 hr and maximal after 4 hr. Levels of CRF-R1 mRNA then returned to control levels after 24 hr. Similar changes were observed when the cells were treated with corticosterone. The down-regulation of CRF-R1 mRNA was dependent on *de novo* protein synthesis as it was blocked by pre-treatment with cycloheximide. Furthermore, half-life studies with actinomycin D suggested that the observed decrease was not due to a change in CRF-R1 mRNA stability and therefore likely the result of a decrease in the rate of gene transcription. This hypothesis is currently being tested. POMC mRNA was also decreased following dexamethasone pre-treatment, however the time-course was much slower with a maximal decrease observed after 24 hr.

These data suggest that glucocortricoid down-regulation of CRF-R1 mRNA is mediated by decreased gene transcription and that this effect involves the induction of a regulatory protein. (DA 08227 Program Project Grant).

41.15

PHORBOL ESTERS DOWN-REGULATE β_1-ADRENERGIC RECEPTOR GENE TRANSCRIPTION IN RAT C6 GLIOMA CELLS. Z. Li[1], V. A. Vaidya[1], L. R. Fitzgerald[1], P. H. Fishman[2], C. A. Machida[3], R. S. Duman*[1]. [1]Dept. Psychiatry, Yale Univ. Sch. Med., New Haven, CT 06508 and [2]Lab. Mol. Cel. Neurobiol., NINDS, NIH, Bethesda, MD 20892 and [3]Div. Neurosci., Oregon Regional Primate Res. Ctr., Beaverton, OR 97006

Protein kinase C (PKC) is known to have multiple effects on the βAR-coupled cAMP system. We have investigated the mechanism by which PKC regulates the expression of β_1AR in rat C6 glioma cells. Treatment of cells with PMA, an activator of PKC, results in a down-regulation of both β_1AR binding sites and mRNA levels in a time- and concentration-dependent manner. Maximal loss (>50%) of β_1AR mRNA levels was observed after 2 h, preceding that of receptor binding sites which occurred by 24 h. An EC_{50} of 15-20 nM PMA was observed for both processes. Pretreatment of the cells with exotoxin A, a protein synthesis inhibitor, had little effect on the PMA-mediated down-regulation of β_1AR mRNA, but slowed the loss of receptor binding sites. Exposure of the cells to PMA did not reduce the half life of β_1AR mRNA, which is ~1 h. In contrast, PMA treatment for 6 h significantly decreased the rate of β_1AR gene transcription as determined by reporter analysis. Treatment with PMA decreased reporter activity by 40~50% as observed with the promoter construct (-3351, -1) bp and with constructs shortened to (-348, -1), but no decrease was seen with constructs shorter than (-331, -1). Consistent with this observation, the construct (-484, -325), as well as mutant CRE or CCAAT reporter constructs which locate upstream of -348, are still responsive to PMA. Based on these results, we conclude that activation of PKC down-regulates β_1AR gene transcription via a small element in the promoter region located between -348 and -331. Studies are currently underway to identify this element. (Supported by DA 08227)

41.16

MODULATION OF NEURONAL ANGIOTENSIN II TYPE 2 (AT_2) RECEPTOR mRNA BY GROWTH FACTORS. X-C. Huang, U. Shenoy, C. Sumners*. Dept. of Physiology, Univ. of Florida, Gainesville, FL 32610.

The expression of neuronal AT_2 receptors demonstrates dramatic spatiotemporal changes before and after birth, suggesting a role for these sites in development. Our previous studies showed that simulation of neuronal AT_2 receptors inhibited mitogen activated protein kinases, which are the key components of growth factor induced signal transduction pathways. Molecular cloning of the AT_2 receptor promoter region demonstrated the presence of potential growth factor regulatory sites, including AP-1 and IRS of PEPCK gene. The present study was undertaken to evaluate the effects of various growth factors on modulation of neuronal AT_2 receptor mRNA levels in neurons cultured from newborn rat hypothalamus and brainstem. Reverse transcriptase polymerase chain reaction (RT-PCR) analysis was employed to evaluate the changes in the mRNA level. The data indicate time-dependent modulation of AT_2 mRNA by growth factors. Incubation of neuronal cultures with nerve growth factor (50 ng/ml, 0 - 2h) increased steady state levels of AT_2 mRNA and this effect was mimicked by the phorbol ester, PMA(100 nM). However, incubation of cultured neurons with nerve growth factor (50 ng, 12 -24h) or insulin (1670 nM, 0 - 24h) decreased AT_2 mRNA levels. Our results provide a link between changes in the growth factor milieu in the developing brain and AT_2 receptor levels. (Supported by NIH grant NS-19441)

41.17

ESTROUS CYCLICITY OF BRAIN ANGIOTENSIN II RECEPTORS E.E. Nilsson and R.C. Speth*, Dept. VCAPP Washington State Univ., Pullman, WA 99164-6520

Although angiotensin II (AII) is best known for its effects on blood pressure and fluid and electrolyte balance, it has also been shown to be a regulator of reproductive function (Phillips *et al.* Adv-Exp-Med-Biol. 1995, p. 357). To further explore the relationship between the brain renin-angiotensin system (RAS) and reproductive function, this study examined ^{125}I-sar^1,ile^8 AII (^{125}I-SI AII) binding in 24 different brain regions of the rat, at the 4 different stages of the estrous cycle. Mature, cycling Sprague-Dawley rats (n = 37) were sacrificed at 1600 hours on either proestrus (P), estrus (E), metestrus (M) or diestrus (D). The brains were frozen and coronally sectioned at 20 microns in a cryostat. The sections were incubated with 500 pM ^{125}I-SI AII, alone or in the presence of 10 μM losartan, 10 μM PD123177, or 3 μM SI AII and subjected to autoradiography. Subsequent densitometric analysis of films then determined total, AT_2 receptor subtype, AT_1 receptor subtype, and nonspecific binding density respectively. Results of this study showed that in virtually all brain nuclei and the anterior pituitary, ^{125}I-SI AII binding to AT_1 and AT_2 receptors was lowest on the day of proestrus, indicating the lowest AII receptor density was on that day. ^{125}I-SI AII binding varied less on the remaining 3 days of the estrous cycle, however AT_2 receptor binding was lower on D than on E or P. These results suggest that female rat brain AII receptors undergo cyclic changes that correlate with the estrous cycle, confirming the linkage between the brain RAS and reproductive function.

Supported by Warner Lambert Co. and Washington State University.

41.18

INHIBITION OF ANGIOTENSIN RECEPTOR (AT_1) EXPRESSION IN NG108-15 CELLS TRANSFECTED WITH AT_1 RECEPTOR ANTISENSE IN AN ADENO-ASSOCIATED VIRAL VECTOR. T. Zelles, D. Mohuczy and M.I. Phillips*. Dept. of Physiology, Univ. of Florida, Gainesville, FL 32610.

We are using gene therapy for hypertension by inhibiting angiotensin II (AngII) receptor genes. The adeno-associated virus (AAV) is a potentially useful vector for non pathogenic delivery of genes into cells. Antisense inhibition is a specific and potentially therapeutic application of gene therapy.

We transfected NG108-15 cells with the AngII type 1 (AT_1) receptor antisense vector pA9, constructed from pTR-UF, containing a recombinant AAV cassette. The cassette also contained a neomycin resistant gene and the transfected cells were selected by G418 in the medium. To investigate the effect of pA9 transfection on AngII receptor level we carried out ^{125}I-[Sar^1 - Ile^8]AngII binding assay on the membranes of undifferentiated, 3 and 5 days differentiated (1.5 % DMSO/0.5 % FBS) NG108-15 cells. The numbers of AT_1 and AngII type 2 (AT_2) receptors were determined using the AT_1 receptor selective antagonist losartan and the AT_2 receptor selective PD123319. Cell differentiation resulted in about a 70x increase in total AngII receptor number which was mainly due to the huge increase of the numbers of AT_2 receptors, reaching the plateau on day 5. AT_1 receptors reached their elevated level (7x) on day 3. Transfected cells had about a 40 % decrease in AT_1 receptor number in the undifferentiated, 3 and 5 days differentiated cells. Simultaneously, the AT_2 receptor number increased 2.5 times in the differentiated stages suggesting an interaction between the two AngII receptors during differentiation.

These results demonstrate that AT_1 receptor antisense DNA, transfected in an AAV cassette, significantly reduces AT_1 receptor binding through several cell passages. AngII receptor antisense delivery in AAV is useful to investigate the role of AngII receptors in neuronal differentiation and apoptosis. *(NIH grant HL27334.)*

41.19

KNOCKDOWN OF THE ANGIOTENSIN TYPE-1 RECEPTOR BY ANTISENSE OLIGONUCLEOTIDES IN CELL CULTURE T. Lesher, Y. Bao, D. Conklin, P. Hartig* and S.P. Ho CNS Diseases Research, DuPont Merck Research Laboratories, Wilmington, DE 19880.

Four phosphorothioate oligonucleotides targeted against the coding region of the angiotensin type-1 (AT_1) receptor mRNA were tested in CHO cells stably transfected with the AT_1 receptor. Complexation with Lipofectin facilitated uptake of a fluorescein-labeled oligonucleotide by 400-fold over controls, (assayed by fluorescence activated cell sorting). The oligo was evenly distributed (fluorescence microscopy) in the cytoplasm but was most prominently visible in the nucleus. Knockdown of the AT_1 receptor was determined using a radioligand binding assay. Using oligonucleotide concentrations as low as 0.25 uM, knockdown with the 4 sequences ranged from 55-75%. Sequences targeting the mRNA downstream of the start codon were more potent than the oligonucleotide at the ATG. Specificity of the antisense knockdown was demonstrated using mismatched sequences (3 mismatches) and reverse sequences.

2'-methoxyribonucleotide analogs of the above antisense sequences hybridize efficiently to the mRNA but do not promote RNase H mediated degradation of the RNA target. These analogs produced good knockdown when targeted at the ATG; however, the knockdown became progressively poorer when 2'-methoxyribonucleotide sequences were targeted against downstream coding regions. This data indicates that antisense knockdown through hybrid arrest is most effective when the oligonucleotide is targeted against the 5'-regions of the mRNA. These results also support the involvement of RNase H in antisense knockdown in this CHO cell line.

41.20

DISTRIBUTION OF BIOTIN-LABELED OLIGONUCLEOTIDES FOLLOWING LATERAL VENTRICLE AND INTRASTRIATAL INJECTIONS: A COMPARISON. G. Dent, S.P. Ho, P. Hartig and R. Grzanna*. The DuPont Merck Pharmaceutical Company, Wilmington, DE 19880-0400.

The successful application of the antisense technology in the CNS requires delivery of adequate amounts of antisense oligonucleotides (ON) to the appropriate target regions. This study was conducted to compare the extent of diffusion of biotinylated random ON delivered into the lateral ventricle (icv) and striatum, respectively. ON were delivered stereotaxically and rats were perfused 24 h later with 4% paraformaldehyde. Transverse sections were processed to visualize the spread of ON. Some sections were stained with cresyl violet to assess the extent of tissue damage.

Following icv injections of 20 nmoles, ON were observed to penetrate up to 2.0 mm into the tissue surrounding the lateral, third and fourth ventricle. Icv injections of 2 nmoles caused the ON to spread approximately 0.8 mm into the tissue. No diffusion into brain tissue was observed when 0.2 nmoles of ON were injected. In sections counterstained with cresyl violet, no tissue damage was detectable in the zone of ON uptake.

Intrastriatal injections of 2 nmoles of ON resulted in a sphere of labeling with a radius of more than 2 mm. At 0.2 nmoles the spread was 0.8 mm; and at 0.02 nmoles the ON diffused approx. 0.4 mm from the center of the injection. This zone was characterized by severe tissue necrosis. With either route of delivery, ON had a granular distribution in the cytoplasm of neurons with no detectable labeling of neuronal nuclei.

The results show that the optimal choice for the route of ON delivery depends on the brain regions to be targeted. Icv injections of nanomolar amounts of ON are preferable when targeting brain regions lying close to the ventricular surface. Regions beyond this periventricular zone are best targeted by parenchymal injections. However, either route of administration will be of limited value in targeting large brain regions such as the cerebral cortex or the cerebellum.

42.1

EXTRACELLULAR MEMBRANE ASSOCIATION OF THE ENDOPEPTIDASE EC 3.4.24.15. T.J Wu*, P.J. Crack, M. Glucksman, J.L. Roberts. Fishberg Research Center for Neurobiology, Mt Sinai School of Medicine, New York, NY 10029.

Endopeptidase EC 3.4.24.15 (EP24.15) is a soluble metalloenzyme abundant in the brain, pituitary and testes. Expression of EP24.15 is ubiquitous in the brain and in the pituitary, having a cellular localization (nuclear and/or cytoplasmic) in both neurons and glia. Our laboratory's interest in this enzyme involves the processing of GnRH released from the median eminence. For the extracellular metabolism of GnRH, the enzyme is expected to be associated in the same compartment, near or at the cell surface. The present biochemical and immunocytochemical study was conducted to ascertain the location of EP 24.15 on the extracellular surface of a pituitary-derived cell line (AtT20). In order to visualize immunostaining on the cell surface in the absence of the cytoplasmic fraction, live AtT20 cells were stained utilizing a specific antibody to EP24.15 and subsequently visualized with confocal laser microscopy. Our results indicate that EP24.15 is sparsely expressed on the surface of the AtT20 cells and bore a punctate appearance with occasional floccular staining in regions with a higher cell density. Cells treated with 1 μM A23187 for 30 min, a known stimulator of EP 24.15 "release" exhibited a decrease in extracellular staining. Biochemical analysis with a thiol-cleavable cross-linker, DTSSP, performed with subsequent western blot analysis of an isolated membrane preparation, also suggests membrane association. In a reduced state (cross-linker broken) a 77 kDa band corresponding to EP24.15 was resolved but in non-reducing condtions (cross-linker intact) this band was absent indicating a complex of membrane proteins and EP24.15 generated by DTSSP. These results suggest that EP24.15 is anatomically situated in a manner consistent with a physiological role in the degradation of GnRH. (Supported by NSF IBN-9512113)

42.2

CHARACTERIZATION OF THE GONADOTROPIN- RELEASING HORMONE RECEPTORS IN THE PITUITARY GLAND OF THE DEVELOPING AMMOCOETE, JUVENILE, AND SEXUALLY MATURING ADULT LAMPREY. O.L.J. Materne and S.A. Sower*, Dept. of Biochemistry and Molecular Biology, University of New Hampshire, Durham, NH 03824-3544.

In our previous study we have shown two classes of high affinity binding sites for gonadotropin-releasing hormone (GnRH) in the pituitary of the adult female sea lamprey, *Petromyzon marinus.* In the current studies, saturation binding assays were done to determine the concentration and affinity of both GnRH binding sites in the pituitaries of lamprey at different developmental stages. In addition, these studies were done in an effort to determine the presence of two GnRH receptors or one receptor having two binding sites, and to detect any cooperativity between the two binding sites. Pituitary sections of 20 μ m were incubated for 3 hrs at 4°C with ten serial dilutions (1.0×10^{-8}M to 1.95×10^{-11}M) of the GnRH agonist D-Ala^{6} , Des Gly^{10} mGnRH. Interestingly no specific-binding was detected in the pituitaries of larval (ammocoetes) and parasitic lamprey that were kept at 2°C water temperature. In comparison, non-parasitic adult lamprey, held at the same water temperature did demonstrate two classes of binding sites: Kd=1.03×10^{-12} M, Bmax=2.5×10^{-15} M, and Kd=2.06×10^{-9}M, Bmax=5.1×10^{-14} M. This work was supported by NSF: 9022834 & 9407767.

42.3

PHOTOPERIODIC DIFFERENCES IN THE IMMUNOREACTIVE GONADOTROPIN-RELEASING HORMONE NEURONAL SYSTEM OF MALE SIBERIAN HAMSTERS D.J. Bernard*, R. Nussbaum, T.H. Horton, and F.W. Turek, Dept. of Neurobiology & Physiology, Northwestern University, Evanston, IL 60208

Previous research on photoperiodic Siberian hamsters (*Phodopus sungorus*) has failed to detect differences in the gonadotropin-releasing hormone (GnRH) neuronal system between animals housed chronically on long and short days. Recent work in our laboratory, however, has identified a transient change in both GnRH mRNA and GnRH peptide content in the preoptic area and hypothalamus during the transition from inhibitory short days to stimulatory long days. We observe an increase in GnRH mRNA containing neurons and GnRH content on the morning of the second long day after transfer from short to long days, but not at time points examined before or after this time. These increases may reflect a transient increase in the number of neurons producing GnRH or an increase in the amount of GnRH produced by an otherwise stable population of GnRH neurons. In this study, we used immunocytochemical methods to help discriminate between these possibilities. Male Siberian hamsters born into long days (16L:8D) were weaned at post-natal day 18 and transferred to short days (6L:18D) or maintained on long days (Long Day Control). Four weeks later, half of the short day hamsters were transferred back to long days (Experimental) while the other half remained on short days (Short Day Control). Experimental animals were perfused two hours after light onset on the morning of the second long day. Age-matched control animals were perfused two hours after light onset on their respective light-dark cycles. Brains were sectioned and processed for GnRH immunoreactivity with the LR-1 antibody (gift of R. Benoit). The number of GnRH-immunoreactive (-ir) neurons was quantified using brightfield microscopy. There was no difference in the number of GnRH neurons between short day control and experimental animals (approx. 330 per brain). Contrary to previously published reports, long day control hamsters had significantly fewer GnRH-ir neurons than both of the other groups (approx. 230 per brain). Based on these data we hypothesize that during the transition from short to long days, male Siberian hamsters experience a transient increase in GnRH production in a stable population of neurons in anticipation of increased secretion on long days. When GnRH secretion increases there is a decrease in the peptide contained within neuronal cell bodies leading to a decrease in their detection with immunocytochemical methods. Supported by NIH Grants P01 HD21921-06 and T32 HD07068.

42.4

CIRCADIAN RHYTHMICITY OF GnRH GENE EXPRESSION IN THE RAT. Andrea C. Gore* and James L. Roberts. Fishberg Research Center for Neurobiology, Mount Sinai Medical Center, Box 1065, NY, NY 10029.

The release of the decapeptide GnRH from neuroterminals in the median eminence undergoes afternoon increases in female rats during the proestrous LH surge and in chronically ovariectomized rats given estradiol. With respect to the biosynthetic events responsible for these rhythms, we have reported that increases in GnRH mRNA levels precede and may be causal to the afternoon proestrous GnRH/LH surge. In the present study, we examined whether male rats exhibit a circadian periodicity in GnRH mRNA levels, and are currently elucidating the mechanism for any such diurnal changes, across a 24-hour period. Methods: Male Sprague-Dawley rats (2-3 months of age) were maintained on a controlled light cycle (12:12 light:dark, lights on 0700 hours). They were sacrificed by decapitation at four hour intervals at 1000, 1400, 1800, 2200, 0200 and 0600 hours. The preoptic area-anterior hypothalamus was rapidly dissected out on ice and frozen in liquid Freon for subsequent analysis of GnRH mRNA levels by RNase protection assay using a rat GnRH cDNA clone. Results: GnRH mRNA levels exhibited significant variation with time of day. They increased two-fold between 1000 and 1400 hours; remained elevated at 1800 hours; and returned to baseline (1000 hour levels) at 2200 through 0600 hours. We are currently determining the mechanism for this circadian rhythm (transcriptional or post-transcriptional) since it is our hypothesis that "chronic" changes in inputs to the GnRH system (e.g. circadian rhythm, stage of estrous cycle) are transcriptionally regulated, while acute inputs (e.g. neurotransmitter stimulation) to the GnRH system appear to affect post-transcriptional mechanisms of regulation. We are also examining whether a circadian rhythm in GnRH gene expression occurs during puberty, when large nocturnal elevations in GnRH release are observed. (FRCN Devel. Grant)

42.5

PROGESTERONE - BLOCKADE OF SYNCHRONIZED PREOVULATORY LH SURGES ARE ASSOCIATED WITH LOW MEDIAN EMINENCE (ME) IN VIVO RELEASE OF LHRH, NPY, AND ß-ENDORPHIN (ßEND). J. Klein, S. Goebbert, J. Rabii, and JP Advis*, Departments of Animal Sciences and Biological Sciences, Rutgers University, New Brunswick, NJ 08903.

We proposed a mechanism by which P4 inhibits synchronized preovulatory LH surges, based on a follicular model of sequential P4 removal and E2 silastic packets implantation into intact cycling ewes. Each animal served as both control (low P4 if P4 implants are removed at the end of a luteal phase, LH surge: 18 ± 2 h after E2) and experimental ewe (high P4, in another estrous cycle, if P4 implants are not removed, no LH surge occurred). We have previously shown that synchronized LH surges are preceded by parallel increases in ME push-pull cannula (PPC) perfusate content of NPY and ßEND, while the LH surge itself is associated with highest LHRH and NPY but decreasing ßEND release. In follicular ewes, in vivo ME-LHRH release is increased by NPY and decreased by ßEND perfusion through the ME-PPC probe. Similarly, ME perfusion of NPY-antiserum stopped the ascending phase of an ongoing synchronized LH surge, while mid-follicular ME perfusion of naloxone (ßEND antagonist) advanced LH surge onset. On the other hand, if P4 is maintained high in another cycle of the same ewe, synchronized LH surges are absent and ME neuropeptide in vivo release is low ($P < 0.01$). LH (mean ± sem in ng/ml) and neuropeptide release (mean ± sem in pg/100 μl of ME-PPC perfusate) at the time of the expected synchronized LH surge in control vs experimental cycles in these ewes (n=6) are as follows, LH: 97 ± 11 vs 0.6 ± 0.1; LHRH 25 ± 4 vs 1 ± 0.5; NPY 331 ± 44 vs 21 ± 9; ßEND 71 ± 18 vs 7 ± 6. Therefore, P4 may prevent preovulatory in vivo release interactions of facilitatory NPY and inhibitory ßEND on in vivo LHRH release from ME neuronal terminals and thus block synchronized LH surges. Support by NJAES-Hatch 06108 & USDA 94-37203-0721 grants to JP Advis.

42.6

COLOCALIZATION OF MU, DELTA AND KAPPA OPIATE RECEPTOR AND LHRH mRNAS IN THE PREOPTIC AREA OF THE RAT BRAIN. S.L. Petersen* and M. Sannella. Biology Department, Neuroscience and Behavior Program, Univ. of Massachusetts, Amherst, MA 01003

Abundant evidence suggests that ovarian steroid regulation of LHRH and LH release is mediated by opiate peptides. The present studies were designed to test the hypothesis that opiates directly regulate LHRH neurons and to determine what opiate receptor(s) mediate this regulation. Female Sprague-Dawley rats were ovariectomized (OVX) and one week later (Day 0) implanted with Silastic capsules of estrogen. At 0900 on Day 2, some estrogen-treated animals were injected with 5 mg progesterone and all animals were sacrificed at 1530 on the same day. Brains were removed, frozen on dry ice and stored at -80° C until cryosectioned. Twelve-micron sections through the preoptic area of the brain were collected and used for dual-label in situ hybridization histochemistry. For these studies, we used a digoxigenin-labeled cRNA probe for LHRH mRNA and ^{33}P-labeled cRNA probes for mu, delta or kappa opiate receptor mRNAs. The digoxigenin-labeled probe was detected with antidigoxigenin conjugated to horseradish peroxidase and diaminobenzidine/hydrogen peroxide. The radiolabeled probes were detected by dipping the slides in NTB-3 emulsion. All three receptor mRNAs were expressed in cells of the preoptic region; however, regardless of steroid treatment, we found no LHRH neurons that expressed mu, delta or kappa opiate receptors. These results suggest that opiate modulation of LH release is not through a direct action on LHRH neurons. (Work supported by NIH grant HD27305 to SLP.)

42.7

POLYCYSTIC OVARY SYNDROME INDUCED BY ESTRADIOL VALERATE IS INDEPENDENT OF BETA-ENDORPHIN. J.L. Smart,C.C. Field, M.J. Low*. Vollum Inst., Ore. Health Sci. Univ., Portland, OR 97201.

Female rats and mice chronically treated with estradiol valerate (EV) exhibit chronic anovulation, persistent vaginal cornification, and polycystic ovaries. EV-treated rats exhibit attenuated pituitary luteinizing hormone (LH) content, LH response to gonadotropin releasing hormone (GnRH), LH replenishment following GnRH-stimulated release, and mean plasma LH concentration. The mechanism that has been proposed to explain these effects is a supersensitivity of GnRH neurons in the medial preoptic area to hypothalamic β–endorphin (BE). Other researchers speculated that μ–opioid receptor supersensitivity is induced by the EV mediated destruction of a subpopulation of arcuate BE neurons (Desjardins G.C., et al. Endocrinol. 132: 86-93, 1993).

To test this hypothesis, we utilized a strain of mice deficient in beta-endorphin produced by gene targeting techniques (Rubinstein M, et al. PNAS, USA 1996, in press). Normal control and BE deficient mice were treated with a single 1 mg dose of EV by i.m. injection. Both groups of mice developed persistent vaginal cornification indicative of anovulation and polycystic ovaries 8 weeks following EV injection. *In-situ* hybridization for POMC message in the hypothalamic arcuate nucleus showed no reduction in POMC mRNA in EV treated wild-type or BE deficient mice. Normal and BE deficient mice given EV injection had a 4-5 fold decrease in follicle stimulating hormone beta subunit mRNA measured by an RNAse protection assay compared to sesame oil treated controls. Daily injections of naltrexone, a stable opiate receptor antagonist, for 3 weeks starting 8 weeks after EV injections were unable to reinstate normal cyclicity in wild-type or BE deficient mice. From these data, we conclude that BE deficient mice have normal onset of puberty, and normal frequency and duration of estrous cycles. Furthermore, BE does not play an important role in the chronic estradiol induced HPG inhibition in mice. These studies were supported by HD30236.

42.8

MEDIAL PREOPTIC μ-OPIOID RECEPTOR DENSITY INCREASES FOLLOWING THE LH SURGE. R. P. Hammer, Jr.* and B. S. Rubin. Depts. of Psychiatry and Anatomy & Cellular Biology, Tufts University School of Medicine, Boston, MA 02111.

μ-Opioid receptors are gonadal steroid hormone-dependent in the medial preoptic area of female rats, wherein endogenous opioids regulate luteinizing hormone (LH) secretion. Therefore, μ-receptor density was examined by quantitative *in vitro* receptor autoradiography following hormone treatment to induce an LH surge. Adult Sprague-Dawley female rats were housed in a controlled environment with a 14:10 h light cycle. Regularly cycling animals were ovariectomized and received estradiol (40 μg/kg) followed by progesterone (8 mg/kg) 48 hr later at 1000 h. Rats were anesthetized and decapitated at 1000, 1400, 1800 or 2000 h, at which time trunk blood was collected for radioimmunoassay, and brains were removed, frozen and sectioned. Sections were incubated in a saturating concentration of [^{3}H]D-Ala^2, MePhe^4, Gly-ol^5-enkephalin (10 nM) to selectively label μ-receptors. Adjacent sections were hybridized with a digoxigenin-labeled 330 bp riboprobe to preproLHRH. Medial preoptic μ-receptor density was significantly greater at 1800 and 2000 h than at 1000 or 1400 h, but was unaffected in the adjacent lateral preoptic area. LH levels increased at 1400 and 1800 h and declined by 2000 h, while the number of neurons expressing LHRH mRNA increased until 1400 h then declined at 1800 hr. A significant correlation ($r = -0.65$, $p \leq 0.005$) was present between μ-receptor density and number of mRNA-expressing neurons across this period. Thus, the upregulation of medial preoptic μ-receptors may provide a substrate for endogenous opioid termination of LHRH synthesis and/or secretion. Supported by USPHS Awards R01 DA06645, HD19174 and P30 HD28897.

42.9

CHARACTERIZATION OF THE MECHANISM THROUGH WHICH ESTROGEN REGULATES POMC GENE EXPRESSION IN THE RAT HYPOTHALAMUS. C. A. Priest* and J. L. Roberts. Fishburg Research Center of Neurobiology, Mount Sinai School of Medicine, New York, NY 10029.

In the rat hypothalamus, estrogen has been shown to regulate proopiomelanocortin (POMC) gene expression in a temporally dependent manner. Gonadectomy produces an up-regulation of POMC mRNA levels that is reversible by chronic estrogen treatment, although acute transcriptional effects of estrogen have been difficult to interpret. Importantly, the promoter region of the rat POMC gene does not contain a consensus estrogen response element. However, as AP-1 activation constitutes a major regulatory pathway for POMC gene expression, we have proposed that estrogen may modulate POMC mRNA levels through a non-classical mechanism that involves AP-1 proteins. To investigate this regulatory pathway further, transient transfections of partial POMC promoter sequences which contain the exon I AP-1 site are being assessed *in vitro* for their ability to modulate transcription in the presence of estrogen. In addition, to establish whether an anatomical substrate exists in the rat hypothalamus for such an interaction, immunohistochemical double-labeling was used to examine neurons in the arcuate nucleus (ARC). Adult female rats were ovariectomized for two weeks and either received no experimental treatment, or were given an i.p. injection of ethanol vehicle or 50 μg estradiol (E2) and were perfused 1, 2, 4, or 48 hrs later. ARC sections were processed for sequential immunohistochemistry using commercially available antisera directed against β-endorphin (βEND) or c-Fos. Cell populations which were immunoreactive (-IR) for βEND, c-Fos or both proteins were quantified using an optical dissector and unbiased stereological methods. Treatment with E2 gradually increased all three populations of cells, with statistically significantly higher numbers of c-Fos-IR, βEND-IR and doubly-labeled cells appearing after 4 hrs. By 48 hrs after E2, numbers of c-Fos-IR and doubly-labeled cells had declined, while the number of βEND-IR cells remained high, indicating population-specific effects of E2 on βEND neurons in the ARC. (Supported by: ADF/HRI-817-5332)

42.10

REGULATION OF PRO-OPIOMELANOCORTIN (POMC) GENE EXPRESSION IN THE HAMSTER ARCUATE NUCLEUS BY PHOTOPERIOD AND TESTOSTERONE. Eric L. Bittman*, Catherine M. Hegarty, Maureen L. Tubbiola, and Gretchen Foltz. Dept. of Biology and Neuroscience and Behavior Program, University of Massachusetts, Amherst 01003.

In seasonal breeders including hamsters, daylength regulates reproduction by altering responsiveness to gonadal steroid hormones. Changes in the action of endogenous opiates which influence gonadotropin secretion and sexual behavior may participate in these effects of photoperiod. Testosterone (T) decreases hypothalamic β-endorphin content in rats and hamsters; effects on POMC mRNA abundance in rat arcuate are controversial. It is not known whether androgens or photoperiod influence POMC gene expression in hamsters. We quantified POMC gene expression in the arcuate of hamsters which were either left intact, castrated without androgen replacement, or castrated and implanted with T either immediately or after a 5-week delay. An additional group was pinealectomized in order to determine whether the pineal gland mediates effects of daylength on POMC expression. Half the animals in each group were moved to short days (SD: 5L:19D); the others remained on 14L:10D (LD). Ten weeks later, brain sections were processed for *in situ* hybridization to detect POMC mRNA using ^{35}S-labeled I13 cDNA probe. The numbers of POMC cells and grains per cell were measured in emulsion-dipped sections.

SD precipitated profound, pineal-dependent testicular regression. T suppressed serum LH more effectively in SD than in LD. ANOVA indicated overall effects of photoperiod and surgical treatments on POMC mRNA (grains/cell; $p=0.01$). Pairwise comparisons (Tukey test) indicated that T significantly reduced POMC mRNA in SD, but not in LD, in the rostral and middle but not the caudal arcuate. T reduced POMC expression in the mid-arcuate whether it was given immediately or 5 weeks after castration, but delayed T replacement was not effective in rostral arcuate. Effects of photoperiod on T's influence on POMC expression may contribute to seasonal breeding. Supported by NIH MH44132, MH00914, and NSF IBN 9319653.

42.11

PARAVENTRICULAR NUCLEUS PLAYS A CRITICAL ROLE FOR FASTING-INDUCED SUPPRESSION OF PULSATILE LH RELEASE IN TESTOSTERONE-TREATED CASTRATED MALE RATS. H. Tsukamura*, S. Yamada, N. Tamaya, M.A.C. Estacio, K.-I. Maeda. Lab. Animal Reproduction, Nagoya University, Nagoya 464-01, Japan.

We have previously revealed that 48-h fasting profoundly suppresses pulsatile LH secretion in female rats in an estrogen-dependent manner. The present study was aimed to determine whether fasting-induced suppression of pulsatile LH secretion in male rats is testosterone-dependent, and whether the paraventriclar nucleus (PVN) is involved in the fasting-induced inhibition of pulsatile LH release. Wistar-Imamichi strain male rats were castrated, and some of them were implanted with various length of Silastic tubings filled with testosterone. Pulsatile LH secretion after 48-h fasting was suppressed only in the testosterone-implanted castrated males, but not in castrated animals without testosterone. Fos protein expression was also determined immunocytochemically in the several brain regions at various time after fasting in castrated males with or without testosterone treatment. Induction of Fos protein was shown in the PVN and supraoptic nucleus only in testosterone-treated animals at the beginning of dark period on the day of fasting, but not in unfasted controls with or without testosterone treatment or castrated animals at any time of the day. Furthermore, PVN lesion blocked the inhibitory effect of fasting on pulsatile LH release. These results clearly suggest that fasting suppresses pulsatile LH secretion in a testosterone-dependent manner, and that the PVN plays a critical role in mediating the fasting-induced suppression of pulsatile LH secretion in male rats.

42.12

PARAVENTRICULAR NOREPINEPHRINE RELEASE MEDIATES GLUCOPRIVIC SUPPRESSION OF PULSATILE LH SECRETION IN OVARIECTOMIZED RATS. S. Nagatani[2], H. Tsukamura[1], K. Murahashi[1], D. C. Bucholtz[2], D. Foster[2], T. Matsushima[1]* and K.-I. Maeda[1], [1]Sch. of Agri. Sci., Nagoya Univ., Nagoya 464-01, Japan; [2]Reprod. Sci. Progr., Univ. Michigan, Ann Arbor MI 48109, USA.

Restriction of glucose availability by 2-deoxyglucose (2DG) suppresses pulsatile luteinizing hormone (LH) release. The purpose of the present study was to determine if norepinephrine (NE) release in the paraventricular nucleus (PVN) is involved in the glucoprivic suppression of LH secretion in ovariectomized rats. Twelve days after ovariectomy, animals were stereotaxically implanted with a unilateral guide cannula for microdialysis in the PVN. Two days later, the PVN was perfused continuously with Ringer's solution or Ringer's solution containing a catecholamine synthesis inhibitor, α-methyl-*p*-tyrosine (α-MPT, 100 μM) through a microdialysis probe inserted in the guide cannula two hours before the beginning of sampling which lasted 3 h. Blood samples were collected every 6 min through an atrial cannula and dialysates were collected every 20 min. One hour after the beginning of sampling, 2DG (400 mg/kg BW) was administered intravenously through the atrial cannula. Paraventricular NE levels significantly ($P<0.05$) increased immediately after 2DG injection, and both mean LH concentrations and the frequency of LH pulses decreased. Administration of α-MPT into the PVN bloked 2DG-induced increase in paraventricular NE release as well as decrease in plasma LH levels. These results suggest that the 2DG-induced suppression of LH pulses is mediated by the NE release in the PVN in ovariectomized rats.

42.13

FASTING-INDUCED FOS EXPRESSION IN HYPOTHALAMIC NEUROPEPTIDE Y (NPY) NEURONS OF ADULT MALE RHESUS MONKEYS: A SEARCH FOR NEURAL SYSTEMS WHICH MAY MEDIATE FASTING-INDUCED SUPPRESSION OF LUTEINIZING HORMONE (LH) SECRETION.

AL Caston-Balderrama*, GE Hoffman, MS Smith and JL Cameron. Depts. of Psychiatry, Cell Biol & Physiol, Neurobiol, and Neurosci, Univ. of Pittsburgh, PA 15213 and the Oregon Regional Primate Research Center, Beaverton, OR 97006

Short-term food restriction suppresses LH secretion in adult male rhesus monkeys (*Macaca mulatta*), with a slowing of LH pulse frequency apparent within the first 6 h after a single missed meal. Conversely, refeeding a normal meal after a brief period of fasting rapidly increases the secretion of LH. In this study, we examined Fos expression in hypothalamic NPY neurons, in response to fasting and refeeding, to determine whether NPY neurons are activated by changes in energy intake, and thus may be playing a role in mediating the fasting-induced suppression of pulsatile LH secretion. NPY neurons are likely candidates because they have been shown to respond to changes in energy availability and are known modulators of the reproductive axis. In this study, 3 monkeys were fasted for 2-3 days and sacrificed at 1000 h, prior to the normal feeding time in our colony (1100 h), to minimize extraneous neural stimulation that accompanies the daily meal (FASTED group). A second group of 3 monkeys was fasted for 2-3 days, a liquid meal was infused through an indwelling gastric cannula between 0630-0830 h, and then this group was sacrificed at 1000 h (REFED group). Intense Fos expression in NPY neurons was observed in the FASTED group along the ventral surface of the hypothalamus, with a 5-fold greater activation when compared to the REFED group. However, NPY neuronal activation in other regions was similar in the two groups. Therefore, only a select population of hypothalamic NPY neurons appear to be activated by short-term fasting, suggesting that these NPY neurons may play a causal role in mediating the fasting-induced suppression of LH secretion.

(Supported by NS09561, HD26888)

42.14

TIME COURSE OF THE SEXUALLY DIMORPHIC RESPONSE IN ESTROGEN RECEPTOR IMMUNOREACTIVITY IN UNDERFED PREPUBERTAL MICE

JN Rocmmich[1], X Li[2], AD Rogol[1,3], and EF. Rissman*[2]. Dept. of Pediatrics[1] Biology[2], and Pharmacology[3], University of Virginia, Charlottesville, VA 22903.

The influence of sex steroids on GnRH release is mediated by the estrogen receptor (ER). We have shown that underfeeding prepubertal female mice for 7 d reduces ER-ir cells in the medial preoptic area (mPOA) and ventromedial nucleus (VMN) but not in the arcuate. Here, we investigated the time course of the reduction in ER-ir in representative sections of the mPOA and VMN. Weanling female (FW) and male (MW) mice were sacrificed at 16 days of age (n=8 FW, n=7 MW). Additional groups were underfed for 24 (n=6 FUF-24; n=7 MUF-24) or 48 (n=7 FUF-48; n=7 MUF-48) h. Brains were perfused, frozen and every fourth coronal section (30 µC) processed and incubated with a monoclonal antiserum for ER (H222; Abbott Laboratories). Using Mocha software one observer, blind to sex and condition of the animal, counted all ER-ir cells on one side of matched sections. Results [mean (SD)] from ANOVA are presented. Like letters ($P \leq 0.05$).

	FW	FUF-24	FUF-48	MW	MUF-24	MUF-48
mPOA ER-ir	717(163)^{ab}	487(198)^{a}	359(197)^{b}	549(166)	538(148)	557(106)
VMN ER-ir	243(88)^{c}	164(83)^{d}	111(35)^{e}	162(53)	129(34)	145(35)

Underfeeding female mice reduced the number of ER-ir in the mPOA within 24 h and VMN within 48 h. Underfeeding had little effect on ER-ir of male mice. Others have shown nutritional alterations modify GnRH pulse generator activity within 24 to 48 h. We have shown changes in ER-ir cell numbers within this same time frame. Thus, underfeeding may stall puberty by impairment of ER protein in brain areas important for modulating the effects of sex steroids on GnRH release.

(Supported by The Genentech Foundation for Growth and Development)

42.15

THE COMBINED USE OF FOS IMMUNOCYTOCHEMISTRY AND LESIONS IN THE STUDY OF NEURAL PATHWAYS UNDERLYING METABOLIC CONTROL OF REPRODUCTION.

B.C. Finnerty, D. Jacobs, M. Szajna , J.M. Swann and J.E. Schneider*. Department of Biological Sciences, Lehigh University, Bethlehem, P.A. 18015

Previous work supports the idea that signals generated by changes in glucose metabolism control reproduction in female Syrian hamsters. Estrous cycles are delayed when glucose utilization is blocked by cerebroventricular or systemic treatment with the glucose antimetabolite, 2-deoxy-D-glucose (2DG) in hamsters. We have examined the effects of anestrus-inducing doses of systemic 2DG (1750 mg/kg) on FOS-like immunoreactivity in a variety of brain areas that have been implicated in metabolic control of food intake and reproduction in other species. These areas include, but are not restricted to the area postrema (AP), nucleus of the solitary tract (NTS), lateral and medial parabrachial nucleus (LPBN and MPBN respectively), central nucleus of the amygdala (CNA), and paraventricular nucleus (PVN). We have noted significantly increased FOS staining in the AP, NTS, LPBN and PVN in hamsters treated with 2DG (1750 mg/kg) compared to vehicle-treated controls ($p < 0.05$). Additionally, we have noted a significant dose-response relationship between 2DG (doses ranging from 750-2000 mg/kg) and number of cells showing FOS-like immunoreactivity in the AP and NTS. A similar trend was apparent in the PVN. To examine whether these areas contain cell bodies or fibers that are important in mediating the effects of 2DG on estrous cycles, we have performed radiofrequency lesions or sham surgeries of these individual areas, treated the hamsters with 2DG or saline, and examined indices of estrous cyclicity. Preliminary results suggest that the effects of 2DG on estrous cyclicity require an intact AP and medial NTS, but not PVN or CNA. Supported by IBN9121056 from NSF,1K02MH01096 from the NIMH, and HD28467 from NICHD.

42.16

EFFECT OF AREA POSTREMA LESIONS ON ESTROGEN RECEPTOR IMMUNOREACTIVITY IN FOOD-DEPRIVED FEMALE SYRIAN HAMSTERS.

A.K. Panicker, J.D. Blaustein and G.N. Wade*. Neuroscience and Behavior Program, University of Massachusetts, Amherst, MA 01003.

Female Syrian hamsters reduce their reproductive activity, including ovulation and estrous behavior, when they are food deprived. Previous studies from this lab showed that 48 hrs of food deprivation caused a decrease in estrogen receptor immunoreactivity (ERIR) in the ventromedial hypothalamus (VMH) and arcuate nucleus (ARC) and an increase in the medial preoptic area (MPOA) and posterior parvocellular paraventricular nucleus (pPVN). One way by which visceral sensory information, including peripheral metabolic status, is conveyed to the brain is believed to be through the area postrema (AP), which is a circumventricular organ in the hindbrain. In order to determine if the AP is involved in mediating metabolic effects on forebrain ERIR, female hamsters were subjected to AP lesions or sham lesions, and the effects of 48 hrs of food deprivation were determined. AP lesions blocked the effect of food deprivation on ERIR in VMH, but had no effect in the pPVN. Previous studies on ERIR in vagotomized animals given metabolic inhibitors like 2-deoxy-D-glucose and methyl palmoxirate showed that vagotomy did not block the decrease of ERIR in VMH, whereas it blocked the increase of ERIR in pPVN. Taken together, these results suggest that ERIR-containing cells in the VMH receive at least some metabolic information via AP, whereas PVN receives it via the vagus. In addition, these results indicate that ERIR cells in the VMH and PVN do not detect metabolic fuel availability directly. Analysis of ERIR in the MPOA and ARC are in progress. Supported by NS 10873, MH00321 and MH00850.

42.17

LEPTIN IS A METABOLIC SIGNAL TO THE REPRODUCTIVE SYSTEM.

I.A. Barash, C.C. Cheung, D.S. Weigle, P. Ren, J.M. Kramer, M. Fallon, E.B. Kabigting, J.L. Kuijper, D.K. Clifton, and R.A. Steiner*, Departments of Ob-Gyn, Physiology and Biophysics, and Medicine, University of Washington and ZymoGenetics, Inc., Seattle, WA 98195

The activity of the reproductive system is gated by metabolic signals that reflect nutritional state; however, the identity of these signals remains unknown. Leptin, a newly discovered hormonal product of the *ob* gene, is expressed by adipocytes and thought to play a role in the regulation of food intake and metabolism. We postulated that, as a derivative of the body's nutritional state, leptin may also signal metabolic information to the reproductive axis. We tested this hypothesis by examining the effects of leptin on the reproductive endocrine system of the *ob/ob* mouse, which has a congenital deficiency in leptin and is thought to be infertile because of a neuroendocrine defect. We treated pair-fed, male and female *ob/ob* mice with leptin (50 µg twice daily, ip) or vehicle (n=10/group) for 14 days, after which the animals were bled and killed. We measured serum levels of LH and FSH, weighed the ovaries, testes, uteri, and seminal vesicles, and performed quantitative histology on all tissues sampled. In females, leptin-treated animals had elevated serum LH levels ($p<0.001$), higher ovarian ($p<0.01$) and uterine weights ($p<0.001$), and increased morphological measures of ovarian and uterine histology ($p<0.001$) compared to controls. In males, leptin-treated animals had elevated serum FSH levels ($p<0.001$), higher testicular ($p<0.05$) and seminal vesicle weights ($p<0.02$), and increased morphological measures of seminal vesicle histology compared to controls. These results suggest that leptin stimulates the reproductive endocrine system in both sexes of the *ob/ob* mouse. *Conclusion:* In the normal animal, leptin may serve as a metabolic "green light" to the activity of the reproductive system. *(Supported by NIH grant RO1 HD27142)*

42.18

EXPRESSION OF LEPTIN RECEPTOR MESSENGER RIBONUCLEIC ACID (mRNA) IN NEUROENDOCRINE AND ENDOCRINE TISSUES OF THE RAT.

P. L. Zamorano, V. B. Mahesh, L. P. Chorich, L.M. DeSevilla and D. W. Brann*. Dept. of Physiology & Endocrinology, Medical College of Georgia, Augusta, GA, 30912.

The obese (ob) gene product, leptin, has recently been shown to be produced by adipocytes and function as a hormone to modulate appetite and metabolism. Intriguingly, the ob/ob mutant female mouse, which does not produce leptin, has been shown to be sterile. This sterility can be reversed by treatment with recombinant leptin, but not by diet restriction - suggesting that leptin is required for normal reproductive function. To determine whether endocrine and neuroendocrine tissues could be targets for leptin action and regulation, we examined whether these tissues express the leptin receptor mRNA transcript by using RT-PCR. The results revealed that the leptin receptor mRNA transcript is highly expressed in the female rat brain, hypothalamus, ovary and uterus, with moderate to low expression in the anterior pituitary and adrenal, respectively. In the male rat, high expression was observed in the brain and hypothalamus, with moderate expression in the anterior pituitary and testis, and low expression in the adrenal. Interestingly, modest expression of the leptin receptor was also observed in immortalized GnRH (GT1-7) neurons. Taken as a whole, the above findings raise the intriguing possibility that reproductive tissues are targets for leptin action, and that leptin may influence reproduction through direct effects on reproductive tissues. *Supported by NIH Grant R29HD28964.*

42.19

INFERTILITY IN THE *fat/fat* MOUSE IS ASSOCIATED WITH A DEFICIT IN PROCESSING THE LHRH PRECURSOR. W.C. Wetsel[1,*] and E.H. Leiter[2]. [1]Lab. of Cellular and Molecular Pharmacology, NIEHS, Research Triangle Park, NC 27709, and [2]The Jackson Laboratory, Bar Harbor, ME 04609.

Mice homozygous for the *fat/fat* mutation are obese, diabetic, and infertile. These mice have a point mutation in carboxypeptidase E (CPE), an enzyme responsible for processing prohormone intermediates to bioactive peptides such as insulin. Recently, immortalized LHRH neurons have been shown to express CPE and this enzyme has been postulated to be involved in processing pro-LHRH intermediates. The purpose of the present study was to determine whether *fat/fat* mice were deficient in processing the pro-LHRH to LHRH. Adult *fat/fat* male and female mice weighed approximately 2-fold more, had elevated plasma insulin levels, and were hyperglycemic compared to their heterozygous littermates. Whole hypothalamic LHRH content was analyzed by RIA with two different antisera. One antiserum (A772) recognized the decapeptide and [Gln^1]-LHRH; a second antiserum (B9) bound all of the different pro-LHRH intermediates including LHRH. Levels of LHRH-like immunoreactivity (IR), as determined with the A772 antiserum, were significantly reduced in hypothalami from male and female *fat/fat* mice compared to those from heterozygotes. Despite this fact, analysis with the B9 antiserum revealed that hypothalami from all mice, regardless of genotype, contained comparable amounts of LHRH-like IR materials. HPLC analyses confirmed that male *fat/fat* mouse hypothalamus was defective in processing the LHRH prohormone. Quantities of [Gln^1]-LHRH-[Gly^{11},Lys^{12}] and LHRH-[Gly^{11},Lys^{12},Arg^{13}] were particularly elevated in hypothalami from these *fat/fat* mice. Moreover, LHRH levels were reduced by approximately 4-fold in the mutant mice. These data indicate that CPE may be the prominent carboxypeptidase involved in processing pro-LHRH intermediates to LHRH. Because LHRH is the major regulator of reproduction in mammals, the mutation in CPE may contribute to infertility in the *fat/fat* mouse. (Supported by the NIEHS Intramural program to WCW.)

NEURAL-IMMUNE INTERATIONS: CNS MECHANISMS

43.1

AREA POSTREMA REMOVAL ABOLISHES STIMULATORY EFFECTS OF INTRAVENOUS INTERLEUKIN-1β ON HPA AXIS ACTIVITY AND *c-fos* mRNA IN THE HYPOTHALAMIC PARAVENTRICULAR NUCLEUS. H. Y. Lee and M. Herkenham*. Section on Functional Neuroanatomy, Laboratory of Cell Biology, NIMH, Bethesda, MD 20892.

This study examined the role of the area postrema (AP) in transducing peripheral immune signals, represented by i.v. interleukin-1 (IL-1), into a neuroendocrine response, manifested by induction of *c-fos* mRNA in the hypothalamic paraventricular nucleus (PVN). The AP, a circumventricular organ with a leaky blood-brain barrier, lies adjacent to the nucleus of the solitary tract (NTS). The AP was removed by aspiration, and two weeks later, AP-lesioned or sham-lesioned rats were injected i.v. with 0.5 μg/kg IL-1β or saline. After 30 min, brains were removed and analyzed for *c-fos* mRNA levels in structures implicated in the HPA axis response to peripheral cytokine challenge. The sham-lesioned animals responded to IL-1 with large elevations in ACTH and corticosterone in the plasma and *c-fos* mRNA in responsive structures. Prior AP removal abolished the IL-1-induced increases in plasma hormone levels and *c-fos* mRNA in the NTS and PVN. However, AP removal had no effect on IL-1-induced increases in *c-fos* mRNA levels in the other areas examined. The selective AP lesion effects suggest that the AP plays a pivotal role in transducing a circulating IL-1 signal into HPA axis activation by a pathway that may be comprised of known anatomical links between the AP, NTS, and corticotropin-releasing hormone (CRH) neurons of the PVN.
(Support: NIMH Intramural Research Program)

43.2

INDUCIBLE CYCLOOXYGENASE mRNA EXPRESSION IN RAT BRAIN FOLLOWING PERIPHERAL ADMINISTRATION OF LIPOPOLYSACCHARIDE. M. Whiteside, N. Quan, X. Wang*, and M. Herkenham. Section on Functional Neuroanatomy, LCB, NIMH, Bethesda, MD 20892.

Inducible Cyclooxygenase (Cox-2) converts arachidonic acid to prostaglandin E2 (PGE2), which is thought to mediate various peripheral LPS-induced central effects, including generation of fever and activation of the HPA axis. To localize PGE2 production in the brain following peripheral LPS administration, cox-2 mRNA expression was examined by *in situ* hybridization histochemistry in rats injected i.p. with 2.5 mg/kg LPS or saline. Cox-2 mRNA levels were not altered in saline-injected animals as compared to non-injected controls. In LPS-injected animals, beginning at 0.5 h, de novo labeled cells appeared; their numbers increased to a peak at 2 h and subsided gradually to basal levels by 24 h. Initially, labeling was observed in cells comprising major, surface-lying blood vessels and meninges. Later, vascular and perivascular cells associated with smaller, penetrating blood vessels, and scattered, small non-vascular cells also became intensely labeled. Changes in cox-2 mRNA expression were not observed in circumventricular organs. These results suggest that peripheral LPS induces a rapid global increase of PGE2 production, which could affect the activity of widespread brain regions. (Support: NIMH Intramural Research Program)

43.3

RAPID INDUCTION OF INTERLEUKIN-1β mRNA IN CIRCUMVENTRICULAR ORGANS AFTER PERIPHERAL ADMINISTRATION OF LIPOPOLYSACCHARIDE. N. Quan*, M. Whiteside, and M. Herkenham. Section on Functional Neuroanatomy, LCB, NIMH, Bethesda, MD 20892.

Peripheral LPS induces IL-1 bioactivity in brain tissue. To identify the neuroanatomical loci of these IL-1-producing cells, we investigated brain IL-1β mRNA expression by *in situ* hybridization histochemistry (ISHH) using a 500 bp ribonucleotide probe on brain sections from rats injected intraperitoneally with 2.5 mg/kg LPS or saline. In control animals, IL-1β mRNA was not detectable by ISSH. In LPS-injected animals, cell labeling appeared at 0.5 h, peaked at 2 h, declined at 8 h and disappeared at 12–24 h. Labeled cells were concentrated in circumventricular organs (CVOs)—organum vasculosum of the lamina terminalis, subfornical organ, median eminence, and area postrema—and were sparsely scattered in blood vessels, choroid plexus, and meninges. Labeling was not observed in any neural structures. In the pituitary, LPS induced strong IL-1β mRNA expression first in the anterior lobe at 0.5 and 1 h, and then in the neural lobe beginning at 2 h, which subsided quickly thereafter. The results show that peripheral LPS induces IL-1β production at the blood brain barrier (BBB) and in CVOs where the BBB is leaky and suggest that these cells are the source of brain IL-1 activity after peripheral LPS injection. (Support: NIMH Intramural Research Program)

43.4

CO-EXPRESSION OF ENKEPHALIN AND GABA IN NEURONS RESPONSIVE TO INTERLEUKIN-1ß IN THE RAT CENTRAL NUCLEUS OF THE AMYGDALA. Heidi E.W. Day*, Eileen J. Curran, Stanley J. Watson and Huda Akil. Mental Health Research Institute, University of Michigan, Ann Arbor, MI 48109.

One brain region which is particularly strongly activated following intraperitoneal (i.p.) administration of IL-1ß is the central nucleus of the amygdala (CeA) [1]. The neurochemical nature of these activated neurons was assessed by a double in situ hybridization (ISH) technique described previously [2].

Male Sprague Dawley rats (300-350g) were treated with IL-1ß (5μg/ml/kg i.p.) or 0.9% saline vehicle (n=5/group). After 30 minutes, animals were killed, the brains frozen and 10μm sections cut and processed for ISH for c-*fos* mRNA with one of the following markers: glutamic acid decarboxylase (GAD65/67 used concurrently as a marker for GABAergic neurons), preproenkephalin (ENK), neurotensin (NT) or corticotropin releasing hormone (CRH) mRNA.

In keeping with published results, relatively few cells within the CeA expressed c-*fos* mRNA following vehicle injection. In contrast, robust labeling for c-*fos* mRNA was observed in the CeA in IL-1ß treated animals. The distribution of c-*fos* mRNA was mainly confined to the lateral subdivision of this nucleus (CeL), with most intense expression observed in ventrolateral regions. Within the CeL, although colocalization with each marker showed regional variations, in general, ~85% c-*fos* positive cells were also positive for GAD 65/67 mRNA. In addition, ~75% cells expressing c-*fos* mRNA expressed ENK mRNA. Fewer cells contained both c-*fos* and NT mRNA (~25%). In contrast to that observed for the paraventricular nucleus of the hypothalamus, very few cells labeled for both c-*fos* and CRH mRNA.

Further studies to determine the projection pathway(s) of these cells may help us to predict the functional significance of activation of the CeA by IL-1ß.

1. Day, H.E.W. & Akil, H. (1996) Neuroendocrinology , **63**: 207-218
2. Curran, E.J. & Watson Jr., S.J. (1995) J. Comp. Neurol. **361**: 57-76

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43.5

RESPONSES OF THE SPLENIC SYMPATHETIC NERVE ACTIVITY TO CENTRAL ADMINISTRATION OF PROSTANOIDS AND CORTICOTROPIN-RELEASING FACTOR IN RATS. T. Katafuchi*, T. Ichijo, T. Ando, S. Take, and T. Hori. Dept. of Physiology, Kyushu Univ., Fac. of Med., Fukuoka 812 Japan.

It has been reported that thesuppression of cellular immunity induced by central administration of interleukin-1β (IL-1β) and interferon α is, at least partly, mediated by an enhancement of the splenic sympathetic nerve activity (SSNA) and that the immunosuppression or the increase in the nerve activity are blocked by pretreatment with corticotropin-releasing factor (CRF)-antagonist and cyclooxygenase inhibitors.

In the present study, we confirmed that an infusion of CRF (0.1- 5.0 μg/10μl/rat) into the third cerebral ventricle (ICV) increased the SSNA in a dose-dependent manner in urethane + α-chloralose anesthetized rats. The responses of the SSNA to CRF (1.0 μg) were completely blocked by ICV pretreatment with α-helical CRF_{9-41} (20 μg/20 μl). No changes in the arterial blood pressure was observed after CRF injection. An enhancement of the SSNA induced by CRF (1 μg) was not blocked by pretreatment with sodium salicylate (1 μg) at a dose of 1000 times more than that for suppressing the IL-1β-induced activation of the SSNA. An ICV injection of prostaglandin E_2 (PGE_2, 1 ng) also produced an increase in the SSNA through an activation of EP1 receptors. In contrast to CRF, the PGE_2- induced enhancement of the SSNA was completely blocked by pretreatment with α-helical CRF_{9-41}. These findings indicate that there is a sequential relationship between actions of CRF and PGE_2, with an activation of PGE_2 followed by that of CRF system. [Supported by Grants-in-Aid for Scientific Research 03670079 (TK) from Monbusho, Japan]

43.6

BRAIN PROSTAGLANDIN E2-INDUCED FEVER AND CHANGES IN NOCICEPTION IN RATS ARE MEDIATED BY DIFFERENT TYPES OF EP RECEPTORS. T. Hori*, T. Oka, M. Hosoi, K. Oka and S. Aou. Dept. Physiol, Fac. Med., Kyushu Univ., Fukuoka 812-82, Japan

To determine which EP receptors are involved in the PGE2-induced fever and changes in nociception, we observed the changes in colonic temperature (Tco), nociceptive behavior and the activities of nociceptive neurons in the trigeminal nucleus in the rat after intracerebral injection of PGE2 and its agonists, i.e., 17-phenyl-ω-trinor (17PT)-PGE2, butaprost and M&B28767 (EP1, EP2 and EP3 agonists, respectively). A microinjection of PGE2 (0.1-10 nmol) and 17PT-PGE2 (1-10 nmol) into the lateral cerebroventricle (LCV) produced a rapid and dose-related rise in Tco. Neither butaprost (0.1-100 nmol) nor M&B28767 (0.01-1 nmol) produced any significant changes in Tco. The PGE2-induced fever was blocked by the LCV pretreatment with SC19220 (EP1 antagonist). On the other hand, non-pyrogenic doses of PGE2 (10 fmol-10 pmol, LCV) and M&B28767 (1-100 fmol, LCV) enhanced nociception as assessed by a hot-plate test, but 17PT-PGE2 and butaprost had no effect on nociception. However, the LCV injection of PGE2 at 1 nmol and 17PT-PGE2 (50 nmol), but not the EP2 and EP3 agonists, induced a rapid and short-lasting hypoalgesia. Nociceptive responses of wide dynamic range neurons in the trigeminal nucleus in urethane-anesthetized rats were enhanced by M&B28767 (10 fmol, LCV) and attenuated by 17PT-PGE2 (10 nmol, LCV). The results indicate (1) that the PGE2-induced fever is mediated by EP1 receptors and (2) that PGE2 at low (non-pyrogenic) doses and high (pyrogenic) doses produces hyperalgesia and hypoalgesia by its actions on EP3 and EP1 receptors, respectively. [Supported by Grant No.06454153 (TH) from Monbusho, Japan]

43.7

PROLONGED EFFECTS ON GENE REGULATION IN THE PARAVENTRICULAR NUCLEUS (PVN) FOLLOWING A SINGLE INJECTION OF INTERLEUKIN-1ß

K. Van Koughnet, O. Smirnova, S. E. Hyman, D. Borsook*. Molecular and Developmental Neurobiology Laboratory, Mass. Gen. Hospital, Boston MA 02114

The proinflammatory cytokine interleukin 1-ß (IL-1ß) has a number of physiological effects on the nervous system, including the paraventricular nucleus (PVN) of the hypothalamus. We have produced transgenic mice expressing an ENK ß-Galactosidase fusion gene containing 3kb of 5' flanking sequences and 1kb of the 3' flanking sequences of the human proenkephalin gene. The transgene is correctly expressed and physiologically regulated in the PVN. Administration of Il-1ß either via i.p. (0.1μg - 5 μg in 0.2ml NS) or i.c.v. (2.5-10 ng) injections produces a significant increase in transgene expression in the PVN. Following a single i.p. injection of IL-1ß (5μg), there is inhibition of stress-induced transgene expression in the PVN and SON in mice receiving a 1.5M stressor 4 or 7 days later. This effect is also observed following a 1.5M saline stressor administered 7 days after mice received i.c.v. injections of IL-1ß (10ng). The long-lived suppression is not due to the initial transgene induction nor due to "tolerance" of the system since a 1.5M stressor administered on day 1 in a separate group of mice did not have any effect on induction by 1.5M saline stressor on day 7. In preliminary experiments, a single i.p. injection of morphine (10mg/kg) had a similar, albeit smaller, effect on transgene induction compared with IL-1ß. Induction of the transgene in the PVN correlated with c-Fos expression following i.p. injections. These results suggest that IL-1ß stimulation may induce prolonged alterations in the response of genes such as the proenkephalin gene to subsequent stressors.

43.8

PERIPHERAL ENDOTOXIN SIGNALS HYPOTHALAMIC CRH NEURONS VIA VAGAL AFFERENTS: EVIDENCE FROM VAGOTOMY AND PVN LESIONS. R.P.A. Gaykema*, G.B. Makara, I. Dijkstra and F.J.H. Tilders. Res. Inst. Neurosciences Vrije Universiteit, Dept. Pharmacology, 1081 BT Amsterdam, The Netherlands, and Inst. Exp. Med. Hung.Acad. Sci., Budapest, Hungary.

Systemic bacterial endotoxin (LPS) as a inflammatory stimulus induces brain-generated illness responses such as fever and activation of the HPA axis. Macrophage-derived IL-1ß is known to be involved, but signalling routes to the brain are not well understood. LPS and IL-1ß induce c-*fos* in defined brain areas including the n. tractus solitarius (NTS), and the n. paraventricularis (PVN). The question emerged whether vagal afferents are involved in the activation of the HPA axis by LPS. Therefore, we assessed whether 1) vagal transection abrogates activation of an ascending pathway via NTS to PVN and 2) the activated substrate as revealed by c-*fos* expression is not part of a descending route from PVN to medulla oblongata. Rats received subdiaphragmatic vagotomies (VGX), PVN lesions, or were sham-operated. Two hours after LPS (20 or 250 μg/kg i.p.) or saline, the rats were decapitated, brains and trunk blood were collected, ACTH and corticosterone levels in plasma, as well as Fos, CRH and TH immunoreactivity in brain sections were determined. VGX suppressed low dose ip LPS-induced Fos expression in NTS and in CRH neurons of the PVN, and suppressed ACTH responses. Responses following the high dose of LPS were much less affected by VGX, but also involved increased Fos expression in the area postrema. PVN lesions attenuated ACTH and cort responses to LPS, but did not influence Fos expression in the NTS. We conclude that low dose of ip LPS leads to activation of a sensitive pathway via vagal afferents that subsequently activate neurons in the NTS, which in turn stimulate neurosecretory CRH cells in the PVN. Additional pathways operating parallel to the vagal route, including the area postrema, may become important in the HPA responses to higher doses of LPS.

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43.9

LOCALIZATION OF IMMUNE AND INFLAMMATORY MARKERS IN THE RAT VAGUS NERVE AND LIVER HILUS. **L.E. Goehler*, R.P.A. Gaykema[1], F.J.H. Tilders[1], S.F. Maier, and L.R. Watkins.**

Dept. Psych, Univ. Colorado, Boulder CO 80309, and [1]Res. Instit. Neurosci., Dept. Pharmacology, Vrije Universiteit Amsterdam, The Netherlands.

Recent evidence implicates the vagus nerve in immune to brain communication. The signal transduction pathways by which immune stimuli may activate the vagus, however, have not been identified. Potential immune/inflammatory mediators include interleukin-1 (IL-1), prostaglandin E2, nitric oxide, and serotonin (5HT). The aims of the present experiment were 1) to identify immune elements, such as macrophages, dendritic cells and mast cells, associated with the abdominal vagus nerve and liver hilus, and 2) to localize potential signalling substances within these cells. To identify immune elements within the vagus nerves, associated paraganglia, and connective tissue, cryostat sections of ventral abdominal vagus nerve, including the hepatic branch and liver hilus, from i.p. endotoxin-treated rats were incubated with antisera raised against either complement receptor 3 (OX42), MHC class II antigen (OX6), or 5HT. In addition, sections were incubated with antisera raised against IL-1, cyclooxygenase 2 (COX2), and inducible nitric oxide synthase (iNOS). Dendritic cells (OX6 positive) extended processes along the nerves and within the paraganglia, whereas macrophages (OX42, IL-1, COX2, & iNOS positive) were found in abundance around the nerve, and within nerve-associated lymphoid tissue. In addition, COX2 antiserum labeled endothelium in whole nerve and liver hilus. Anti-5HT antisera labeled numerous mast cells throughout the tissue, as well as glomus cells in the paraganglia. These results indicate that cells producing inflammatory mediators are located in close association with vagal nerve fibers and paraganglia. As such they provide an anatomical basis for immune to brain communication via vagal afferents.

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43.10

RESPONSES TO INTRAVENEOUS CYTOKINES (IL-1β & TNF-α) ARE ATTENUATED BY SUBDIAPHRAGMATIC VAGOTOMY. **B. A. Schwartz*, M. Fleshner, M. McGorry, L. E., Goehler, D. Martin[1], S. F. Maier, & L. R. Watkins.** Dept Psychology, Univ Colorado at Boulder, Boulder, CO 80309 & [1]Amgen, Boulder, CO.

Sudiaphragmatic vagotomy blocks fever & corticosterone (CORT) responses produced by intraperitoneally (i.p.) administered IL-1β & TNF-α. There is some evidence that transection of the vagus does not block these responses if cytokines are administered intraveneously (i.v.). Thus the present studies tested whether sudiaphragmatic vagotomy will block either fever or CORT responses to i.v. IL-1β & TNF-α. Dose response studies were conducted to match the levels of fever produced by i.p. IL-1β, as well as to match the CORT response produced by i.p. TNF-α. In Exp 1, rats (8/grp) received either subdiaphragmatic vagotomy or sham surgery. Rats (4-6wks after surgery) were then implanted with thermisters i.p.. Three days after thermister implantation, rats were injected i.v. with either 0 or 500ng IL-1β. Core body temperatures (CBT) were taken prior to injection (BL) & then each 30 min for 6 hrs. Complete sudiaphragmatic vagal transections were verified using fluorogold. Subdiaphragmatic vagotomy **attenuated** the elevation in CBT produced by i.v. IL-1β . In Exp. 2, rats (6/grp) received either subdiaphragmatic vagotomy or sham surgery. Rats (4-6wks after surgery) were injected i.v. with either 0 or 75μg TNF-α. Blood samples were taken at 0, 1, 2 & 4 hrs after injection & serum CORT levels measured by RIA. Complete sudiaphragmatic vagal transections were verified using fluorogold. Subdiaphragmatic vagotomy **attenuated** the TNFα induced increase in CORT. Thus the vagus is involved in mediating peripheral (both i.p. & i.v.) cytokine communication to the brain. Supported by NIH MH55283, NIH MH45045, & UROP.

43.11

SUBDIAPHRAGMATIC VAGOTOMY SELECTIVELY BLOCKS TNFα EFFECTS ON CARRIER PROTEINS & SERUM CORTICOSTERONE. M. Fleshner*, L. Silbert, T. Deak, , L.E. Goehler, D. Martin[1],S.F. Maier, & L.R. Watkins. Dept Psych, U. Colorado, Boulder, CO 80309 & [1]Amgen, Boulder, CO.

We have previously reported that subdiaphragmatic vagotomy blocks corticosterone (CORT) elevation & hypothalamic NE depletion induced by peripheral IL-1β [Brain Res. Bull. 37 (1995): 605-610]. The present studies tested whether TNFα: 1) produces the same pattern of changes as that produced by IL-1β; 2) signals the HPA response via the vagus; & 3) signals liver protein synthesis changes via the vagus. In Exp. 1, rats (8/grp) received 0, 250 (x2) or 500 (x2) μg i.p. TNFα. Blood samples were taken at 0.5, 1, 2 & 4 hrs later & serum CORT levels measured by RIA. After the final blood sample, hypothalami were removed. Tissue NE levels were assayed using HPLC. Both doses of TNFα produced an elevation in serum CORT at 1, 2 & 4 hrs after injection similar to that reported with IL-1β. In contrast, TNFα did not produce hypothalamic NE depletion. Exp. 2 examined the role of the vagus in TNFα mediated effects. Rats (6/grp) received subdiaphragmatic vagotomy or sham surgery. Rats (4-6wks after surgery) were injected with either 0 or 250 (x2) μg i.p. TNFα. Blood samples were taken at 0, 1, 2 & 4 hr after injection & serum CORT measured. In addition, liver protein synthesis was assessed by measuring total serum protein & corticosterone binding globulin (CBG; the CORT carrier protein). Subdiaphragmatic vagotomy blocked the TNFα induced increase in CORT but did not block the TNFα induced decrease in serum protein & CBG. Thus the vagus plays a selective role in TNFα mediated communication to the brain. Supported by NIH MH55283, NIH MH45045, & UROP.

43.12

LEUKOCYTE TRAFFICKING INTO THE RAT CENTRAL NERVOUS SYSTEM AFTER VIRAL INFECTION OF VISUAL CIRCUITRY. S. Rassnick*, L. W. Enquist[1], A.F. Sved, and J. P. Card. Dept. of Neuroscience, University of Pittsburgh, Pittsburgh, PA 15260 and [1]Dept. of Molecular Biology, Princeton University, Princeton, NJ 08544.

The temporal characteristics of leukocyte infiltration into the rat CNS was studied using a well-characterized model of viral-induced neuropathogenesis. Rats received an intra-vitreal injection of a virulent neurotropic alpha herpesvirus (pseudorabies virus, PRV-Becker) to produce a productive infection of central visual circuitry that arises from transynaptic passage of PRV from retinal ganglion cells. A powerful aspect of this model is that temporally separate stages of infection emerge: the onset of productive viral replication is evident in the dorsal geniculate nuclei and tectum approximately one day prior to that in retino-recipient regions of the circadian timing system (e.g., suprachiasmatic nucleus). Immunocytochemical procedures were used to identify infected sites 72 hrs post-inoculation using antibodies to virion proteins. Non-neuronal cells that were localized around these sites of infection were detected with antibodies that recognize the leukocyte common antigen (i.e., mononuclear leukocytes and microglia), a cytoplasmic antigen on monocytes and macrophages (ED-1+), or the ß chain of CD8 antigen on cytotoxic T-lymphocytes. Viral infection and leukocyte recruitment first occurred in the dorsal geniculate and tectum, then in the suprachiasmatic nucleus. Localization of subclasses of cells with specific antibodies showed focal recruitment of reactive microglia, monocytes, and CD8ß+ lymphocytes to regions of viral infection. Quantitative analysis revealed that the magnitude of this recruitment correlated with the number of infected neurons and pathological changes in each retino-recipient area examined and that ED-1+ and CD8ß+ cells were most numerous in areas of advanced infection. Because the transport of PRV through the visual circuitry preceded leukocyte infiltration, these findings suggest that leukocyte trafficking is spatially and temporally-linked to the transynaptic passage of virus and the severity of infection in the nervous system [Supported by RO1 MH53574 (JPC) and RO1 NINDS33506 (LWE)].

CARDIOVASCULAR REGULATION: BRAINSTEM MECHANISMS

44.1

PERIAQUEDUCTAL GRAY MATTER PROJECTIONS TO AUTONOMIC PREGANGLIONIC NEURONS, E. Farkas, A. S. P. Jansen and A. D. Loewy*. Dept. of Anatomy and Neurobiology, Washington University, School of Medicine, St. Louis, MO 63110

Stimulation of the periaqueductal gray (PAG) produces a variety of autonomic and somatomotor responses. In order to analyze the anatomical substrate of the visceral responses, we have used pseudorabies virus (PRV), a transneuronal retrograde marker and *Phaseolus vulgaris* leucoagglutinin (PHA-L), an anterograde axonal tracer to determine the specific PAG projections to autonomic preganglionic nuclei. Two sympathetic systems (adrenal gland and stellate ganglion) and two parasympathetic systems (trachea and pancreas) were studied with the PRV tracing method. After inoculating these targets with PRV, the lateral, ventrolateral and dorsomedial PAG subnuclei contained virally infected neurons. In a second experiment, specific subregions of the PAG were injected with PHA-L. The PAG projects to the dorsal vagal nucleus, the nucleus ambiguus and the salivatory nuclei. We suggest that the PAG coordinates autonomic output via direct pathways to autonomic preganglionic nuclei, in addition to known projections to pontine and medullary premotor areas.

This research was supported by the National Institute of Heart, Lung, and Blood (HL-50527) and Juvenile Diabetes Foundation.

44.2

PERIAQUEDUCTAL GRAY NEURONAL RESPONSES TO HINDLIMB MUSCLE CONTRACTION IN THE CAT. J.M. Kramer*, M.D. Jarboe and T.G. Waldrop. Depts. of Molecular and Integrative Physiology and Kinesiology, College of Medicine and the Neuroscience Program. University of Illinois at Urbana-Chapaign, Urbana, IL. 61801.

The cardiovascular and respiratory responses to muscle contraction have been well defined; however, the brain substrates responsible for these responses have not been completely mapped. Therefore, the goal of this study was to examine the possible involvement of the periaqueductal gray region of the midbrain (PAG), an area known to play a role in blood pressure and respiratory control, in exercise regulation. Static contractions of hindlimb muscles were evoked by stimulation (40HZ, 3X MT) of the peripheral, cut ends of the L7 and S1 ventral roots in anesthetized cats. The Achilles tendon was isolated and attached to a force transducer for force measurement. Single unit activity of PAG neurons was recorded with tungsten microelectrodes; signal averaging was performed to characterize basal rhythms of the neurons relative to the cardiac cycle, cervical sympathetic nerve and phrenic nerve activities. Static muscle contraction altered the discharge rate of 60% of the PAG neurons studied. Most (78%) neurons responded to contraction with an increase in firing rate; 22% neurons were inhibited by contraction. Neurons in more caudal regions of the PAG appeared to respond with a greater increase in firing rate than neurons in rostral regions. The basal discharge of most neurons stimulated by muscle contraction was related to cardiac, sympathetic or respiratory rhythms with cardiac rhythms being most prevalent. Thus, these findings indicate that PAG neurons receive input from skeletal muscle receptors responsive to muscular contraction. Since the basal discharge of these contraction-sensitive neurons have cardiovascular/respiratory related rhythms, the PAG may play a role in eliciting cardiorespiratory adjustments observed during exercise. (Supported by NIH 06296 and American Heart Association- Illinois Affiliate).

44.3

LOCUS COERULEUS ACTIVITY AND SYMPATHETIC NERVE DISCHARGE DURING CLONIDINE WITHDRAWAL. M. Fisher*, R.L. Stornetta and P. G. Guyenet. Pharmacology Dept., Univ. of Virginia, Charlottesville, VA 22908.

The present experiments sought to determine the discharge pattern of locus coeruleus (LC) neurons and a sympathetic nerve (SND, splanchnic) during antagonist precipitated clonidine (CLO) withdrawal. Rats were treated chronically with CLO via osmotic minipumps (200 μg/kg/day, s.c.) or received sham operation. After 7-13 days CLO-treted rats or sham rats were anesthetized with halothane, artificially respirated, and instrumented for i.v. drug administration, arterial pressure (AP), SND measurement and LC unit recording. After baseline measurements were obtained the α_2-adrenergic receptor antagonist atipamezole was administered (1.5 mg/kg, i.v.). Chronic treatment with CLO did not alter resting mean AP and heart rate (CLO: 103±5mmHg, 359±14bpm; sham: 106±4mmHg, 338±12bpm). In addition baseline LC unit firing was unaltered by chronic CLO treatment (CLO: 2.0±0.1 spikes/s, n=82: shams:1.6±0.2, n=54). CLO withdrawal with atipamezole produced tachycardia (+86±9bpm vs +3±8bpm for shams, p<.001) but no change of mean AP (p<0.46). CLO withdrawal increased SND (163±14%, p<0.001) and produced abrupt swings between 2 levels of activity (SND upswings of 30-120 s duration ocurring every 1-4min). Administration of the α_2 antagonist to sham rats produced little effect on LC firing (1.7±0.1 spikes/ s after drug, n=51). In rats treated chronically with CLO atipemazole silenced LC units for several min(n=7). Then units fired selectively during the SND upswings (n=32). In conclusion, antagonist precipitated CLO withdrawal in halothane-anesthetized rats exhibits several features reported previously in awake spontaneously CLO withdrawing rats including tachycardia but little change in mean AP. Under anesthesia the syndrome included increased SND with periodic upswings synchronized with episodes of LC activity. These abrupt fluctuations may underlie the characteristic AP upswings present in awake rats that undergo spontaneous CLO withdrawal. Supported by NIH RO1 HL28785.

44.4

Somatostatin Activates An Apamin-Sensitive Potassium Conductance in Parabrachial Neurons *In Vitro*. Samuel B. Kombian[1]*,Tarek M. Saleh[1], Jeffrey A. Zidichouski[1&2], and Quentin J. Pittman[1]. [1]Neuroscience Research Group, University of Calgary and [2]Ciba-Geigy Canada Ltd., Calgary, Alberta, Canada T2N 4N1

The peptide somatostatin 1-28 (SOM) has previously been shown to modulate the flow of visceral afferent information through the parabrachial nucleus (PBN) to the cortex in the rat *in vivo*. However, the cellular mechanism by which SOM produces this effect is not known. We therefore examined the effects of SOM on PBN cells and on synaptic transmission in this nucleus using a pontine slice preparation and the nystatin perforated-patch recording technique. Bath application of SOM over a wide concentration range, affected neither the CNQX-sensitive EPSC evoked by perinuclear stimulation nor the frequency of TTX resistant mEPSCs. However, SOM (10 μM) attenuated the inward current induced by a brief bath application of AMPA. While in many neurons, SOM activates an M-current, we did not find evidence for the presence of this current in PBN cells. SOM reversibly induced an apamin-sensitive (charybotoxin-insensitive), calcium-activated potassium conductance leading to an outward current in these cells. It also blocked an outwardly rectifying conductance activated above -50 mV. These effects were mimicked by the SOM fragment 1-14 but not by the fragment 1-12. Furthermore, SOM did not affect the charybdotoxin-sensitive AHP recorded in these cells. When slices were incubated overnight in pertussis toxin, the SOM-induced effects were abolished, suggesting that a pertussis toxin-sensitive G-protein couples the SOM receptor to its effectors in PBN cells. These results show that SOM modulates a potassium conductance in PBN cells. This postsynaptic effect of SOM may decrease the excitability of PBN cells and modulate their response to other inputs. (This work is supported by MRC, Alberta Heart and Stroke and AHFMR).

44.5

Peptidergic modulation of synaptic transmission in the parabrachial nucleus *in vitro*: Importance of degradative enzymes in regulating synaptic efficacy.

TM. Saleh[1]*, SB. Kombian[1], JA. Zidichouski[1&2] and QJ. Pittman[1]

[1]NRG, University of Calgary and [2]Ciba-Geigy Canada Ltd.,Calgary, Alberta, Canada

This study examined the effects of substance P (SP) and calcitonin gene-related peptide (CGRP) on synaptic transmission in a pontine slice preparation containing the parabrachial nucleus (PBN). Stimulation of the ventral, external lateral portion of the PBN elicited glutamate-mediated, excitatory postsynaptic currents (EPSC) in cells recorded using the nystatin perforated-patch recording technique in the external lateral, external medial and central lateral subnuclei of the PBN. Bath application of SP or CGRP dose-dependently and reversibly attenuated the evoked EPSC. The attenuation of the EPSC induced by both of these peptides was not accompanied by changes in input resistance of PBN cells over a wide voltage range, nor did these peptides alter the inward current induced by a brief bath application of AMPA. Both peptides did cause a decrease in the frequency on spontaneous, miniature- EPSCs. The combined application of subthreshold concentrations of these two peptides revealed a synergistic interaction in reducing the evoked EPSC. The substance P neurokinin-1 (NK-1) receptor antagonist, CGP49823, completely and reversibly blocked **both** the SP- and CGRP-induced attenuation of the EPSC. However, the rat-CGRP receptor antagonist, human-CGRP ($HCGRP_{8-37}$) did not block the actions of CGRP or SP on the EPSC. Using a metabolically stable analogue of SP, SP (5-11), or an endopeptidase inhibitor, phosphoramidon, we were able to demonstrate that CGRP enhances the SP effect by inhibiting a SP endopeptidase. These results suggest that SP (and CGRP indirectly through an inhibition of the SP endopeptidase) acts on presynaptic, NK1-receptors to cause an inhibition of excitatory transmission in the PBN. These results indicate an important role of endopeptidases in regulating synaptic modulation by peptides. (This work is funded by the MRC and Alberta Heart and Stroke and AHFMR).

44.6

Cholecystokinin Inhibits and Neurotensin Enhances Excitatory Synaptic Transmission in the Parabrachial Nucleus *In Vitro*.

JA. Zidichouski*[1&2], TM. Saleh[1], SB. Kombian[1], and QJ. Pittman[1]. [1]Neuroscience Research Group, University of Calgary and [2]Ciba-Geigy Canada Ltd., Calgary, Alberta, Canada T2N 4N1

Cholecystokinin (CCK) and neurotensin (NT) are present in fibres innervating the parabrachial nucleus (PBN) and have previously been shown to modulate the flow of visceral afferent information through the PBN to the cortex in the rat. This study examined the effects of CCK and NT on synaptic transmission in the PBN using a pontine slice preparation and the nystatin perforated-patch recording technique. Stimulation of the ventral, external lateral portion of the PBN elicited glutamate-mediated, excitatory postsynaptic currents (EPSC) in cells recorded in the PBN. Bath application of NT dose-dependently and reversibly enhanced, while CCK attenuated, the evoked EPSC. In addition, the frequency of spontaneous, miniature-EPSCs recorded in PBN cells was significantly increased by NT and decreased by CCK application. Paired-pulse depression was also enhanced and decreased by NT and CCK, respectively. These synaptic changes induced by NT and CCK were not accompanied by changes in input resistance of PBN cells over a wide voltage range (although NT reduced an outwardly rectifying conductance at potentials positive to -20 mV), nor did these peptides alter the inward current induced by a brief bath application of the glutamate agonist, AMPA. The NT antagonist, SR48692 (100 μM), completely and reversibly blocked the NT-induced enhancement of the EPSC. The non-selective CCK receptor antagonist, proglumide (100 μM), completely and reversibly blocked the CCK-induced attenuation of the EPSC. In addition, the selective CCK-A receptor antagonist, L-364,718 (10 μM) , but not the selective CCK-B receptor antagonist, L-365,260 (100 μM), blocked the effect of CCK on synaptic transmission. These results suggest that NT and CCK act at presynaptic NT and CCK-A receptors respectively to modulate excitatory synaptic transmission through this nucleus. (This work is supported by MRC, Alberta Heart and Stroke and AHFMR).

44.7

MODULATION OF ARTERIAL CHEMORECEPTOR INPUTS IN THE RAT NTS BY ACTIVATION OF THE LATERAL PARABRACHIAL NUCLEUS (LPBN).

L.F Hayward*, and R.B. Felder. Dept. of Internal Med., University of Iowa College of Medicine, Iowa City, IA 52242

There is increasing evidence that the LPBN may be an important modulator of cardiorespiratory reflexes, including the arterial baroreflex and the arterial chemoreflex. Previous studies have demonstrated that descending projections from the LPBN to the nucleus tractus solitarius (NTS) are involved in LPBN attenuation of baroreflex function. The present study was undertaken to investigate whether interconnections between the LPBN and the NTS may also be involved in LPBN modulation of arterial chemoreflex function. Extracellular recordings were made from urethan anesthetized male Sprague Dawley rats (300-350 gm). Arterial chemoreceptors were stimulated by intracarotid injection of CO_2 saturated bicarbonate. LPBN neurons were activated with a monopolar stimulating electrode placed in the ipsilateral LPBN. Ten of 21 NTS neurons were responsive to arterial chemoreceptor stimulation, including 5 which were chemoreceptor excited (mean increase in discharge rate 40±17%; mean±SEM) and 5 which were chemoreceptor inhibited (mean decrease in discharge rate -47±10%). Electrical stimulation of the LPBN inhibited the spontaneous discharge of 6/10 chemoreceptor responsive NTS neurons (mean decrease in baseline activity 65±9%), including 3 chemoreceptor inhibited and 3 chemoreceptor excited neurons. These preliminary results demonstrate that activation of the LPBN may modulate central processing of chemoreceptor inputs at the level of the NTS, supporting previous work which implicates the LPBN in the central modulation of the arterial chemoreflex. Supported by AHA (LFH) and HL14388 (RBF).

44.8

SUBSTANCE P MAY EXCITE BARORECEPTIVE UNITS OF THE LATERAL PARABRACHIAL COMPLEX.

J.A. Lovell and S.L. Stuesse*. Kent State University, Tuscarawas Campus, New Philadelphia, Ohio 44663 and Neurobiology Dept., NEOUCOM, Rootstown, Ohio 44272.

Cardiovascular information ascends from the nucleus tractus solitarius of the medulla to the Lateral Parabrachial Complex (LPB). Substance P-containing fibers and cells are found throughout the rostrocaudal extent of the LPB. Although commonly associated with transmitting painful stimuli, substance P is found in brain regions not associated with processing information about pain. To explore whether substance P might play a role in the processing of baroreceptive information within the LPB, we identified areas of the LPB containing neurons that displayed blood pressure-dependent changes in activity and determined if exogenously applied substance P affected the activity of these neurons.

Substance P and norepinephrine were pressure ejected into the lateral parabrachial complex in areas that contained units responsive to acute changes in systemic blood pressure. The effects of these drugs on multiple unit activity was ascertained. Substance P (10 ng/μl and 100 ng/μl) increased multiple unit activity of blood pressure-sensitive units of the lateral parabrachial complex, while a lower dose of substance P (1 ng/μl) had no effect. Neither norepinephrine (1μg/μl) nor phosphate buffer changed the activity of blood pressure-sensitive neurons. Thus, substance P may play a role in processing of baroreceptive information in the lateral parabrachial complex.

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44.9

CHOLINERGIC BLOCKADE ELIMINATES THE HEART RATE PAUSES OF TONIC REM SLEEP.

R.T. Lau, U.T. Wallooppillai, B.D. Nearing, J. Quattrochi*, J.A. Hobson, and R.L. Verrier. Laboratory of Neurophysiology, Program in Neuroscience, Harvard Medical School; Institute for Prevention of Cardiovascular Disease, Deaconess Hospital, Boston, MA 02115 and Harvard College, Cambridge, MA 02138.

Abrupt changes in cardiac rhythm reflect the high degree of autonomic variability associated with REM sleep but the precise CNS and peripheral autonomic mechanisms mediating these changes are unclear. In order to investigate state-dependent changes in heart rate, respiration, and the autonomic mechanisms responsible for these events during REM sleep, cats were chronically implanted under surgical anesthesia with EEG, EMG, EOG, LGB, hippocampal CA1, diaphragmatic, and ECG electrodes.

Following control recordings, 0.3 mg/kg of the β_1 adrenergic blocker atenolol and 0.1 mg/kg of the muscarinic blocker glycopyrrolate (neuroactive compounds with polar hydrophilic properties which do not cross the blood-brain barrier) were administered intravenously in freely moving, unanesthetized animals, both alone and in combination. R-R intervals were computer analyzed and correlated with pontine, cortical, hippocampal, and diaphragmatic activity.

Normal REM sleep was marked by distinct pauses in heart rate defined as decelerations in rate of ≥ 20% lasting 1.2 to 5 sec. Pauses occurred principally during tonic and low-level phasic REM sleep and were shown to be respiratory-independent as measured by diaphragmatic activity. Parasympathetic blockade with glycopyrrolate selectively eliminated pauses with or without concomitant autonomic blockade with atenolol.

These results suggest that cholinergic mechanisms play a major role in heart rate variability during REM sleep. Specifically, we conclude that: 1) tonic activity during REM is associated with pauses in heart rate; and 2) pauses are the result of vagal cholinergic influence as opposed to sympathetic withdrawal.

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44.10

SYMPATHETIC BLOCKADE ELIMINATES THE HEART RATE SURGES OF PHASIC REM SLEEP.

J. Quattrochi, U.T. Wallooppillai, R.T. Lau, B.D. Nearing, J.A. Hobson*, and R.L. Verrier. Laboratory of Neurophysiology, Program in Neuroscience, Harvard Medical School; Institute for Prevention of Cardiovascular Disease, Deaconess Hospital, Boston, MA 02115 and Harvard College, Cambridge, MA 02138.

REM sleep is characterized by intense phasic central activation and surges in heart rate coupled with elevations in coronary blood flow reflecting marked variability in autonomic regulation. The neural mechanisms underlying these phasic alterations in CNS state and cardiac rhythm remain to be defined. In order to investigate state-dependent changes in heart rate, respiration, and the autonomic mechanisms responsible for these events during REM sleep, cats were chronically implanted under surgical anesthesia with EEG, EMG, EOG, LGB, hippocampal CA1, diaphragmatic, and ECG electrodes.

Normal REM sleep was marked by episodes of surges in heart rate lasting >3.5 sec with a mean R-R interval <300 msec. Surges were closely associated with phasic PGO activity and theta rhythm, but independent of respiratory diaphragmatic activity. Following a control series of recordings, atenolol (β_1 adrenergic blocker, 0.6 mg/kg i.v.) and glycopyrrolate (muscarinic blocker, 0.1 mg/kg i.v.) were administered in freely moving, unanesthetized animals, both alone and in combination. R-R intervals were computer analyzed and correlated with pontine, cortical, hippocampal, and diaphragmatic activity. Sympathetic blockade with atenolol selectively eliminated surges.

These results suggest that an ascending degree of cholinergic phasic activity in the pontine tegmentum and hippocampal theta during REM sleep promotes an increase in sympathetic innervation of the heart leading to surges in heart rate.

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44.11

DO LATERAL PARABRACHIAL NEURONES PROJECT TO THE INTERMEDIOLATERAL CELL COLUMN IN THE THORACIC SPINAL CORD?

John H. Cootet, Kamal Motaweit¶, Susan Pynert, Richard Ransont and Maher Kamel¶.†Department of Physiology, University of Birmingham, Birmingham B15 2TT UK and ¶Department of Anatomy, University of Minia, Minia, Egypt. SPON: Brain Research Association

The lateral subnuclei of the parabrachial complex [LPBN] located in the dorsolateral pons are considered to play an important role in modulating cardiorespiratory activity. Several studies have shown this region has the appropriate connections both to and from the medulla and higher brain regions, to carry out this role, but so far no direct projections to sympathetic nuclei in the spinal cord have been described. In an anterograde tracing study using biotin dextran amine injected into a highly discrete site in the external LPBN we have traced axons to the thoracic cord where terminal branches entered the intermediolateral cell column [IML] and central autonomic area. However, these branches did not make synaptic contact with sympatho-adrenal preganglionic neurones which had been pre-labelled with the retrograde tracer cholera B toxin HRP. Furthermore the density of terminals was greater in the upper thoracic segments T_1-T_6 than in the the lower thoracic segments. Therefore it is possible that the LPBN neurones project to the thoracic cord and their axon terminals synapse with interneurones in or close to IML or preferentially synapse with non adrenal medullary sympathetic neurones.

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44.12

PRESSOR RESPONSES ELICITED BY NATURAL STIMULATION OF VESTIBULAR RECEPTORS IN THE CAT. B.J. YATES*, S.F. WOODRING and C.D. ROSSITER. Depts. of Otolaryngology and Neuroscience, Univ. of Pittsburgh, Pittsburgh, PA 15213.

Considerable evidence suggests that the vestibular system has influences on the sympathetic nervous system and participates in compensating for posturally-related hypotension. Increases in sympathetic outflow are produced by nose-up vestibular stimulation in the cat to *presumably* offset a drop in blood pressure that could accompany movements that place the animal's long longitudinal axis against the force of gravity, such as vertical climbing. The purpose of the present study was to determine the characteristics of the pressor response elicited by natural vestibular stimulation.

Experiments were conducted on decerebrate, paralyzed, and artificially-ventilated cats. Selective vestibular stimulation was produced by trapezoidal movements of the head (with the body fixed) in animals whose C_1-C_3 dorsal roots and IXth and Xth cranial nerves were transected. Nose-up trapezoidal rotations (50° amplitude, 2 sec rise time) routinely produced pressor responses in animals with systemic blood pressure > 100 mm Hg. The median amplitude of the response was ~20 mm Hg, and the latency from stimulus peak was ~1.4 sec. The responses disappeared when blood pressure dropped below 100 mm Hg, drugs that produced peripheral vasoconstriction were injected, or the VIIIth cranial nerves were cut. No changes in blood pressure were elicited by ear-down rotations.

These data demonstrate that vestibular receptors activated by nose-up tilt can elicit a large increase in blood pressure that is appropriate to offset orthostatic hypotension.

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44.13

ROSTRAL PARA-AMBIGUUAL FIELD NEURONS HAVE ACTIVITY CORRELATED TO THE 8-13 HZ RHYTHM IN SYMPATHETIC NERVE DISCHARGE IN CAT. C. W. Dempesy*, D. E. Richardson, C. J. Fontana, and J.-H. Song. Lab. of Neurosurgery, Tulane University School of Medicine, New Orleans, LA 70112.

The 8-13 Hz rhythm of the sympathetic nerve discharge (SND) has previously been reported (J. Neurophysiol. 72:106-120, 1994) to correlate to unit activity in the rostral caudal ventrolateral medulla (0-2.5 mm rostral to the obex, 4-4.25 mm lateral, and within 2 mm of the ventral surface) in cat, but not to any region more anterior. We have now shown that such correlation can be obtained for neurons in the rostral para-ambiguual field, extending 3.8 mm rostral to the obex and over a wider range of laterality (3.5 to 4.2 mm), and at similar depth. Twenty-one of 78 examined cells in baroreceptor-denervated, urethan-anesthetized cat correlated with a 9.3-12.2 Hz peak in the SND, while firing at an average rate of 2.8 ± 0.6 spikes/sec. The power spectra of all neurons displayed a sizable peak corresponding to the correlating frequency. Several neurons had power spectra containing additional peaks that were comparable in size to the peak of the correlating frequency, but were not cardiac related or relevant to any known phenomena. Activation of the baroreflex (by aortic obstruction) in these baroreceptor-denervated animals regularly shifted the 8-13 Hz rhythm of the SND to the cardiac frequency, the higher frequency returning promptly when the blood pressure returned to normal. (Funding provided by the Tulane Department of Neurosurgery.)

44.14

EFFECTS OF CO_2 DRIVE ON 10-HZ COHERENT RHYTHMS IN MULTIPLE SYMPATHETIC DISCHARGES AND ON HFOS IN PHRENIC DISCHARGE. Q.P. Yu*, M.I. Cohen and W-X. Huang. Dept. of Physiol., Albert Einstein Col. Med., Bronx, NY 10461.

In 6 midcollicular decerebrate, vagotomized, baro-denervated, gallamine-paralyzed cats, recordings were taken from both phrenic nerves and from 3-4 sympathetic (symp.) nerves (cervical, splanchnic, inferior cardiac) at different levels of end-tidal CO_2. Spectral coherences between both phrenic activities and between pairs of symp. activities were computed. During hypercapnia and normocapnia, all symp.-symp. coherences had distinct peaks around 10 Hz. In 5 cats, phrenic apnea (disappearance of respiratory rhythm) was produced at sufficiently low CO_2 (2-3%). As CO_2 level was lowered, the spectral frequency of the HFO peaks in phrenic discharge was reduced. However, during phrenic apnea distinct 10-Hz peaks were still present in symp.-symp. coherences, although they were reduced in both amplitude and frequency. The degree of reduction was variable between cats and also between signal pairs in the same cat. These results indicate that, although symp. rhythms are reduced with reduction of CO_2 drive, they are influenced less strongly than respiratory activity. (Supported by N.I.H. Grant HL-27300.)

44.15

DIFFERENTIAL RELATIONSHIPS AMONG RESPIRATORY-RELATED RHYTHMIC DISCHARGES OF SYMPATHETIC NERVES WITH DIFFERENT TARGETS. S.-Y. Zhou*, G.L. Gebber, S. Zhong and S.M. Barman. Dept. Pharmacol./Toxicol., Michigan State Univ., E. Lansing, MI 48824

Partial coherence analysis was used to study the relationships among the respiratory-related rhythmic discharges of the left and right inferior cardiac nerves (CNs), left renal nerve (RN) and splenic nerve (SN) in vagotomized, baroreceptor-denervated cats anesthetized with pentobarbital. Whereas the ordinary coherence values (near 1.0) relating the respiratory-related discharges of pairs of these nerves were statistically indistinguishable, mathematical removal of the portion of the linear relationship between two signals common to a third (i.e., partialization) revealed that the relationships were differential. For example, the coherence value relating the respiratory-related discharges of the left CN and SN was reduced to a significantly greater extent by partialization using right CN activity than RN activity. Thus, the rhythmic discharges of the left CN and SN had more in common with right CN activity than RN activity. This might be explained by 1) episodes of selective gating of respiratory-related activity to the RN, 2) differential influences of the respiratory oscillator on circuits controlling the four sympathetic nerves, 3) differential crosstalk among circuits receiving inputs from a common rhythm generator or 4) nonuniform coupling of multiple rhythm generators, each controlling a different sympathetic nerve. The existence of multiple sympathetic rhythm generators is supported by instances when the rhythm in phrenic nerve activity became locked 1:2 to that in one sympathetic nerve and 1:1 to that in another. These observations suggest that the respiratory-related rhythmic discharges of sympathetic nerves with different targets are derived from separate although normally coupled oscillators which are themselves distinct from the respiratory oscillator. (Supported by NIH Grant HL13187).

44.16

COUPLED OSCILLATORS ACCOUNT FOR THE SLOW RHYTHMS IN SYMPATHETIC NERVE DISCHARGE (SND) AND PHRENIC NERVE ACTIVITY (PNA). S. Zhong*, G.L. Gebber, S.-Y. Zhou, and S.M. Barman. Dept. Pharmacol/Toxicol., Michigan State Univ., E. Lansing, MI 48824.

Phase-locked slow rhythms in SND and PNA at the frequency of breathing in vagotomized animals are believed to arise from a common brain stem oscillator. The results obtained in vagotomized, baroreceptor-denervated cats anesthetized with phentobarbital do not support this view. First, whereas the coherence values relating PNA to either inferior cardiac or renal SND were close to 1.0 at the frequency of the respiratory cycle, the discharges of the two sympathetic nerves often remained significantly correlated at this frequency after partialization by using PNA. Partialization is equated to mathematical removal of the portion of the linear relationship between inferior cardiac and renal SND common to PNA. Thus, the residual coherence suggests that the slow rhythm in SND is dependent upon central mechanisms in addition to those responsible for rhythmic PNA. Second, during hypercapnia, the strong 1:1 relation between the slow rhythms in SND and PNA was disrupted. In such cases, the rhythms in SND and PNA became coupled in a 2:1 relation. Third, during hypocapnia, the slow rhythm often was maintained in SND when the phrenic nerve became quiescent. In other cases, the rhythms in SND and PNA became uncoupled (zero coherence). These observations suggest that the slow rhythms in SND and PNA arise from separate although normally coupled oscillators rather than from a common brain stem cardiorespiratory generator. (Supported by NIH grant HL13187).

44.17

INTERRELATIONS OF LUNG INFLATION AFFERENT ACTIVITY (LIA), PHRENIC NERVE ACTIVITY (PNA) AND SYMPATHETIC NERVE DISCHARGE (SND). G.L. Gebber*, S. Zhong, S.-Y. Zhou and S.M. Barman. Dept. Pharmacol./Toxicol., Michigan State Univ., E. Lansing, MI 48824.

Partial coherence analysis was used to study the relationships among intratracheal pressure (ITP; index of LIA), PNA and inferior cardiac SND in normocapnic, artificially respired cats anesthetized with urethane or pentobarbital. This method mathematically removes the portion of the relationship between two signals common to a third signal. The three signals were related 1:1 at the frequency of artificial respiration, and the ordinary coherence values relating any two signals approached unity. Under urethane anesthesia, partialization of the ITP → SND relationship by using PNA reduced the coherence value from 0.87 ± 0.02 to 0.35 ± 0.03. Since the partial coherence value was significantly different from zero, these data suggest that 1:1 locking of ITP and SND is dependent, in part, on a pathway from the lungs that bypasses the respiratory oscillator. The ITP → SND coherence value was reduced from 0.87 ± 0.01 to 0.15 ± 0.01 after partialization by using PNA under pentobarbital anesthesia. Thus, the "direct" pathway from the lungs to sympathetic networks was less important in pentobarbital-anesthetized cats. As expected, vagotomy eliminated the coherence of ITP to SND as well as PNA. Partilization of the PNA → SND relationship by using ITP (vagus intact) reduced the coherence value from 0.85 ± 0.02 to 0.23 ± 0.04 (urethane) and 0.86 ± 0.01 to 0.13 ± 0.02 (pentobarbital). These low partial coherence values suggest that either direct coupling of central respiratory and sympathetic networks was weak or ITP and PNA were almost mathematically equivalent. (Supported by NIH Grant HL13187).

44.18

THE DISCHARGE PATTERN IN RENAL SYMPATEHTIC NERVE ACTIVITY RELATED TO FLUCTUATION OF BEAT TO BEAT BLOOD PRESSURE IN THE CONSCIOUS RAT. T. Kunitake, A. Shimokawa, Y. Ishizuka, K. Kato, M. Saita, Y. Ishida*, T. Hanamori, H. Kannan Department of Physiology, Miyazaki Medical College, Miyazaki, 889-16, JAPAN

The periodicity in the sympathetic nerve activity has been discussed in many reports. We also have examined by using the spectral analysis the periodicity in renal sympathetic nerve activity (RSNA) in the conscious rat. The cardiac-related component had larger power than others. Therefore we have studied the temporal relationship between the cardiac-related activity in RSNA and the R wave of ECG in more detail. After detecting the peak in integrated RSNA by using the differential method, the peak to peak interval was measured. The time lag of each peak from the preceding R wave of electrocardiogram was also measured. The scatter diagram of peak to peak interval against time after R wave was made. On this diagram, four discrete areas that included almost all points were demonstrated. The result suggested that the discharge pattern (DP) could be subdivided into four classes depending on the number of bursting discharge during one cardiac cycle. (Kunitake, et al., Soc. Neurosci. Abstr., 21: 1399, 1995) In the present study, we examined the relationship between the DP in RSNA and the spontaneous fluctuations of blood pressure and its derivative (dP/dt). The cross correlation analysis indicated that beat to beat blood pressure (BBP) and dP/dt prior to two or three cardiac cycle have close relationship with DP. The differences of BBP or dP/dt among four classes of DP at second or third previous beat were larger than those at current beat, i.e., BBP and dP/dt in DP which showed two bursting discharges were significantly lower than those in other DPs which had no or one bursting discharge. Therefore, the DP could be simulated by the multiple regression with BBP, dP/dt and those previous values. The result suggests the RSNA at rest is regulated as the "beat to beat baroreflex"

44.19

ADRENAL NERVE RESPONSES TO ELECTRICAL VESTIBULAR STIMULATION. I.A. Kerman* and B.J. Yates. Depts. of Otolaryngology and Neuroscience, Univ. of Pittsburgh, Pittsburgh, PA 15213.

There is abundant evidence to suggest existence of vestibulo-sympathetic reflexes in the cat. These reflexes appear to counteract effects of postural changes on blood pressure to prevent orthostatic hypotension. Responses to natural and electrical vestibular stimuli have been observed in the abdominal splanchnic nerve (SN). Since SN carries some of the sympathetic preganglionic fibers innervating the adrenal gland, it is possible that adrenal function is influenced by the vestibular system. To investigate this hypothesis we recorded adrenal nerve (AN) responses to electrical stimulation of vestibular afferents.

Experiments were conducted in 4 decerebrate cats. A pair of stimulating ball electrodes was implanted in the labyrinth near vestibular afferents bilaterally. In 3 of the animals cerebellectomy was performed prior to start of recording. In the other animal recordings were performed with cerebellum first intact and then removed. ANs selected for recording were either terminal branches of SN or adrenal branches of surrounding ganglia. AN responses to stimulation of the vestibular nerve (1-5 shocks, 0.2 ms width, interpulse interval 3 ms, duty cycle 1 sec) were recorded using bipolar hook electrodes. In two animals bilateral AN dissection was performed, in the other animals only contralateral adrenal fibers were dissected. Modulating effects of electrical stimulation were observed in all animals. In three of the animals responses consisted of excitation, and in one animal the response was a combination of excitation and inhibition. Average response threshold (5-shock trains) was 214±28 (SEM) mA with the average latency from the effective shock of 28±4 (SEM) msec. Average duration of the response in all of the animals was 54±5 (SEM) msec. In three of the animals vestibular lesions were performed: bilateral VIIIth nerve resection, aspiration of vestibular nuclei, and kainate injection into vestibular nuclei, respectively. Each of these lesions abolished previously-recorded responses.

The present data suggest that vestibular inputs may influence adrenal medullary function and contribute to vestibulo-sympathetic reflexes. Supported by NIH grant DC00693.

44.20

ACETYLCHOLINE EXCITES VAGAL CARDIAC NEURONS IN THE NUCLEUS AMBIGUUS BY BOTH POST-SYNAPTIC AND PRE-SYNAPTIC NICOTINIC MECHANISMS. D. Mendelowitz*, R.A. Neff and M. Mihalevich. Dept. Physiology and Biophysics, Univ. Tenn., Memphis, TN 38163.

Neurons in the Nucleus Ambiguus (NA) are known to receive dense cholinergic innervation. However the population of neurons in the NA that receive these synapses and the evoked responses are unknown. In this study we examined the direct post-synaptic responses, as well as changes in spontaneous synaptic events, that occur in identified vagal cardiac neurons in the NA in response to different cholinergic agonists.

The soma of these neurons were visualized in an in-vitro 250μ thick brainstem slice and identified by the presence of a fluorescent tracer that had been previously applied to their peripheral terminals surrounding the heart. DIC optics, infrared illumination and detection cameras were used to guide and position the patch pipette onto the surface of the identified neuron. Whole cell perforated patch clamp recordings were obtained by including nystatin in the patch pipette solution.

Both acetylcholine (Ach) and nicotine, but not bethanechol, evoked post-synaptic inward currents in identified vagal cardiac neurons (control -186±42.2 pA, bethanechol -191±42 pA, nicotine -381±75.4 pA, recovery -164±42 pA). The Ach and nicotinic responses reversed close to 0 mV and were accompanied by a decrease in membrane resistance. Ach and nicotine also elicited an increase in spontaneous glutamatergic synaptic events, presumably via a presynaptic mechanism. Surprisingly, the membrane resistance recovered to greater levels than during control. These results suggest that nicotine excites vagal cardiac neurons in the NA by activating post-synaptic receptors, and in addition, increases the spontaneous activity of glutamatergic synapses that innervate these vagal cardiac neurons.

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UROGENITAL REGULATION: BLADDER

45.1

SENSORY ASPECTS OF MICTURITION CONTROL IN THE HUMAN: A POSITRON EMISSION TOMOGRAPHY (PET) STUDY.
Bertil F. M. Blok* and Gert Holstege. Department of Anatomy and Embryology, University of Groningen, The Netherlands .

Previously, we have shown using the PET-scanning method that certain cortical and brainstem areas of the human brain are specifically active during micturition and urine storage. The micturition related areas in the human brainstem are located in the same regions as those in the cat. The next step was to define the brain areas involved in bladder filling.

Cerebral blood flow was monitored in 18 right-handed male volunteers (22-51 years) using a CTI ECAT 951/31 whole body positron emission tomograph. Changes in regional cerebral blood flow (rCBF) were measured using the intravenous radioactively labeled water ($H_2{}^{15}O$) bolus technique. An injection of 1.85 MBq $H_2{}^{15}O$ was given for each run. Scanning was made during 4 successive conditions: 1) with a filled bladder, 2) during micturition, 3) and 4) with an empty bladder.

During the condition with a filled bladder (scan 1) in comparison to the other conditions (scan 2, 3 and 4), a significant increase ($p<0.001$) in rCBF was found in the medial cerebellum and the left and right temporal lobe. Significant decreases in rCBF were found on the left in the rostral striatum and dorsolateral prefrontal cortex and in the right anterior cingulate gyrus. No activation was found in the thalamus or periaqueductal gray (PAG). The results suggest that the suppression of the sense of a filled bladder is regulated at the sacral cord level, and not at the level of the PAG, thalamus or more rostrally. The involvement of the cerebellum and the striatum can be explained in terms of inhibition of movement to avoid the sense of a filled bladder. Understanding the central control of bladder filling in humans might help to understand the pathophysiology of sensory urge, an important problem in many women.

45.2

INNERVATION AND NERVE GROWTH FACTOR IN THE EARLY POST-NATAL RAT BLADDER. W.D. Steers*, J.D. deHoll, J.M. Spitsbergen and J.B. Tuttle. Departments of Urology and Neuroscience, University of Virginia, Charlottesville, VA 22908.

The adult pattern of visceral innervation is often not present at birth and post-natal development of bladder innervation has not been thoroughly described. We examined the appearance of adrenergic bladder innervation and how this relates temporally to measures of bladder function and expression of nerve growth factor (NGF), upon which the development of many autonomic neurons depends. Adrenergic fibers, visualized by glyoxylic acid fluorescence, first appeared in the bladder just after birth and increased post-natally. Norepinephrine (NE) content was low at 1 wk but tripled between 2 and 3 post-natal wks. Bladder tissue NGF, assayed by ELISA, dropped significantly at 3 wks and remained low at 6 wks post-natal while NGF in the major pelvic ganglion rose from 3-6 wks, suggesting uptake by NGF-dependent neurons. Ganglionic cell counts showed no sigificant decline indicating a mild or absent cell death period. Levels of bladder NGF mRNA assayed by RT-PCR correlated with NGF protein. Immediately post-natal rat pups were unable to void spontaneously, requiring perineal stimulation, and had grossly abnormal cystometrogram. By 3 wks, animals voided spontaneously but retained some perineal responsiveness. Compliance improved from 1-6 wks when it showed a rise. The results suggest adrenergic innervation of the bladder develops post-natally during the period of shift from a primarily somato-visceral to viscero-visceral micturition reflex. NGF is implicated in regulating neural innervation. A recapitulation of this ontological process may accompany neuroplasticity following obstruction or denervation of the adult bladder. Supported by grants from NIDDK.

45.3

ELECTROPHYSIOLOGICAL EVIDENCE FOR POTENTIAL SUB-GROUPS OF BLADDER PREGANGLIONIC NEURONS IN THE CAT. C.W. Morgan* Anatomy and Neurobiology and Urology, Eastern Virginia Medical School, Norfolk, VA 23501

Extracellular and intracellular recordings were obtained from 19 bladder preganglionic neurons (BPGN) in the S2 spinal cords of chloralose anesthetized female cats. BPGN were identified in the lateral lamina VII by responding antidromically to ventral root stimulation and by their response to bladder distension generated from filling or to reflex bladder contractions. These BPGN had thresholds of 0.4-0.7V, conduction velocities of 3.5-7 M/sec, and fired at bladder pressures of 10-20 cm water. Individual BPGN showed similar firing patterns (in terms of numbers of spikes and numbers of bursts) across successive contractions. No BPGN was observed to fire constantly over an entire contraction but bladder pressure rose steadily even when a neuron was silent (in between bursts). Comparison of different BPGN over the same bladder contractions indicated (1) different firing patterns across neurons during each contraction and (2) some neurons were firing during the time others were silent.

Though conduction velocities and thresholds were not discriminating, the firing pattern data suggest there may be separate and specific roles for PGN innervating the bladder. If this proves to be true it this would suggest sub groups of BPGN, each having specific functions in bladder contraction.

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45.4

MUSCARINIC FACILITATION OF ACETYLCHOLINE (ACh) AND NOREPINEPHRINE (NE) RELEASE IN THE HUMAN URINARY BLADDER. G.T. Somogyi*, H.D. Flood, M.F. Bellinger and W.C. de Groat. Department of Pharmacology, Division of Urologic Surgery, University of Pittsburgh, Pittsburgh PA, 15261.

Previous studies revealed a facilitation of ACh and NE release in the rat bladder by M1 muscarinic receptors (Somogyi et al. J.Physiol. 480,81, 1994). In this study presynaptic facilitation was examined in bladder specimens obtained from patients aged 4-8 years undergoing ureteral reimplantation. Strips weighing 10-15 mg were taken from the bladder body and were prelabelled with ^{14}C-choline and ^{3}H-NE. One period of electrical stimulation was used and the release was expressed as percent fraction of the tissue ^{3}H or ^{14}C content. The release of ACh and NE which was measured in the presence and absence of the choline uptake inhibitor hemicholinium-3 (HC-3) (10 μM) was dependent on the pattern of stimulation. Continuous stimulation (CS) with 20 Hz 200 shocks evoked a large release of ACh and NE (5.88±1.4% and 1.81±0.35%, respectively), in comparison to intermittent stimulation (IS) with the same number of shocks (10 trains every 5 sec), which produced a small release of 0.79±0.19% and 0.19±0.03%, respectively, (n=7).The non-specific muscarinic blocker, atropine (0.1 μM) significantly inhibited ($p<0.05$) the CS induced release of ACh and NE (0.56±0.15% and 0.65±0.2%, respectively, n=4). However, the M1 muscarinic blocker, pirenzepine (50-100 nM) inhibited the ACh and NE release (1.76±0.6% and 0.51±0.13%, respectively, n=7) only in the absence of HC-3. Interestingly, atropine was effective even in the presence of 10 μM HC-3. The cholinesterase inhibitor, eserine (5 μM) enhanced both the CS and IS induced release of ACh and NE (CS: 13.03±1.99% and 3.34±0.77%, respectively, n=3 IS: 5.63±2.1% and 1.4±0.32%, respectively). Our present results indicate that muscarinic M1 receptor mediated presynaptic facilitation is operating in the human urinary bladder. The fact that the specific M1 receptor blocker, pirenzepine, is ineffective in the presence of HC-3, but that the non-specific muscarinic blocker, atropine, is still effective raises the possibility of additional non-M1 muscarinic presynaptic facilitation in the human urinary bladder. (Supported by the NIH grant NIDDK DK-45741 and by a grant from the Interstitial Cystitis Association (ICA).

45.5

ACUTE EFFECTS OF DIMETHYL SULFOXIDE ON C-FOS INDUCTION AND NITRIC OXIDE RELEASE IN SENSORY NEURONS. M.A. Pezzone*, A.J. Kanai, W.C. de Groat, and L.A. Birder. Departments of Pharmacology and Medicine, University of Pittsburgh, School of Medicine, Pittsburgh, PA 15261 and Department of Physiology, University of North Carolina, Chapel Hill, NC 27599.

Intravesical administration of dimethyl sulfoxide (DMSO) has been used to provide symptomatic relief in patients with interstitial cystitis, a chronic, painful disorder of the urinary bladder. To determine if DMSO directly affects sensory pathways, the induction of c-Fos immunoreactivity in the rat spinal cord was measured following intravesical administration of DMSO. In urethane-anesthetized, adult female, Wistar rats (150-200 g), intravesical instillation of a 50% solution of DMSO for 2 hours resulted in an up-regulation of c-Fos immunoreactivity in laminae I, II, and X, as well as, lamina VII near the sacral parasympathetic nucleus of the spinal cord. DMSO also induced firing on the pelvic nerve. These findings paralleled those produced by capsaicin-sensitive bladder afferents activated by chemical irritation of the bladder.

Given that capsaicin-sensitive afferent neurons in dorsal root ganglia (DRG) express neuronal nitric oxide synthase (nNOS), the release of nitric oxide (NO) from sensory neurons was also investigated following local administration of a 0.1% DMSO solution on individual, acutely-dissociated, lumbo-sacral DRG cells. A nafion-coated porphyrinic microsensor (tip dia, 0.5-1.5 μm; NO detection limit, 5 nM ; response time, 1 msec) was placed directly on the surface of individual neurons to directly measure NO release. The calcium ionophore, A23187, elicited a transient release of NO from DRG cells, demonstrating the presence of a constitutive NOS in these neurons. DMSO elicited a smaller release of NO from individual DRG neurons (range 25-75 nM). These observations suggest that DMSO may release NO in DRG cells via increased $[Ca^{2+}]_i$. In conclusion, direct stimulation of sensory neurons and release of NO may be the initial event involved in the de-sensitization of pain pathways by DMSO. Supported by NIH grant NIDDK 49430.

45.6

FREQUENCY DEPENDENCE OF MUSCARINIC FACILITATION OF TRANSMITTER RELEASE IN URINARY BLADDER STRIPS FROM NEURALLY INTACT (NI) OR CHRONIC SPINAL CORD TRANSECTED (SCT) RATS. G.V. Zernova, M. Yoshiyama, A.M. Booth*, W.C. de Groat and G.T. Somogyi. Dep of Pharmacology, Univ of Pittsburgh, Pittsburgh PA, 15261.

Frequency dependent facilitation of ^{14}C-acetylcholine (ACh) and ^{3}H norepinephrine (NE) release was studied in the urinary bladder strips of NI and chronic (30 days) SCT rats. After prelabelling the strips with ^{14}C-choline and ^{3}H-NE, one period of electrical stimulation was applied (100 shocks at frequencies of 2,5,10 and 40 Hz), which increased the release of ACh and NE from nerve terminals in a frequency dependent manner (maximal facilitation: 7-10 fold) . In SCT bladders the ACh frequency response curve was shifted to the left and reached a maximum at 10 Hz, whereas, in NI bladders 40 Hz produced the maximal release. In NI bladders the non-specific muscarinic blocker atropine (ATR) (0.1μM) and the M1 muscarinic receptor blocker pirenzepine (PIR) (0.05μM) were ineffective at 2 Hz but between 5-40 Hz significantly decreased ACh release by 35-80%. However, in SCT bladders PIR did not significantly decrease ACh release at any frequency except 2 Hz, whereas, ATR produced significant inhibition at all frequencies ($p<0.05$; 2 Hz: by 83%, 40 Hz: by 66%). In NI bladders ATR increased NE release at 5 Hz (by 113%) but decreased it at 40 Hz (by 45%), whereas, PIR was ineffective at 5 Hz and decreased NE release at 10 and 40 Hz (by 26% and 37%, respectively). In SCT bladders ATR decreased the release of NE at every frequency (2 Hz: 74% and 40 Hz: 60 %). After ATR in both NI and SCT bladders, ACh and NE release was 2-8 times higher at 40 Hz than at 2 Hz indicating the existence of a non-muscarinic facilitatory mechanism. This was more prominent in SCT bladders. These results indicate that M1 muscarinic facilitation, which is the main facilitatory mechanism of ACh and NE release in NI bladders is down regulated in SCT rats, whereas, non- M1 muscarinic and non-muscarinic facilitatory mechanisms are upregulated. (Supported by the NIH grant NIDDK DK-45741 and by a grant from the Interstitial Cystitis Association (ICA).

45.7

SUPRASPINAL AMPA AND NMDA GLUTAMATERGIC TRANSMISSION IN THE MICTURITION REFLEX IN THE RAT. M. Yoshiyama* & W.C. de Groat. Dept. Pharmacol., Univ. of Pittsburgh, Pgh., PA 15261.

Our previous studies revealed that glutamatergic transmission mediated by AMPA and NMDA receptors in the lumbosacral spinal cord plays an essential role in the micturition reflex pathway. In the present study, the effect of intracerebroventricular (i.c.v.) as well as intrathecal (i.t.) administration of LY215490 (LY), a competitive AMPA receptor antagonist and MK-801 (MK), a non-competitive NMDA receptor antagonist on the micturition reflex were evaluated in the urethane-anesthetized rat, to determine if glutamatergic mechanisms in the brain as well as the spinal cord are important for the control of micturition. Bladder activity was monitored during continuous infusion cystometrograms. I.c.v. or i.t. injection of LY215490 in small doses (0.01-0.03 μg) did not change bladder contraction amplitude (BCA) or external urethral sphincter (EUS) EMG activity. However, larger doses (0.1-0.3 μg) abolished BCA and rhythmic EUS EMG activity. In some rats, tonic EUS EMG activity persisted. MK administered i.c.v. in a dose of 0.6 μg had no effect, but 1.8 μg decreased BCA and EUS activity by 30-40% and 6 μg completely abolished these responses. I.t. injection of MK in similar doses (0.6-6 μg) did not change BCA or EUS EMG activity but a large dose (18 μg) totally suppressed bladder and EUS activity. In some rats, baseline bladder pressure markedly increased after i.c.v. injection of either drug. This was not as prominent after i.t. injections. Combined i.c.v. administration of doses of MK (0.6 μg) and LY (0.03 μg) which individually had no effect on BCA and EUS EMG activity completely suppressed these responses. Combined i.t. administration of MK (6 μg) and LY (0.03 μg) in doses which individually had no effect completely suppressed EUS EMG activity and decreased BCA by 30%. These data indicate that glutamatergic mechanisms in the brain as well as the spinal cord are essential for controlling micturition and that the interaction between AMPA and NMDA glutamatergic transmission is important at both sites. (Supported by NIH grant DK-49430)

45.8

EFFECT OF INTRAVESICAL ADMINISTRATION OF ZD6169 ON THE MICTURITION REFLEX AND ON C-FOS EXPRESSION IN THE SPINAL CORD INDUCED BY NOXIOUS BLADDER STIMULATION IN THE RAT. Y. B. YU*, M. O. FRASER, W. C. de GROAT. Department of Pharmacology, University of Pittsburgh, Pittsburgh, PA 15261.

The effects of ZD6169, an ATP-sensitive K+ channel opener, on reflex urinary bladder (UB) activity and on spinal processing of afferent input from the UB were evaluated in urethane anesthetized female Wistar rats. Continuous transvesical cystometrograms (0.04mL/min) were performed in untreated (U-T), capsaicin-treated (C-T; 125 mg/kg, s.c.) and capsaicin-vehicle-treated (V-T) rats. ZD6169 administered intravesically in concentrations of 2, 5, 10, and 100 ng/mL increased by 30% to 161% the intercontraction interval (ICI) in a concentration dependent manner in U-T and V-T rats but not in C-T rats. ZD6169 did not alter the ICI in C-T animals. The effects appeared within 30 min after administration. ZD6169 did not alter peak intravesical pressure during voiding. Pretreatment with ZD6169 (10 ng/mL) intravesically for 1 hr also suppressed C-fos gene expression in the L6-S1 spinal cord induced by a 2 hr infusion (0.04 mL/min) of 0.25% acetic acid (AA) into the urinary bladder. Irritation of the bladder with AA elicited a large increase in the numbers of Fos positive cells in the dorsal commissure (DCM), sacral parasympathetic nucleus (SPN) and dorsal horn (DH) in L6 (average 117 cells/section) and S1 spinal cord (68 cells/section). ZD6169 reduced the numbers of Fos cells by 48% in the DCM, 50% in DH, and 33% in SPN of L6-S1 segments of spinal cord in AA-irritated rats. Capsaicin pretreatment also markedly decreased the numbers of AA-induced Fos positive cells by 81% in DCM, 62% in DH and 49% in SPN. These results suggest that ZD6169 can influence bladder capacity by suppressing capsaicin-sensitive, C-fiber afferent receptors in the bladder wall and thereby alter the afferent limb of the micturition reflex. (Supported by a Contract from Zeneca Corporation and NIH grant DK 49430).

45.9

ELECTRICAL STIMULATION OF THE MEDIAN PREOPTIC AREA OR THE LATERAL SEPTAL NUCLEUS INHIBITS BLADDER CONTRACTIONS IN ANESTHETIZED FEMALE RATS. P. L. Vera*, Dept of Neurosurgery, George Washington University, Washington DC 20037

The medial preoptic area (MPA) projects to the pontine micturition center in the rat (Nadelhaft et al., Neurosci. Lett., 143:271-274). The purpose of this study was to determine the role of the MPA in micturition. Female Sprague-Dawley rats (n=6;200-300 g) were anesthetized with halothane and then urethane (1.1 g/kg; iv) through the jugular vein. The carotid artery was cannulated for blood pressure recording. The bladder was exposed and cannulated either through the urethra or through the dome of the bladder. Cystometrograms were performed by infusing saline either through the urethra or the dome of the bladder. Following a craniotomy, electrical stimulation (50 Hz, 3 sec, .2 msec, 25-300 uAmps) of the MPA or the lateral septal nucleus (LSN) resulted in inhibition of bladder contractions and a small depressor response. Continuous stimulation (20 Hz; 5 min; 50 uAmp) completely abolished contractions in the case of transurethral CMG or resulted in high resting pressure and overflow incontinence in the case of transvesical CMG. This inhibition was present even after sectioning the hypogastric nerves bilaterally, and the sympathetic chains bilaterally, between the L4 and L5 ganglia. Stimulation during periods of bladder quiescence failed to elicit bladder contractions.

These results suggest that the MPA and LSN directly inhibit bladder contractions. This inhibition is not mediated by the sympathetic nervous system.

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45.10

ELECTRICAL STIMULATION OF THE SUBSTANTIA NIGRA OR VENTRAL TEGMENTAL AREA INHIBITS BLADDER CONTRACTIONS IN ANESTHETIZED FEMALE RATS. D.R. Liskowsky* & P.L. Vera, Universities Space Research Association, Washington, DC, 20024; Dept. of Neurosurgery, George Washington University, Washington, DC 20037.

The substantia nigra has been shown to inhibit bladder contractions in cats (Yoshimura et al., Neurourology & Urodyn., 11:535-545). In contrast, systemic activation of dopaminergic receptors in rats results in bladder hyperreflexia (Kontani et al., Jpn. J. Pharmacol., 54:482-486). The aim of this study was to determine the effect of electrical stimulation of the substantia nigra pars compacta (SNpc) and the ventral tegmental area (VTA) on micturition in the rat. Female Sprague-Dawley rats (n=8;200-300 g) were anesthetized with halothane and then urethane (1.1 g/kg; iv) through the jugular vein. The carotid artery was cannulated for blood pressure recording. The bladder was exposed and cannulated either through the urethra or through the dome of the bladder. Cystometrograms were performed by infusing saline either through the urethra or through the dome of the bladder. Following a craniotomy, electrical stimulation (50 Hz, 3 sec, .2 msec, 25-300 uAmps) of the SNpc (n=8) or the VTA (n=1) resulted in bladder inhibition. This inhibition persisted even after bilateral sectioning of the hypogastric nerves and the sympathetic chains at the level of the L4 ganglia. In addition, haloperidol (2 mg/kg, i.v.) did not prevent the inhibition. These results suggest that stimulation of the SNpc or VTA inhibits bladder contractions. This inhibition is not mediated by sympathetic pathways or dopaminergic systems.

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45.11

CENTRAL NERVOUS SYSTEM SITES LABELED FOLLOWING INJECTION OF PSEUDORABIES VIRUS (PRV) INTO THE DISTAL COLON OR BLADDER OF ADULT RATS. W.C. de Groat*, J.P. Card and M.A. Vizzard. Univ. of Pittsburgh, Depts. of Pharm. and Neurosci., Pittsburgh, PA 15261 and the Univ. of Vermont, Depts. of Neurol. and Anat. and Neurobiol., Burlington, VT 05405.

PRV (10^6-10^8 pfu/ml) was injected into the wall of the distal colon (n=14) or urinary bladder (n=15) and the distribution of PRV-infected neurons (PRV-IN) examined following survival times of 52-96 h. After colon injection, PRV-IN in L6-S1 spinal cord (SC) were mainly in S1 whereas after bladder injection, PRV-IN were in both segments. Following bladder injection, PRV-IN were present in supraspinal sites: pontine micturition center (PMC), raphe, A5, A7, periaqueductal gray (PAG), parabrachial nucleus (PBN), locus coeruleus (LC), subcoeruleus, red nucleus (RN), hypothalamus and cerebral cortex. Following colon injection, PRV labeling in supraspinal sites included those sites labeled following bladder injection as well as the vagal dorsal motor nucleus (DMV), N tractus solitarius (NTS), N ambiguus (NA) and area postrema (AP). To determine if labeling at these sites (DMV, NTS, NA and AP) was mediated by vagal pathways, PRV was injected into the colon of T8 SC transected (SCT) animals (n=5) and allowed to survive for 96 h (n=2) or 120 h (n=3). At 96 h in SCT animals, PRV-IN were present in the thoracolumbar and lumbosacral SC. A few PRV-IN were also present above the SC transection site in T7 in the intermediolateral cell column and dorsal horn. However, at 96 h, no PRV-IN were present in any supraspinal site. At 120 h in SCT animals, the most heavily PRV-infected supraspinal sites were the DMV, NTS, NA and AP. Few if any PRV-IN were present in the PMC, PAG or PBN. A few PRV-IN were present in A5 and A7 regions. In one animal with more extensive PRV labeling in the SC above the transection, PRV-IN were present in A5, A7, LC and RN. These results demonstrate that: (1) the PMC may have multiple functions; (2) PRV labeling in the DMV, NTS, AP and NA following colon injection is most likely mediated via vagal pathways; (3) PRV labeling in the PMC, PAG and PBN following either colon or bladder injection is most likely mediated via lumbosacral spinal pathways. [Support: PVA-SCRF (1461-01), APA (VB1-9402-1), ICA]

45.12

PSEUDORABIES VIRUS (PRV) DOES NOT ESCAPE TO THE NEUROPIL FROM INFECTED NEURONS. DOUBLE LABELLING OF MAJOR PELVIC AND DORSAL ROOT GANGLION NEURONS FROM THE BLADDER AND COLON OF RAT. I. Nadelhaft*, G. Smith and S. C. Nuding, Research and Development Service. Veterans Administration Medical Center, Bay Pines, FL. 33504.

The use of pseudorabies virus (PRV) as a neuroanatomical tracer is based on its ability to be transported from the initial site of infection to a neuron, its subsequent replication within that neuron and, most importantly, its export from this neuron to other neurons only via the synapses they make with the infected neuron. To test this property, PRV was injected into either the urinary bladder or the distal colon of male rats and, at the same time, fast blue was injected into either the distal colon or the bladder. After a two to four day incubation, the animal was perfused and the major pelvic (MPG) and L1-L2 and L6-S1 dorsal root (DRG) ganglia examined for PRV-IR cells, dye labelled cells and double labelled cells.The number of double labelled neurons ranged between 2% and 10% of the number of PRV-labelled cells. This number is about the same as is found when two different dyes are used for injections into the colon and bladder [Keast et al., Cell Tissue Res 256:105(1989)]. Therefore, it appears that the pseudorabies virus is confined to the infected neurons and is not exported to the surrounding neuropil.

These results also suggest the there are single neurons in the MPG as well as the DRG that send processes to both the bladder and the colon.

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45.13

SUPRAMEDULLARY INNERVATION OF THE URETHRA
B. Erokwu, W.M. Grill, and M.A. Haxhiu*
Case Western Reserve University, Cleveland, Ohio, 44106

The supraspinal CNS cell groups that innervate spinal neurons controlling functions of the urethra were identified by the viral retrograde transneuronal labeling method. Pseudorabies virus (PRV) was injected into the wall of the pre-prostatic urethra of male Sprague-Dawley rats. After five to eight days survival, spinal cord and brain tissue sections from these animals were processed for immunohistochemical detection of PRV. Labeled cells were found in the parapyramidal area, lateral paragigantocellular and gigantocellular nuclei, and in the midline neurons. Within the dorsal aspect of the pons, labeling was found in Barrington's nucleus. A few labeled neurons were observed within the locus ceruleus and subceruleur nuclei, as well as Kolliker-Fuse nucleus. In the ventral aspect of the pons, labeled neurons were found within the nucleus raphe magnus, and in the A5 cell group. In the mesencephalon, PRV labeled cells were found in the ventrolateral part of the central gray matter. A few labeled cells were observed in the gigantocellular division of the red nucleus. In the diencephalon, PRV labeled cells were seen in the hypothalamus: lateral dorsal and paraventricular nuclei. A few cells were observed in retrochiasmatic region. At seven and eight days following PRV injection, intense labeling was seen in the medial nucleus of amygdala, less intense in central and lateral nuclei of amygdala complex. Labeling was observed in lateral septal nucleus, hippocampus, primary motor cortex, and in piriform cortices. The overall distribution of labeled neurons was similar to that observed following PRV injections into the wall of the bladder. These results indicate that the CNS neurons controlling the genitourinary system are found to be located within many of the same regions that are involved in autonomic and neuroendocrine functions.

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45.14

CHEMICAL CODING OF NEURONS IN THE HYPOGASTRIC (HG) AND MAJOR PELVIC GANGLION (MPG) OF ADULT MALE RATS. R.W. Hamill* and M.A. Vizzard. University of Vermont, College of Medicine, Departments of Neurology and Anatomy and Neurobiology, Burlington, VT 05405.

The MPG and HG of the rat pelvic plexus contain postganglionic neurons that innervate the urinary bladder, colon, reproductive organs (MPG) or reproductive organs primarily (vas deferens, prostate gland, seminal vesicles) (HG). To identify potential chemical coding in MPG and HG neurons, single and double fluorescence immunohistochemistry were employed to assay for putative neurotransmitters. MPG and HG were removed under sterile conditions from male Sprague-Dawley rats (n=20; 180-200 g) and incubated in culture medium for 48 h at 37 °C without colchicine prior to fixation. Cryostat sections (25 µm) of ganglia were mounted directly onto gelatinized slides. Immunoreactivities for enkephalin-endorphin (ENK-END), neuronal nitric oxide synthase (NOS), vasoactive intestinal peptide (VIP), neuropeptide Y (NPY) and tyrosine hydroxylase (TH) were demonstrated within the MPG and HG using standard techniques. In double-labeled preparations, many neurons in both the MPG and HG that exhibited TH-immunoreactivity (IR) also exhibited NPY-IR whereas many additional NPY-IR neurons did not contain TH-IR and may have been cholinergic. TH/NPY-IR neurons tended to be larger than NPY-IR only neurons and TH-IR only neurons tended to be among the largest cells in either ganglion. NPY-IR nerve fibers and pericellular baskets of varicosities were present throughout both ganglia whereas pericellular TH-IR varicosities were rare. In double-labeled preparations, many neurons in both the MPG and HG that exhibited NOS-IR also exhibited VIP-IR whereas many additional VIP-IR neurons did not contain NOS-IR. NOS-IR and VIP-IR neurons were among the smallest cells in either ganglion. Numerous NOS-IR and VIP-IR nerve fibers and pericellular varicosities were also present throughout each ganglion. In single-labeled preparations, pericellular ENK-END-IR varicosities were numerous and distributed throughout both the MPG and HG. Very few neurons contained detectable ENK-END-IR in either ganglion. These studies indicate substantial neurotransmitter heterogeneity of pelvic autonomic ganglia, probably reflecting functional diversity. [Support: PVA-SCRF (1461-01), APA (VB1-9402-1), ICA, NINDS NS22103]

45.15

PUTATIVE NEUROTRANSMITTER INPUTS TO RETROGRADELY LABELED NEURONS IN THE PONTINE MICTURITION CENTER OF THE RAT.

A. Nadel* & P.L. Vera, Dept. of Neurosurgery, Washington, DC 20037.

The aim of this study was to describe the neurotransmitter content of projections to neurons in the pontine micturition center (PMC). Sprague-Dawley female rats (n=4; 200-250 gm) were anesthetized with sodium pentobarbital (50 mg/kg; IP) and a L1 laminectomy was performed to expose the L6/S1 spinal cord. Bilateral injections of Fluorogold (2%;250 nl) were made in the spinal cord. The rats were allowed to recover for 1 week and then perfused transcardially with 4% paraformaldehyde fixative. 10 or 14 um thick sections through the PMC, containing retrogradely labeled neurons were exposed to the following antisera: leu-enkephalin (L-enk), met-enkephalin (m-enk), serotonin (5-HT), substance P (SP), tyrosine hydroxylase (TH) and calcitonin gene related peptide(CGRP). The primary antisera were visualized using a secondary antibody labeled with rhodamine or indocarbocyanine.

Immunoreactive processes containing these putative neurotransmitters were detected in the PMC, except for CGRP. Some of these terminals were also seen in close apposition to retrogradely labeled neurons in the PMC.

These results suggest that terminal processes containing putative neurotransmitters are present in the PMC and in close apposition with retrogradely labeled neurons. Thus, it is possible that these transmitter may modulate the output of PMC neurons to affect bladder function.

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45.16

PELVIC NERVE STIMULATION INDUCES FIELD POTENTIALS IN PERIAQUEDUCTAL GRAY OF CAT. J.W. Downie*, M. Duong, J.L. Martin and H.J. Du. Dept. Pharmacology, Dalhousie Univ. Halifax, Nova Scotia, Canada B3H 4H7.

Neuroanatomical studies have indicated that the periaqueductal gray (PAG) may be a target for projecting sacral cord neurons conveying afferent information from the urinary bladder. As a prelude to studies on these projecting cells, we have mapped the PAG for areas responding to electrical stimulation of bladder afferents in the pelvic nerve (PLN) of cat, and compared that map to ones obtained from stimulation of the pudendal (PDN) and superficial perineal (SPN) nerves. Under halothane anesthesia, electrodes were implanted on the PLN close to the bladder and PDN and SPN were dissected through a parasaggital incision near the base of the tail. Laminectomy was done to permit placement of a cord dorsum (CD) electrode. Field potentials in the PAG (A6-P2) were recorded under chloralose anesthesia using a monopolar tungsten electrode (9-12MΩ). The area from A6-P2 (0=intraural line) was sampled. The cats were perfusion-fixed with cold 4% paraformaldehyde. Histological localization of lesion sites and tracks was carried out in 40μ sections stained with thionin. The data were obtained in 8 cats. PAG field potentials were multiphasic, with occassional single unit discharges observed on the early phase. Mean central conduction velocities (CD-PAG) for the beginning of the early phase were 33 (range: 16-45) m/s for the PLN evoked response, 32 (17-51) for PDN and 41 (32-51) for SPN. The sites of maximum PLN-evoked field potentials for each experiment lay between A3.5 and P1.5 and overlapped the rostrocaudal extent of PDN-evoked potentials. SPN maxima were found from A6-P0.2. In conclusion, the PAG may be involved in processing bladder afferent information for perception of fullness or reflex micturition.

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45.17

OPTICAL IMAGING DEMONSTRATION OF THE SPONTANEOUS NEURONAL ACTIVITIES IN THE MALE RAT MAJOR PELVIC GANGLION (MPG) FOLLOWING PELVIC NERVE TRANSECTION.

*M. Imaizumi and M. Kawatani** Dept. of Physiology, Akita Univ. Sch. of Med., Akita, Japan 010.

Membrane potential changes were measured in the MPG by optical imaging system. The MPG was isolated from Nembutal anesthetized rats (40–50 mg/kg) and perfused with modified Tyrode's solution. After identified the MPG, 128×128 photopixel-array-multi-channel system was used for detection of voltage-sensitive dye (RH–975). In normal ganglia (n=12), KCl (300 mM) and ACh (3.3×10^{-7} M) excite the ganglion cells dose dependently. Area distribution of the excited cells was different for these two drugs. The effect from the formal drug was 724% larger than the latter. No rhythmic excitation was observed. Following pelvic nerve transection(2–6 weeks period to the experiments) rhythmic voluntary excitation (CVE) was observed (14/21). The excitation occurred more than one spotted area and spreading out the ganglion cells. The frequency of excitation was 1.8–3.4 Hz. TTX (6.0×10^{-7} M) abolished the CVE. Following hypogastric nerve transection, CVE was not recorded (n=4). These data suggest that spontaneous neural finding was developed following pelvic nerve transection that could have major excitation for emptying the urinary bladder during pelvic nerve injury.

45.18

THE ROLE OF GLUTAMINERGIC TRANSMISSION ON DETRUSOR-SPHINCTER DYSSYNERGIA IN UNANESTHETIZED RATS. T. Satoh, K. Sugaya, H. Noto*, O. Nishizawa, M. Kawatani. Dept. Phisiol. and Urol., Sch. Med., Univ. Akita, Japan. 110

Suprasacral spinal cord injury in human result in inappropriate contractions of the external urethral sphincter muscle during micturition (detrusor-sphincter dyssynergia : DSD). This study was undertaken to examine the role of glutaminergic neurotransmission on DSD in unanesthetized chronic spinal rats (CSR). Chronic spinal cord (Th 7-8) transection (under inhalation anesthesia) in female Wistar rats were used. MK-801 (0.03-100 μg), a noncompetitive NMDA receptor antagonist and LY293558 (0.03-100 μg), a competitive AMPA receptor antagonist were administered intrathecally. The effects of antagonists were also examined in acute capsaicin rats (ACR) (capsaicin was applied topically on the bladder dome) and chronic capsaicin rats (CCR) (capsaicin was administered subcutaneously 4 days before experiments). DSD developed in all CSR and the amplitude of urethral contraction (UC) was 6.8±1.1 cmH2O (mean±S.E., n=14). MK-801 and LY293558 slightly inhibited bladder activities and markedly reduced intraurethral pressure (IUP) during bladder contractions (DBC). 10 μg of LY293558 moved IUP DBC below zero (-1.2±0.6 cmH2O, n=7) which synegestic pattern. Acute capsaicin treatment reduced UC. The antagonists did not alter UC in ACR. The antagonists reduced the UC in CCR, which is similar to the result from the CNTR. These data suggest that capsaicin-sensitive C-fiber afferents have role of both promoting and reducing DSD. Capsaicin sensitive Aδ-afferents are essential for the development of DSD, and glutamate convey its transmission.

45.19

PROJECTIONS OF THE PONTINE MICTURITION CENTER TO THE SACRAL PARASYMPATHETIC NUCLEUS IN THE RAT, S.C. Nuding*, G. Smith and I. Nadelhaft. Research and Development Service, VA Medical Center, Bay Pines, FL 33504.

Experiments in our laboratory have previously established a connection between the sacral parasympathetic nucleus (SPN) of the intermediolateral region of the L6-S1 spinal cord segments and the pontine micturition center (PMC) [Neurosci. Abstr. 13: 734 (1987)]. After small (20 - 60 nL) **unilateral** injections into the SPN, retrograde transport of fluorescent microspheres labelled neurons in the PMC **bilaterally**, suggesting that individual PMC neurons might have projections to both SPNs. To test this hypothesis, a small injection of rhodamine labelled latex microspheres was made into the SPN on one side of the rat spinal cord; a similar injection of fluorescein tagged microspheres was made into the other. After several days, the animals were perfused. Inspection of 40 μm cord sections confirmed that the placement of these injections was similar along the rostro-caudal axis of the cord and that neither of the tracers had spread across the midline. 30 μm brain sections were examined for rhodamine (red) and/or fluorescein (green) labelled PMC neurons. Red, green and **double labelled** PMC neurons were found on both sides of the brain. We therefore conclude that there is a redundancy in the connections from the PMC to the spinal cord and that at least some PMC neurons send axons to parasympathetic preganglionic neurons in the SPN on both sides of the lumbosacral spinal cord.

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45.20

VAGINAL RECEPTIVE FIELDS MODULATE THE BLADDER REFLEX. **B.W. Tedeschi* and C.W. Morgan** Anatomy and Neurobiology and Urology, Eastern Virginia Medical School, Norfolk, VA 23501

Experiments were done on 10 female cats anesthetized with α-chloralose, a suprapubic tube installed to record bladder pressure, and a laminectomy done to allow recording from preganglionic neurons in the sacral spinal cord. Reflex bladder contractions were initiated by increasing inflow to the bladder from raising a reservoir 10-20 cm above the bladder. Under conditions of constant volume repeated slow, rhythmic contractions up to 45 cm water were observed on a chart recorder over 5-10 min periods. These detrusor contractions were inhibited by deep vaginal/cervix stimulation with a lubricated glass rod. The latency for the inhibition varied from 1-10 sec and the effect lasted for minutes. In contrast, stimulation in the vestibule region of the vagina, possibly involving the urethral meatus, elicited bladder contractions similar in amplitude and rhythm to a normal reflex. This reflex occurred from a baseline accommodation pressure of 5-7 cm water with a latency of 1-3 sec and continued for many seconds beyond the stimulus similar to a normal reflex.

The excitatory response may be part of a urethra to bladder reflex that facilitates detrusor contraction when urine enters the urethra. The detrusor inhibitory reflex may serve sexual function, working along with the sphincter muscles to maintain continence during copulation.

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45.21

REGIONAL AND SEX DIFFERENCES IN GUINEA PIG BLADDER NITRIC OXIDE SYNTHASE ACTIVITY. M. Sales, A. Cowan*, M. R. Ruggieri and M. A. Pontari. Temple University School of Medicine, Philadelphia, Pennsylvania.

Preliminary evidence indicates a possible alteration of nitric oxide synthase (NOS) in the bladder mucosa of patients with interstitial cystitis. The function and distribution of NOS in the bladder is unknown. The purpose of this study is to compare NOS levels in the mucosa and muscle of the bladder dome and base from male and female guinea pigs. Results of assays performed in triplicate are shown in the table below (mean ± sem for n=3-6). Both calcium dependent (CD) and independent (CI) NOS activity was measured by the conversion of ^{14}C-L-arginine to ^{14}C-L-citrulline in soluble fractions of bladder homogenates. Overall, levels of NOS were higher in the males than females. In the muscle, equal amounts of both CI and CD activity were found in the dome of both sexes, while in the base, levels of CI activity were roughly double that of CD activity. In the mucosa, CI NOS activity was greater than CD in the base of both sexes. In the mucosa of the dome, CI activity was greater than CD in the male but not in the female, where the CD NOS activity was greater. These data show greater amounts of CI than CD NOS activity throughout the bladder base in both sexes. These differences may give a structural basis for regional differences in bladder physiology.

NOS ACTIVITY: pM/min/mg protein		Males		Females	
		CD	CI	CD	CI
DOME	Mucosa	5.37±0.01	12.5±0.13	5.12±0.01	1.07±0.05
	Muscle	2.45±0.06	2.47±0.27	1.25±0.03	1.51±0.02
BASE	Mucosa	4.36±0.21	10.7±0.31	0.84±0.05	1.47±0.08
	Muscle	2.45±0.15	4.00±0.09	0.43±0.05	0.83±0.05

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45.22

FUNCTIONAL CLASSIFICATION OF MUSCARINIC RECEPTOR SUBTYPES IN THE RAT URINARY BLADDER, A. S. Braverman, I. J. Kohn, and M. R. Ruggieri*. Temple University School of Medicine, Philadelphia, Pennsylvania.

Muscarinic agonists (e.g. bethanecol) and antagonists (e.g. oxybutinin) have been used clinically to treat a variety of voiding disorders, although the muscarinic control of bladder function remains incompletely understood. We investigated the effects of subtype selective muscarinic antagonists on both carbachol induced (direct muscle stimulation) and electrically stimulated (nerve evoked) contractile responses of rat bladder body strips *in-vitro*. Two electric field stimulation paradigms were performed after purinergic desensitization. In the first paradigm, the tissue was stimulated with 100 shocks at each frequency. In the second paradigm, the tissue was stimulated at each frequency until maximum tension was achieved. Both paradigms yielded similar results. The affinity of a series of muscarinic antagonists derived from analysis of cumulative carbachol dose response curves indicates M3 mediated bladder contraction. Quite different affinities were observed with inhibition of electrically stimulated contractions. The M2 selective antagonist methoctramine (10 nM, 30 nM) increased nerve evoked contractions by 10% (p,0.05) whereas the M1 selective antagonist pirenzepine (10 nM) inhibited contractions by 15% (p,0.05). These doses of antagonists had no effect on carbachol induced contractions. These findings indicate presynaptic M1 facilitory and M2 inhibitory receptors and post-synaptic M3 receptors mediating bladder smooth muscle contraction.

Unlike M2 and M3 receptors, the presence of m1 receptors in the rat bladder body could not be demonstrated by immunological methods nor could the presence of m1 receptor mRNA be demonstrated by Northern blot analysis. RT-PCR utilizing subtype specific primers confirmed the presence of the m1, m2, m3, and m4 receptor mRNA. No m5 mRNA was detected. The function of the M4 receptor is unknown. RT *in-situ* PCR localized m1 receptor mRNA in what appeared to be nerve fibers surrounding blood vessels and bladder smooth muscle, particularly adjacent to the serosal surface.
Supported by NIH grants DK43333, DK40579, and DK39086

SUBCORTICAL SOMATOSENSORY PATHWAYS I

46.1

LIGHT AND ELECTRON MICROSCOPIC EXAMINATION OF PRIMATE TRIGEMINAL MESENCEPHALIC NEURONS. N.F. Capra†*, D. Dessem†, P. Luo† and P.J. May‡. Dept. of Oral and Craniofacial Biological Sciences†, U. of Maryland Dental School, Baltimore, MD 21201, Depts. of Anatomy, Ophthalmology and Neurology‡, U. of Mississippi Med. Ctr., Jackson, MS 39216.

The trigeminal mesencephalic nucleus (Mes V) supplies critical information on the state of the masticatory muscles to the brainstem nuclei controlling jaw movements. Despite this important role, the morphological features of these primary sensory neurons are not well described in primates. In macaque monkeys, a number of neurons were retrogradely labeled following injections of 10-15 µl of WGA/HRP into the masseter or pterygoid muscles. Large spherical neurons were found among small clusters of neurons along the mesencephalic tract in the pons, and along the margins of the periaqueductal gray in the midbrain. The subsequent experiments sampled only this latter region for labeled neurons. Injections of 0.1-0.2 µl of biotinylated dextran amine (BDA) placed in the exiting trigeminal nerve produced homogeneous staining of presumptive MesV neurons. These cells generally had large, round somata that ranged up to 40 µm in diameter. Although many showed no dendritic processes, numerous short processes, which extended up to 50 µm and sometimes branched, emerged from other labeled somata. EM examination of labeled and unlabeled presumptive Mes V neurons revealed somata with pale nuclei and copious cytoplasm containing numerous mitochondria and scattered stacks of rER. Their plasma membranes were largely bare of synapses, but showed numerous protrusions, and small processes that sometimes enclosed nearby neuronal elements. A few small terminals contacted these neurons. They contained clear pleomorphic vesicles, although clear and dense-cored spherical vesicles were also present. Many of these features have been reported in non-primate species. However, the number and complexity of dendritic processes on BDA labeled macaque Mes V neurons is striking. Furthermore, ultrastructural confirmation of synaptic input suggests that central modulation of primate trigeminal mesencephalic neurons occurs.
Supported by : DE06027 (NFC), DE10132 (DD & PL) and EY09762 (PJM)

46.2

SOMATICALLY-EVOKED RESPONSES IN THE PONS DETECTED MAGNETICALLY OUTSIDE THE BRAIN OF THE JUVENILE SWINE. Y. C. Okada*, N.S. Papuashvili[1], and C. Xu. Depts. Neurol. & Physiol., Univ. New Mexico Sch. Med., Albuquerque, NM 87131. [1]Depts. Neurosurg. & Pathophysiol., Inst. Clin. & Exp. Neurol., Tbilisi 380092, Georgia.

Subcortical activities generated by stimulation of the snout can be detected magnetically above intact brain in the juvenile swine (Hashimoto et al., Neurosci. Lett., 1996). The generator for the somatic evoked field (SEF) was identified here. Based on our earlier study, we hypothesized that the generator was located in a region inferior to the thalamus, possibly in the midbrain or brainstem. Thus, these regions in the brain of the swine (3-5 weeks old, 5-12 kg) were exposed laterally under anesthesia (ketamine 9.0 mg/kg/hr i.v. and xylazine 3.6 mg/kg/hr i.v.) by removing the facial muscles and the mandibular bone. The location in the snout near the dorsal portion of a nostrile was stimulated with a bipolar needle electrode (5-6 mA, 300 µs, 2 sec/stim). Somatic evoked potentials (SEPs) recorded on the surface of the pons consisted of a spike having the same morphology and latency as the spike recorded simultaneously within the cortex and thalamus. The polarity reversed along the rostral-caudal direction with the reversal occurring about 1 cm posterior to the trigeminal nerve root in the pons. The polarity was positive rostrally as in the responses in the cortex and thalamus. The SEF recorded laterally over the pons was stronger (as much as 20 pT) than that recorded above the decorticated brain surface (about 5 pT). It consisted of a spike having the same latency as the simultaneously recorded SEPs. Its generator was in the pons close to the reversal point for the SEP and its underlying current was directed rostrally, consistent with the SEP polarity. Neuronal currents in the pons, possibly from the spinal trigeminal complex, seem to be capable of generating SEFs that are strong enough to be detected even above the vertex of the intact brain. Supported by NIH grant RO1-NS30968.

46.3

A COMPARISON OF THE TERMINATION PATTERN OF THE CERVICO-THALAMIC TRACT AND THE DISTRIBUTION OF CAT301-IMMUNO-REACTIVITY. J. Broman* and M. Zhang. Department of Cell Biology, Faculty of Health Sciences, Linköping University, S-581 85 Linköping, Sweden.

The distribution of cervicothalamic tract (CTT) terminations, labeled with anterogradely transported tracers (WGA-HRP or biotinylated dextran) injected into the lateral cervical nucleus of cats, was compared with the distribution of immunoreactivity for a cell-surface antigen detected with the monoclonal antibody Cat301 (kindly provided by Dr Susan Hockfield) in the same or adjacent sections.

The most abundant CTT termination was detected in the ventroposterior nucleus (VP), mainly in its lateral part (VPL) and sparsely in its medial part (VPM) and in the ventral periphery of VP. In the VPL, CTT terminations were seen in a peripheral rim of the nucleus and in between its lateral and medial subdivisions (VPLl and VPLm). A larger field of termination was evident in the dorsal part of VPLl and dorsolateral part of VPLm rostral to the caudal pole of VPLl. The regions of the VPL receiving CTT terminations were characterized by sparse occurrence of Cat301 immunoreactivity (Cat301-ir). In the central parts of the VPL containing dense Cat301-ir, anterograde labeling had mainly the appearance of fibers of passage. Dorsal to the VP, scattered CTT terminations were seen in the medial division of the posterior complex, which was virtually devoid of Cat301-ir. Dense focussed CTT termination was detected in the medial extension of the magnocellular medial geniculate nucleus (MGmc) but absent from its main lateral part. The termination was centered upon clusters of cells in MGmc displaying dense Cat301-ir.

Our present observations demonstrate that the CTT terminates in a compartment of the VPL characterized by sparse occurrence of the antigen recognized by the Cat301 antibody. Thus, the CTT is related to a chemically defined compartment of the VPL that may be the origin of specific thalamocortical channels, the organization of which remains to be elucidated. In contrast, anterograde labeling in the medial MGmc is associated with clusters of Cat301 immunolabeled cells. The significance of this difference between CTT recipient cells in the VPL and MGmc is unclear.
(Supported by the Magnus Bergvall Foundation.)

46.4

ANATOMY AND PHYSIOLOGY OF CORTICO-TECTAL PROJECTIONS FROM THE SOMATOSENSORY NEOCORTEX OF THE RAT: GOLGI-LIKE RETROGRADE LABELING USING DEXTRANAMINE. R. M. Brown*, G. Paschall, M. Burton, D. Geron, B. Clancy and L.J. Cauller. GR 41, Cognition and Neuroscience Program, University of Texas at Dallas, Richardson, TX 75083-0688.

Pressure injections of biotinylated or rhodamine dextranamine (10kMW) into deep layers of the superior colliculus (SC) produced retrograde Golgi-like filling of the dendrites of layer Vb pyramidal neurons in rat neocortex including: primary and secondary somatosensory areas (SI and SII); medial areas including FR2 and cingulate cortex; and lateral areas including polysensory and perirhinal cortex. Only cells in layer Vb characteristic of intrinsically bursting neurons were labeled. And all labeled dendrites involved profuse apical tufts extending to layer I. Therefore the cortico-tectal neurons are of the morphological type that is very sensitive to "backward" corticocortical projections (Cauller and Connors, 1994, *J. Neurosci. 14:751)* indicating top-down cortical influences are extended to superior colliculus. Injections of dextranamine into SI or SII anterogradely labeled fibers terminating in the deep layers of superior colliculus. Accordingly, electrical stimulation of SI or SII evoked peak-negative field potentials in deep layers of the superior colliculus and positive potentials in upper layers. Antidromic activation of the collaterals of these layer Vb neurons by stimulation in superior colliculus evoked widespread cortical activation which may represent the general function of the layer Vb pyramidal neurons. This fluorescent dendrite technique also offers the unique opportunity to locate and analyze the apical dendrites of layer Vb neurons *in vitro*.
Supported by a grant from the Whitehall Foundation.

46.5

GABA-MEDIATED INHIBITION SHAPES PHASIC RESPONSES IN THE RAT'S THALAMIC VENTROBASAL COMPLEX (VB). C. Vahle-Hinz[1] and T.P. Hicks*[2], [1]Physiol. Inst., UK Eppendorf, D-20246 Hamburg, Germany, & [2]Inst. Biol. Sci., Natl. Res. Council, Ottawa, Canada.

The role of GABAergic inhibition in thalamic processing of information from whisker mechanoreceptors was studied in urethane-anaesthetised rats. VB neurones were activated by trapezoidally shaped mechanical displacements of whiskers in the presence of GABA receptor subtype-selective agonists and antagonists. Apart from an increase in discharge rate, phasic neuronal responses remained phasic in the presence of levels of bicuculline (BIC) sufficient to block muscimol- but not (-)baclofen-induced suppressions of firing. However, in phasic neurones responding solely with on/off discharges to the transitional phases of trapezoidal stimuli, BIC induced temporally increased responses which lasted for the entire duration of the dynamic portions of the whisker displacement, thereby inducing responses where previously none had occurred. Higher levels of BIC (or activation by the amino acid excitant, AMPA) that resulted in ongoing activity outside of the stimulus period, revealed a BIC-resistant phase of suppression lasting for several hundred milliseconds during the statically maintained, plateau phase of the stimulus. There was no reliable action of 2-hydroxysaclofen consistent with a contribution of $GABA_B$ receptors. The results show that $GABA_A$ receptors do contribute significantly to the form of dynamic aspects of the responses of phasic VB neurones. Other events appear to contribute further to the modification of responses in VB and are mediated through neither $GABA_A$ nor $GABA_B$ receptors. Supported by DAAD.

46.6

FAST AND TIME-DEPENDENT ANOMALOUS RECTIFICATION IN THALAMOCORTICAL NEURONES. S.R. Williams and V. Crunelli.* Physiology Unit, School of Molecular and Medical Biosciences, University of Wales Cardiff, Cardiff, CF1 3US, UK.

Thalamocortical (TC) neurones are known to express a Na^+/K^+ hyperpolarisation-activated current, I_h, that is thought to exclusively mediate anomalous rectification of the voltage-current (V/I) relationship. We report that anomalous rectification properties of TC neurones are more complex, and are a product of three different ionic mechanisms. Intracellular recordings were made from TC neurones of the cat ventrobasal complex under current- and discontinuous voltage-clamp. Under control conditions V/I relationship were non-linear when measured at peak and steady-state, similarly under voltage-clamp current-voltage relationship were non-linear (n=13). ZD 7288 (100-300 μM) (n=24) irreversibly abolished time-dependent rectification mediated by I_h, whilst sparing fast rectification. In some neurones in the presence of ZD 7288 voltage responses demonstrated a time-dependent increase in input resistance, that was apparent from potentials negative to -80 mV and was reflected as a fading inward current under voltage-clamp (n=6). Ba^{2+} (0.1-2 mM) reversibly abolished fast rectification (n=9) and this time-dependent increase in input resistance. Ba^{2+} however, revealed a non-inactivating inward current with very slow activation kinetics, first apparent at potentials negative to -90 mV: this current was sensitive to Cs^+ (5 mM) (n=4) and had a reversal potential between -60 and -50 mV, indicative of a mixed cationic current. These data indicate that TC neurones express I_h, an inwardly rectifying K^+ current, I_{KIR} and a slow form of I_h termed I_{hslow}. *SRW is a Wellcome Prize Student.*

46.7

Single channel properties of neuronal $GABA_A$ receptors in rat nucleus Reticularis thalami (nRt) and ventrobasal relay complex (VB). J. Kang, J.R. Huguenard*, D.A. Prince. Dept. Neurol. & Neurol. Sci., Stanford Univ. School of Medicine, Stanford University, CA 94305.

Synaptic inhibition within the thalamus plays a critical role in sensory processing and in generating thalamocortical rhythms, such as those occurring during sleep and generalized absence epilepsy. We have previously demonstrated differences in inhibitory post-synaptic currents (IPSCs) recorded in thalamic relay vs nRt neurons - IPSC duration in nRt cells was approximately 3 times longer than in relay cells. These functional differences, in conjunction with the known heterogeneity in $GABA_A$ receptor subunits in various thalamic nuclei, led to the hypothesis that single channel properties would be diverse. $GABA_A$ channels in nRt, where there appears to be a predominance of α3 and modest levels of γ2 subunits, would have single channel properties distinct from those in relay neurons, where δ, β2, α4, and α1 levels are high, with low amounts of γ2.

Single channel recordings were made in rat thalamic slices from nRt and VB neurons. With cell-attached patches, 2 μM GABA activated single or multiple channel events with similar unitary conductances (γ) in both cell types (nRt: 16.0±0.5 pS; VB: 17±1.7 pS). Channel activity was clustered into bursts in patches from both cell types, but single channel open times were much longer in nRt cells (biexponentially distributed; τ_os= 1.5&14 ms) compared to VB cells (monoexponentially distributed; τ_o = 1.9 ms). Similar results were obtained with inside-out patches under symmetrical [Cl^-] conditions (γ = 27 pS). Further, bath application of 100 μM Zn^{2+} preferentially depressed the activity in patches from nRt (84±2% suppressed; n=6), but had much less effect on VB channels (24±4% suppressed, n=4).

These data further support the proposal that functional differences in $GABA_A$ receptor-mediated inhibition in the thalamus are the result of diverse heteromultimeric receptor combinations. Thus GABA modulatory compounds might have relatively selective actions in a subset of thalamic nuclei. Supported by NIH grants NS06477 and NS07280.

46.8

Functionally diverse inhibitory innervation of ventrobasal thalamus by thalamic reticular neurons. C.L. Cox*, J.R. Huguenard and D.A. Prince, Dept. Neurol. & Neurolog. Sci., Stanford Univ. Sch. Med., Stanford, CA.

The thalamic reticular nucleus (nRt) provides the primary source of inhibition in rodent ventrobasal thalamus (VB), influences receptive fields of VB cells, and has an essential role in generating intrathalamic rhythms. We previously showed that nRt axonal arborizations in VB range from dense clusters to diffuse projections (J.Comp. Neurol.,366:416). In the present experiments we tested the hypothesis that these arborization types mediate different inhibitory influences on VB neurons.

Dual intracellular recordings were obtained from pairs of synaptically-connected nRt and VB neurons using whole cell patch techniques with biocytin-filled electrodes. Single action potentials (APs) from nRt neurons evoked inhibitory postsynaptic currents (IPSCs) without failure in 4 of 6 VB neurons (>100 trials/cell). In the remaining pairs, single APs failed to evoke IPSCs in 44% and 74% of trials.

Amplitude distributions of IPSCs in 2 neurons with failures consisted of a single peak averaging 18.5±8.8 and 20.6±9.6 pA, whereas IPSCs were larger and more variable (mean range: 28-514 pA) in the remaining pairs. To estimate the number of release sites required to account for the evoked IPSCs, we recorded miniature IPSCs (mIPSCs) in VB neurons. The mIPSCs amplitude distributions consisted of a single, slightly skewed peak which was fit by a single gaussian (6/8 cells), and the remaining distributions were best fit by two gaussians. Mean mIPSC peak amplitude was 12.2±1.3 pA, suggesting that 1-3 release sites could account for IPSCs in neurons with failures, whereas ~2-70 release sites would be required to produce IPSCs in the non-failure neurons. Burst discharge (3-7 APs) specifically augmented IPSCs in non-failure neurons.

Following axonal reconstruction, nRt neurons that produced no postsynaptic failures had cluster arborizations (n=2), whereas a nRt neuron that produced failures had a diffuse arbor with few branches. Our data suggest that nRt neurons produce at least two types of inhibitory influence in VB: large IPSCs result from discharge of neurons with focal arbors and many synaptic contacts, while less potent IPSCs are evoked by nRt cells with diffuse projections and fewer release sites. Supported by NIH grants NS06477 and NS07280, and the Pimley Training Fund.

46.9

NMDA RECEPTOR SUBTYPE 1 IS LOCALIZED TO A SUBPOPULATION OF LOCAL CIRCUIT NEURONS IN THE CAT VB COMPLEX. X. Meng* and H. J. Ralston, III. Department of Anatomy, W.M.Keck Foundation Center for Integrative Neuroscience, University of California, San Francisco, CA 94143-0452.

Previous studies have indicated that GABAergic local circuit neurons (LCNs) modulate glutamate mediated excitatory activity in the ventrobasal thalamus (VB). Nitric oxide synthase (NOS) resides within a sub-population of GABA-immunoreactive (GABA-ir) LCNs in the cat VB. The purpose of the present study was to explore the distribution of n-methyl-d-aspartate receptor subtype 1 (NR1) on GABA-ir and NOS LCN neuronal profiles in the cat VB.

Deeply anesthetized adult cats were euthanized by an intracardiac perfusion with mixed aldehydes. Serial vibratome sections of the thalamus were cut in the coronal plane. Fluorescence-labeled antibodies were used to study the distributions of GABA, NOS and NR1 with a confocal laser scanning microscope. Many GABA-ir and NOS positive LCNs were also immunoreactive for NR1, as were the non-GABA-ir projection neurons. Immunoelectron microscopic studies in which a pre-embedding NR1 immunoreactive technique was combined with a post-embedding GABA-immunogold method, indicated that many thalamic somata and postsynaptic dendrites exhibited NR1 immunoreactivity, some of which also contained immunogold labeling for GABA. In the LCN presynaptic dendrites, GABA labeling was occasionally colocalized with NR1. NR1 immunopositivity was rarely detected in axons, although GABA-immunogold was frequently seen in many axon terminals and numerous dendrites. These data provide additional evidence that glutamate acting through NR1, such as occurs at the terminals of medial lemniscal axons, may alter responsiveness of thalamic relay neurons through nitric oxide facilitatory and/or GABA inhibitory mechanisms. Furthermore, these organizations could provide a circuitry wherever excitatory afferent activities mediated by NR1 can influence the GABAergic LCN appendages in VB, resulting in feed-forward GABAergic inhibition of thalamic relay neurons. Supported by NIH grant NS 23347.

46.10

CHARACTERIZATION OF THALAMIC TERMINAL ARBORS FROM THE PRIMATE TRIGEMINAL PRINCIPAL SENSORY NUCLEUS. D.L. Andrew*[1,2], P.J. May[1,3] and S. Warren[1]. Depts. of Anatomy[1], Occupational Therapy[2] and Ophthalmology[3], Univ. of Mississippi Med. Ctr., Jackson, MS 39216

Previous studies of the trigeminal system have focused on the barreloids in the rat thalamus. However, there is limited information describing the trigemino-thalamic projections in primates. Using biotinylated dextran amine (BDA), we previously demonstrated the pattern of the terminal arbors arising from the spinal trigeminal nucleus in macaques. The present experiments used BDA injections in *M. fascicularis* monkeys to characterize the trigeminal principal nuclear projections to the ventral posterior medial (VPM) nucleus. Labelled trigemino-thalamic terminal arbors are located throughout the rostral-caudal axis of VPM. The contralateral label extends dorsal-ventrally, adjacent to the arcuate lamina. The ipsilateral label is located more medially within the nucleus. Terminal fields in VPM are arranged in a honeycomb pattern. Axons of different sizes intermingle and contribute terminal arbors to this complex. The terminal arbor characteristics of thin and thick fibers appear to differ. Thinner fibers predominate in the dorsally located terminal fields. They are studded with *en passant* boutons that have a variety of shapes. These long, undulating fibers lack a consistent relationship to the somata. This suggests the VPM neuropil is the primary target of the thin arbors. Thicker fibers are more numerous in the core of the nucleus. They appear fragmented, lack clear branches and display fewer conventional boutons. These thick fibers form a complex, three-dimensional cluster near cell bodies. This arrangement suggests that the proximal dendrites receive a dense termination from the thick arbors. EM analysis reveals irregularly shaped labelled profiles with clear spherical vesicles that make asymmetric synaptic contacts on dendritic profiles. In general, the principal nuclear projections are more centrally located than those arising from the spinal trigeminal nucleus. These variations in terminal field organization may provide a morphological substrate for differential processing of trigeminal signals within the primate VPM. Supported by NIH Grants: NS27996 (SW); EY09762 (PJM)

46.11

THE ORGANIZATION OF SPINAL AND CORTICAL PROJECTIONS TO THE INTRALAMINAR *CENTRALIS LATERALIS* (CL) OF THE PRIMATE THALAMUS. Henry J. Ralston III*, Antonia M. Milroy and Diane Daly Ralston. Department of Anatomy and the W.M. Keck Foundation Center for Integrative Neuroscience, University of California, San Francisco, CA, 94143-0452.

The somatosensory zone of CL in the non-human primate receives afferents from the dorsal horn of the spinal cord (STT) for processing nociceptive information. It also receives projections from the primary somatosensory cortex. Four adult male *M. fascicularis* monkeys were used in this study, three for spinal projections and one for cortical projections. Wheatgerm agglutinin conjugated to horseradish peroxidase (WGA-HRP) was injected into the dorsal horn of the spinal cord in the cervical enlargement or into the primary somatosensory cortex, area 3 in the hand representation. After 2 days survival the reanaesthetized monkeys were euthanized by intracardiac perfusion with mixed aldehydes. Vibratome sections of thalamus were reacted with TMB and labeled regions of CL were prepared for electron microscopy using post-embedding GABA immuno-gold methods.

STT terminals were large, with rounded synaptic vesicles (RL) and formed asymmetric contacts with dendrites of thalamic neurons. Only occasionally did these terminals contact vesicle-filled profiles that were GABA immunoreactive (GABA-ir). Cortical projections also end as RL profiles as well as smaller terminals with rounded synaptic vesicles (RS). Both types of cortical terminals contact GABA-ir presynaptic dendrites as well as non-GABA-ir dendritic shafts.

We conclude that: 1- the spinal projections to macaque CL are similar to the projections to VPL in that few spinal terminals contact GABA-ir neurons. This is in contrast to STT projections to VPL and CL in the cat which commonly contact GABA-ir neurons; 2- the macaque CL has properties of the two classes of thalamic nuclei, "First order" and "Higher order" (Guillery, J.Anat. 187: 583, 1995), in that it exhibits features of both sensory relay and of association types of thalamic nuclei. Supported by NIH/NS 23347.

46.12

DEAFFERENTATION AND LOCAL DAMAGE GIVE RISE TO BURSTING ACTIVITY IN THALAMUS OF AWAKE PATIENTS. J. Tsoukatos*, K.D. Davis, R.R. Tasker, A.M. Lozano, and J.O. Dostrovsky. Departments of Physiology and Surgery, University of Toronto, Toronto, Ontario, Canada, M5S 1A8.

Thalamocortical relay neurons fire in a bursting pattern mediated by a low threshold calcium current during sleep but not during wakefulness. However, a number of studies have reported the existence of bursting activity in thalamus of awake chronic pain patients and have hypothesized that this activity might be related to their pain. In order to examine this hypothesis we have analysed the incidence of bursting neurons in motor and sensory thalamus of 63 movement-disorder, 8 multiple sclerosis, and 55 pain patients during microelectrode recordings performed to assist in localizing the target for a lesion or implantation of a stimulating electrode. The average incidence of bursting cells per track (bursting index-BI) was higher in multiple sclerosis and pain patients (BIs: 1.7 and 3.5 respectively) than in Parkinson disease (PD), essential tremor, and cerebellar tremor patients who had no deafferentation (BIs: 0.6, 0.6 and 0.7 respectively). In a subgroup of PD patients who had previously undergone thalamotomy many bursting cells were encountered when the electrode traversed the borders of the previously lesioned region (BI: 1.6 for subgroup). In a phantom limb pain patient (BI: 7.7) we identified regions which contained bursting cells with no receptive fields and where electrical stimulation evoked pain or paraesthesia in the phantom. In a dystonia patient with a traumatic lesion to the thalamus (BI: 2.5) we encountered bursting cells near the borders of damaged regions. In many cases the intra-burst firing pattern was analysed and was consistent with an underlying calcium spike. These findings suggest that neurons in damaged and/or deafferented regions of motor and sensory thalamus switch to a bursting firing pattern and this activity is not necessarily related to pain. However, if such bursting were to occur in thalamic pain relay sites following deafferentation, it might cause chronic pain.
(Supported by Canadian MRC, Parkinson Foundation of Canada, NIH)

46.13

THE INFLUENCE OF ELECTROMAGNETIC WAVES OF MILLIMITER-RANGE ON SOMATOSENSORY EVOKED POTENTIALS PARAMETERS IN RESIDUAL SPASTIC CEREBRAL PALSY. S.Nikitin*, P.Sokolov (1),V.Desnizza (2).Lab. of Neurophysiol., Inst. of Neurol., Volokolamskoe sch., 80, Moscow, Russia(1);Pain Res.Gr,Langestr.40-2,76530 Baden-Ba en,Germany(2).

The influence of electromagnetic wave (EMW) of millimeter-range (7,1 mm) on somatosensory evoked potentials parameters (SSEP) - the duration of N0+P1 - was analysed in 21 patients (23,0±4,5 years old, 13 male, 8 female), with residual spastic cerebral palsy (RSCP) before/after the treatment. SSEP were registered after the stimulation of left:right n.ulnaris. With standart physiotherapeutic protocol patients were treated at cervical, thoracic and low back paravertebral projection levels with EMW-radiator (IAV-1) for 10 min from the distance of 1 cm. There were no side effect of the treatment, in 14(66%) cases the spasticity decreased subjectively and objectively,but there were no correlation with the SSEP changes. There were no statistical difference between left:right sides for N0+P1 duration. The N0+P1 duration was increased in all patients - 12,9±3,9:12,6±3,3 /16,9±4,6:16,1±3,8 ms ($p<0,05$).The results reflect the dispersion because of the desynchronization of sensory afferents functional activity in RSCP.The latencies of the analysed components were normal.EMW may have an influence on the sensory afferent axon ionic channels.Supported by the Inst. of Neurology, Moscow,Russia.

SUBCORTICAL SOMATOSENSORY PATHWAYS II

47.1

INNERVATION DENSITIES ON THE CAT HINDLIMB. J. Culberson*, R. Millecchia, and P. Brown. Depts. of Anatomy and Physiology, West Va. Univ., Morgantown, WV 26506.

In order to examine the relationship between innervation density and other physiological and psychophysical variables as a function of position on the hindlimb, we have devised a method for visualizing cutaneous innervation density (ID) using data accumulated from several animals.

In anesthetized cats, low threshold mechanoreceptive innervation fields (IFs) of cutaneous nerves were mapped electrophysiologically; the nerves' myelinated axons were counted in histological sections. Each nerve's IF was digitized from a standard leg drawing and all points on a 1 mm grid within an unfolded skin representation of the IF were assigned a value equal to the nerve's ID (axons/IF area). Combining data across all nerves in all animals, the average ID was computed for each grid point. Exponential smoothing was applied across the grid. IDs were plotted in pseudo-color on an unfolded skin view and three standard leg views. The highest innervation density is on the plantar foot, rather than on the toes. These data will be correlated with map scale estimates to determine whether there are regional departures from constant convergence/divergence ratios.

Supported by NIH grants 1 R01 NS30725 and 1 R01 NS29997.

47.2

A MODEL OF LAMINA III - IV CELL RECEPTIVE FIELDS. P. Brown*, R. Millecchia, J. Lawson, J. Culberson, and S. Stephens. Depts. of Physiol. and Anat., W. Va. Univ., Morgantown, WV 26506.

Low threshold mechanoreceptive fields (RFs) of 500 dorsal horn neurons recorded in anesthetized cats were drawn on standard views of the leg. Recording sites were marked with microlesions. The recording locations were shifted rostrocaudally to compensate for interanimal variation in pre- and postfixation of the somatotopic map. RF outlines were characterized by RF center in cylindrical coordinates; area; length/width ratio; and orientation of long axis. Exponentially smoothed mean and variance surfaces were estimated for these five variables, on a grid of 40 points mediolaterally (ML) by 20/segment rostrocaudally (RC). Number of cells per grid point was estimated by exponential smoothing of cell counts in laminae III and IV.

Elliptical RFs were simulated for 100,000 cells, using the mean and variance data. When RFs were simulated at the locations of the original 500 cells, the RFs of real and simulated cells overlapped by approximately 80% (cross-correlation of real and simulated RF points on a grid on the skin with 0.5-mm spacing).

The density of cell representation of skin points on the hindlimb was represented as contour plots on dorsal view maps and dermatomes were similarly plotted on the standard views of the leg. Overlap of modeled and real data was at the 80% level.

Supported by NIH grants 1 R01 NS30725 and 1 R01 NS29997.

47.3

VISUALIZATION OF SIGNIFICANT DIFFERENCES OF CELL PROPERTIES IN SOMATOTOPIC MAPS. J. V. Odom*, R. Millecchia, and P. Brown. Depts. of Ophthalmol. and Physiol., W. Va. Univ., Morgantown, WV 26506

In order to test for differences between two populations of cells as a function of location in a somatotopic map, we must compare data sets in which sampled cells are randomly scattered throughout the map. We describe cell properties as exponentially smoothed surfaces fitted to data in the plane of the map. All data contribute to the computation of the value of each grid point, but each datum is weighted with a coefficient which declines exponentially with distance from the grid point. Both means and variances are represented using standard computations, substituting the sum of weights for the number of data at each point. Student's t values are computed at all grid points, keeping in mind the fact that grid points' t values are not independent of each other. Values of p are then derived, and significant difference surfaces are equal to differences of means where differences are statistically significant (e.g., $p < 0.01$) and zero where differences are not significant. We used Monte Carlo simulation methods to test this approach using two populations of 100,000 values at 4,000 grid locations. Correlations between calculated population and sample surfaces were 0.96 (mean1); 0.97 (mean2); 0.91 (variance1); 0.91 (variance2); 0.99 (difference); 0.97 (t); 0.78 (p), and 0.70 (significant difference).

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47.4

CORRELATION OF DORSAL ROOTLET RFs AND DORSAL HORN CELL RFs AT THE LEVEL OF ENTRY OF DORSAL ROOTLETS. J. Lawson*, R. Millecchia, J. Culberson, S. Stephens, C. Holbert and P. Brown. Depts. of Physiology and Anatomy, West Va. Univ,. Morgantown, WV 26505

We have proposed that spinal dorsal horn cells assemble their prototype receptive fields (RFs) during development by receiving input from primary afferent fibers entering the cord at their rostrocaudal levels, and that these initial RFs are subsequently modified by activity dependent mechanisms. If this is the case, then the combined RFs of adult dorsal horn cells at any rostrocaudal level should closely match the aggregate RFs of primary afferents entering at the same level.

In anesthetized adult cats, RFs of dorsal horn cells confined to a band as wide as an adjacent dorsal rootlet were determined. Individual rootlet primary afferent RFs were obtained by teasing apart the rootlet with fine forceps, and then suspending small fasicles on a hook electrode. Rootlets rostral and caudal to the cell band were also recorded. Aggregate dorsal horn cell RFs and rootlet RFs were correlated using degree of overlap on the skin. The correlation between rootlets and the cell band declined with rostrocaudal separation. While this suggests that cell band and adjacent rootlet RFs are highly correlated, undersampling of rootlet RFs made analyses difficult.

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47.5

ROSTROCAUDAL DISTRIBUTIONS OF CORD DORSUM POTENTIALS AND OBSERVED AND MODELED SINGLE-UNIT RESPONSES. Stephens, S., Millecchia, R., Culberson, J., Lawson, J., Gladfelter,W.*, and Brown, P. Depts. of Anatomy and Physiology, West. Va. Univ., Morgantown, WV 26505.

Previous studies showed rostrocaudal (RC) distributions of cord dorsum potentials (CDPs) evoked by intra-axonal stimulation reflect the RC distributions of the axons' boutons. This study compared RC distributions of CDPs and responding dorsal horn cells.

Single tactile afferents were stimulated at thirty-two standard sites (SSs) on legs of anesthetized cats while signal averaging from 15 sites spanning the RC extent of the hindlimb representation. CDP data for each stimulation site were combined across animals by exponential smoothing. The RC distributions of CDPs were compared to the distributions of responding cells, determined by overlap of each SS and the RFs of 500 recorded neurons and 100,000 simulated neurons. Correlations were highest for the model population: mean ± SD 0.76 ± 0.18, and there was no difference of distribution centers. Standard deviations of CDP RC distributions (a measure of RC spread) were significantly greater than the SDs of responding cell RC distributions: 1.07 ± 0.21 vs 0.88 ± 0.42, respectively, $t = 3.64$, $p < 0.001$.

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47.6

NEURAL CORRELATES OF SPATIAL DISCRIMINATIONS IN THE DORSAL HORN. R. Millecchia* and P. Brown. Physiol. Dept., W. Va. Univ., Morgantown, WV 26506.

Variations of two-point discrimination and localization thresholds on the skin have been attributed to variations of CNS receptive field (RF) size and map scale. Variation of two-point discrimination with orientation has been attributed to longitudinal elongation of RFs.These hypothesized correlations were tested with a model of the 100,000 neurons in the cat dorsal horn laminae III-IV representation of the hindlimb. Stimuli were simulated as single or paired skin contacts, 2mm x 2mm. Each stimulus was tested for overlap with the RF of each of the 100,000 neurons, and responding cell density was depicted on dorsal view grids. For each discrimination, responses to a reference and a comparison stimulus were compared by cross-correlation of the distributions of responding cells. As comparison and test stimuli were varied from identical to more and more different, the correlations declined monotonically from 1.0 toward 0. Correlation criteria were used as discrimination thresholds; as criteria were decreased from 1.0 toward 0, difference limens (DLs) increased. Two point discrimination and localization DLs varied with position on the skin in parallel with map scale and dorsal horn cell RF size. Two point discrimination was also better for transverse than for longitudinal stimulus orientation.

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47.7

PLASTICITY IN THE SPINAL DORSAL HORN FOLLOWING PERIPHERAL NERVE REGENERATION. H.R. Koerber* and K. Mirnics. Dept. of Neurobiology, University of Pittsburgh, School of Medicine, Pittsburgh, PA 15261.

Adult cats were anesthetized with α-chlorlaose 5-12 months following transection and self union of the left tibial and sural nerves. In each experiment a regenerated fiber was impaled in the dorsal columns with electrodes containing 5% Neurobiotin (NB) in 1M K-acetate. The impaled fiber's adequate stimulus was determined and the fiber activated by passing brief (200μs) current pulses through the microelectrode. The resulting cord dorsum potentials (CDPs) were recorded at 8 sites in response to shocks delivered every 56ms and to pairs of shocks (50ms ISI) delivered every 2s, and the fiber injected with NB. In the same preparations electrodes containing 3 M K-acetate were used to penetrate dorsal horn cells. The impaled cells' receptive fields (RFs) were mapped taking note of areas of skin from which the most vigorous responses were elicited. Needle electrodes inserted into these cutaneous "hot spots" were used to electrically activate individual or small numbers of regenerated fibers while simultaneously recording the resulting CDPs and intracellular EPSPs (if any). This allowed the determination of the incidence of connectivity between regenerated fibers and dorsal horn cells with overlapping RFs. At the completion of the recording experiments the animals were perfused, the cord removed and processed to recover the injected fiber. In agreement with findings in intact animals, NB revealed many long-ranging collaterals which were not seen using intracellularly injected HRP. Although there was no qualitative difference in their morphology compared to those seen in controls, the correlation between spatial distribution of boutons and amplitudes of the monosynaptic CDPs revealed significant shifts in the functional efficacy of the central connections. Transcutaneous stimulation revealed a significantly higher incidence of connectivity between regenerated fibers and cells with overlapping RFs with time after regeneration (5-6 mo. 20%; 9 mo. 67%; 12 mo. 86%). Qualitatively, there was no obvious anatomical reorganization in the nucleus proprius, however, functional changes observed with time following transection suggest the formation new somatotopically appropriate central connections. NS23725 (HRK).

47.8

ULTRASTRUCTURE OF LESION-INDUCED CHANGES IN SYNAPTIC ORGANIZATION IN TRIGEMINAL SUBNUCLEUS CAUDALIS: NEUROTRANSMITTERS AND RECEPTORS.
L. E. Westrum*, N. L. Anderson, P. A. Irish, and M. A. Henry. Dept. of Neuro. Surgery, U. of Washington, Seattle, WA 98195.

Synaptic reorganization following primary deafferentation of trigeminal nucleus might be associated with remodeling of the neurotransmitter patterns and their receptors. We are using lesions of the trigeminal nerve in conjunction with pre- and postembedding electron microscopy immunolocalization protocols to study resultant synaptic reorganization emphasizing patterns of immunoreactivity for glycine (Gly), GABA and the NMDA subtype of glutamate receptor. Normally primary afferents are glutamatergic, form type I contacts onto dendrites and receive type II contacts from GLY positive and GABA positive axons. Following lesions, primary afferent terminals degenerate but retain contacts by Gly and GABA positive terminals. The postsynaptic type I specializations persist. Some of these type I postsynaptic sites, vacated by the degenerated terminals are reoccupied by Gly positive terminals in addition to the previously reported GABA positive terminals. These "reinnervated" type I postsynaptic sites are being studied to determine if they retain or have lost their NMDA receptor reactivity. Thus deafferentation of excitatory postsynaptic sites results in reoccupation by inhibitory axons which could affect the receptor characteristics of these postsynaptic sites.

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47.9

QUANTITATIVE ELECTRON MICROSCOPIC STUDY OF GLUTAMATE AND GABA IN DEVELOPING FELINE SPINAL TRIGEMINAL NUCLEUS. P.S. Irish, A.L. Marquardson, L.E. Westrum, M.A. Henry*. Dept of Neurological Surgery, Univ. of WA, Seattle, Washington 98195.

Information on the amino acid neurotransmitters involved in nociception is important for understanding the specific roles of excitatory and inhibitory neurotransmitters in modulation of pain. We examined tissue from kittens of 3 and 17 days, when the trigeminal system shows major periods of increase in axo-axonic synapses. Double-labeling with different sized colloidal gold particles was used for the excitatory neurotransmitter glutamate (GLU) and the inhibitory neurotransmitter gamma aminobutyric acid (GABA). Synaptic vesicle-containing terminal profiles were identified in electron micrographs of ultrathin sections from trigeminal subnucleus caudalis, lamina II. A total of 252 μm^2 was analyzed per age. Results comparing 3 day to 17 day show: 1) the number of small terminal profiles (<1μm^2) decreases (91%-88%), while the number of large terminal profiles (>3μm^2) increases (0.9%-4%), approximately 4-fold; 2) the increase in the terminal profiles size is not correlated with increase in number of synaptic vesicles; 3) the number of terminal profiles with high GLU-and GABA- immunoreactivity increases approximately 2-fold. These data suggest that the increase in amino acid immunoreactivity reflects the developmental role of GLU and GABA during synaptogenesis and in pain modulation.

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47.10

NATURE OF SYNAPTIC TRANSMISSION IN THE COCULTURED RAT TRIGEMINAL SYSTEM. W. Guido*, F-S Lo & R. S Erzurumlu, Dept of Anatomy and Neuroscience Center, LSU Medical Center, New Orleans, LA, 70112.

In explant cocultures of embryonic rat trigeminal ganglion (TG) with same age whisker pad and early postnatal brainstem slices (containing brainstem trigeminal nuclei, BSTC), TG axons grow and arborize in both targets. Furthermore, central TG axon arbors form functional connections with brainstem target neurons in BSTC. Here, we examined various aspects of synaptic transmission in this *in vitro* sensory system. Excitatory postsynaptic potentials (EPSPs) in BSTC cells were reliably evoked by electrical stimulation of the ganglion explant. EPSPs had short latencies (0.9-3 msec) and variable durations (20-80 ms), and were occasionally followed by an IPSP (EPSP/IPSP pairs). Recordings of postsynaptic activity over a wide range of membrane potentials revealed several important features about these responses. At depolarized levels, EPSPs triggered action potentials, while at hyperpolarized levels they evoked low threshold Ca^{2+} spikes. The amplitude of EPSPs peaked early and declined with membrane depolarization, a pattern consistent with glutamatergic nonNMDA receptor activation. The IPSP component of the EPSP/IPSP pair was short (40 ms) and reversed polarity at -70 mV, a pattern consistent with the activation of a $GABA_A$ mediated Cl- conductance. Bath application of the $GABA_A$ antagonist, bicuculline produced two changes. First, spontaneous EPSP activity became prevalent. In some cells, synchronous barrages of spontaneous EPSP activity led to periodic waves of depolarization and high frequency firing in BSTC cells. Second, the duration of EPSPs became longer (200-800 msec). EPSP duration increased with membrane depolarization, a pattern consistent with NMDA receptor activation. Taken together, these results indicate that developing BSTC cells in coculture form both excitatory and inhibitory connections and the latter may play a role in regulating the pattern and nature of excitatory postsynaptic activity. *Support by NSF 9396270 (WG) and NIH NS32195 (RSE).*

47.11

DEVELOPMENTAL CHANGES IN MEMBRANE PROPERTIES OF BRAINSTEM TRIGEMINAL NEURONS DURING PATTERN (BARRELETTE) FORMATION. E. Gunhan-Agar*, R.S. Erzurumlu & W. Guido. Anatomy & Neuroscience Center, LSU Medical Center New Orleans, LA 70112.

Little is known about the electrophysiological properties of brainstem trigeminal neurons during development and refinement of connections in the rat trigeminal pathway. For example, the functional state of first-order central relay neurons in brainstem trigeminal nuclei (BSTC) during whisker-specific pattern (barrelette) formation has yet to be explored. Using intracellular recordings in a brainstem slice preparation, we examined the membrane properties of BSTC cells from rats during PND 0-21. At all ages tested, neurons were capable of generating trains of action potentials in response to intracellular injection of depolarizing current pulses. Increasing the amplitude of the depolarizing current also reduced the latency of the first spike and increased firing frequency. During times when barrelletes develop (PND 0-5), spikes were shorter and longer and showed frequency accommodation; the latter was due to the activation of a large two component after-hyperpolarization (AHP). After the consolidation of barrelettes (PND 5), several voltage-dependent conductances emerged which greatly altered signal transmission. These included a large low threshold (LT) Ca^{2+} conductance which led to Ca^{2+} spikes and burst firing, a transient K+ conductance (I_A) which delayed the onset of spike firing, and a mixed cation conductance (I_H) which prevented strong hyperpolarization and contributed to the establishment of intrinsic burst activity. Cells from earlier ages (PND 0-5) lacked I_A and I_H, and had a weak LT Ca^{2+} conductance that gave rise to small Ca^{2+} spikes devoid of bursts. Taken together, these results suggest that the electrophysiological properties of BSTC neurons play a significant role in pattern formation by minimizing signal distortion and insuring that excitatory responses from sensory periphery are accurately received and transmitted according to stimulus strength. *Support by NIH NS32195 (RSE) and NSF 9396270 (WG)*

47.12

POSSIBLE FEATURE DETECTION IN THE SOMATOSENSORY BRAINSTEM D. W. Doherty* and H. P. Killackey, Department of Psychobiology, University of California, Irvine, 92717

Rat trigeminal ganglion neuron responses are tuned to the direction of vibrissa deflection. Recent data demonstrate that directional tuning is also prominent in nucleus principalis neurons. Directionally tuned response profiles may reflect a deflection direction response probability profile. The structure of the particular surface sampled by the rat results in vibrissa deflections that correspond to a unique probability profile. Probability profiles built up over several small vibrissa movements, or "tremors," can result in signals ideally suited for discriminating textures or detecting surface orientation and contour. Specifically, signals utilized for texture discrimination could result from responses to small vibrissa deflections, between approximately 0.3^o and 0.6^o. Small deflections usually result in single vibrissa receptive fields and relatively tight directional tuning in nucleus principalis neurons. In contrast, signals leading to surface orientation and contour detection could result from relatively large vibrissa deflections, between 1.2^o and 2.3^o. Large deflections usually result in multi-vibrissa receptive fields and broader directional tuning. Our hypothesis predicts that signals in brainstem neurons, specifically neurons in nucleus principalis, could play a role in feature detection in the somatosensory system. Supported by Human Frontiers Grant RG55/94.

47.13

BRAINSTEM TRANSNEURONAL LABELING AFTER HSV-1 INJECTION INTO THE ETHMOIDAL NERVE OF THE MUSKRAT. W.M. Panneton* and P.F. McCulloch. Dept. of Anatomy and Neurobiology, Saint Louis University, St. Louis MO, 63104

When the nasal cavity of muskrats are stimulated with either ammonia vapors or water there are dramatic cardiorespiratory responses similar to those of diving. To define the brainstem circuitry of the diving response, we innoculated the ethmoidal nerve of muskrats with Herpes Simplex Virus 1 (HSV-1), strain 129, and waited for the anterograde transneuronal transport to occur. As a transneuronal tracer, HSV-1 crosses synapses and therefore can label secondary and tertiary neurons in a circuit. The ethmoidal nerve, which innervates the nasal mucosa, was injected with 1 µl of virus (1×10^8 PFU) and the animals survived 3 to 6 days. The animals were then perfused, their brains harvested and processed immunohistochemically using a commercial antibody against HSV-1. After short survival times only sparse label was found in the superficial medullary dorsal horn (MDH) ipsilaterally and the ventrolateral medulla. Longer survival times showed increased label in the ventrolateral parts of the superficial MDH, in lamina V, as well as in the ventral paratrigeminal n. Neurons were found in the rostral and caudal ventrolateral medulla, the dorsal reticular formation (RF), and the dorsolateral n. tractus solitarius (NTS). The area around the A5 group and the locus coeruleus were well labeled bilaterally. The ventrolateral part of the parabrachial nucleus and the Kolliker-Fuse n. contained neurons mostly ipsilaterally. The median raphe also contained label. With longest survival times there was increased label in the MDH, and all parts of the NTS, raphe, RF and dorsolateral pons. The anterograde transport of HSV-1 shows promise as a method for defining the brainstem circuitry of the diving response.

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47.14

FOS-DETERMINED NEURONAL ACTIVATION WITHIN THE BRAINSTEM OF THE MUSKRAT AFTER NASAL STIMULATION. P.F. McCulloch, W.M. Panneton and P.A. Young*. Dept. of Anatomy and Neurobiology, Saint Louis University, St. Louis MO, 63104

The immunohistological detection of FOS, the protein product of *c-fos*, was used as a marker of neuronal activation within the brainstem of muskrats after repeated stimulation of the nasal passages with either ammonia vapors or retrogradely-flowing water. Both methods of nasal stimulation produced the same cardiorespiratory events (an immediate 62% decrease in heart rate, 29% increase in mean arterial blood pressure, and sustained expiratory apnea). After ammonia stimulation of the nasal passages, increased FOS label was detected within the spinal trigeminal nucleus (ventral laminae 1 and 2 of the medullary dorsal horn (MDH), ventral paratrigeminal nuclei, and spinal trigeminal nucleus interpolaris), an area just ventromedial to MDH, the caudal dorsal reticular formation and the area of the A5 catecholamine group compared to control animals. Water stimulation of the nasal passages produced increased FOS label only in the A5 catecholamine group. There was increased FOS label in the ammonia group in the ventral laminae 1 and 2 of MDH and the ventral paratrigeminal nuclei compared with the water group. We conclude that ammonia stimulation of the nasal passages produces a different pattern of neuronal activation within the brainstem compared with water stimulation. In addition, we found that FOS immunohistochemistry did not detect significant changes in neuronal activation within known cardiorespiratory control centers, even though stimulation of the nasal passages produces both apnea and changes in heart rate and blood pressure.

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47.15

CHRONIC CHANGES IN S100ß CONCENTRATION WITHIN THE RAT TRIGEMINAL BRAINSTEM FOLLOWING ADULT INFRAORBITAL NERVE TRANSECTION. B.G. Klein*, and D. Jones, Dept. of Biomedical Sciences and Pathobiology, College of Veterinary Medicine, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061.

We have previously demonstrated increases in serotonin (5-HT) content and immunoreactivity within rat trigeminal subnucleus interpolaris (SpVi) correlated with somatosensory alterations following adult infraorbital nerve (ION) transection. No such lesion-induced increases in 5-HT afference were observed within subnucleus caudalis (SpVc). We have also demonstrated that ION transection is not associated with a chronic change in the density of SpVi cells immunoreactive for S100ß, a 5-HT growth regulator. This does not preclude that lesion-induced changes in S100ß content or release in those S100ß immunoreactive cells might influence alterations of 5-HT afference following nerve transection. Here, we investigated whether ION transection was associated with a change in S100ß concentration within the trigeminal brainstem. Eleven adult male rats were decapitated approximately one year after ION transection and brains were rapidly removed and frozen on dry ice. Nine normal rats were subjected to a similar procedure. Transverse sections were then made through SpVi and SpVc and samples from each section were taken according to previously described methods. S100ß content was then analyzed by an enzyme-linked immunosorbent assay (Griffin et al., 1993). Concentrations of S100ß were calculated following protein assay of the brain samples. Within SpVi, no significant differences in S100ß concentration were found between the medians of the lesioned and intact sides of the lesioned rats nor between the intact side and normal rats. However, in SpVc, median S100ß concentration on the lesioned side (0.11 ng/mg) was significantly less ($p<.005$) than on the intact side of lesioned rats (0.94 ng/mg), while the intact side did not significantly differ from normal. Support: Virginia Center on Aging ARDRA Fund & VA-MD Coll. Veterinary Medicine.

47.16

ANODALLY-FOCUSSED POLARIZATION ALLOWS DIFFERENTIATION OF LARGE AND SMALL PRIMARY AFFERENT INPUT TO CAUDAL BRAINSTEM. J.C. Petruska*, C.H. Hubscher, and R.D. Johnson. Depts. of Neurosci. and Physiol. Sciences, University of Florida, Gainesville, FL, 32610.

Various techniques are currently used to study the processing of nociceptive information conveyed centrally by small diameter afferent fibers (SDAF; thinly myelinated Aδ and unmyelinated C). These fibers are activated by noxious stimuli (mechanical, thermal, chemical, electrical) applied directly to the nerves or their somatic or visceral territories. These stimulation methods are confounded by (i) damage to the tissue, receptors, or peripheral nerves and (ii) large diameter afferent fiber (LDAF; Aβ and large Aδ) activation (i.e., LD+SDAF stimulation). Alternatively, application of anodally-focussed polarization to a peripheral nerve blocks activity of LDAF and thus allows selective propagation of strictly SDAF action potentials (Petruska and Johnson, Soc. Neurosci. Abst., 1994) without these confounding problems. In the present study, the applicability of this technique for accurately differentiating between large and small peripheral fiber input to neurons in the CNS was assessed using electrophysiological techniques. Electrical stimuli were applied to the tibial and caudal cutaneous sural (CCS) nerves in urethane-anesthetized rats. Polarization electrodes were placed on the sciatic nerve at the hip. Recordings were made from neurons in the nucleus gracilis (NG) and nucleus reticularis gigantocellularis (Gi) having receptive fields innervated by the tibial or CCS nerves. Responses of these single NG and Gi neurons were tested in response to both LDAF and LD+SDAF stimulation, with and without polarization. As expected, most NG neurons responded preferentially to LDAF stimulation alone, although preliminary data suggest SDAF mediated affects on some neurons in NG. Neurons in Gi responded to LD+SDAF stimulation. Polarization blocked LDAF mediated but not SDAF mediated neuronal responses in both nuclei. Thus, this technique allows for the identification of small versus large afferent fiber mediated input to CNS neurons. Supported by UF DSR and NIH Center MH15737 grants.

47.17

POSTSYNAPTIC EFFECTS PRODUCED BY MOTOR CORTEX AND PRIMARY AFFERENT FIBRES IN THE CUNEATE NUCLEUS OF THE CAT. L Martinez*, J Mariño and A Canedo. Dep of Physiol. Fac of Med. Univ of Santiago de Comp. 15705, Santiago.SPAIN.

The experiments were carried out in anaesthetized (α-chloralose) and paralyzed (pavulon) cats. The records were restricted to the medial cuneate nucleus (0-4 mm caudal to the obex) by using the whole-cell patch recording technique. Cuneothalamic neurons (CTNs) were identified by stimulating the contralateral medial lemniscus (ML) at A2. In addition, stimulating electrodes were placed in the ipsilateral dorsal columns (DC) at C2; in the forearm representation area of the motor cortex (MCx); and, once determined, in the center of the cutaneous receptive field (RF) for each neuron. Both MCx and afferent stimuli at DC or RF evoked postsynaptic inhibition or excitation on cuneate cells (CNs). Electrical stimulation of the RF elicited doublets of spikes (mean latency 6.2, range 5.6-7.2), doublets or bursts of discharges (mean latency 6.4, range 4.8-9.8) in CTNs and interneurons respectively; and also evoked inhibitory postsynaptic effects lasting 20-120 ms (IPSPs, mean latency 6.7 ms, range 5-9 ms) in CNs. MCx stimulation elicited excitatory postsynaptic effects (EPSPs) in all the cuneate interneurons tested (n=30; mean latency 5.4, range 2-10 ms), and IPSPs of 60-120 ms duration in all the tested CTNs (n=31; mean latency 9 ms, range 7.3-15 ms). The EPSPs seemed to be generated at the dendritic arbor distant from the soma, since they were not affected by the polarization of the membrane; and the IPSPs appeared to be generated at somatic level for they reversed polarity with injections of negative current. Responses evoked by stimulation of the RF, DC and MCx were also found at hyperpolarized membrane potentials, suggesting activation of a low threshold calcium conductance in analogy with other places (e.g. thalamus). Presumed calcium dendritic spikes were also recorded following RF and MCx stimuli. Protocols specifically conducted to reveal presynaptic inhibition gave negative results. Thus, trains of long duration (up to 750 ms) applied to the DC at several frequencies (from 10 up to 500 Hz) were consistently followed in a one-to-one manner by the CTNs, but the interneurons failed at stimulus frequencies of 100 Hz or higher. The frequency-following failure of the interneurons was not due to the amplitude decrease of the EPSPs, indicating that the release of neurotransmitter was normally produced. Instead, the spike generating mechanism was disrupted probably due to the inactivation of the postsynaptic sodium channels. Finally, paired pulses (stimulating DC as conditioning and RF as test, at different intervals) did not reveal presynaptic processes over CNs. It is concluded that, in our experimental conditions, both MCx and primary afferent stimuli evoked postsynaptic effects on cuneate neurons without evidence of presynaptic influences.
Supported by DGICYT. JM is a fellow of the Xunta de Galicia.

47.18

RAPID CHANGES IN PRIMATE CUNEATE NUCLEUS MAPS AFTER HAND NERVE INJURY. J. Xu* and J.T. Wall. Department of Neurobiology & Anatomy, Medical College of Ohio, Toledo, OH 43699.

Cortical maps of the hand begin reorganizing within minutes-hours after injury of hand nerves. The main cuneate nucleus (CN) contains a map of the hand that is the origin of lemniscal projections to cortical hand maps. To assess potential CN contributions to cortical reorganization, this study mapped the CN of monkeys during the first minutes-hours after distal forearm transection of the ulnar and median nerves. Functional organization in CN regions that normally represent the hand was clearly altered following this injury. Cutaneous fields of neurons at some recording sites underwent rapid shifts from, for example, glabrous hand locations innervated by the sectioned nerves, to hairy hand or forearm locations innervated by the intact radial nerve. Due to these changes, normal discontinuous and small representations of hairy hand skin innervated by the radial nerve became more continuous and larger (normal mean = 16% [+/-3] versus postinjury mean = 28% [+/-4] of CN transverse area; $t[11] = 5.70; p < 0.001$). Within these postinjury representations, inputs from proximo-radial to more disto-ulnar locations on the hairy hand were represented at ventral to dorsal locations in the CN. Neurons at other CN locations lost cutaneous responsiveness, and became either unresponsive or only responsive to tap stimuli (normal mean = 3% [+/-2] versus postinjury mean = 18% [+/-5] of CN transverse area; $t[11] = 7.21; p < 0.001$). Overall, normal CN organization patterns characterized by a continuous representation of the glabrous hand and discontinuous, small representations of the hairy hand, were transformed into abnormal patterns characterized by no representation of the glabrous hand, larger continuous representations of the hairy hand, and large cutaneously unresponsive regions. These transformations resemble previously reported cortical map transformations seen during the first minutes-hours after this injury. Thus, initial central reorganization after this injury entails CN and cortical changes that are similar in nature and that appear with a similar rapid time course. Supported by NIH Grant NS21105.

47.19

SIZE RELATIONSHIPS OF THE HAND AND CUNEATE NUCLEUS IN HUMANS AND NONHUMAN PRIMATES. X-F Wu*, J. Nguyen, M.J. Forgues, and J.T. Wall. Department of Neurobiology & Anatomy, Medical College of Ohio, Toledo, OH 43699.

Recent studies in monkeys describe size relationships of the hand and the main cuneate nucleus (CN), the brainstem nucleus receiving cutaneous inputs from the hand. Extrapolation of these relationships to humans presumes similarities across species; however, given the large differences in body and brain sizes of humans and monkeys, this presumption may not be valid. To evaluate size relationships of the hand and CN in human and nonhuman primates, this study compared peripheral volumes of the hand skin, and central volumes of the CN, in adult humans and squirrel monkeys. Hand skin thickness and area were measured from hand dissections to determine hand skin volume. CN volumes were determined from three dimensional reconstructions of cytochrome oxidase stained sections that spanned the CN. In humans, the hand skin occupied a mean volume of 62,569 mm^3 (range = 58,647-66,143). The CN in humans occupied a mean volume of 57 mm^3 (range = 52-65). In contrast, the hand skin in monkeys occupied a smaller mean volume of 1,852 mm^3 (range = 1,438-2,379). Likewise, the CN in monkeys occupied a smaller mean volume of 2.3 mm^3 (range = 2.1-2.5). From these findings, it is clear that CN volumes are much smaller than hand skin volumes in both humans and monkeys. To quantify this scaling, compression factors were calculated from the ratio: hand skin volume (mm^3) / CN volume (mm^3). Compression factors were similar in humans (mean = 1,098; range = 902-1,272) and monkeys (mean = 805; range = 575-1,132). Thus, as would be expected, hand skin and CN volumes are larger in absolute size in humans. This difference aside, the compression factors indicate that scaling of the relative sizes of the hand and CN is similar in humans and monkeys. Supported by NIH Grant NS21105.

47.20

"REAL-TIME" MONITORING OF WHISKING KINEMATICS IN THE HEAD-FIXED RAT. R. Bermejo*, D. Houben and H. P. Zeigler, *Biopsychology Program, Hunter College, City University of New York, NY.*

Neurobehavioral studies of the rat's vibrissa system require isolation of individual vibrissae, experimental control of vibrissa movements, and measurement of the kinematics of these movements. We have previously described a head-fixed preparation for isolation and experimental control of individual vibrissa movements. To increase the utility of this preparation we have developed an inexpensive system for the "real-time" monitoring of individual vibrissa movements with high spatial and temporal resolution (13µ; 1 msec). The system utilizes an optical sensor composed of an array of 2048 light-sensitive elements (CCDs). The sensor is mounted below the whisker pad and aligned with the axis of the body. Illumination of the array by a collimated light source generates a constant baseline voltage in each element. Interruption of the light by an object generates a voltage shift in a subset of elements. Movement of the whisker with respect to the sensor produces successive displacements in the *position* of that voltage shift which are linearly related to the whisker position. The entire CCD array is repeatedly scanned to read the voltage at each of its elements. A comparator circuit identifes the successive positions of voltages above a preset threshold and outputs the data to a microprocessor for computation of the whisker movement trajectory. The system may be used to monitor the kinematics of a single vibrissa movement with all other whiskers clipped, or the relative detectability of a selected single whisker may be differentially increased. Because amplitude and velocity measurements are made "on-line", preselected output values of these kinematic variables may be used to trigger differential reinforcement in conditioning paradigms. The system should facilitate behavioral, electrophysiological and functional marking studies of the rodent's whisking behavior.
(Supported by Research Scientist Award MH-00320 to H. P. Z.).

48.1

ACTIVITY ASSOCIATED EXPRESSION OF ZIF268 IN THE RAT WHISKER BARREL FIELD. L. Rioux[1*], J. Nissanov[2], A. Chaudhuri[3].
[1]Dept. Pharmacology, Univ. Pennsylvania, Philadelphia, PA 19104.
[2]Biomed. Eng. & Sci. Inst., Drexel Univ., Philadelphia, PA 19104.
[3]Dept. Psychology, McGill Univ., Montreal, PQ H3A 1B1, Canada

Activity dependent expression of the immediate early genes (IEG) has been widely utilized for functional brain mapping. Due to the punctate characteristic of labeling, a consequence of the nuclear compartmentalization of these proteins, immunodetection of these gene products can support visualization of neuronal activity at spatial resolution of individual neurons. We exploited this feature to examine the relation between sensory stimulation of the whiskers and expression of Zif268 in the somatosensory cortex. Previous studies (Melzer and Steiner, 1994, Neurosci Abst 20:566.9; Steiner & Gerfen, 1994, J. Comp. Neurol. 344:297-304), utilized *in situ* hybridization to demonstrate that an increased level of expression of the *zif268* mRNA is observed in response to whisker stimulation. Is this due to increased level of expression by individual cells only or is there an increase in the number of *zif268* expressing cells? We evaluated this by comparing the contralateral and ipsilateral somatosensory cortex following first clipping of the whiskers on one side and then three hours of light brushing of the whiskers on the intact side. Whisker barrel fields were visualized using cytochrome oxidase and the number of Zif268 labeled cells within the barrel field counted on adjacent sections. A 50% to 100% greater number of labeled cells was observed on the side contralateral to the stimulated whiskers over the ipsilateral side. The increase in Zif268 immunopositive neurons suggests that the increase in *zif268* mRNA abundance is also due in part to increase in the number of expressing cells. (Supported by NIH P41RR01638, MRC Canada [MA 12685]).

48.2

ACTIVITY DEPENDENT CHANGES IN THE DISTRIBUTION OF CORTICO-CORTICAL AFFERENTS TO SI IN ADULT MONKEY.
M. Costa*, A. Nuñez, J.A. Gandía and E. Rausell. Dept. of Morphology, School of Medicine, Autónoma University, 28029 Madrid, Spain.

The modifications that occur in the extent of partial body SI representations under activity dependent conditions can be explained on the basis of sprouting of cortico-cortical axons from other somatosensory areas. We have explored the possibility of this being one of the underlying mechanisms for the extension of the representation of digit 2 (D2) into the representation of digit 1 (D1) after peripheral selective denervation of D1.

The lateral border of D2 representation in area 3b was identified by means of single unit extracellular recording in normal adult anesthetized monkeys (Macaca Fascicularis), and in monkeys that had been subjected to peripheral selective cutaneous denervation of the first digit fifteen days earlier. Small paired injections of Fast Blue (FB) and Diamidino Yellow (DY), or fluorescent Texas Red and Fluorescein labeled dextrans (Molecular Probes) were made at each side of this border. Retrogradely single and double labeled cortico-cortical cells were localized in areas 2, 1 and SII, then plotted and counted, using the Neurolucida system and software. 3-D reconstructions of the "clouds" of labeled cortico-cortical cells were made at each cortical area.

In normal monkeys, pairs of fluorescent injections separated about 100 µm resulted in less than 10% neurons retrogradely labeled with both dyes in areas 1,2 and SII. In denervated monkeys, pairs of fluorescent dyes separated 150-250 µm, resulted in a range of 15-20% neurons retrogradely labeled with both dyes, particularly in areas 1 and 2. We analyzed the degree of overlapping between the two sets by calculating the "segregation" and "probability" indices (cf. Agmon et al., J. Neurosci., 15: 549-561, 1995), in each case. We then calculated the degree of correlation between significative variations in those indices and the experimental denervation, to explore the presence of pre-existing collaterals or the occurrence of sprouting of new cortico-cortical terminals.

Our results suggest that sprouting of cortico-cortical terminals might have occurred in the deafferented cortical region, and could be considered as an additional mechanism underlying the plastic changes observed in the SI cortical representation under activity dependent conditions.

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48.3

INTRACORTICAL MICROSTIMULATION INDUCES LONG-TERM DEPRESSION OF FIELD POTENTIALS IN THE RAT SOMATOSENSORY CORTEX *IN VITRO*. G. Boehmer*, P. Heusler, H.R. Dinse. Dept. of Physiology and Pathophysiology, Johannes Gutenberg University, 55099 Mainz, Germany.

Intracortical microstimulation (ICMS), i.e. repetitive electrical pulse trains delivered to the somatosensory cortex of the rat *in vivo*, results in cortical reorganization of skin field representation. The present *in vitro* study aimed at examining mechanisms underlying this type of cortical plasticity. Experiments were performed on brain slices (500 µm) including the rat somatosensory cortex. Brain slices were superfused with carbogen-saturated artificial cerebro-spinal fluid (ACSF) at 36 °C. Field potentials (FP) were evoked by electrical stimulation in cortical layer IV/V using steel-needle electrodes. FP were recorded from cortical layer II/III using ACSF-filled glass-microelectrodes. ICMS was delivered to cortical layer IV/V by a steel-needle electrode. For ICMS pulse trains of 40 ms duration (13 pulses of 100 µs and 400 µA to 1 mA at 300/second) were repetitively delivered at a rate of 1.5/second. Duration of ICMS was 100-150 minutes. FP were composed of an antidromic component (neither blocked by APV, NBQX, or low-calcium ACSF) and several synaptic components (S1-Sn). S1 mainly depended on activation of AMPA receptors (blocked by NBQX), while the contribution of NMDA receptors (blocked by APV) was small. Contribution of NMDA receptor activation increased with increasing order number of the synaptic component. ICMS induced a reduction of the amplitude of the synaptic components as well as an increase of the synaptic delay. After cessation of ICMS a transient strong effect was observed during the first minutes reaching a lower but significant level of depression outlasting the observation period of 90 minutes. When ICMS was delivered during continuous superfusion of the brain slice with ACSF containing bicuculline methochloride (1 µM) the reduction of S1 amplitude and the increase of S1 latency was strongly enhanced. Effects of ICMS were interpreted in terms of the induction of long-term depression (LTD) of synaptic efficacy. Results suggest an involvement of LTD in ICMS-induced reorganization of representational maps of the somatosensory cortex.

48.4

TRAINING IMPROVES SOMATOSENSORY DURATION DISCRIMINATION IN HUMANS **S.S. Nagarajan*, D.T. Blake, B.A. Wright and M.M. Merzenich, Keck Center, UCSF, CA 94143-0732.**

The purpose of this study was to determine whether or not and how specifically normal adult humans learn to improve somatosensory duration discrimination with practice. In a two-alternative forced choice paradigm, subjects were presented with one pair of vibratory pulses separated by a base duration (BD) and another pair by a target duration (TD). Subjects indicated which pair was the TD which was always greater than the BD by a variable amount and was adjusted adaptively over 60 trials to determine discrimination thresholds expressed as Weber fractions [($TD_{threshold}$-BD)/BD)]. The flutter-vibration pulses were sinusoidal displacements (100µm, 25ms, 40Hz) delivered with a probe (1.5mm diameter with a rigid surround) either to the thenar eminences (n=4) or the distal segment of the ring finger (n=6). Each subject was trained for 900 trials/day with a BD of 100ms for 10-15 days. Significant improvements (mean change from 0.35 to 0.17) were observed between mean Weber fractions measured before and after training ($F_{1,9}$=37.04, p<0.0005). Before and after training, Weber fractions were also measured for different BDs, skin-sites and pulse frequencies. Observed improvements at the trained BD transferred to adjacent digits ($F_{1,4}$=39.5,p<0.005) and to the same skin-site in the contralateral hand ($F_{1,9}$=6.28,p<0.05). By contrast, when the pulse frequency was changed to 200Hz there was no significant learning at the trained duration ($F_{1,3}$=0.01,p>0.9). Interestingly, improvements transferred to BDs of 50 ms ($F_{1,9}$=10.4,p<0.01) but not to BDs of 200ms ($F_{1,9}$=3,p>0.1) or 500ms ($F_{1,3}$=7,p>0.05). Control subjects tested over comparable periods without training showed no significant changes on any condition ($F_{1,4}$=0.231, p>0.65), indicating that training accounted for all observed changes. These results indicate that (1) somatosensory duration discrimination improves with training and (2) this learning generalizes widely across skin-sites, even to the untrained hand and that it also generalizes to a shorter (50ms) duration, but not across stimulus frequencies and to durations longer than 100ms. This data suggests channel and duration specificity in learning somatosensory durations. **[Supported by the Charles A. Dana Foundation and NIH Grant NS-10414]**

48.5

ENVIRONMENTAL ENRICHMENT COUNTERACTS DECLINE OF SENSORIMOTOR PERFORMANCE AND DETERIORATION OF CORTICAL ORGANIZATION IN AGED RATS. L. Churs, F. Spengler, M. Jürgens, H.R. Dinse*. Inst. Neuroinformatik, Ruhr-Univ. RUB, Bochum, Germany

We have shown that aging results in severe deterioration of a number of parameters reflecting cortical organization such as receptive field (RF) size, RF overlap, temporal integration (TI) properties and response latencies of somatosensory cortex (SI) in old rats: Here we report that an enriched environment has a beneficial effect on the development of age-related cortical deterioration.

Starting at 26 months, FBNF1 rats were housed under enriched environmental conditions (EC) for 6-8 months. Age-matched controls were kept under social but input deprived conditions (SC). Sensorimotor performance was scored based on a on a pole-running test and on analysis of footprints and video recordings during walking. We recorded single- and multiple unit activity in layer IV of the fore- and hindpaw representations of SI of urethane anaesthetized EC and SC animals (31-35 months) and in normal adult, SC housed animals (3-7 months). RF size, response strength and latencies and TI properties were analyzed. Behavioral tests revealed significant improvements of sensorimotor performance and coordination in all enriched animals including prolonged pole-running, increased step length and decreased step width. The maintenance of behavioral fitness was paralleled by profound effects on cortical organization. In comparison to SC controls which showed the typical age-related enlargement of RF size, RFs in the EC-group were significantly smaller by about 50 %, thereby reaching an intermediate state between old SC and normal adult animals. The typical decline of TI properties in old animals due to a decreased ability to follow high frequency stimulation was not observed in old EC animals. Response latencies were lengthened with chronological age in both SC and EC animals.

The results demonstrate that enforced stimulation and exercise based on unspecific and multifactorial cues as provided by an enriched environment has a profound effect on cortical organization in old rats, thereby supporting the concept of "use it or loose it". The age-related lengthening of latencies in both EC and SC animals indicates that this parameters seem not to be involved in plastic reorganizational processes during aging (cf. the effects of a CA^{2+} blocker in old animals, this meeting).

We acknowledge the supply of FBNF1 rats by Tropon, Germany. Supported by Sandoz Foundation for Gerontol. Research

48.6

EFFECTS OF REPETITIVE SENSORY STIMULATION ON HUMAN CORTICAL RHYTHMS L. Narici*[2], K. Portin[1], R. Salmelin[1] and R. Hari[1] [1]Brain Research Unit, Low Temperature Laboratory, Helsinki University of Technology, 02150 Espoo - Finland; [2]Department of Physics, University of Rome "Tor Vergata", 00133 Roma - Italy

The effect of short trains of somatosensory, visual, and auditory stimuli on the human cortical rhythms was studied by recording MEG signals from 9 healthy subjects with a 122-channel Neuromag-122™ whole-scalp neuromagnetometer. Electric 0.3-ms pulses to the median nerve at intensity above motor threshold, 30-ms light pulses with red 1 mm-diameter LEDs covering 1 cm^2 at an eccentricity of 7 deg in the left hemifield, and 15-ms monaural noise bursts were delivered in separate sessions in trains of 15 stimuli, interleaved with 1.6-s pauses. The stimulus repetition rates within the trains varied randomly from 6 to 14 Hz. Two different phenomena were observed in cortical signals in the 5 to 25 Hz frequency band: driving of rhythms during the stimulus trains and global suppression of all cortical spontaneous signals after the stimulus trains. Driving was seen as a clear enhancement of cortical activity at the stimulus frequency in the sensory-specific cortex of the stimulated modality. After stimulus trains, spontaneous activity was suppressed below resting level for a few tens of seconds. At least three stimuli in a train were needed to induce the post-stimulus suppression. The suppression did not depend on the stimulated modality, nor did it show lateralization. Our results indicate that repetitive stimuli may alter the functional state of human cerebral cortex for relatively long periods.

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48.7

SPATIOTEMPORAL REPRESENTATION OF MULTI-WHISKER STIMULI IN THE THALAMOCORTICAL LOOP. A. A. Ghazanfar*, L.M.O. Oliveira, V.S. Votaw, and M.A.L. Nicolelis. Dept. Neurobiology, Box 3209, Duke University Medical Center, Durham, NC 27710.

Although rats use rhythmic movements of most of their whiskers to discriminate surfaces, few studies have applied multiple whisker stimulation to characterize neuronal responses in the VPM and SI cortex. Since distributed representations have been observed at both levels for single whisker stimuli, we hypothesized that neuronal ensembles in these structures should be optimally organized to process object attributes based on patterns of multiwhisker stimulation. To explore this possibility, we have combined ensemble recordings in the VPM and layer V of SI cortex with stimulation of whisker rows and columns at 1-10Hz. We recorded from 263 SI cortical neurons and 177 VPM neurons in 12 different animals under pentobarbital anesthesia while stimulating sets of three whiskers, either in rows or columns. A large proportion of SI and VPM neurons showed a summated response following multiwhisker stimulation that was greater than the arithmetic sum of the individual whisker responses. Furthermore, some neurons only responded to multiwhisker stimuli. In cortex, the amount of non-linear facilitation covaried with the receptive field of the neuron: summation was greatest for rows in the surround receptive field and neurons with RF centers in caudal whiskers showed greater summation for rostral than caudal whisker columns. In some instances, multiwhisker stimuli recruited more cortical tissue faster, and the responses lasted longer, than those obtained for single whisker stimuli. Some of these cortical properties were altered by neonatal facial nerve section which disrupted whisker movements. Thus, we suggest that, during active exploration, patterns of multiwhisker contact continuously shape representations in the thalamocortical loop and that this process is computationally useful. *Supported by the Whitehall Foundation.*

48.8

IMMEDIATE AND SIMULTANEOUS REORGANIZATION IN THE BRAINSTEM, THALAMUS, AND CORTEX INDUCED BY PERIPHERAL DEPRIVATION. B.M. Faggin*, K.T. Nguyen and M.A.L. Nicolelis. Dept. Neurobiology, Duke University, Durham, NC 27710.

Previously (Nicolelis et al, 1993), we have demonstrated that small injections of local anesthetics in the rat face induce an immediate reorganization in the ventral posterior medial nucleus of the thalamus (VPM). Since these changes involved both short and long-latency components of neuronal responses, we suggested that both ascending and descending pathways should contribute to this reorganization. Here, we tested this hypothesis by recording the simultaneous activity of up to 100 neurons, located in the principal (PrV) and spinal (SpV) subnuclei of the trigeminal brainstem complex, the VPM, and the infragranular layers of the primary somatosensory cortex (SI). The same set of neurons were recorded before and after lidocaine (1%, 0.01-0.04 ml) was injected around the whisker pad. Stimulation of at least 15 single whiskers were used to quantify changes in receptive fields (RF) induced by the facial anesthesia in 12 Long-Evans rats. Simultaneous reorganization of the whisker representation in the PrV/SpV, VPM, and SI cortex was observed a few minutes after the lidocaine injection. This global reorganization was characterized by changes in both the spatial and temporal domains of RFs. In each structure, we identified a silent area, where most responses were abolished; a transition area, where responses either increased or decreased; and an unmasking area, where new sensory responses emerged. In this latter region, we observed unmasking of both short-latency (SpV = 0-5 ms, VPM = 4-8 ms, SI = 8-15 ms) and long-latency components (SpV = 5-10 ms, VPM = 15-25 ms, and SI cortex = 15-40 ms). These results support the hypothesis that plastic reorganization observed at each level of somatosensory system depends on changes occurring at other levels of the pathway. *Supported by NIH:DE-11121-01.*

48.9

DEPTH PROFILES OF THE RECIPROCAL CORTICOCORTICAL AND THALAMOCORTICAL RESPONSES IN SI AND SII NEOCORTEX OF RATS. K. Paul, P. Hamilton, and L.J. Cauller*, GR 41, Cognition and Neuroscience Program, University of Texas at Dallas, Richardson, TX 75083.

This study employed depth profiles (0.1 mm increments) of evoked field potentials, multiple unit activity (MUA) and current source density to examine the cortical responses to somatosensory, thalamocortical or corticocortical inputs recorded in the forepaw areas of the primary (SI) and secondary (SII) somatosensory areas of anesthetized rats. The SI response profile evoked by VPl thalamic stimulation was similar to the profile evoked by forepaw stimulation: peak-negative field potentials, MUA and current sinks beginning in layer IV followed by current sinks and unit activity ascending to supragranular layers. In addition, VPl stimulation evoked a short latency layer VI response. The response profile evoked in SII by stimulation of SI resembled the somatosensory or thalamocortical SI response consistent with the characteristic termination of "forward" cortical projections from SI to layers III/IV of SII. In contrast, the SI response to stimulation of SII involved superficial peak-negative field potentials and current sinks with MUA distributed through deeper layers consistent with the characteristic termination of "backward" cortical projections from SII to layer I/II of SI. Similarly, the response of SI and SII to stimulation of the VM thalamus involved a more superficial activation consistent with the superficial termination of the VM thalamocortical projections. In sum, these electrophysiological profiles in anesthetized rats correspond to the established laminar patterns of thalamocortical and reciprocal corticocortical projections between areas of the somatosensory cortex (Clancy, *et al.* 1995, *Soc. Neurosci. Abst.*; Mitchell and Cauller *ibid*). Supported by a grant from the Whitehall Foundation.

48.10

LAMINAR ANALYSIS OF SOMATOSENSORY ACTIVITY IN AREAS SII, 5, and 7b OF THE AWAKE MONKEY: I. EVIDENCE FOR PARALLEL THALAMO- AND SERIAL CORTICO-CORTICAL INPUT. **M.C. Lee* and J.C. Arezzo. Departments of Neuroscience and Neurology, Albert Einstein College of Medicine, Bronx, NY 10461.**

Recent studies have suggested an evolutionary transition from parallel to serial modes of somatosensory processing in lower to higher mammals. The present study was designed to investigate the issue of hierarchical versus parallel models of cortical organization in primates. Contralateral somatosensory evoked potentials (SEPs) and multiunit activity (MUA) were recorded intracortically from neuronal ensembles with a linear array, multicontact (15 channels, 200 μ intercontact spacing), low-impedance electrode (300-500 kOhm at 1kHz) in three male monkeys *(M. fascicularis)*. One-dimensional current source density (CSD) profiles were calculated and used to determine the sequence of activity onset: i) across laminae within each cortical "column" sampled, and ii) across equivalent laminae from discrete cortical areas.

Initial activity, determined by earliest onset cortical sinks and MUA, indicates that the granular laminae of these areas are activated about 2 ms within that of SI. This latency difference is not consistent with feedforward input from SI, given that the separation of initial activity in layer IV versus that in layers II/III of SI itself is on the order of 4 ms. Later activity in SII and portions of 5 and 7b, determined by longer latency cortical sinks, are usually associated in time with long-latency, long-duration MUA. This activity is localized in the supragranular laminae and may reflect intrinsic feedforward or feedback cortical processing or continuous afferent input.

The timing and laminar pattern of activity apparent in these data provide evidence to support both early, parallel, thalamic input and later, serial, cortical input to the non-primary somatosensory areas SII, 5, and 7b. In addition, the present results suggest that the previously anatomically defined thalamic afferents to these areas are indeed activated by passive stimulation. Finally, the demonstration of multiple, independent, temporally overlapping generators precipitates the question of what is the contribution of these nonprimary elements of the somatosensory network to the surface response? Supported by MH06723.

48.11

LAMINAR ANALYSIS OF SOMATOSENSORY ACTIVITY IN AREAS SII, 5, and 7b OF THE AWAKE MONKEY: II. CONTRIBUTION TO LONG-LATENCY SURFACE SEPs. **J.C. Arezzo* and M.C. Lee. Departments of Neuroscience and Neurology, Albert Einstein College of Medicine, Bronx, NY 10461.**

The epidural somatosensory evoked potential (SEP) recorded over the dorsolateral convexity in primates is characterized by early components occurring at 12, 20, and 45, and by compound late responses peaking at approximately 110 and 160 ms. Previous studies isolated the generators of the initial components to the anterior and posterior banks of the central sulcus, and the crown of the postcentral gyrus. The present study was designed to investigate the contribution of the non-primary areas SII, 5, and 7b, whose activity is described in the accompanying study, to the later components of the scalp-recorded responses.

Contralateral, ipsilateral, and bilateral SEPs and multiunit activity (MUA) were recorded in three awake male monkeys *(M. fascicularis)*, as described in the previous abstract. Laminar profiles in the contralateral non-primary cortical areas sampled are characterized by complex patterns of polarity inversions and associated regional transmembrane current flow (one-dimensional CSD), with prominent activity ranging from 50 to 200 ms post-stimulation. In contrast, stimulus driven increases or decreases in MUA are completed by 100 ms. Ipsilateral and bilateral response profiles are similarly characterized by long-latency, long-duration SEP and CSD activity. In most sites, the magnitude of transmembrane current flow and associated long-latency activity exceeds that of the initial responses. Analysis of both the intracortical and volume conducted signals confirms the presence of several "open-field" generator configurations with potentials traceable from the epidural surface to their deep intracortical dipole sources (e.g. banks of the intraparietal and lateral sulci).

These findings suggest that SII, 5, and 7b contribute to the late components of the epidural SEP, and further, that the surface response also represents activity in select ipsilateral cortical regions. Long-latency SEP responses in humans may thus provide an index of the functional integrity of bilateral non-primary cortical somatosensory areas. Supported by MH06723.

48.12

THE PROPERTIES OF THALAMOCORTICAL AND INTRACORTICAL SYNAPTIC TRANSMISSIONS IN THALAMOCORTICAL SLICES OF THE RAT BARREL CORTEX STUDIED BY OPTICAL RECORDING. H. INOKAWA*, S. HIGASHI, T. KUROTANI, E. AKASE, & K. TOYAMA Department of Physiology Kyoto Prefectural University of Medicine, Kyoto 602, Japan

Our previous optical recording study of rat thalamocortical slices revealed patchy excitation occurring in layer 4, which corresponded to morphologically identified barrels, after electrical stimulation of ventrobasal nucleus (VB) of the thalamus. In the present study, we analyzed laminal differences in the responses evoked by VB stimulation and the receptors contributing to thalamocortical and intracortical synaptic transmissions. VB stimulation produced steeply rising responses (fast responses) in layers 4 and 6 (latency 4.8 ms ± 0.66) followed by slow responses appearing in layers 2/3 and 5 (latency 7.8 ms±0.76 and 6.7 ms ±0.70, respectively) as well as in layers 4 and 6. The differences in the latency between the fast and slow responses suggests that the former represents monosynaptic excitation, while the latter represents the polysynaptic excitation transmitted via layers 4 or 6. Consistent with this supposition, high divalent cations (6 mM Ca^{2+} and 6 mM Mg^{2+} in krebs), which reduce polysynaptically activated responses, selectively reduced the slow responses in all layers. The NMDA receptor antagonist APV (50 μM) only slightly reduced the slow responses in all of the layers without affecting the fast responses in layers 4 and 6 when bath applied, while the AMPA receptor antagonist DNQX (40 μM) almost completely abolished both the fast and slow responses in all of the layers. In addition, when intracortical inhibition was blocked by the $GABA_A$ receptor antagonist bicuculline (20 μM) preferentially enhanced the slow responses. These results suggests that fast and slow responses are mediated by thalamocortical and intracortical connections, respectively, that the AMPA receptors dominate over the NMDA receptors in the thalamocortical as well as the intracortical transmission, and that the intracortical transmission is under strong control of GABA inhibition.

48.13

LOCAL INHIBITORY RESPONSES IN THALAMOCORTICAL RELAY NEURONS: COMPARISON OF RAT AND GUINEA PIG IN VITRO

C.-Q. Kao*, D.A. Coulter, Department of Neurology, Medical College of Virginia, Richmond, Va 23298

GABAergic inhibition originating from nucleus reticularis thalami (NRT) synchronizes thalamocortical rhythms. However, the role of thalamic interneurons in generating rhythms is unknown. This is complicated by use of rodents in many of thalamocortical rhythm-generation studies. Rodents differ from primates and carnivores in that many of the thalamic nuclei lack GABAergic interneurons. We have begun to characterize local thalamic stimulation-evoked IPSPs within the ventrobasal complex (VB), comparing properties between rats and guinea pigs. These two species differ in that rats lack interneurons within VB, while guinea pig VB contains 15-20% interneurons, which synapse with relay neurons in the typical synaptic triad arrangement (Spreafico et al. *Neuroscience* 59:, 961, 1994).

Whole cell patch recordings of VB neurons in thalamocortical slices were employed to characterize responses evoked in the presence of D,L APV (50 μM) and CNQX (10 μM). Local stimuli activated IPSPs in VB neurons at a lower threshold in guinea pig than in rat, presumably due to the lower threshold activation of interneuron cell bodies compared to NRT-originating fibers. These low-threshold IPSPs had two components: an early, bicuculline-sensitive component, and a later, 2-hydroxysaclofen-sensitive component, with a reversal potential of approximately -110 mV. Further differential quantitative and pharmacological comparison of GABAergic inputs to VB neurons in these species could provide useful information clarifying the role of local circuit interneurons in generation of thalamocortical rhythms.

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48.14

THALAMOCORTICAL RHYTHMS *IN VITRO*: CELLULAR CONTRIBUTIONS OF CORTICAL, THALAMIC, AND THALAMIC RETICULAR NEURONS.

Coulter, D.A.* and Zhang, Y.-F. Department of Neurology, Medical College of Virginia, Richmond, VA 23298-0599

The thalamocortical (TC) system supports phasic oscillations as one of two main states of function. We have developed a TC slice which supports spontaneous TC oscillations when exposed to a medium containing no added Mg^{2+}. In the present study, we examine cellular contributions of thalamic, cortical, and thalamic reticular (NRT) neurons to TC rhythms.

Slice patch recording techniques were employed to assess cellular contributions to ongoing rhythms, recorded by an extracellular electrode located in cortex or thalamus. Extracellular stimulation was via an electrode located in NRT. Some neurons (20%) did not exhibit activity correlated with ongoing TC rhythms. These neurons were not considered further. All of the "participating" cortical neurons recorded exhibited strong excitation during TC rhythms (58/58 cells). Both NRT and thalamic neurons evidenced two populations which contributed to TC rhythms in contrasting ways. For NRT neurons, 58/86 recorded neurons were strongly excited during ongoing rhythms, with little evidence for inhibition contributing to activity. In these neurons, lateral stimulation within NRT rarely evoked IPSPs. In a second group of NRT neurons (28/86 cells), large IPSPs were evident during ongoing activity, often triggering rebound bursts. Lateral NRT stimulation usually evoked large IPSPs in these neurons. In thalamus, similar groups were seen, with 42 cells strongly excited, and 40 cells strongly inhibited (with rebound bursting) during ongoing rhythms. These data suggest that there are populations of NRT and thalamic neurons which contribute to TC rhythm generation in distinct ways. Support by NIH grant NS-31000.

48.15

REGIONALLY SELECTIVE BLOCKADE OF GABAergic INHIBITION BY ZINC IN THE THALAMOCORTICAL SYSTEM: FUNCTIONAL SIGNIFICANCE.

John W. Gibbs, III[2,3], Yun-Fu Zhang[1], Melissa D. Shumate[2], and Douglas A. Coulter[1,2,3] Depts. of Neurology[1], Physiology[2], and Anatomy[3], Medical College of Virginia, Richmond, Va. 23298.

GABAergic inhibition originating in the thalamic reticular nucleus (NRT) directed onto the thalamus serves to synchronize and drive thalamocortical (TC) rhythms. Timm's stain of coronal sections of rat brain demonstrated zinc-containing terminals in the cortex, thalamus, and particularly in NRT. To explore the role of endogenous zinc in modulation of rhythms generated within the TC system, we have begun to functionally characterize zinc effects on $GABA_A$-mediated currents in the thalamus, NRT, and the cortex. Effects of zinc were also investigated on TC rhythms in TC slices.

$GABA_A$-evoked chloride currents were recorded using whole-cell voltage clamp techniques in acutely isolated adult (>p60) rat NRT, thalamic, and cortical neurons. Zinc blocked $GABA_A$-mediated responses noncompetitively. Zinc concentration/response curves showed zinc effects to be strongest in thalamic, intermediate in NRT, and weakest in cortical neurons. Bath application of zinc 300 μM had differential effects on distinct types of TC rhythms. Zinc exacerbated rhythms resembling spike wave discharges, while it blocked rhythms resembling generalized tonic-clonic seizure discharges. The differential effects of zinc on TC rhythms suggests distinct neuromodulatory roles on differing seizure types supported by the TC system. Supported by NIH-NINDS Grant NS 31000.

48.16

CHARACTERISTICS OF CONDITIONS FOR PROPAGATING NEURONAL ACTIVITY IN NEOCORTEX. D. Golomb* and Y. Amitai. Zlotowski Center for Neuroscience and Dept. of Physiology, Ben-Gurion University, Beer-Sheva 84105, Israel.

In neocortex, evoked neuronal population discharges propagate over long distances when $GABA_A$ inhibition is blocked. Using field potential recordings and pharmacological manipulations in slices, together with a computational model, we have examined how the duration, shape and velocity of these discharges depend on the strength of excitatory conductances g_{AMPA} and g_{NMDA}. Complete blockade of NMDA receptors by APV (25-50 μM) reduced the velocity, but did not abolish propagation. Increasing the concentration of the AMPA blocker CNQX was followed by a parallel decrease in propagation velocity, until it ceased altogether. In the computational model, pyramidal cells were coupled via AMPA and NMDA synapses with a one-dimensional, spatially-decaying connectivity pattern. Brief local stimulus evoked traveling activity if g_{AMPA} was above a threshold $g_{AMPA,c}$. Activity was terminated by a slow outward current and by synaptic depression. Away from the stimulus, the activity profile maintained its shape and propagated at a constant velocity. Each cell fired n spikes during the discharge. The number n and the system's parameter set uniquely determined the discharge shape . Larger-n discharges were associated with a stronger initial stimulus. The model predicts: 1. For $g_{AMPA} \gg g_{AMPA,c}$, propagation velocity is linearly dependent on g_{AMPA} and is independent of n. 2. For $g_{AMPA} \gtrsim g_{AMPA,c}$, multiple discharge shapes with different n occur, depending on the stimulus strength. Propagation is faster for a larger n.

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SOMATOSENSORY CORTEX AND THALAMOCORTICAL RELATIONSHIPS III

49.1

MODULATION OF EVOKED SOMATOSENSORY ACTIVITY IN RAT BARREL CORTEX BY REMOTE NOCICEPTIVE STIMULI. R.T. Stevens*, W.W. Chuang and C.J. Hodge, Dept. Neurosurgery, SUNY Health Sci. Center, Syracuse, N.Y. 13210

Using intrinsic cortical optical imaging (COI), the rat barrel cortex was studied to determine whether the cortical response to low threshold inputs (whisker movement) would be altered when competitive stimuli are introduced at a remote cutaneous site. Optical images were obtained following single whisker movement and compared when the same whisker is activated coincident with stimulation of tail or hindlimb.

Using either thinned skull or partial craniotomy preparations, anesthetized rats were fixed in a stereotactic apparatus and skin was tied back to form a saline well over the barrel cortex. COI was done using a 850nm reflected light. Pairs of data files were collected and compared between whisker stimulation alone (W-alone) and whisker stimulation combined with a competitive stimulus applied to tail or hindlimb, i.e., vibration (W-v), warming (W-w), heat (W-h), or pinch (W-p).

When no competitive stimuli are introduced, the resulting paired W-alone images are similar in size and location. When a non nociceptive competitive stimulus was applied (W-v or W-w) the resulting COIs were not discernibly different from W-alone COIs. Painful heat (>60°C) applied to the tail or hindlimb produced some decrease in the whisker COI when compared to its paired W-alone COI although this effect was variable. A 3 second noxious pinch of tail, hindlimb or forelimb, however, dramatically decreased the whisker barrel COI. These results demonstrate a modulation of cortical response to peripheral inputs by remote noxious stimuli which is reminiscent of the process of Diffuse Noxious Inhibitory Controls previously described at the spinal cord level.

Supported by Dept. of Neurosurgery, SUNY HSC and by a gift from the Perkins Foundation.

49.2

THE CONNEXION BETWEEN MEDIAL THALAMUS AND ANTERIOR CINGULATE CORTEX: AN ELECTROPHYSIOLOGICAL STUDY. M.-M. Hsu[1] and B.-C. Shyu *. [1]Graduate Inst. of Life Sci., National Defense Medical Center and Inst. of Biomedical Sci., Academia Sinica, Taipei, Taiwan, R. O.C.

We attempted in the present study to characterize the neuronal properties of anterior cingulate cortex (ACC) evoked by electrical stimulation of medial thalamus (MT) and to verify the synaptic connection between these two structures.

Male Sprague Dawley rats (body weight 250 to 350g) were maintained in general anesthesia by α-Chloralose (50mg/kg, i.v.). Tungsten microelectrodes were used for electric stimulation in the MT (CL & Pf nuclei) and for recording in the ACC. The field potentials, multiple or single units activities in the ACC were evoked by electric stimulation of MT where the nociceptive responses were identified.

An evoked negative potential with 24 ms latency was recorded by MT stimulation on the surface of ACC. An early negative potential (15 ms latency) could be recorded between 0.5 and 1.0 mm from the surface. The polarity of the surface negative potential was reversed between 1.0 and 1.5 mm in the depth of the ACC. The evoked multiple units activities were occurred at about the latency of 15 ms or 24 ms at the depth between 1.0 mm to 2.0 mm of the ACC. A total of 68 units was identified histologically in the ACC. The mean ampltidue of the action potentials was 0.8 ± 0.1 mV (Mean ± S. E.). All of the responsive neurons were discharged spontaneously and about 32.3% (21/68) of them were firing in a burst pattern. The majority (93.7%. 63/68) of MT-evoked ACC units were activated trans-synaptically at the Cg1, Cg3, IL areas of ACC. The mean latency of response was 20.1 ± 1.4 ms. About 7.3 % (5/68) of units were activated antidromically at the Cg1 area and their mean latency was 9.6 ± 1.1 ms.

The electrophysiological findings in the present study have confirmed the neuroanatomical connection between MT and ACC. The nociceptive information in the MT is transmitted to ACC and may be modulated reciprocally by the activities from ACC. (Supported by grant from the National Science Council, ROC)

49.3

RAT SOMATOSENSORY CORTEX: AN ANATOMICAL SUBSTRATE FOR BILATERAL INTEGRATION Victor Pegado, David Froc, Emily Spironello & Kathryn M. Murphy*, Departments of Psychology, Neural Organization & Plasticity Laboratory, McMaster University, Hamilton ON Canada L8S 4K1

Primary sensory areas demonstrate physiological, metabolic and cellular compartmentalization that reflects their division into function modules. Within the rat somatosensory cortex inputs from the contralateral vibrissae form the barrel field which is comprised of dense thalamic inputs representing individual vibrissae and a surrounding lattice-work called the septa. The septa regions does not receive direct thalamic input and instead is involved in cortico-cortical connectivity, including callosal connections. Therefore, the septa region is where sensory information from the vibrissae on both sides of the snout is integrated. We have investigated the features of the septa that could provide a substrate for those neurons to integrate bilateral somatic stimulation. Using immunohistochemical techniques the distribution of large pyramidal neurons (SMI-32) and the NMDAR1 subunit of the voltage-gated NMDA receptor was examined in tangential sections through the rat primary sensory cortex. In supragranular layers, both large pyramidal cells and the NMDAR1 receptor subunit were found to be most dense in the septa region. Previous studies have demonstrated that large pyramidal cells have *active dendrites*, these processes respond in a non-linear fashion and can act as coincidence detectors or temporal integrators. In addition, the NMDA receptor is involved in the transmission of correlated activity. Thus, large pyramidal neurons with apical dendrites that extend up through the barrel and septa, and that have active dendrites and NMDA receptors, are ideally suited to integrate bilateral behaviourally relevant sensory information. Based on our findings, we propose that these features of the septa in rat barrel field provides the substrate for bilateral integration of somatic information that could be involved in certain orienting behaviours.

Funded by an NSERC grant to KMM

49.4

CONNECTIONS FROM CINGULATE, POSTERIOR PARIETAL AND MOTOR CORTEX TO ANTERIOR PARIETAL CORTEX (APC) IN MACAQUES K.A. Findlay, M.M. Glasier, E.R. Ergenzinger, V.J. O'Boyle Jr. and T.P. Pons*, Department of Neurosurgery, Bowman Gray School of Medicine, Winston-Salem, NC 27157-1029.

Topographic representations of the body surface are located in APC of macaques. APC receives inputs from multiple cortical and subcortical regions. In this study, we examined the pattern of projections from cortical areas other than the secondary somatosensory cortex and insula. Microelectrodes were used to delineate the representation of various body parts in APC. The retrograde tracers fast blue (FB), diamidino yellow (DY), and fluororuby (FR) were then injected into electrophysiologically defined representations of the hand, face and occiput/shoulder/neck regions in APC. Analysis of areas 5 and 7b of posterior parietal cortex revealed neurons projecting to the occiput/shoulder/neck representation. Neurons labeled from each of the separate injection sites were found in the same general region of primary motor cortex though none was double or triple labeled. This pattern of label is interpreted as indicating a lack of topographic projections from this region to APC. In cingulate cortex, neurons projecting to the occiput/shoulder/neck representation were found in both banks of the cingulate sulcus in layers V and VI. Neurons projecting to the face and hand representations in APC were found in layers V and VI immediately lateral to the cingulate sulcus. These findings suggest a topographical pattern of connections from the cingulate region to APC.

T. P. Pons was an Alfred P. Sloan Fellow (Supported by NIMH grant RO1 MH53369-02)

49.5

DYNAMIC POPULATION CODING IN RAT SOMATOSENSORY CORTEX. T. Kalt, A.C. Akhavan*, D. Jancke, and H.R. Dinse. Institut für Neuroinformatik, Theoret. Biol. Ruhr-Univ. Bochum RUB, Bochum, Germany

We present an analysis of the neural population behavior recorded in rat primary somatosensory cortex following tactile stimulation based on the population coding approach. The activity of single neurons was recorded extracellularly in urethane anesthetized rats. Single or paired stimuli were applied to a number of fixed locations along the distal-proximal extension of the hindpaw. Two-point stimulation at two loci was applied either simultaneously or with time-delays between 35 to 70 ms. The population representations of activity were constructed in the parametric (stimulus) space of the hindpaw skin area with each neuron contributing at the location of its RF.

When single tactile stimuli were used, their location could be reconstructed by the population with high precision. When composite stimuli were presented, we found distance-dependent global nonlinear behavior of the entire neural population with respect to the amplitude and to the location of the population representation. The amplitudes of the representations were reduced compared to the linear superposition indicating distant-dependent inhibitory interactions. The shift of the population representation compared to the calculated linear superposition can be interpreted as an attraction of one stimulus towards the other. Generally, this effect was specific and asymmetric, resulting in an attraction of activity towards the tip of the digits. Both effects may be considered as correlates of psychophysical phenomena.

In order to resolve the dynamic aspects of the population responses, population representations were calculated for separate time-slices of 3 ms duration. The resulting sequences of representations provided a coherent and continuous image of the emergence and fading of neuronal activity over time. Interaction effects as outlined before resulted in latency shifts and changes of inhibitory and excitatory components.

The results indicate that the population coding approach allows accurate reconstruction of tactile stimuli and the time-resolved analysis of global, nonlinear interactions due to two-point stimulation of entire neural populations.

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49.6

SPATIAL ANALYSIS CAN INFORM ON THE SOCIOLOGY OF CELL POPULATIONS: A STUDY OF EXTRA-RELAY AFFERENTS TO PRIMARY SENSORY AREAS. A. Granato* and D. Minciacchi‡, Inst. of Anat., Catholic Univ., Rome, ‡Dept. of Neurol. and Psychiat. Sci., Univ. of Florence, Italy.

The informational flow through the cortical mantle starts from the primary sensory cortices, whose computational frame is fed substantially via thalamo-cortical projections. The projection from the thalamic relay nucleus supplies the competent information and the extra-relay projections provide accessory information; the meaning of this latter input is largely unknown. We approached this issue by studying the distribution and the "social" interactions of extra-relay cell populations projecting upon single cortical representations. We analyzed, in the cat, the projections from the thalamic anterior intralaminar nuclei (AIN) and from the claustrum (Cl) to different representations of the primary somatosensory (S1) and visual (V1) cortices. After electrophysiological identification, fluorescent tracers were injected into discrete regions of S1 and V1. We already analyzed the fine topography of Cl and AIN projections (Minciacchi et al., J. Comp. Neurol., 362: 46-70, 1995).

Here we use spatial analysis tools to gain further insights on the geometrical attributes of cell populations. Three-dimensional reconstructions and rotations revealed that cortical projections of AIN display a high level of complexity: our findings indicate that it is plausible to recognize at least two separate sets of input, originating from the medial AIN sector and from the central lateral nucleus. The use of spatial tessellation (Voronoi diagrams) further strengthens our result: in the lateral sector of AIN neurons projecting to a given representation (e.g. to V1 retinal periphery representation) are strongly aggregated into clusters, whereas in the medial sector this tendency is present for neurons projecting to the vertical meridian representation.

The use of spatial analysis has proven fruitful both for suggesting peculiar modes of thalamo-cortical communication, and for refining the taxonomic subdivision of thalamic entities.

(Supported by CNR and MPI)

49.7

DIFFERENTIAL CHANGES OF AFFERENT SENSORY TRANSMISSION TO THE SI CORTEX DURING TRANSIENT LOWERING OF BODY TEMPERATURE BETWEEN RAT AND HAMSTER C.K. Won[1], B.K. Kim[1], N.-P. Jung[2], Y.K. Oh[3], I.H. Choi[3], H.W. Park[2], H.K. Jeon[2] & H.C. Shin*[1] Dept. of Physiology[1], Coll. Med., Hallym Univ., Dept. of Biology[2], Yonsei Univ., Wonju, Dept. of Biology[3], Yonsei Univ. Seoul, Korea

We have compared the effects of acute lowering of BT on the afferent sensory transmission to the primary somatosensory (SI) cortex between hamster and rat. Quantitative determination of the effect of hypothermia on the afferent sensory transmission was carried out by generating poststimulus time histograms of single unit responses to the subcutaneous electrical stimulation of the receptive field located in the whisker area of the face. Rats showed no change of afferent sensory transmission until BT 27°C, but dramatic suppression of sensory transmission from 26°C to 22°C, reaching 100% inhibition at BT 21°C, while hamster exhibited gradual suppression of sensory transmission from 34°C till 18°C, reaching 95% of inhibition. Differential effects were also oberved while elevating BT up to 37°C. Rats began to show steep disinhibition from BT 26°C, while hamster exhibited immediate, but gradual recovery from BT 19°C. Response latencies of evoked unit responses were also differentially affected during hypothermic condition. Significant delay of the latencies was observed from 29°C in rat, but from 22°C in hamster. These results suggest the presence of inherently different neural mechanisms to process somatosensory information during transient lowering of body temperature between hibernator and non-hibernator. (This study was supported by the BSRI-95-4418, Ministry of Education to NP Jung)

49.8

LEARNING INDUCED FORMATION OF CORTICAL POPULATIONS INVOLVED IN TACTILE OBJECT RECOGNITION. F. Spengler*, T. Hilger, X. Wang# and M. M. Merzenich#. Inst. für Neuroinformatik, Ruhr-Universität Bochum, 44780 Bochum, Germany; #Keck Center for Integrative Neuroscience, UCSF, San Francisco 94143, USA

We designed a behavioral training task for somatic discrimination to study reorganizational mechanisms involved in tactile object recognition in adult owl monkeys. The task was designed as a stimulation train of alternating and successive vibrations (75 Hz, 80 ms duration, 300 ms interstimulus interval) applied to the glabrous surface of the hand by two objects, spatially specified as bars. Object A touched the tips of finger 2-3-4 and object B touched all three segments of third finger. Monkeys were trained to hold the hand on the stimulation bars during the alternating background stimulation (A-B-A-B) and to retract the hand when two vibrations occurred successively at bar A or B within a distinct time window.

After the behavioral training, a detailed electrophysiological mapping procedure and quantitative data collection in the middle layers of the hand representation in the primary somatosensory cortex, Area 3b in the trained and control hemisphere was done in the anesthetised monkeys. In two monkeys the cortical hand representation contralateral to the trained hand revealed in striking contradiction to the ipsilateral control hemisphere two representational domains of reorganized receptive fields of simultaneously stimulated skin fields segregated by non-cutaneous or deep input representations. This results support the hypothesis of neuronal plasticity based on coincident input-dependent synaptic changes, which may be mediated mainly by hebbian mechanisms, and on an additional, possibly non-hebbian mechanism, which results in the spatial separation of sequentially perceived inputs. Both active input-driven network processes may provide the neuronal bases of the learned object recognition and discrimination.

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49.9

IMPORTANCE OF STIMULUS LOCATION ON THE TIME COURSE AND MAGNITUDE OF MOVEMENT-RELATED SUPPRESSION OF TACTILE DETECTION IN HUMANS. Williams S.R., Shenasa J. and Chapman C.E*. CRSN, Université de Montréal, Montréal, Québec, Canada, H3C 3J7

As part of an ongoing study of the effects of active movement on the perception of tactile stimuli, the spatial extent of the perceptual suppression caused by a simple active movement (abduction of the right index finger) was evaluated using data collected from 42 human subjects. Electrical stimuli were delivered to one of 10 stimulation sites (right index finger, dorsum of right hand, right digit 5, right dorsal forearm, right dorsal arm, right shoulder, right pectoral area, left shoulder, left index finger, right thigh) in separate blocks of trials. At each site, the intensity of stimulation was initially set at a level where 90% of the stimuli were detected at rest. The time course and amplitude of any movement-related suppression of tactile perception was examined at each stimulation site in relation to two peripheral events: the onset of movement and the onset of movement-related electromyographic (EMG) activity.

At all but the three most distant sites, a significant and sustained decrease in perceptual performance was observed during movement. The time course and the magnitude of the observed perceptual suppression were both found to depend on the site of stimulation. As distance between the stimulation site and the site of movement increased, the inital decrease in perceptual performance occurred later, occurring 70 ms prior to EMG onset when stimuli were applied to the moving digit and 50 ms after EMG onset when stimuli were applied to the chest. The maximal decrease in perceptual performance was smaller at more distant sites. These results indicate that a relatively constrained active movement can decrease tactile detection over a wide area of the skin, and that the timing and amplitude of these effects depend on the distance between the site of stimulation and the body part in motion. Supported by the MRC, FRSQ, GRSNC and Unimédia.

49.10

DIFFERENTIAL TEXTURE SENSITIVITY IN SI AND MI CORTICAL AREAS OF MONKEYS DURING PASSIVE TACTILE DISCRIMINATION. W. Jiang* and C. E. Chapman. CRSN, Université de Montréal, Canada.

The present study compared the texture sensitivity of neurones in two cortical structures, the primary somatosensory (SI) and primary motor (MI) cortices, in two monkeys performing a passive tactile discrimination task. The animals were trained to discriminate a standard surface (raised dots, 2 mm spatial period (SP) over the entire length) from 3 other surfaces in which the SP was proportionally increased to 3, 4 or 5 mm over the second half of its length (surfaces physically continuous). Following the presentation of each surface to the 3rd and 4th digit tips (D3 and D4), the monkeys indicated the presence or absence of a change in texture by, respectively, pulling or pushing a lever with the opposite hand to obtain a juice reward (Task). The monkeys were also trained to maintain the same hand position without paying attention to the presentation of the surfaces (monkey not working, distracted with random drops of juice) (No-Task). Single unit recordings were made from both MI and SI of one monkey and MI of the other. 72 units with a cutaneous receptive field on D3 and/or D4 were tested in both conditions (34, MI; 38, SI). Graded texture-related responses were obtained for 28/38 (74%) SI neurones. Only 11/28 (39%) MI neurones showed graded responses to texture. All of the texture-related SI neurones encoded the roughness changes in both conditions, Task and No-Task. In contrast, only 2/11 MI neurones showed similar texture-related activity in both conditions. The remaining 9 MI neurones displayed texture-related discharge only in the No-Task condition. The results suggest that SI is more sensitive than MI to peripheral texture information. Moreover, the presence of texture-related discharge in MI, but not SI, neurones was dependent upon the experimental conditions, indicating the existence of powerful state-related controls over sensory transmission to motor cortex. Supported by the MRC and Université de Montréal.

SOMATOSENSORY CORTEX AND THALAMOCORTICAL RELATIONSHIPS IV

50.1

THE DEVELOPMENT OF SYNAPSES IN MOUSE BARRELS, EMPHASIS ON BARREL D4. E.L. White*, D. Lev and E. Weinfeld Center for Brain Research, Faculty of Health Sci., Ben-Gurion Univ., Beer Sheva, ISRAEL.

This abstract reports on a continuing, comprehensive study of the development of synaptic patterns in mouse PMBSF barrels. One aspect of the study focuses on the distribution of synaptic profiles in the neuropil as assessed in EM montages taken of thin sections cut in the tangential plane through the entire extent of barrel D4 at the mid-layer IV level. To date, two samples of roughly 10,000μm^2 each have been analyzed at ages P6, P9, P11, P13 and at 65 days (P1=the first 24 hours after birth). Results show a steady increase with age in the number of axodendritic and axospinous, synapse profiles. In parallel, the development of synaptic input to cell bodies is being followed. Most somata received symmetrical synapses only and are presumed to be spiny stellate cells. At P6, nearly all these somata receive no synapses; from P7 on there is a steady growth in the numbers of synapses received by these somata with the mode of one synapse per cell body well established by P12 and the trend towards larger numbers of synapses per cell body becoming more pronounced subsequently. Distributions of axo-somatic synapses similar to the adult are observed by P15. Supported by Israel Acad. of Sciences 618/93 to E.L.W.

50.2

SYNAPTIC ZINC IN THE DEVELOPING BARREL CORTEX OF THE MOUSE. J. Skangiel-Kramska* and A. Czupryn. Department of Neurophysiology, The Nencki Institute, 02-093 Warsaw, Poland.

It has been suggested that synaptic zinc may be involved in the processes that underlie developmental and functional plasticity. One of useful model systems to study cortical plasticity is the whisker-to-barrel system of the mouse. We sought to establish whether the distribution of synaptic zinc developmental pattern is correlated with the critical periods of morphogenetic and functional plasticity. In order to examine the distribution of zinc-containing terminals in the barrel cortex of the mouse during postnatal development, the selenite method of Dansher was used. Zinc staining during the first postnatal days was very low. Laminar variations in zinc staining in SI cortex emerged from PND 6. At PND 8 these variations were clearly visible. The intense zinc staining was found in layers I, II, III and V. Layers IV and VI demonstrated weaker staining. These pattern persisted in adulthood. Section through layer IV from PND 6 revealed association between the cytoarchitectonically visible modular organization of barrels and the distribution of zinc-containing terminals due to higher zinc concentration in septa between barrels and surrounding cortex. Barrel sides showed the relative scarcity of zinc in comparison to the barrel hollows. The difference in zinc staining between barrel side and barrel hollow diminished with age but was still present in adult mouse (up to PND 70). Serial tangential sections through layer IV, obtained from animals at different ages, demonstrated that zinc staining within barrel hollow firstly appeared in the deep part of layer IV (PND 6). As the brain matured, the upper part of layer IV showed the presence of zinc-containing terminals.

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50.3

CHANGES IN THE DISTRIBUTION AND DENSITY OF NADPH-d-POSITIVE CORTICAL NEURONS DURING THALAMOCORTICAL CONNECTIVITY. R. Erzurumlu* and E. Ulupinar. Department of Anatomy, Louisiana State University Medical Center, New Orleans, LA 70112.

Nitric oxide is a messenger molecule involved in activity-dependent refinement of synaptic connections during development and in some forms of learning. Nitric oxide synthase (NOS) is an essential enzyme in the synthesis of nitric oxide. We used NADPH-diaphorase histochemical staining to visualize NOS-positive cells in the mouse somatosensory cortex during the establishment of thalamocortical (TC) connectivity (as revealed by DiI labeling). We then quantified the distribution and density of stained cells in relation to developing TC projections using Magiscan Image Analyzer. Between the ages postnatal day 0 (PND0) to two weeks of age (PND14), the distribution and density of NOS-positive cells changed dramatically. On PND0, NOS-positive cells were found in the ventrolateral parts of the somatosensory cortex and in deep layers. The distribution of NOS-positive cells corresponded to the location of TC axons at this age. Gradually NOS-positive cells appeared in dorsomedial cortex and in more superficial layers. As TC axons developed arbors in layers VI and IV (between PND0 and 3), the number of NOS-positive cells almost doubled. Following the establishment of whisker-specific patterning of TC axon arbors and formation of barrels in layer IV, diffuse neuropil staining demarcated the barrel hollows. The density of NOS-positive cells also decreased and reached a plateau between PND7-14. These results suggest a role for nitric oxide in activity-dependent stabilization of patterned connectivity between the somatosensory thalamus and neocortex. *Supported by NIH (NS32195)*

50.4

THE DEVELOPMENT OF GLUTAMIC ACID DECARBOXYLASES (GAD) IN RAT SOMATOSENSORY CORTEX WITH RESPECT TO BARREL FIELDS. P.J. Kiser, N.G.F. Cooper, and G.D. Mower*. Department of Anatomical Sciences and Neurobiology, University of Louisville School of Medicine, Louisville, KY 40292.

The purpose of this study was to detect correlations between the development of barrel fields in rat somatosensory cortex (SI) and the development of the GABA synthetic enzymes GAD65 and GAD67. Developing barrel fields were identified in coronal sections (40μm) from 1, 3, 4, 5, 6, 9, 13, 19 and 31-day-old rats using cytochrome oxidase (CO) and acetylcholinesterase (AChE) staining methods. Adjacent sections were then immunoreacted with antibodies to GAD65 or GAD67. From day three onward, CO staining shows a darkly stained band in the lower cortical plate that corresponds to the barrels that are evident by days five to six in layer IV. At day one, a dark band of GAD67 stained cells and processes in the superficial subplate is the dominant characteristic. More cells and processes are present below this darker band than are present superficially. A second band is apparent on day six that overlaps the barrels in layer IV. The original band persists in layer V at this age approximately 100μm below layer IV. By day nine, the barrel septa are evident by their diminished immuno-staining clearly demarcating the barrels present in layer IV. The staining intensity of the lower band has diminished and cell staining is clear in all cortical layers. By day 13 the lower band is no longer detected and this pattern is maintained into adulthood.

These results show the presence of GAD67 in a defined layer of the subplate before the formation of barrels as evidenced by Nissl or CO staining (day's 1-3). These GAD67 elements (which are not evident after day 9-13) could represent an embryonic remnant GABA system which interacts with segregating thalamocortical afferents in the formation of mature barrels.

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50.5

COMPLEXITIES IN THE THALAMOCORTICAL AND CORTICOTHALAMIC PATHWAYS. N.C. Adams‡*, D.A. Lozsádi† and R.W. Guillery†. ‡Deptartment of Developmental Neurobiology, UMDS, Guy's Hospital, London, SE1 9RT, England; †Department of Human Anatomy, Oxford University, South Parks Road, Oxford, OX1 3QX, England.

The complex reorganization of fibres passing between the telencephalon and diencephalon early in development was previously demonstrated in the rat (Adams and Guillery (94) *Soc. Neurosci. Abs.*, **20**, p1685). In the adult this latticework lies in the thalamic reticular nucleus, derived from the ventral thalamus, and lateral to that in the perireticular nucleus. We now report, in the developing chick and adult turtle, a comparable latticework in the fibres in the ventral thalamus.

We suggest that the fibre reorganization produced in this lattice is a fundamental requirement for linking topographically organized maps in the thalamus to the several corresponding cortical maps. Since one thalamic nucleus can connect to several cortical maps, and cortical maps are often mirror reversals of each other, complex crossings in the two way thalamocortical and corticothalamic connections are necessary. Two transient groups of cells, the perireticular nucleus (located in the internal capsule lateral to the reticular nucleus) and the cells of the cortical subplate, are prominent along the course of axons linking cortex and thalamus early in development. The functions of these two cell groups are not known. However, since complex patterns of reorganization, defasciculation and crossings occur in the regions of these cells, it is likely that they play a role in creating the latticework of the adult.

We suggest that the ubiquitous presence of such a zone of fibre reorganization is integral to the functioning of the thalamocortical pathways, and that the complexity of these pathways, with multiple cortical representations may have been central to the evolutionary success of the thalamotelencephalic system.

Supported by the Wellcome Trust and MRC.

50.6

WIDESPREAD PROJECTIONS FROM LAYER VII (VIb, MATURE SUBPLATE) NEURONS TO LAYER I IN ADULT RAT CORTEX REVEALED SELECTIVELY WITH FLUORESCENT TRACERS. B. Clancy* and L.J. Cauller GR 41, Cognition and Neuroscience Program, University of Texas at Dallas, Richardson, TX 75083.

Projections to cortical layer I originating from a tangential band of neurons (layer VII) at the interface of the cortex and the white matter were demonstrated with surface applications of retrograde fluorescent tracers, confirming the persistence of this circuit in the adult (100-550 g) rat cortex. Ten-minute surface applications of diamidino yellow (DY) or Fast Blue (FB) placed on primary sensory cortices (<1 mm^2; application confined to layer I, *i.e.*, no layer IV cells labeled) identified both local and widespread (>4 mm) ipsilateral projection neurons subjacent to cortical layer VIb which send axons to the superficial layer. Applications of fluorescent retrograde tracer on the surface of primary somatosensory cortex (S1) labeled layer VII neurons directly below the application site, rostromedially above the forceps minor, and caudolaterally in a continuous band to secondary somatosensory cortex (S2). Application to layer I of M1 labeled layer VII neurons from the application site discontinuously as far as S2. Applications on layer I of the auditory cortex labeled layer VII neurons in auditory areas and in V1, while applications on V1 labeled layer VII neurons in auditory cortices as well as in V1 and V2. The entire sub-set of cortical layer VII neurons failed to label with local or interareal applications or injections of non-fluorescent conventional tracers (e.g., HRP, biocytin). The layer VII neurons, with dendrites in the white matter and in the corticocortical fiber system (deep cortical layers) demonstrate progenitive, morphological and immunopositive correspondence to interstitial neurons as well as to neurons of the claustrum. The persistence of the developmental projection pattern from NADPHd+ layer VII (adult subplate) neurons to cortical layer I in rat cortex provides anatomical evidence for their participation in mature cortical function.

Supported by a grant from the Whitehall Foundation.

50.7

THE TOPOGRAPHIC ORGANIZATION OF SOMATOSENSORY AREA 3A IN THE MARMOSET MONKEY (*CALLITHRIX JACCHUS*). K. J. Huffman*, L. Krubitzer, J. Clarey and R. Tweedale. Center for Neuroscience and Department of Psychology, University of California, Davis, USA, and Vision, Touch and Hearing Research Centre, University of Queensland, Australia.

In four marmosets, the topographic organization of somatosensory area 3a, the area of cortex immediately rostral to the primary somatosensory area, SI or 3b, was examined using electrophysiological recording techniques. Neural activity was recorded at a number of closely spaced recording sites; receptive fields for neurons at these sites were determined; and stimulus preferences for neurons were identified. In all cases, neurons in area 3a consistently responded to deep stimulation of the contralateral body surface. Most neurons responded exclusively to taps to a particular body part, but in some cases neurons also responded to cutaneous stimulation. Although receptive fields for neurons in area 3a were somewhat larger than for those in area 3b, the details of the digit and face representation could still be obtained. By examining sequences of receptive field progression and order in area 3a, maps of the body surface were constructed, and the internal organization of the field ascertained. Like area 3b, the mediolateral organization of area 3a progressed from the toes and foot, to the hindlimb, trunk and forelimb, and more laterally to the hand, digits and face representations. The representations of the hand and digits assumed the largest portion of area 3a. Area 3a was observed to form a mirror reversal of area 3b such that the ventral surface was represented caudally and the dorsal surface rostrally. When electrophysiological results were related to myeloarchitecture in cortex that was flattened and cut parallel to the cortical surface, it was found that the map in area 3a was coextensive with a 1 - 2 mm strip of lightly myelinated cortex just rostral to the darkly myelinated area 3b. These results represent the first complete description of area 3a in any primate, and indicate that somatosensory cortex is composed of a number of separate representations of the sensory epithelium.

50.8

THE SOMATOTOPIC ORGANIZATION OF THE PARIETAL MEDIAL AREA IN THE CALIFORNIA GROUND SQUIRREL (*SPERMOPHILUS BEECHEYII*). D. Slutsky, P. Manger, K. J. Huffman, and L. Krubitzer*. Center for Neuroscience & Department of Psychology, University of California, Davis, USA.

In the somatosensory cortex of rodents such as mice and rats, a primary somatosensory area, SI, and a second somatosensory area, SII, have been consistently identified. Studies of rodents from other major lineages including Sciuridae, have demonstrated that cortex in these rodents is more complexly organized, and contains multiple representations of the sensory epithelium. For instance, in the eastern gray squirrel, three complete representations, SI, SII, and the parietal ventral area, PV, have been identified. Connectional data indicate the presence of two additional fields, the parietal medial area, PM, and the parietal rhinal area, PR. The present investigation in which electrophysiological recording techniques were combined with analysis of cortical architecture demonstrates that PM contains a complete representation of the contralateral body surface. Multiple recording sites were made in the cortex of three squirrels; receptive fields were obtained and stimulus preferences ascertained for neurons at these sites. Recording results were related to architectonic distinctions in cortex that was flattened, cut parallel to the cortical surface, and stained for myelin. The mediolateral organization of the body surface was similar to that described for SI. The hindlimb was represented most medially, followed by the trunk, forelimb, hand, vibrissae, and the face representations most laterally. The hand and vibrissae representations occupied a relatively large portion of the entire field. Neurons in PM responded to stimulation of deep receptors and often habituated to the stimulus. When electrophysiological recording results were related to cortical myeloarchitecture, it was found that a 1 mm strip of lightly myelinated cortex was coextensive with PM. These results in rodents indicate that cortex caudal to SI contains neurons which are responsive to somatosensory stimulation. This cortex corresponds to areas 1 and 2 in primates and SIII in cats. Thus, it is possible that a caudal field is part of a basic somatosensory processing network present in all or most mammals.

50.9

BEHAVIORAL, CORTICAL, AND MECHANOSENSORY SPECIALIZATIONS IN THE STAR-NOSED MOLE (*CONDYLURA CRISTATA*) K. C. Catania* and J. H. Kaas. Dept. of Psychology, Vanderbilt University, Nashville, TN 37240

The nose of the star-nosed mole consists of 22 fleshy appendages (11 per side) covered with thousands of epidermal sensory organs called Eimer's organs. Each appendage is innervated by a single nerve branch and is represented in cortex by a stripe of tissue that can be visualized by flattening cortex, sectioning parallel to the pia, and processing the sections for cytochrome oxidase. In 4 moles, the number of Eimer's organs, the number of myelinated afferents, and the area of the cortical representation in primary somatosensory cortex was quantified for the 11 appendages from one half of the nose. These anatomical measurements were related to behavioral measurements (analysis of slow motion video), which revealed preferential use of specific nasal appendages.

Although the structure and form of the Eimer's organs is uniform across the different appendages, the preferentially used appendages have higher innervation densities. In addition, these appendages have greater cortical magnification per Eimer's organ, and larger cortical areas per afferent, compared to larger but less used appendages. Observations of newborn star-nosed moles suggest these appendages also develop earliest. The results indicate some of the ways in which peripheral receptors and central nervous structures may be specialized to enhance input and processing for behaviorally important areas in a sensory array.

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50.10

CORTICAL AND THALAMIC CONNECTIONS OF THE SECOND SOMATOSENSORY AREA, S2, IN THE PROSIMIAN PRIMATE, *GALAGO GARNETTI*.

Wan-hsun Carolyn Wu*, Pamela D. Beck and Jon H. Kaas
Department of Psychology, Vanderbilt University, Nashville, TN 37240

The present study was part of an effort to compare the organization of the somatosensory system in different primates. Injections of fluorochromes (DY, FB and FR) or WGA-HRP were placed into portions of S2 identified by microelectrode recordings. After 7 days survival, the perfused cortex was flattened and cut parallel to the surface. The thalamus was cut coronally. Alternative sections were mounted for fluorescence or reacted for TMB, CO, myelin or Nissl substance. The dense ipsilateral cortical connections were with S1 (3b), presumptive 3a, PV and cortex caudal to S1. The major callosal connections were with S2. Thalamic connections differed from those of S1 in that S2 injections revealed more label in the ventroposterior inferior nucleus and less in the ventroposterior nucleus. Labeled neurons were also in the presumptive posterior nucleus. These results are consistent with the distribution of label in simian primates. Taken together, they indicate that many of the major connections of S2 in simian and prosimian primates are similar. Supported by NIH grant NS 16446.

50.11

INDIVIDUAL DIFFERENCES IN THE SIZE OF THE HAND AND SOMATOSENSORY CORTICAL REPRESENTATION OF THE HAND. J.T. Wall*, R.C. Kolarik, A.C. Silva, X-F Wu, and S.K. Rasey. Department of Neurobiology & Anatomy, Medical College of Ohio, Toledo, OH 43699.

Hands vary significantly in size. Longstanding views suggest that the size of the cortical representation of the hand is dictated by peripheral factors relating to size or innervation of the hand. Thus, individuals with large hands and large numbers of afferents might be expected to have large cortical representations of the hand. This study analyzed interindividual differences in skin areas of hands, and areas, lengths, and widths of the related area 3b somatosensory cortical representations of these hands, in 15 normal adult squirrel monkeys. Hand skin areas ranged from 1837 - 2449 mm^2, a 33% difference between largest and smallest hands. Cortical hand representations ranged in area from 10.5-14.6 mm^2, a 39% difference between largest and smallest representations. The hand representation was a much compressed representation which, in different individuals, ranged from 126-210 times smaller than its hand skin area. There was no significant correlation between hand skin area and cortical representation area (τ=-0.22; p=0.42). The length of the hand representation, defined by functional borders with the face and forelimb representations, and the width of the hand representation, defined by cytoarchitectonic borders of area 3b, varied independently. The area of the hand representation was significantly correlated with the length (τ=0.65; p=0.009), but not width of the representation. These results suggest that individual differences in the size of the hand representation are more strongly correlated with variability in the functional, as opposed to cytoarchitectonic, borders of this representation. The results further indicate that cortical representation size is not directly related to hand size. This perspective differs from views that suggest cortical representation size is dictated by peripheral factors relating to size of the hand. Supported by NIH Grant 21105.

50.12

LIMITS ON INTRACORTICAL CONNECTIVITY WITHIN THE SENSORY-MOTOR CORTEX OF MACAQUE MONKEYS. A. Munoz, T.M. Woods*, P.R. Manger and E. G. Jones. Department of Anatomy and Neurobiology, University of California, Irvine, CA 92717.

Representational maps of the hand and foot have been electrophysiologically identified in the different cytoarchitectonical areas of the macaque sensory-motor cortex, and correlated with its differential connectivity patterns. Connections within and between parts of the representation may form a basis for map plasticity but have not been thoroghly explored. In macaque monkeys (*Macaca mulata* and *M.fuscata*) microelectrode multiunit mapping of neuronal responses to tactile stimulation identified the representations of individual fingers and toes in areas 3b and 1. Small extracellular iontophoretic injections of biocytin were made at single identified sites. Parasagittal or tangential sections were processed for biocytin histochemistry, electrode tracks were identified and Nissl staining matched cytoarchitectonic boundaries. Injections at D1 and D2 hand digit representations in area 3b showed a patched pattern of anteroposterior reciprocal connections with areas 3a and 1, and efferent projections to area 2, along a limited mediolateral band perpendicular to the central sulcus. Only limited connections were found between representations of adjacent digits and connections beyond the representations of the digits were sparse or absent. There was a sharp boundary corresponding to the face representation. Area 1 injections in D1 representation showed efferent projections to area 4 and reciprocal connections with areas 3a, 3b, 2 and 5, with a similar mediolateral restriction as in the case of area 3b injections. The present data suggest the presence of mediolaterally limited, but anteroposteriorly elongated connectivity patterns concentrated within the representations of individual digits at different cytoarchitectonical regions within the primate sensory-motor cortex. The degree of overlap into adjacent digit representations should limit activity-dependent plasticity. Supported by NIH NS21377 and HFSP

50.13

CORTICAL REPRESENTATION OF VISCERAL AND SOMATIC SENSATION IN HUMANS. A. Schnitzler*, J. Volkmann, P. Enck[1], T. Frieling[1], H.-J. Freund and O.W. Witte. Dept. of Neurology and Dept. of Gastroenterology[1], Heinrich-Heine-University, D-40225 Düsseldorf, Germany.

It is well established that somatic afferents project to the contralateral primary (SI) and bilateral secondary somatosensory cortex (SII). However, very little is known about cortical representation and localisation of consciously perceived visceral sensory stimuli. The aim of the present study was to delineate the spatiotemporal characteristics of cortical responses elicited by electrical stimulation of the human esophagus as compared to stimulation of somatic afferents. Cortical magnetic activity to electrical stimulation of the distal and proximal esophagus, the lips, and median nerves on both sides were recorded from six healthy, right-handed adults using a whole-head neuromagnetometer. The distributions of the responses were adequately explained by time-varying multi-dipole models, and the localisation of the current sources was determined by superposition on individual MRI scans. In contrast to responses evoked by stimulation of somatosensory afferents (median nerves, lips), no discernible signals could be detected in SI after esophageal stimulation. Well reproducible responses peaking at 90 to 130 ms after stimulation of esophageal afferents were obtained bilaterally in SII in close vicinity to SII sources evoked by median nerve and lip stimuli. Strengths of the esophageal SII sources increased with increasing interstimulus intervals (0.5s, 1s, 2s, 4s). Source activity was positively correlated with the psychophysical perception of distinct stimuli as determined on a subjective rating scale. At an ISI of 0.5s perceptive temporal discrimination of successive stimuli was poor and SII response amplitudes were small. Similarly, stimuli were peceived dull and spatially diffuse when shorter ISIs were used.

Our results suggest that visceral afferents from the esophagus project to SII probably bypassing SI. This may represent a neurophysiological basis for the relatively low temporal and spatial resolution of conscious visceral sensation. (Supported by the Deutsche Forschungsgemeinschaft, SFB 194, Z2)

50.14

SYNAPTIC INACTIVITY INDUCES STRUCTURAL PLASTICITY IN AXOSPINOUS SYNAPSES OF CORTICAL DENDRITIC SPINES. G. Benshalom* and V. Glukhman. Dept. of Morphology, Fac. of Health Sciences, Ben-Gurion Univ. of the Negev, Beer-Sheva, Israel.

This study was aimed at determining if structural alterations are induced in cortical dendritic spines by synaptic inactivity of their axospinous synapses, as was previously hypothesized (Benshalom, 1989, Synapse 4: 210-222). Ultrastructural observation focused on a population of dendritic spines along dendrites of spiny stellate neurons in layer IV of the barrel region of mouse somatosensory cortex. Approximately 10-23% of all axospinous synapses on these neurons are thalamocortical. Sensory neural input conducted via the thalamus to each of the two cortical barrel regions originates in a set of vibrissae at the contralateral side of the face. Deprivation of thalamocortical synaptic activity in the barrel cortex of one hemisphere was accomplished by removal of vibrissae unilaterally. 1-3 days later, mice were intracardially perfused with aldehyde solution and the Golgi-gold toning technique applied for light and electron microscopic identification of spiny stellate neurons. Ultrastructural examination revealed that the mean frequency of dendritic spines along the identified spiny dendrites was the same for both hemispheres. The percentage of dendritic spines exhibiting flat contour of axospinous synapses was similar in both hemispheres, but the percentage of dendritic spines with "postsynaptic-concave" spine heads was 4.5X higher, and the percentage of spines with "postsynaptic-convex" spine heads was 0.84X lower in the activity-deprived cortical hemisphere than in the control hemisphere. These results support the hypothesis that neural activity-related structural plasticity is an ongoing process within the mature brain. Supported by The Israeli Academy of Sciences, BRF grant.

PAIN PATHWAYS: HIGHER CENTERS

51.1

BRAIN NUCLEI AND TRANSMITTERS INVOLVED IN RESPONSE TO PROXIMAL COLON DISTENTION IN CONSCIOUS RAT. L. Wang*, Y. Martínez and Y. Taché CURE/DDRC, Dept. of Med. and Brain Res. Inst., UCLA, Los Angeles, CA 90073

Colon distention activates neurons in the brain autonomic regulation centers, as demonstrated by *c-fos* expression. Oxytocin (OT) vasopressin (AVP) and corticotropin-releasing factor (CRF) are the major transmitters in the hypothalamic nuclei influencing visceral functions. Central catecholaminergic (CA) neurons have been identified for their role in stress related autonomic and neuroendocrine circuitry. The aim of this study was to determine the neural pathways and neurochemical coding related to proximal colon distention by detecting the co-existence of the *c-fos* gene protein (Fos) expression with OT, AVP, CRF and CA. Visceral stimulation was performed in conscious SD male rats by proximal colon distention (10 ml of air, 30 s on/off for 10 min, approximally 80 mmHg) through a balloon chronically implanted in the proximal colon. Dual immunohisto-chemical staining were performed one hour after the stimulation by processing immunohistochemistry first for Fos with ABC-nickel intensified DAB technique and then for OT, AVP, CRF and tyrosine hydroxylase (TH) with ABC-DAB. For CRF immunohistochemistry rats were pretreated with a low dose of colchicine (20 µg/rat). Fos expression induced by proximal colon distention in conscious rats was observed in the paraventricular nucleus (PVN), supraoptic nucleus (SON) and accessory neurosecretory nuclei of the hypothalamus, locus coeruleus (LC), Barrington's nucleus, nucleus tractus solitarius (NTS), A1/C1 and A5 areas. Double stained cells of Fos with OT or AVP were observed mostly in the magnocellular division of the PVN (81% of OT- and 18% of AVP-positive cells), in SON (36% of OT- and 16% of AVP-positive cells) and in accessory neurosecretory nuclei. The Fos expression after colon distention was lower in the rats treated with colchicine than those without colchicine. However, it was still increased when compared with sham distention, and the double stained neurons with Fos and CRF were located in the parvocellular part of the PVN. Most of the Fos-expressing neurons in the pons and medulla after the proximal colon distention were detected in TH-positive nuclei, such as A1/C1, NTS, LC and Barrington's nucleus. In A1/C1, 74% of TH-containing neurons and in the NTS 42% were Fos-expressing. The results indicate that stimulation of the proximal colon by repetitive distention in conscious rats activates CA-, OT-, VP-and CRF-producing neurons in the brain and that these transmitters may be involved in processing information related to noxious visceral mechanical stimulations and in modulating pain-related visceral reflexes.

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51.2

THE NUCLEUS GRACILIS (NG): A CROSS ROAD FOR PELVIC VISCERAL AND CUTANEOUS INPUTS INTO THE THALAMUS. K. N. Westlund, E. D. Al-Chaer and W. D. Willis*. Department of Anatomy and Neurosciences, University of Texas Medical Branch, Galveston, TX 77555-1069.

The role of the nucleus gracilis (NG) in processing viscerosensory information arising from pelvic visceral organs is a major concern of researchers investigating mechanisms of visceral pain. In an earlier report and in the accompanying abstract, we have shown that the dorsal column (DC) mediates colonic input into the ventral posterolateral (VPL) nucleus of the thalamus and that NG cells respond to colorectal distension (CRD) and colon inflammation induced by mustard oil. This study focuses on the role of the NG per se as the mediator or the main gate for pelvic visceral information to go into the VPL nucleus. Experiments were made on male Sprague Dawley rats anesthetized with pentobarbital (40 mg/kg ip; 5 mg/kg/h iv). In one group, recordings were made from single cells in the VPL nucleus in response to CRD and cutaneous stimuli before and after consecutive microlesions of the contralateral and sometimes of the ipsilateral NG. The results obtained indicate that lesion of the contralateral NG interrupts the majority and sometimes all of the colorectal input into the VPL; at the same time, it reduces dramatically the responses of VPL cells to cutaneous stimuli mainly of innocuous nature. Occasionally, new cutaneous receptive fields are unmasked that would activate the same VPL cell with different stimulation modalities. In group 2, recordings were made from postsynaptic dorsal column (PSDC) cells at L6-S1 spinal segments in response to CRD and cutaneous stimuli. The projections of these cells were followed into the NG by antidromic activation of their axons using microstimulation (< 40 µA). These inputs and outputs through the nucleus gracilis put it at a major cross-road of visceral and cutaneous information between the periphery and higher brain centers. (Supported by NS09743, NS32778, NS11255).

51.3

MODULATION OF VISCEROSOMATIC INTERACTIONS IN THE THALAMUS BY DORSAL COLUMN INPUT. E. D. Al-Chaer*, K. N. Westlund and W. D. Willis. Department of Anatomy and Neurosciences, University of Texas Medical Branch, Galveston, TX 77555.

The role of the dorsal column (DC) in visceral pain processing is becoming more evident as recent studies have elucidated its importance over that of other spinal tracts in mediating nociceptive visceral input into the ventral posterolateral (VPL) nucleus of the thalamus (Al-Chaer et al. 1995; 96). On the other hand, increased responses of VPL nucleus, nucleus gracilis (NG) and postsynaptic dorsal column (PSDC) cells to graded colorectal distension after colon inflammation were also recently reported (Al-Chaer et al. 1996). This study focuses on the interactions between simultaneous visceral and cutaneous inputs before and after selective lesions of different spinal tracts. Experiments were done in anesthetized male Sprague Dawley rats. Responses of viscerosensitive cells in the VPL nucleus, the NG and of PSDC cells to cutaneous stimuli (Brush, Pressure and Pinch) and to graded colorectal distension (CRD) were obtained before and after colon inflammation induced by intracolonic injection of mustard oil (MO). The results obtained so far, indicate that colon inflammation with mustard oil, not only potentiates the responses of single cells to CRD but also modifies their responses to cutaneous stimuli. Similarly, cutaneous stimuli applied during inflammation alter the increased background activity of viscerosensitive cells observed following MO injection into the colon. These interactions between visceral and cutaneous inputs are differentially affected by various spinal lesions involving the DC or pathways in the ventrolateral columns. (Supported by NS09743, NS32778, NS11255.)

51.4

ANATOMICAL EVIDENCE FOR CELLS OF ORIGIN OF A POSTSYNAPTIC DORSAL COLUMN VISCERAL PATHWAY: SACRAL SPINAL CORD CELLS INNERVATING THE MEDIAL NUCLEUS GRACILIS. M.D. Christensen*, W.D. Willis and K.N. Westlund. Marine Biomedical Institute, Dept. Anatomy & Neursociences, UTMB, Galveston, TX 77555-1069.

The goal of this anatomical study is to characterize the neurons of the lumbosacral spinal cord which project through the dorsal column to the medial portion of the medullary nucleus gracilis. The retrograde tracer, Fluoro-ruby, tetratramethyl rhodamine conjugated dextran (10,000MW, Molecular Probes) was pressure-injected into the medial nucleus gracilis of male Sprague-Dawley rats (n-4). Eight to ten days later, colchicine (10µl, 10µg/ l saline) was injected subdurally at a laminectomy site over the lower lumbar enlargement. After 10-15h, the animals were transcardially perfused with 4% buffered paraformaldehyde. Cryosections of L6-S1 were processed immunocytochemically with substance P antibody (1:10,000; Incstar) and subsequently with fluorescein conjugated anti-rabbit IgG. Cells of origin of terminals innervating the medial nucleus gracilis were primarily localized medially in lamina III. Additional cells were localized medially in laminae X, VII and IV or more centrally in lamina III. Substance P co-localization will be examined. Midline dorsal column lesions will eliminate effective retrograde labeling. These data provide detailed anatomical descriptions of the localization of neurons in the sacral spinal cord which may be responsible for transmission of nociceptive visceral input through the dorsal column to the nucleus gracilis and ultimately to the ventrolateral nucleus of the thalamus observed in electrophysiological studies. (Supported by NIH grants NS11255, NS32778 and NS09743).

51.5

ORIGIN OF SPINOTHALAMIC INPUT TO VMpo IN THE MONKEY. E.-T. Zhang* and A.D. (Bud) Craig. Div. of Neurobiology, Barrow Neurological Institute, Phoenix, Arizona 85013.

The posterior part of the ventral medial n. (VMpo) is a cyto- and chemo-architectonically distinct nucleus in monkey and human thalamus that receives dense, topographic lamina I spinothalamic (STT) input, contains a concentration of nociceptive- and thermoreceptive-specific neurons, and projects topographically to the dorsal anterio. insular cortex (Nature 372:770, 1994). In this study, we have used cholera toxin subunit B (CTb) to retrogradely label cells that project to VMpo.

Recordings were used to localize VMpo. A single iontophoretic injection of de-salted 0.3% CTb (2 µA, 20 min) or large hydraulic injections of 2% CTb were made (6-14 µl). Animals survived 3-4 wks, and an immunohistochemical ABC/DAB reaction was performed. Thionin and calbindin staining verified injection placement. The entire brainstem and spinal cord was examined.

Injections that covered all of thalamus labeled cells contralaterally in lamina I (31-49%), laminae IV-VI (31-47%), and laminae VII-X (17-38%). In contrast, in cases with a single injection in VMpo, 85-90% of the labeled cells were in lamina I, 10-15% were in laminae IV-VI, and none were found in laminae VII-X.

The finding that STT input to VMpo originates almost exclusively from lamina I neurons confirms that VMpo is a lamina I spino-thalamo-cortical sensory relay nucleus. This is consistent with the fundamental concept that lamina I projections relate the sense of the physiological condition of the body, which includes the specific sensations of pain and temperature. (Supported by NS 25616)

51.6

SPONTANEOUS ACTIVITY OF MEDIAL AND LATERAL THALAMIC NEURONS IN RATS. B.J. Haworth, V.M. Tronnier, D. Sterio* and P.C. Rinaldi. Dept. of Neurosurgery, University of California, Irvine, CA 92717.

Hyperactivity and bursting have been studied in lateral thalamic nuclei in patients during stereotactic procedures for relief from pain and in animals, typically in response to stimuli or in relation to sleep. Less is known of spontaneous activity and bursting in the medial thalamic nuclei. The purpose of this study is to characterize spontaneous thalamic activity, focusing on bursting, from medial and lateral thalamic nuclear groups in somatosensory and pain related structures. In 23 chloral hydrate sedated male rats 115 neurons were isolated and analyzed from extracellular recordings from the medial (CM, DM, Pf, Cl) and lateral (VPL, VPM) thalamus. Cells were classified in terms of fields and response to noxious and non-noxious stimuli, periodicity, interval histogram and burst analyses. In medial nuclei, 39% (16/41) and in lateral nuclei, 83% (39/47) of the cells responded to pain and/or touch. Spontaneous bursting was exhibited by 74% (39/53) of medial cells and 87% (41/47) of lateral cells. The cells from the medial nuclei exhibit a longer average interval duration and fewer spikes per burst than cells from lateral nuclei. Activity of lateral cells exhibits a tendency for spikes within a burst to organize in a pattern of shorter to longer successive intervals, while this relationship breaks down for medial cells. This study supports the concept that not only do the medial and lateral nuclei differ with respect to response to somatosensory stimulation, but that spontaneous activity appears specific to each region, again paralleling differences in function. These findings also provide a framework for evaluating the hyperactivity and bursting which appear as modulation in the activity of the network of these nuclei following deafferentation.

51.7

RECEPTIVE FIELD EXPANSION AND FUNCTIONAL CHANGES OF VENTROBASAL THALAMUS (VB) NEURONS IN RATS WITH STRYCHNINE-INDUCED ALLODYNIA, S.E. Sherman*, L. Luo and J.Q. Dostrovsky, Department of Physiology, University of Toronto, Toronto, Ontario, Canada M5S 1A8

Spinal strychnine (STR; glycine receptor antagonist) produces allodynia, as evidenced by nocifensive behavioural and autonomic responses to otherwise innocuous tactile stimuli (e.g. touch, brush, air jet). The aim of the current study was to determine if spinal STR causes changes in thalamic somatosensory processing that might contribute to the production of allodynia. Extracellular, single-unit recordings were conducted in the VB of 19 urethane-anaesthetized, male, Wistar rats (342 ± 44 g). Receptive fields (RFs) and responses to noxious and innocuous cutaneous stimuli were determined for 19 units (1 per animal), prior to, and immediately following intrathecal (i.t.) STR (40 µg). Eighteen animals, developed allodynia with a mean onset time of 5 (± 3) minutes after i.t. STR, as evidenced by the ability of innocuous brush or air jet to evoke cardiovascular and/or motor reflexes. All (3) nociceptive specific (NS) units became responsive to brush stimulation within 4 minutes after the onset of allodynia, and 1 became sensitive to brushing over an expanded RF. RF expansion was also noted for all of the low threshold (LT; 14) and wide dynamic range (WDR) neurons (2), either concurrently with allodynia (1 NS; 5 LT; 2 WDR) or within 10 minutes after the onset of allodynia. These RF changes were substantial for the WDR neurons, whose small, contralateral RFs expanded to include extensive bilateral regions. Nine LT units displayed similar dramatic RF expansion, and 10 LT units with contralateral RFs, developed bilateral RFs after i.t. STR. One NS and 9 LT neurons had increased spontaneous firing rates. The present results demonstrate that the direct actions of STR on spinal processing of sensory information are reflected by changes in the receptive fields and response properties of nociceptive and nonnociceptive thalamic neurons, and infer that thalamocortical mechanisms may be operative in the expression of STR-induced allodynia. (Supported by the Gifford-Jones and W. Ward Awards)

51.8

ARE RESPONSES TO VISCERAL STIMULATION IN THE CAT'S LATERAL THALAMUS ORGAN SPECIFIC? A.C. Horn, J. Brüggemann*, M. Petersen, C. Vahle-Hinz[1] and K.-D. Kniffki. Physiologisches Institut, Universität Würzburg, D-97070 Würzburg, and [1]Physiologisches Institut, UK Eppendorf, D-20246 Hamburg, Germany.

In squirrel monkeys and macaques there exists a high degree of viscero-visceral convergence in the lateral thalamus (72% and 41%, respectively). We studied the representation of a thoracic (esophagus) and an abdominal visceral organ (distal colon) in the lateral thalamus of α-chloralose-anesthetized cats. Distension of the viscera with pressure intensities of up to 80 mm Hg was effected via balloon catheters. In addition, somatic receptive fields (RFs) were determined with non-noxious and noxious mechanical stimuli. Location of recording sites were verified histologically. Visceroceptive neurons were located in the dorsal and lateral parts of the posterior complex (POd/l). Most (80%) of the neurons responded either to colon or to esophagus stimulation. These responses were nociceptive, and consisted about equally often of discharge increases and decreases. Both types of visceroceptive neurons, however, had similar low-threshold somatic RFs, located on the lower back, hindleg or foot. Somatic RFs of the visceroceptive neurons were equally often of hetero- or homo/perisegmental origin. Only one neuron was activated by noxious mechanical stimulation of the foot as well as both kinds of viscera. All neurons with viscero-visceral inputs had excitatory responses. The results indicate that a convergent representation of thoracic and abdominal viscera may be rare in the cat's POd/l, implying organ specific processing of visceral information.

Supported by DFG grant Kn 177/5-3.

51.9

DYNAMICS OF POPULATIONS OF NEIGHBORING NEURONS FOR INNOCUOUS AND NOXIOUS STIMULI IN THE VENTROPOSTERIOR LATERAL NUCLEUS. T. Shi*, J. Brüggemann, L. R. Airapetian and A. V. Apkarian, Dept. of Neurosurgery at SUNY Health Science Center, Syracuse, NY 13210

With a multi-microelectrode recording technique, we examined the dynamics of populational neurons in the ventroposterior lateral nucleus (VPL) of the monkey. Earlier we reported that with this technique the incidence of nociresponsive neurons was much higher than that recorded with single electrodes (10% vs 30%) and that the cross-correlations between neurons were different between the noci-sites (defined as some neurons responding to a noxious stimulus online) and the rest i.e., non noci-sites. In the present study the changes in the firing rate and in cross-correlation and relative locations of neurons were further analyzed.

Individual neuronal activity of mutiple neurons were recorded from VPL of the halothane-anesthetized squirrel monkey with a matrix microelectrode. Four-channel neuronal signals were collected and then offline clustered with Datawave. Innocuous and noxious stimuli were applied. It was found that some of the recorded neurons repeatedly responded to a stimulus but others did not. Both excitatory and inhibitory cross-correlations were found in noci- and non noci-sites. Some neurons did not change their firing rates for a stimulus but their cross-correlations did. During noxious stimuli the number of exitatory cross-correlation pairs were increased compared to that during innocuous stmuli at both noci- and non-noci-sites even if there were much less nociceptive responses at non noci-sites. Pressure stimulus caused more inhibitory cross-correlation pairs than did the other innocuous stimuli at noci-sites. The averaged distance between recorded neurons at noci-sites was longer than that at non noci-sites. The results suggest that local neuronal networks in VPL constantly adjust to incoming signals by changes in firing rates and connectivities.

Funded by Department of Neurosurgery at SUNY HSC.

51.10

A MECHANICAL STIMULATOR FOR PSYCHOPHYSICAL AND NEUROPHYSIOLOGICAL STUDIES OF PAIN AND TOUCH. L. TenBokum, R.C. Kupers, C.-C. Chen, G.H. Duncan, and M.C. Bushnell*. Départ. Stomatol. & Cent. Rech. Sci. Neurol., Univ. Montréal, Montreal, Canada H3C 3J7.

Most commercially available mechanical stimulators lack the displacement and force characteristics to adequately stimulate the skin across a wide range of innocuous and noxious forces. We describe here a small, inexpensive stimulator which addresses these limitations; its application to human and monkey psychophysical and neurophysiological studies is reported.

The computer-controlled stimulator advances a mechanical probe against the skin until a target force is achieved and maintains that force until retracting the probe at the end of stimulation. A single load cell (resolution 1g; range 250g) monitors the probe force and provides feedback to a servo-controller which adjusts probe displacement (max 25mm), thus maintaining the target force in spite of variable skin compliance. Plastic tips differing in size and shape can be mounted on the probe; a conical tip with a 0.5mm spherical point was tested in human psychophysical and monkey behavioral and neurophysiological experiments.

A monotonic relationship was found between perceived stimulus intensity and probe force across a range of 5 to 200 grams applied to the maxillary face. Forces greater than approximately 100 grams were perceived as painful by the humans and were frequently escaped by the monkey. Single neurons in VPM thalamus of the monkey produced monotonic response functions when receptive fields were stimulated with the probe at different forces.

This small, inexpensive mechanical probe provides great versatility in its application to human and animal psychophysical, behavioral and neurophysiological studies. Reliable human psychophysical ratings, as well as monkey escape behavior and single-unit responses, demonstrate the usefulness of this apparatus for studies of pain and tactition.

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51.11

NEURAL CORRELATES OF STATIC MECHANICAL HYPERALGESIA IN VPM THALAMUS OF THE BEHAVING MONKEY. C.-C. Chen*, R.C. Kupers, and M.C. Bushnell. Départ. Stomatologie and Centre de Recherche en Sciences Neurologiques, Université de Montréal, Montreal, Canada H3C 3J7.

Spinothalamic tract cells show changes in activity after tissue damage consistent with their involvement in hyperalgesia. Yet, little is known about thalamic processes underlying hyperalgesia and allodynia. We studied the role of VPM thalamic neurons in the production of mild mechanical hyperalgesia.

One rhesus monkey pressed a lever to escape a mechanical stimulus or to receive food reward for detecting a light. A plastic probe (tip diam.=0.5mm; displacement=2cm) was placed 1-cm from the facial skin. At 6-10s intervals, the mechanical stimulator was applied at a force of 25, 50, 75 or 150g for 5 sec or the light was illuminated for 1.5 sec. Single VPM neurons were isolated while the monkey performed the tasks. The neuronal and behavioral responses to mechanical stimulation were tested before and after 20 min topical application of capsaicin (0.02M, dissolved in 5.0ml 70% ethanol) to the receptive field.

The monkey escaped more mechanical stimuli after capsaicin application than before ($p<0.05$), suggesting a mild mechanical hyperalgesia to punctate stimuli. Of four cells that responded maximally to noxious pressure (150g), three were more responsive to mechanical stimulation after capsaicin ($p<0.05$). In all cases, the increased responsiveness was reversed by cold application to the receptive field ($p<0.05$). Of four cells that responded maximally to innocuous mechanical stimuli, two showed *decreased* responsiveness to mechanical stimulation after capsaicin ($p<0.05$), whereas two were unaffected.

These data indicate that activity in ventroposterior thalamus could subserve mechanical hyperalgesia. The increased excitability of cells that normally code noxious stimuli is similar to that found in STT neurons of anesthetized monkeys. The decreased excitability of some tactile thalamic neurons supports other reports of C fibre modulation of low threshold somatosensory transmission.

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51.12

MICRO-INJECTIONS OF MUSCIMOL INTO LATERAL SUPERIOR COLLICULUS DISRUPT ORAL ORIENTATION TO THE SITE OF INJECTION IN THE FORMALIN MODEL OF PAIN. P. Redgrave* and S. Wang
Dept. Psychology, University of Sheffield, Sheffield, S10 2TP, U.K.

Nociceptive neurones in the basal ganglia, cerebellum and related structures are probably involved in generating nocifensive behaviour. However, little is known of the circuits through which pain can affect movement. The present study used the comparatively well understood output projection from striatum to superior colliculus (SC) via substantia nigra pars reticulata, to study the mechanisms used by rats to locate a persistent source of pain. Our specific aim was to test whether interrupting basal ganglia output at the level of the SC, by local injections of the GABA agonist muscimol, is critical for directing oral behaviour to a region of the body injected with formalin. C-fos immunohistochemistry was used to determine the regional spread of the inhibitory effect of muscimol on neuronal activity.

Oral behaviour elicited by 50µl of 4% formalin injected into the hindpaw was suppressed in a dose-related manner by micro-injections of muscimol into the lateral-SC (12.5-50ng: 0.5µl/side); injections in medial SC had little effect. Bilateral inhibition of lateral-SC caused animals to re-orient their attention and activity from lower to upper regions of space. Unilateral injections of muscimol into lateral-SC elicited contralateral turning independent of which hindpaw was injected with formalin. Oral behaviour was suppressed when injections of muscimol and formalin were contralaterally opposed. However, when formalin was injected into the perioral region, micro-injections of muscimol into contralateral lateral-SC had no effect on the ability of animals to localise the injected area with either foot.

These findings suggest that basal ganglia output via the lateral SC is critical for nocifensive responses which entail the mouth moving to and acting on the foot, but not when the foot is the active agent applied to the mouth. The data also suggest that pain produces a general facilitation of units throughout collicular motor maps, which can be converted into a spatially inappropriate signal by locally suppressing parts of the map with muscimol.. *(Supported by Wellcome Trust grant 038011/Z/93 to PR)*

51.13

PHYSIOLOGICAL PROPERTIES OF VENTROLATERAL ORBITAL (VLO) CORTEX IN AWAKE RATS WITH THERMAL HYPERALGESIA. M-M Backonja* and C. Levenick, Department of Neurology, Medical School, University of Wisconsin, Madison WI, 53792

It has been postulated, on the basis of anatomic and physiologic investigations that Ventrolateral Orbital (VLO) cortex of frontal lobe participates in pain related processes. This study was conducted for the purposes of further characterization of this cortical area in awake behaving rats. An array of 6 stainless steel electrodes was advanced according to stereotactic coordinates. There was continued monitoring of electrical activity at each electrode. Once an acceptable number of units was identified at the stereotactically determined depth, the electrodes were fixed with dental acrylic. Activity in at least two electrodes per rat remained stable for a period of several weeks. That allowed longitudinal recordings from the same group of units. Within this time period, recordings were performed before and after thermal hyperalgesia was induced by loose ligation of the sciatic nerve with chromic gut, as per Bennett and Xie (1988). Analysis of the recordings have revealed thus far that thermal stimuli produce an increase in neuronal activity, especially when stimuli are in the noxious range. VLO units responded to bilateral stimuli. The unit activity during the time when rats exhibited thermal hyperalgesia was not significantly different from the unit activity during the time prior to hyperalgesia state. This preliminary finding suggests the possibility that although VLO may be involved in pain perception, is unlikely that it is the cerebral region is involved in genesis or maintenance of neuropathic processes underlying thermal hyperalgesia.

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51.14

MAPPING PAIN CIRCUITS: LIMBIC STRUCTURES AFTER NOXIOUS STIMULATION OF VISCERA. A Solodkin[1], GF Gebhart[2] and RJ Traub*[2]. Departments of Anatomy & Neurology[1] and Pharmacology[2]. University of Iowa. Iowa City, IA 52242.

The perception of pain has been subdivided into a discriminative and an affective component. While the discriminative component of visceral pain is under much study, the latter is poorly understood. Since emotion and memory are important aspects in the perception of visceral pain, an understanding of the pathways involved is relevant. Hence, using c-Fos expression as a marker, we have studied the participation of limbic areas after noxious colorectal distention (CRD) in the rat.

Our observations showed that: i) In all cortical areas studied, layers III and V consistently had the largest density of Fos-labeled nuclei. In addition, some of these cells were identified as pyramidal by double labeling with SMI-32; ii) Within the cortical and subcortical areas studied, the distribution of Fos showed preferential localization to certain subregions and nuclei. Among the areas with the largest density of Fos-labeled nuclei were: Cingulate (Cg3), agranular insula and infralimbic cortices at the prefrontal cortex level; posterior retrosplenial and ventral perirhinal cortices, subicular complex, central nucleus of amygdala, anterior-dorsal and medio-dorsal nuclei in thalamus and ventral periaqueductal gray.

It is suggested that: 1) A subpopulation of neurons expressing Fos after noxious CRD are either cortico-subcortical projection neurons or cortico-cortical association neurons; 2) The variation in the density of Fos-labeled nuclei within and between areas provides an indication of the complexity and importance of events taking place in limbic structures during visceral pain. In addition, it provides a good model for the prediction and testing of the circuitry involved during higher cognitive functions such as emotion or memory. Supported by NS 30604 and NS 19912.

51.15

ANATOMY OF A CORTICAL ANTINOCICEPTION TRIGGERING SITE (CATS-1) IN THE AGRANULAR INSULAR CORTEX OF THE RAT

Adam R. Burkey * and *Luc Jasmin* Georgetown University Medical Center, Departments of Neurosurgery and Cell Biology, Washington, DC 20007

We have recently identified a cortical antinociception triggering site (CATS-1) in the agranular insular cortex of the rat which contributes to systemic morphine-induced antinociception in association with a descending inhibition of spinal nociceptive neurons. The anatomical connectivity of CATS-1 was determined using anterograde tracing with biotin dextran and retrograde tracing with Fluoro-Gold. Those connections with most relevance to nociception include reciprocal projections to and from the ventral lateral orbital cortex and infralimbic cortex; a bilateral projection to the nucleus accumbens; reciprocal connections with the ipsilateral basolateral amygdaloid nuclei; and reciprocal connections with the midline thalamic nuclei, lateral hypothalamus and medial parabrachial area. CATS-1 also receives afferents from the ventral tegmental area and substantia nigra. Direct injection of the CATS-1 efferent pathway through the ventral striatum filled CATS-1 neurons and their dendrites. The multipolar morphology of these cells, with somata 40 µm and extended dendrites measurable to 600 µm, strongly resembles that of the mu-opioid receptor bearing cells defined by mu-opioid receptor immunocytochemistry in CATS-1. This study suggests possible routes by which morphine acting within CATS-1 could result in descending inhibition of nociceptive neurons. This route appears to be through the rostral ventral medulla via the lateral hypothalamus, and through the periaqueductal gray via the infralimbic cortex. The connections of CATS-1 to the midline thalamus and limbic structures further imply that CATS-1 could modulate nociception through mechanisms other than descending inhibition.

This work supported by a Howard Hughes Fellowship Training Grant.

51.16

NOCICEPTIVE NEURONS IN PRIMATE INSULAR CORTEX. J.O. Dostrovsky* and A.D. (Bud) Craig, Dept. of Physiology, Univ. of Toronto, Ontario, Canada, and Div. of Neurobiology, Barrow Neurological Institute, Phoenix, AZ 85013.

Previous work indicates that a distinct region of the dorsal anterior insula, posterior to gustatory cortex, receives topographic input from the posterior part of the ventral medial n. (VMpo), a lamina I spinothalamic relay nucleus in monkey and human thalamus that contains a concentration of nociceptive- and thermoreceptive-specific neurons (Nature 372:770, 1994; SNA 21:1165, 1995). In this study, we examined the response properties of neurons in this cortical region.

Recordings were made with tungsten microelectrodes using a dorsal approach in pentobarbital-anesthetized cynomolgus monkeys. Histological reconstructions from Nissl sections verified penetrations through the appropriate region of the insula. In one animal anterograde dextran labeling from VMpo (injected one month prior to recording) confirmed the recording sites within the VMpo projection field. In such sites responses to noxious mechanical and in some cases also thermal stimuli could be evoked (22 background or multi-unit clusters and 19 single units in a total of 16 tracks). Most of these neurons responded tonically (or phasically) only to noxious stimulation. A somatotopic organization was found with face most anterior and arm posterior, consistent with anatomical findings. Interestingly, in one monkey a region in dorsal insula anterior to the VMpo projection field was found where noxious stimulation produced a profound inhibition of spontaneous firing.

This demonstration of a concentration of nociceptive neurons in primate anterior insula confirms that VMpo is a lamina I spino-thalamo-cortical sensory relay nucleus and establishes a solid basis for the EEG and PET activation of human insular cortex by noxious stimuli. This supports the fundamental concept that lamina I projections relate the sense of the physiological condition of the body, which includes the specific sensations of pain and temperature. (supported by DE 05404 and NS 25616)

51.17

PERSISTENT NOXIOUS INPUT VIA INFLAMMATION PREFERENTIALLY AFFECTS THE EVOKED RESPONSES OF NOCICEPTIVE SI NEURONS TO MECHANICAL STIMULI. M. Sholas* and D.R. Kenshalo, Jr. Neurobiology & Anesthesiology Branch, NIDR, NIH, Bethesda, MD. 20892, * Program in Neuroscience, DMS, Harvard Medical School, Boston, MA. 02115.

Subcutaneous injection of 30 mg of carrageenan into the glabrous skin of the hand of a monkey produces a profound increase in volume(72±24%) lasting 24 to 48 hours. We examined the effects of inflammation on the population of SI neurons. Male rhesus monkeys were sedated with ketamine 24 hours prior to the recording session for the carrageenan injection. We recorded from nociceptive units in the hand representation of SI. Once a nociceptive neuron was isolated, the receptive field of each neuron 500 µM above and below it were categorized as: low threshold mechanoreceptor (LTM), wide-dynamic range (WDR), nociceptive specific (NS), deep/joint receptor (DT), or as a hybrid between WDR and LTM (HB). The categorization was based on the responses of the neuron to a graded series of mechanical stimuli and an intradermal capsaicin injection (50 µg).

Eighty neurons were studied in the inflammed state and compared to a control population of 88 neurons. The group after inflammation had similar proportions of cell types as the control group. The receptive field sizes were comparable as well. However, the evoked responses of nociceptive units to pressure and pinch stimuli in the group after inflammation were significantly (ANOVA, $p<0.05$) increased as compared to those in the control population. No differences were found in the evoked responses to brush stimuli, or in the background activity of the neurons. The hyperalgesia associated with inflammation reflect a central hyperexcitability of nociceptive units without global changes in the number of neurons responsive to noxious stimulation.

51.18

ANTERIOR CINGULATE PROJECTION FROM MDvc (A LAMINA I SPINOTHALAMIC TARGET IN THE MEDIAL THALAMUS OF THE MONKEY). A.D. (Bud) Craig* and E.-T. Zhang. Div. of Neurobiology, Barrow Neurological Institute, Phoenix, Arizona 85013.

Earlier PHA-L results demonstrating topographic lamina I trigemino- and spinothalamic terminations in the ventral caudal part of the medial dorsal n. (MDvc, a distinguishable region in both monkey and human thalamus) suggest a primate medial pain pathway. We have examined the cortical projections of MDvc, which prior studies have not established.

Injections in MDvc of anterograde tracers (TRITC- and biotin-labeled dextrans, 7 µA x 20 min, or 1-3 µl; barbiturate-anesthetized cynomolgus monkeys; 3-4 wks survival) produce a discrete band of middle layer terminal labeling in the fundus and both banks of the anterior cingulate sulcus at the level of the genu of the corpus callosum (area 24c). Cortical injections (FB, DY and CTb) retrogradely label MDvc only from this sulcal area, confirming the anterograde finding. Detailed analyses of the neighborhood relationships of this area with regard to connections with other regions (VL/VA, AM, Csl, Cem; motor and insular cortex; spinal cord; amygdala) have begun.

This result identifies an anatomical substrate that could be the basis for the involvement of anterior cingulate cortex in pain (and itch) indicated by clinical and PET evidence. This lamina I spino-thalamo-cortical pathway could underly the thermal grill pain illusion and central pain, consistent with a fundamental role in the integration of pain, temperature and homeostatic activity. (Supported by NS 25616)

51.19

OVERLAP OF MEDIAL WALL fMRI ACTIVITY BETWEEN PAIN, MOTOR, AND VIBROTACTILE TASKS IN HUMANS, P.A. Gelnar[1], B.R. Krauss[1]*, N.M. Szeverenyi[2], A.V. Apkarian[1], Depts. Neurosurgery[1] and Radiology[2], SUNY HSC; Syracuse, NY.

A recent comparison of medial wall activity in human PET studies to monkey data (Picard and Strick, 1996) revealed corresponding subdivisions in supplementary motor area (SMA and pre SMA), and rostral (anterior and posterior divisions; RCZa, RCZp) and caudal cingulate (Cing) zones (CCZ). The present study compares the medial wall functional magnetic resonance imaging (fMRI) pain activations to the above subdivisions. fMRI studies were done in which motor, vibratory, and painful stimuli were utilized in the same subject.

Multislice fMRI (echoplanar BOLD) was utilized to investigate cortical activity in 28 subjects. Eight slice locations were imaged beginning at either the anterior commissure (AC) and extending rostrally 45.5mm or 6.5mm posterior to the AC extending caudally 45.5mm. Significant medial wall clusters were identified and their slice locations and dorsoventral position noted.

For all three tasks activity was seen bilaterally in SMA and Cing. In the motor task contraSMA and contraCing were more active than ipsiSMA and ipsiCing. However in the pain task the ipsiSMA was more active than contraSMA. There was greater activity in contraSMA with the motor task as compared to noxious heat and vibratory stimuli ($p<0.005$). In the motor task, 2 subdivisions were found in SMA and 3 subdivisions in Cing, corresponding respectively to preSMA, SMA and RCZa, RCZp and CCZ. In the pain task, specific portions of contra-preSMA and contra- SMA were active, while Cing regions were more homogeneously activated bilaterally.

The results show substantial overlap between tasks in the medial wall cortex. Although there may be specialized segregations within these subdivisions.

This research was funded by the Department of Neurosurgery at SUNY HSC.

52.1

ADULT RATS SPINALIZED AT BIRTH EXHIBIT INAPPROPRIATE WITHDRAWAL REFLEXES.

J. Schouenborg, H. Holmberg*, X.-L. Luo and A. Levinsson. Dept. of Physiology and Neuroscience, Lund University, Lund, Sweden.

Aim: In normal adult rats, single hindlimb muscles receive cutaneous excitatory input from well defined receptive fields whose distribution of sensitivity reflects the withdrawal action of the muscle. This spatial organization is attained through a postnatal tuning process. The underlying mechanisms are not known. To study the role of descending pathways in this context, withdrawal reflexes were studied in adult rats which had been spinalized at birth.

Methods: Electromyography was used to record CO_2-laser evoked reflexes (20-30 sites were stimulated on the plantar surface of the right hindpaw) of peroneus longus, extensor digitorum longus and gastrocnemius muscles in adult decerebrated rats (n=5) which had been spinalized at the level of Th 8-10 at birth. In addition, compound hindlimb reflex responses evoked by CO_2-laser stimulation were characterized in four awake rats (recorded on videotape).

Results: Hindlimb reflexes were typically highly excitable, being evoked even by light touch. Reflexes of gastrocnemius, which are abolished after spinalization in normal adult rats, were consistently evoked. The functionally adapted spatial organization of the withdrawal reflexes seen in normal rats was lacking.

Conclusions: Withdrawal reflexes in rats with chronic spinal transection do not become functionally adapted as in normal rats, indicating that the normal postnatal tuning of withdrawal reflex function depends on intact descending connections from the brain.

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52.2

ADRENALECTOMY CAUSES EXAGERATED PERIPHERAL AND NEURONAL RESPONSE TO NOXIOUS STIMULATION THAT IS REVERSED BY DEXAMETHASONE. M.A. Ruda*, K. Malsnee and R.X. Zhang. NAB, NIDR, NIH, Bethesda, MD 20892.

Glucocorticoids (GC) function peripherally to suppress inflammation by acting on the inflammatory/immune response and centrally at neuronal steroid receptors to modify gene expression. GC receptors include subtypes with corticosterone (CORT) having a high affinity for type I and dexamethasone (DEX) having a high affinity for type II. This study examines the role of GC in a model of peripheral inflammation and hyperalgesia. Male Sprague-Dawley adrenalectomized (ADX) rats with, and without, GC replacement by CORT or DEX were compared to normal (N) and sham (SH) surgery rats. CORT or DEX was delivered by osmotic minipumps (10-20 µg/µL/hr) beginning 4 days prior to unilateral hindpaw injection of complete Freund's adjuvant (CFA) (4 weeks after ADX). Paw withdrawal latency to radiant heat was measured at 2 hr, 1, 2, and 3 days. The post-CFA withdrawal latency in ADX rats was significantly shorter than N and SH rats at 2 hr; ADX rats receiving CORT were similar to control rats, while DEX rats demonstrated significantly less hyperalgesia. Edema in ADX rats resulted in significantly larger paw diameters than N and SH rats; ADX rats receiving CORT were similar to control rats, while those receiving DEX had substantially reduced edema. Day 3 after CFA injection the spinal lumbar enlargement was removed, total RNA extracted, and RNA blots hybridized with a preprodynorphin (PPD) probe. ADX markedly increased the induction of PPD mRNA related to inflammation and hyperalgesia compared to that in N and SH rats. CORT reversed the PPD enhancement by ADX back to N and SH induced levels; DEX rats showed no PPD induction. These data demonstrate profound effects of GC on both the peripheral and central response to inflammation and hyperalgesia. Since DEX acts mainly at the type II GC receptor, these results suggest a major role for this receptor in the response to a noxious inflammatory agent. The substantial attenuation of PPD gene induction after DEX treatment suggests that DEX may be clinically useful to prevent excess neuronal activity in nociceptive neuronal circuits and prevent their alteration.

Funded by IRP, NIDR, NIH.

52.3

NEONATAL RATS EXHIBIT AN EARLIER DEVELOPMENT OF PEAK RESPONSE TO PERIPHERAL INFLAMMATION AND HYPERALGESIA THAN ADULT RATS. R.X. Zhang*, K. Malsnee and M.A. Ruda. NAB, NIDR, NIH, Bethesda, MD 20892.

Spinal nociceptive neural circuits undergo considerable postnatal (P) changes. Although peptide-containing primary afferents are present in the spinal cord at birth, their synaptic circuits continue to develop postnataly. This study examines neonatal rats' peripheral response to noxious stimulation and spinal neuronal gene induction in comparison to that of adult rats in a model of peripheral inflammation and hyperalgesia. Complete Freund's adjuvant (CFA) was injected unilaterally into the hindpaw of P1, P3, P9, P13, P21 and adult rats. Paw diameter and withdrawal latency to radiant heat were examined at 2hr, 1, 2, and 3 days after CFA injection and preprodynorphin (PPD) mRNA expression was measured at 4hr, 8hr, 1, 2, and 3 days. The extent of edema, based on difference scores between the ipsi and contra hindpaws, was different in each age group but all peaked at day 1 (0.16 cm at P1 and P3, 0.26 at P9 and P13, 0.3 at P21 and 0.45 for adult rats). In neonates thru P21 the withdrawal latency in the injected paws was shortest at 2hr and approached control latencies by day 3, except for P21 rats which continued to show significant hyperalgesia. Adult rats exhibited peak hyperalgesia at day 1, which was reduced at day 3 but still significant. Baseline withdrawal latency of neonates was 1 sec faster than adults. The constitutive expression of PPD was higher in neonatal than adult rats. At P9 there was significant induction of PPD in ipsilateral spinal cord, which was near peak levels by 8hrs after CFA and significantly reduced by day 3. In contrast, the PPD increase in adult rats reached peak levels at day 1 after CFA and persisted at high levels through day 3. Neonates and adult rats had the same peak percent increase in PPD mRNA expression. These data demonstrate a robust inflammation induced hyperalgesia in the neonates and a corresponding dramatic response in spinal nociceptive neuronal circuits that occurs faster than seen in the adult. The fact that in neonates the PPD mRNA induction occurs at the same percent increase as seen in adults stresses the importance of pain control in neonates to prevent alterations in spinal neural circuits due to excessive activity in pain pathways. Funded by IRP, NIDR, NIH.

52.4

HORMONAL AND BEHAVIOURAL EFFECTS OF GONADECTOMY IN FORMALIN-TREATED MALE AND FEMALE RATS A.M. Aloisi* and C. Lupo. Istituto Fisiologia Umana, Univ. degli Studi di Siena, I-53100 Siena Italy

Sex hormones play a role in the hormonal and behavioural responses to pain. In the present experiment we evaluated the interaction between gonadectomy (gdx) and persistent pain induced by formalin in male and female rats. Twelve males and 12 females were anesthetized and then half were gonadectomized and half were sham-operated. Twenty days later they were s.c. injected with formalin (50 µl, 10%) and tested in the hole-board for 60 min. Rats were then anesthetized and blood was collected from the abdominal aorta. ACTH, corticosterone, testosterone and estradiol plasma levels were determined by RIA. Gdx increased paw-jerk frequency in males but decreased it in females. In addition gdx increased flexing duration in both sexes. Gdx did not affect ACTH and corticosterone, while it decreased testosterone and estradiol in both sexes. These data indicate that the gdx-induced effect on pain-evoked responses is not related to significant modifications in ACTH and corticosterone plasma levels.

This research was supported by MURST, Italy (60% funds)

52.5

CHEMICAL LESIONS TO BRAINSTEM DOPAMINERGIC NEURONS FACILITATE NOCICEPTIVE REFLEXES (NR) AND ACCELERATE AUTOTOMY (AT) BEHAVIOR IN RATS. S.F. Atweh*, S.J. Jabbur, N.B. Bahuth and N.E. Saadé. Fac. of Med., American Univ. of Beirut, Lebanon.

Previous work from our laboratory has shown that kainic acid lesions of striatal neurons inhibited NR and reduced AT in rats (Saadé et al. Soc. Neurosci. Abstr. 21:1166, 1995 and *Physiol. & Behav.*, in Press). In view of the important input to the striatum from the substantia nigra (SN), the aim of this study was to investigate the effects on NR and AT of selective chemical lesions to the dopaminergic supply to the striatum.

Various groups of rats were subjected to pain tests including paw pressure (PP), hot plate (HP) and tail flick (TF) latencies one week prior to injection of saline (sham group) or 5-hydroxydopamine (20µg in 3.3µl) either in the striatum or in the SN. After one week recovery, NR were tested for another 2 week period. Then the animals were subjected to a leg denervation contralateral to brain injection site.

Latencies of TF, HP and PP on the contralateral leg were significantly decreased (30%, 40% and 45%, respectively) while the AT of the denervated legs were significantly accelerated. We conclude that the dopaminergic nigrostriatal system plays a role in triggering central mechanisms involved in acute and chronic deafferentiation pain.

(Supported by grants from the *DTSabbagh Fund* and the *University Research Board.*)

52.6

THE EFFECTS OF NMDA ANTAGONIST, AP5, INJECTED INTO THE DENTATE GYRUS AND PERIAQUEDUCTAL GREY ON FORMALIN-INDUCED PAIN IN RATS. J.E. Magnusson*, K.L. Ruck, G.J. Mader, Jr., H. R. Clemmons & A. L. Vaccarino, Department of Psychology, University of New Orleans, LA 70148.

Several lines of evidence show a critical role of the *N*-methyl-D-aspartate (NMDA) receptor in the development of formalin-induced pain. In the present study we examined the role of NMDA activity in the dentate gyrus and periaqueductal grey in the development of formalin-induced. Under sodium pentobarbital anesthesia rats were implanted unilaterally with a guide cannula aimed at the dentate gyrus or periaqueductal grey. Following a 7-14 day recovery period rats received an infusion of the NMDA antagonist, AP5 (5 ug/0.5 ul/1 min), or saline into the dentate gyrus or periaqueductal grey. Five min after the infusion of AP5 rats received a sc injection of 1% formalin into the plantar surface of the paw contralateral to the cannula. The rats were then observed for pain-related behaviours (paw elevation) for the subsequent 70 min. The results showed that AP5 had differential effects when injected into the dentate gyrus and periaqueductal grey. Supported by a Louisiana Education Quality Support Fund (LEQSF).

52.7

HUMAN Aδ AND C-FIBER NOCICEPTOR RESPONSES TO LOW INTENSITY PAINFUL HEAT. R. H. Gracely and D. R. Kenshalo Jr.*. NAB, NIDR, NIH Bethesda MD 20892.

Peripheral pain suppression and central summation have been demonstrated with frequent (0.3 Hz), intense (51°C) heat pulse stimulation. This study used low intensity, infrequent heat stimuli applied to the volar forearm to evaluate temporal effects of Aδ and C-fiber mediated pain sensations.

Forty-two subjects performed both pain rating and reaction time tasks to pain sensations evoked by trains of 8 3s-duration heat stimuli delivered at 15s intervals via a 0.8 cm diameter contact thermode. In random order, stimulus trains were delivered to each of two sites 3 cm apart (SAME), or to a new site for each stimulus in each of two trains (CHANGE). Stimulus temperatures in each train were 47;47,47,47,45,45,47,47°C

Responses to 47°C were stable in the CHANGE condition. In the SAME condition, responses (stimulus 1 rating=13.9, latency=1657 msec) were altered to the second stimulus (rating decrease to 10.3, P<.0001; latency increase to 2738 msec, p<.0001) and stable thereafter. Correcting for increased latencies observed with less intense sensations, the increased latency to stimulus 2 (0.4s) is consistent with a shift in conduction velocity from Aδ to C-fibers. Stimulus control was demonstrated by increased latencies and decreased ratings to 45°C in each condition (p<0.0001).

These results show that repetitive 47°C stimuli suppress Aδ activity, isolating Aδ and C-fiber nociceptor function.

Funded by IRP, NIDR, NIH.

52.8

EXTEROCEPTIVE SUPPRESSION OF MASSETER MUSCLE ACTIVITY EVOKED BY PAINFUL HEAT STIMULI IN HUMANS. J. Ellrich[1], H.C. Hopf [2] and R.-D. Treede[1] (SPON: European Neuroscience Association). Johannes Gutenberg-University, [1]Institute of Physiology and Pathophysiology, Saarstr. 21, D-55099 Mainz, [2]Department of Neurology, Langenbeckstr. 1, D-55101 Mainz, Germany.

Electrical stimulation of the mental nerve evokes two suppression periods (SP) in masseter muscle activity. It is controversial whether these periods are evoked by activation of tactile (Cruccu et al., Exp. Brain Res., 77 (1989): 447-450) or nociceptive fibers (Miles et al., Exp. Brain Res., 65 (1987): 331-336).

We investigated 10 healthy volunteers (20 to 26 years) using an infrared laser, that selectively activates nociceptors in hairy skin. Perceptual and pain thresholds of mental nerve area were determined. While volunteers clenched their teeth at maximum strength 20 painful laser stimuli (600 mJ) were applied. Latencies of SP were measured from the rectified and averaged signal. A SP was defined as more than 50% suppression of the background EMG, measured in a 100 ms pretrigger interval. Latencies and durations of SP were measured at the point when EMG activity reached 80% of the background level. SP was also elicited by electrical pulses (200 μs) with intensities twice the individual pain threshold.

With a mean pain threshold of 366 ± 68 mJ the applied intensity was certainly painful in all volunteers. In 9 of 10 subjects the SP could bilaterally be evoked by painful laser stimuli. The onset latency was 46.9 ms, the duration 58.8 ms. In only one subject SP consisted of two separate periods. The electrical thresholds of detection were 1.3 mA, of early SP 9 mA, of late SP 4.7 mA, and of pain 11.6 mA. The onset latencies were 11.8 ms and 44.9 ms for electrically evoked SPs, respectively.

Comparing the latencies of laser and electrically evoked SP one has to consider the nociceptor activation time of about 40 ms. The adjusted latency of laser evoked SP agrees well with that of the electrically evoked early SP, but not late SP. The high electrical threshold of the early SP could point to a nociceptive origin as well.

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PAIN MODULATION: ANATOMY AND PHYSIOLOGY—HIGHER CENTERS I

53.1

EFFECTS OF SEROTONIN RECEPTOR AGONISTS ON NEURAL ACTIVITY IN THE ROSTROVENTRAL MEDULLA (RVM). R. Vallabhapurapu, M. Dewberry, C.L. Thurston*. Dept. Biomedical Sciences, Univ. South Alabama, Mobile, AL 36688.

I.v. administration of serotonin (5-HT) inhibits the tail flick (TF) reflex and produces triphasic effects on mean arterial pressure (MAP) and ON and OFF cell activity of the RVM. The current study compared the effects of i.v. administration of a 5-HT_1 receptor agonist (5-carboxyamidotryptamine maleate) and a 5-HT_3 receptor agonist (2 methyl 5-HT maleate) to the effects of 5-HT on TF latency, MAP, and ON and OFF cell activity in rats.

The 5-HT_1 receptor agonist produced a dose-dependent (0.1 - 40 nmole) decrease in MAP for 1-15 min that was tightly correlated to an increase in ON cell activity and a decrease in OFF cell activity. The greater doses produced a delayed increase in TF latency. The 5-HT_3 receptor agonist (10 - 720 nmole) produced a brief decrease in MAP (30-120 sec), a dose-dependent excitation of ON cells that lasted less than 30 sec, and inhibition of OFF cells that lasted up to 7 min at the greater doses. The 5-HT_3 receptor agonist also inhibited the TF reflex 10 sec after injection of the greater doses, similar to 5-HT. These data suggest that the inhibition of the TF reflex observed within 10 sec after 5-HT administration may involve activation of 5-HT_3 receptors. The 5-HT-induced decreases in MAP, excitation of ON cells, and/or inhibition of OFF cells may involve activation of 5-HT_1 and/or 5-HT_3 receptors. Further studies are underway to determine the effects of 5-HT_2 and 5-HT_4 receptor agonists on MAP and ON and OFF cell activity, and to determine the role of vagal afferents in any of the observed effects.

Supported by NIH grant NS31495

53.2

CHEMICAL STIMULATION OF THE ROSTRAL VENTROMEDIAL MEDULLA: ANALYSIS OF CONDITIONED PLACE AVOIDANCE AND STRESS HORMONE RELEASE. M. M. Morgan*, P. K. Whitney, J. S. Boyer and A. M. Strack. Dept. of Psychology, Washington State University, Vancouver, WA 98686 and Dept. Physiology, UCSF, San Francisco, CA 94143, USA

Stimulation of the rostral ventromedial medulla (RVM) produces both antinociception and immobility. Because RVM stimulation reduces pain it is assumed to be rewarding. In contrast, because immobility is a defensive behavior it is assumed to be aversive. The objective of the present study was to determine if stimulation of the RVM is aversive by assessing conditioned place aversion and the release of stress hormones.

Male Sprague-Dawley rats were tested in one of two ways: 1) Conditioned Place Aversion--microinjection of kainic acid (40 pmol/0.2 μl) and saline into the RVM were paired with different sides of a conditioning chamber over 4 days. On the fifth day, the rat was placed in the center of the conditioning box and the amount of time spent on each side was measured. 2) Stress Hormones--rats were decapitated 5 min after microinjection of kainic acid or saline into the RVM so that blood plasma could be collected and assayed for ACTH and corticosterone levels.

Following training, rats neither preferred nor avoided the side of the chamber paired with kainic acid microinjections. Likewise, there was no difference in ACTH (mean = 351 vs 362 pg/ml) or corticosterone levels (27 vs 31 μg/dl) for rats receiving kainic acid and saline, respectively. As reported previously, locomotion was significantly reduced in rats receiving kainic acid compared to saline controls (mean of 6.6 vs 19.1 squares crossed in 30 s; $t(42) = 5.62$, $p < .05$).

These data indicate that despite the production of immobility, activation of RVM neurons is not aversive. This is surprising given that the RVM and periaqueductal gray (PAG) are two parts of the same descending pathway, and stimulation of the dorsolateral PAG produces place aversion and an elevation in stress hormones.

This investigation was supported in part by funds provided for medical and biological research by the State of Washington Initiative Measure No. 171.

53.3

IDENTIFICATION OF RAPHE MAGNUS NEURONS ACUTELY ISOLATED FROM DEVELOPING RAT MEDULLA. S. C. Nam,* S. S. Cho, W. Lim and J. Kim. Dept. of Physiology, Chonnam University Medical School, Kwangju and Depts. of Anatomy and of Physiology & Biophysics, College of Medicine, Seoul National University, Seoul, Korea

The nucleus raphe magnus(NRM) is one of the major nuclei in the rostral medulla. It is known that neurons in the NRM are involved in the descending modulation of nociceptive processing from supraspinal levels. Cellular mechanisms in these neurons, however, are poorly understood. In this study, as a tool to study its cellular mechanism, we tried to identify NRM neurons acutely isolated from medial and ventral medulla at the level of facial nerve compared to those in postnatal rat medulla *in situ*. Immunohistochemical method using antibody against serotonin was used to identify NRM neurons because these neurons contain serotonin. Positively stained neurons with the antibody were observed *in situ* as well as in neurons isolated enzymatically and mechanically from medial and ventral medulla. A small number of neurons were immunostained and displayed either bipolar or multipolar neurites in both preparations. Viability of these neurons was also checked using electrophysiological method. High-threshold and low-threshold, voltage-activated calcium currents were recorded in the acutely isolated multipolar neurons. These results indicate that NRM neurons acutely isolated from postnatal rat medulla are intact and can be used for studying cellular mechanisms of their descending modulation of nociceptive procgessing.

53.4

INTRINSIC FIRING RHYTHM IN RAPHE MAGNUS. T.R. White and I.D. Hentall*. University of Illinois College of Medicine, Rockford, IL 61107.

We explored the source and statistical structure of the roughly periodic activity found in nociceptively-inhibited raphe magnus cells. Techniques included cathodal excitation (artifact <1 ms) or anodal block via the extracellular recording microelectrode, and iontophoresis of clomipramine (a 5-HT re-uptake blocker). In pentobarbital-anesthetized rats (female, 250-300 g), spontaneous intervals (I) were fitted to the standard gamma probability-density distribution,

$$P(I) = \beta^{-\alpha} I^{(\alpha-1)} \exp(-I/\beta)/\Gamma(\alpha).$$

Group means for estimates from 46 cells were 6.4 for unitless "delay" parameter α, 31 ms for "rate" parameter β, 90 ms for the mode of I (= αβ−β), and 147 ms for mean I (= αβ). Correlation of adjacent intervals was weak but positive in most cells ($p<0.05$); mean r was 0.072 in 15 cells with 4,000 spikes/cell.

Oscillation can be defined parametrically from the theoretical autocorrelation A(I) for gamma-distributed, serially uncorrelated, normalized (β=1) intervals,

$$A(I) = \exp(-I)\Sigma I^{(\alpha n-1)}/\Gamma(\alpha n)$$

(summing over n = 1, 2, 3,...∞). Multiple, evenly-spaced autocorrelogram peaks, to which intuitive definitions appeal, exist if α>2. A definition with acceptably conspicuous peaks is α>4 (coefficient of variation = $\alpha^{-0.5}<0.5$).

Spike-triggered pulses at excitatory threshold (0.3 ms, -0.4 to -3.1 μA), given about 50% out of firing phase, caused a mean wait until the next spike of 1.2 mean spontaneous intervals (13 cells). The comparable wait was 0.4 (6 cells) after anodal block timed to erase one expected spike (30-60 ms, 0.13 to 0.67 μA). The post-block interval was fitted by a much lower α (2.3, control 6.1), with β relatively unchanged (22 ms, control 27). Conversely, steady clomipramine (1 to 20 nA, 2 min, 7 cells) markedly altered β (376 ms, control 87), but not α (4.6, control 4.9); buspirone (a 5-HT1a agonist) was similarly selective.

This resetting of the rhythm implies an "oscillator" intrinsic to the cell. We suggest that random excitatory input is low-pass filtered by dendritically released 5-HT, which inhibits the activated cell at its 5-HT1a autoreceptors.

Supported by NIH (NS26116)

53.5

INTERFERENCE WITH EXCITATORY AMINO ACID (EAA) TRANSMISSION WITHIN THE ROSTRAL VENTROMEDIAL MEDULLA (RVM): EFFECTS OF BLOCKING ON-CELL DISCHARGE. V. Tortorici*, S. McGaraughty and M.M. Heinricher, Div. Neurosurgery, Oregon Health Sciences University, Portland, OR 97201.

Two classes of neurons with distinct responses to opioids have been identified in the RVM, a region with a well-documented role in nociceptive modulation. "Off-cells," activated by opioids, are likely to exert a net inhibitory effect on nociceptive processing. This activation is indirect, via disinhibition. "On-cells" are directly inhibited by opioids. This cell population thus provides a means through which opioids can gain access to the modulatory circuitry of the RVM, and it has been suggested that on-cells act as inhibitory interneurons within this region.

We have previously shown that EAA neurotransmission is crucial to the nocifensor reflex-related on-cell burst. The aim of the present study was to examine the consequences of blocking this input for neuronal interactions within RVM, and for nociceptive responsiveness, by infusing the non-selective EAA receptor antagonist kynurenate (1-2 nmol, 200-500 nl) into the RVM while recording activity of on-, off- and neutral cells in lightly anesthetized rats. Kynurenate infusions led to a significant decrease in on-cell firing, including suppression of the on-cell burst. Off-cells continued to pause. Tail flick latency was unaffected by kynurenate infusions.

That the on-cell burst is blocked without affecting the off-cell pause indicates that on-cells do not have an important role as inhibitory interneurons within the RVM. Moreover, under the conditions of these experiments, the role of this cell class in controlling ongoing nociceptive responses is not critical.

Supported by grants from NIDA, the National Headache Foundation, and the Fogarty International Center (VT).

53.6

THE EFFECTS OF MORPHINE IN THE BASOLATERAL NUCLEUS OF THE AMYGDALA ON THE ON, OFF, AND NEUTRAL CELLS IN THE ROSTRAL VENTROMEDIAL MEDULLA OF ANESTHETIZED RATS. S. McGaraughty* and M.M. Heinricher, Div. of Neurosurgery, Oregon Health Sciences University, Portland, OR, 97201.

The amygdala is implicated in fear-motivated defensive responses, one of which is hypoalgesia. Freezing and analgesia defensive responses require the periaqueductal grey (PAG), a site which has dense reciprocal connections with the amygdala. However, PAG-mediated antinociception occurs via the rostral ventromedial medulla (RVM), which has direct projections to the dorsal horn. Three classes of physiologically characterized neurons have been previously identified in the RVM. Morphine given systemically, in the PAG, or locally in the RVM activates OFF cells, inhibits ON cells and does not affect NEUTRAL cells. In order to show that hypoalgesia induced by intra-amygdaloid morphine involves RVM neurons, a microelectrode was placed in the RVM of lightly anesthetized rats to record the activity of ON, OFF, and NEUTRAL cells while morphine (10 μg in 0.4μl) was infused bilaterally into the basolateral amygdala (BLa). Following the BLa morphine, tail flick latency increased significantly from baseline as did OFF cell activity; ON cell activity was depressed and NEUTRAL cells were unaffected. All effects were reversed by naloxone (0.4 mg/kg, iv). These results demonstrate that the infusion of morphine into the BLa causes hypoalgesia and recruits RVM ON and OFF cells in a similar manner to systemic, PAG, or locally administered opioids.

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53.7

SPECTRAL ANALYSIS OF ARTERIAL BLOOD PRESSURE AND PONTOMEDULLARY RAPHE NEURONAL ACTIVITY IN THE ISOFLURANE-ANESTHETIZED RAT. C.G. Leung* and P. Mason, Committee on Neurobiology and the Dept. of Pharmacological and Physiological Sciences, The University of Chicago, Chicago, IL 60637

In order to determine whether the discharge of neurons in the nucleus raphe magnus (RM) and adjacent nucleus reticularis magnocellularis (NRMC) reflects autonomic as well as nociceptive processes, spectral analyses were used to determine component frequencies common to both arterial blood pressure (abp) and the activity of RM/NRMC neurons in rats lightly anesthetized with isoflurane. The abp contained extremely low frequency (period length: 6.4-18.5 min) oscillations that have not previously been described in the rat. Oscillations of the same frequency were also found in the activity of several classes of RM/NRMC neurons. These oscillations corresponded to the alternating bursts of activity and periods of inactivity exhibited in the records of ON, OFF, and NEUTRAL cells or the rhythmic changes in REGULAR and SEROTONERGIC-LIKE cell activity. The discharge of all ON cells increased during periods of low abp, whereas the discharge of OFF cells and SEROTONERGIC-LIKE cells tended to increase during periods of high abp. In contrast, the discharge of NEUTRAL and REGULAR cells did not have a consistent relationship to abp. ON, OFF and SEROTONERGIC-LIKE cells may participate in the modulation of both autonomic and nociceptive processing. The role of NEUTRAL and REGULAR cells in both autonomic and nociceptive processing remains unclear. This research was supported by grants NS33984 & DA07861 (PM) & 1F31DA05698 (CGL).

53.8

PHYSIOLOGICAL CHARACTERIZATION OF SEROTONERGIC CELLS IN THE PONTOMEDULLARY RAPHE AND RETICULAR FORMATION Peggy Mason*, Dept. of Pharmacological & Physiological Sciences and Committee on Neurobiology, University of Chicago, Chicago, IL 60637.

Although serotonin is an important neuromodulator of nociceptive, autonomic and motor processing in the spinal cord, little is known about the physiological characteristics of SEROTONERGIC neurons in the inferior raphe nuclei. After characterizing neurons by their responses to noxious stimulation and their unstimulated discharge pattern, 47 cells were intracellularly labeled with Neurobiotin in the lightly anesthetized rat; all labeled cells were tested for serotonin immunoreactivity. Labeled neurons were located in raphe magnus, pallidus and obscurus and in the adjacent ventral reticular nuclei. SEROTONERGIC cells discharged, without long pauses, at an average interspike interval of 738 ms with a range of 320 - 2039 ms. Most SEROTONERGIC cells cells were unaffected or slightly excited by pinch and unaffected by noxious heat. Non-serotonergic cells were physiologically heterogeneous and differed from SEROTONERGIC cells in that they 1) were consistently excited or inhibited by noxious heat; 2) had a "bursty" discharge pattern and/or 3) had a rapid discharge rate. A discriminant function was derived which allows for the identification of pontomedullary SEROTONERGIC neurons based solely on their unstimulated discharge characteristics. The physiological characteristics of pontomedullary SEROTONERGIC neurons suggest that these neurons 1) have state-dependent activity and 2) are not directly affected by opioid administration. This research was supported by grants DA07861 and NS33984 and the Brain Research Foundation.

53.9

EVIDENCE THAT THE ANTINOCICEPTION PRODUCED BY CARBACHOL IN THE NUCLEUS RAPHE MAGNUS IS MEDIATED BY NORADRENERGIC NEURONS IN THE A7 CATECHOLAMINE CELL GROUP. K. Nuseir*, B.A. Heidenreich, and H.K. Proudfit, Dept. of Pharmacology, Univ. of Illinois at Chicago, Chicago, IL 60612.

Activation of neurons in the nucleus raphe magnus (NRM) produces antinociception that may be mediated in part by the activation of spinally-projecting noradrenergic neurons. Anatomical studies have demonstrated that neurons in the NRM project to noradrenergic neurons in the A7 catecholamine cell group that innervate the spinal cord dorsal horn, while pharmacological studies have demonstrated that stimulation of A7 neurons produces antinociception that can be reduced by intrathecal injection of α_2-adrenoceptor antagonists. The present studies were designed to provide more direct evidence that activation of NRM neurons produces antinociception by activating noradrenergic neurons in the A7 cell group. NRM neurons were stimulated by microinjecting the cholinergic agonist carbachol (5 μg) into sites in the ventromedial medulla of lightly anesthetized Sprague Dawley rats (Sasco). The antinociception produced by carbachol was assessed using the tail flick test. Microinjection of carbachol into sites in the NRM produced antinociception that lasted more than 60 min. However, the effects of carbachol were attenuated by microinjection of the local anesthetic tetracaine (10 μg) into sites within the A7 cell group. Similar injections of tetracaine more than 500 μm from A7 neurons, identified by tyrosine-hydroxylase immunoreactivity, did not alter the effect of carbachol. These results support the conclusion that the antinociception produced by activating neurons in the NRM is mediated by the subsequent activation of spinally-projecting noradrenergic neurons in the A7 cell group. Supported by PHS grant DA03980 from NIDA.

53.10

RELATIONSHIP OF μ-OPIOID RECEPTORS TO GABA-SYNTHESIZING NEURONS WITHIN THE PAG, RVM, AND SPINAL CORD. A. Kalyuzhny* and M. Wessendorf, Dept Cell Biol & Neuroanat, Univ Minnesota, Minneapolis, MN 55455

In previous studies we have reported immunoreactivity for the cloned μ-opioid receptor (MOR1) within the PAG, NRM, and spinal cord. Since activation of μ-opioid receptors has been reported to affect the sizes of GABA-mediated synaptic potentials, we investigated the distribution of MOR1-staining in relation to neurons synthesizing GABA within these regions. To allow double-labeling immunocytochemistry, we combined MOR1 antiserum with monoclonal antibodies to either GABA- or GAD. Secondary antibodies were donkey anti-rabbit IgG conjugated with cyanine 3.18 and donkey anti-mouse IgG conjugated with cyanine 5.18. In the midbrain, dense GAD-ir varicosities were observed in the caudal PAG. Both GABA-ir and MOR1-ir were observed in the area adjacent to the aqueduct, and the cells were intermingled. In the RVM and spinal cord dorsal horn, GAD-ir varicosities were found apposed MOR1-ir somata. In addition, MOR1-ir processes apposed many GABA-ir somata in the spinal cord. We conclude that μ-opioid receptors have the potential to interact with GABAergic neurons at a number of sites relevant to the modulation of nociception.

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53.11

ANALYSIS OF INTRINSIC RAT PERIAQUEDUCTAL GRAY NEURONS: MODULATION OF OPIOID PEPTIDE mRNA'S BY PERIPHERAL NEUROPATHY F. G. Williams* and A. J. Beitz
Dept. of Pathobiology, University of Minnesota, 1988 Fitch Ave., St. Paul, MN 55108

Our previous studies in the rat periaqueductal gray (PAG) using an experimental model for neuropathic pain have demonstrated increases in the number of enkephalin-immunoreactive terminals, and increases in both cellular levels and frequencies of neurons expressing proenkephalin A mRNA. However, it was unknown whether the PAG neurons that respond to the nociceptive state have intrinsic connections, or whether they project outside the PAG. We previously demonstrated that local axon collaterals are rare, hence local circuit neurons can be identified to an acceptable probability by small, local injections of retrogradely-transported dyes. In the present study, we combined hybridization histochemistry of proenkephalin A mRNA with retrograde identification of intrinsic PAG neurons to learn how chronic nociception affected PAG-intrinsic neurons. Six days after loose ligation of the sciatic nerve with chromic gut suture, 30-50 nl of rhodamine-dextran was placed in the ventrolateral PAG. Ten days after ligation the animals were sacrificed and the brains were sectioned, fixed, alkylated and delipidated. 48-mer probes were end-labeled using biotin-16-dUTP and terminal transferase, hybridized using standard conditions, and then visualized using FITC avidin and epifluorescence microscopy. In the ventrolateral PAG, mononeuropathy increased by 63% per unit area the number of double-labeled neurons, i.e. local circuit neurons that contained proenkephalin A mRNA. This demonstrates that the "nociceptive-specific" region of the PAG contains enkephalinergic intrinsic neurons, and suggests that the intrinsic neurons are a significant source of the enkephalin participating in disinhibitory anti-nociceptive descending circuits. Supported by NIH grants NS 28016, DA 06687, and DE 06682.

53.12

ACTIONS OF SEROTONIN ON IDENTIFIED NEURONES IN THE MIDBRAIN PERIAQUEDUCTAL GREY MATTER V.V. Stezhka and T.A. Lovick (SPON: Brain Research Association)
Dept. Physiology, Univ. Birmingham, Birmingham B15 2TT, U.K.

Recent evidence suggests that 5HT1A and 5HT2-mediated effects are involved in the antiaversive effect of serotonin in the periaqueductal grey matter (PAG) (Noguiera, R. and Graeff, F. Pharmacol. Biochem. Behav. 52 (1995) 1-6). We have therefore investigated the effects of serotonin and agonists at 5HT1A and 5HT2 receptors on neurones in the "defence area" of the PAG.

Whole cell patch recordings were made from 29 neurones in the dorsolateral PAG in coronal midbrain slices from 150-300g rats. In 14 cells (48%), close application of 5HT evoked depolarising responses of 4.3-17mV, mean duration 461.7±46.9s, accompanied by a 8-109% increase in input resistance. These responses were mimicked by α-methyl 5HT. In 10 cells (35%) hyperpolarising responses of 4.2-21mV, mean duration 250±80.1s, were mimicked by 8,OH-DPAT. Five cells (17%) were unresponsive.

Morphological reconstructions of 9 cells filled with biocytin (7 depolarised by 5HT, 2 unresponsive) revealed round, triangular and fusiform-shaped somata, diameter 14-29μm with up to 5 varicose primary dendrites which arborised within the PAG. In 6 of the responsive cells, the axon coursed dorsally or dorsolaterally out of the PAG into the tegmentum or superior colliculus after giving off collaterals dorsally and ventrally in the PAG. The 2 unresponsive cells were bipolar fusiform cells with intrinsic axons.

These results suggest that 5HT can excite a population of projection neurones in the PAG via an action at 5HT2 receptors.

53.13

THE MEDIAL PREOPTIC NUCLEUS OF THE HYPOTHALAMUS (MPO) MODULATES ACTIVITY OF NITRIC OXIDE (NO) SENSITIVE NEURONS IN THE MIDBRAIN PERIAQUEDUCTAL GRAY (PAG) Charles W. Hall, T.M. Da Costa Gomez, and M.M. Behbehani*, Department of Molecular and Cellular Physiology, University of Cincinnati, Cincinnati, OH 45267-0576.

Synaptic activities in the presence and absence of the NO donating compound sodium nitroprusside (SNP) were recorded in PAG slices using gramicidin perforated patch electrodes. SNP increased the number of synaptic events in 6 out of 9 cells. This response was attenuated in 4 of these 6 cells by glutamic acid receptor antagonists (CNQX and MK801). This experiment suggests that NO enhances the presynaptic release of glutamic acid in the PAG.

In chloral hydrate anesthetized rats, the characteristic depressor response elicited by MPO stimulation was attenuated by microinjection of the nitric oxide synthase (NOS) inhibitor nω-nitro-L-arginine (NLA) into the dorsal PAG (2/2 rats). Furthermore, microinjection of NLA into the dorsal PAG independently produces increases in MAP (2/2 rats), while microinjection of SNP into the dorsal PAG produces a slight depressor response (1/1 rat). These experiments suggest that the depressor effect observed secondarily to MPO stimulation is due, at least in part, to NO-producing PAG neural circuits.

The effect of MPO stimulation on PAG neuronal firing activity was evaluated in the presence and absence of the NOS inhibitor 7-nitroindazole (7NI). Before the application of 7NI, 2 cells were inhibited, 1 cell was excited, and 1 cell did not respond to MPO stimulation. In the presence of 7NI however, MPO stimulation excited 1 cell that was previously inhibited, and the responses of the remaining cells to MPO stimulation were blocked. This final series of experiments confirms that the MPO alters the activity of NO responsive neurons in the PAG. (Supported by NIH grant # NS-20643).

53.14

SOMATIC AND VISCERAL INPUTS TO NEURONES IN THE ANTERIOR HYPOTHALAMUS WITH IDENTIFIED AFFERENT AND EFFERENT PROJECTIONS. R.K. Snowball and B.M. Lumb. SPON: Brain Research Association.
Dept. of Physiology, University of Bristol, Bristol, BS8 1TD, U.K.

Activation of neurones in the anterior hypothalamus (AH) can evoke antinociception and autonomic changes. Direct projections to the AH from nociceptive neurones in the spinal cord, and descending projections from AH neurones to the midbrain periaqueductal gray (PAG) and midline caudal medulla have been described. The aim of the present study was to determine whether descending systems originating in AH and relaying in the PAG and/or caudal medulla might be triggered by nociceptive inputs from the spinal cord.

Extracellular single unit recordings were made from neurones in the AH of 12 α-chloralose-anaesthetised and paralysed rats. AH neurones were tested for their responses to noxious visceral and somatic stimuli. The central connections of the same AH neurones were determined by their responses to electrical stimulation in the spinal cord and in the PAG and midline caudal medulla, at physiologically-identified pressor and depressor sites. Of 36 AH neurones tested with the peripheral stimuli, 13 responded to visceral and/or somatic stimuli. Seven of these received excitatory inputs from the spinal cord and 5 of the 7 were driven orthodromically from the PAG and midline medulla. Of 9 AH neurones that were antidromically activated from the PAG and/or medulla, only 2 responded to peripheral stimuli and none responded to spinal cord stimulation.

These results do not support the idea of a simple 'loop system' since most of the AH neurones that responded to peripheral stimuli and spinal cord stimulation were driven orthodromically and not antidromically from the PAG and medulla. However, the data raise the possibility that the PAG and/or caudal medulla may act as relay sites in an ascending projection from the spinal cord to the AH.
Supported by the Wellcome Trust. RKS is a Medical Research Council scholar.

53.15

PARABRACHIAL AREA-INDUCED MODULATION OF CORNEAL-RESPONSIVE NEURONS IN TWO REGIONS OF THE SPINAL TRIGEMINAL NUCLEUS. I.D. Meng*, J.W. Hu, A.P. Benetti and D.A. Bereiter. Depts. Neuroscience & Surgery, Brown Univ./RI Hospital, Providence, RI 02903 & Faculty of Dentistry, Univ. of Toronto, Ontario.

The aim of this study was to compare the inhibitory effects of PBA conditioning stimulation (CS) on electrically evoked A- and C-fiber input from characterized corneal-responsive neurons in two regions of the spinal trigeminal nucleus: the subnucleus interpolaris/caudalis transition region (Vi/Vc) and laminae I/II of the subnucleus caudalis/cervical cord transition region (Vc/C1). In chloralose anesthetized rats, Vi/Vc (40) and Vc/C1 (20) corneal-responsive neurons were functionally classified according to their cutaneous receptive field properties as either LTM, WDR, NS, deep (D) or cornea only (CO). At the Vi/Vc transition region, LTM (9), WDR (2), NS (1), D (3), and CO (25) neurons were studied, whereas at Vc/C1 all neurons were either WDR (11) or NS (9). Projections to PBA were determined, followed by testing the effect of PBA CS (20-40 μA, 400 Hz, 0.1 ms pulse duration for 20 ms) delivered 50 ms prior to test stimulation (1.1-1.5 x T) of the cornea or cutaneous receptive field. No Vi/Vc and 15/20 Vc/C1 neurons projected to ipsi- or contralateral PBA (mean latency = 5.3±5.7 ms). Inhibition of evoked responses (<75% of control) occurred in >70% of Vi/Vc and Vc/C1 neurons regardless of neuronal class. Facilitation was found in only 2 neurons. At the Vi/Vc transition region, no difference was found in the extent of A- and C-fiber inhibition (means of 16% and 18% of control, respectively). However, at the Vc/C1 transition region the A-fiber input was inhibited more than the C-fiber input (means of 32% and 65% of control, respectively; $P<0.05$). Also, PBA CS inhibited both A- and C-fiber evoked activity to Vi/Vc neurons more than to Vc/C1 neurons ($P<0.05$). Glutamate injections into PBA (500 nl, 200mM) inhibited both A- and C-fiber evoked responses, including those from Vc/C1 neurons in which electrical stimulation produced inhibition of only A-fiber responses. These results suggest unequal descending control of corneal input to Vi/Vc and laminae I/II of Vc/C1 by local excitation of PBA neurons. RIH funded.

53.16

ALTERATION IN OPIOID mRNA IN THE PARABRACHIAL NUCLEUS IN RESPONSE TO PERIPHERAL NOXIOUS INFLAMMATION. L.L. Bellavance*, F.G. Williams, M.F. Jacquin & A.J. Beitz. Neuroscience Program, University of Minnesota, St. Paul, MN 55108; Dept. of Neurology & the Center for the Study of Nervous System Injury, Washington University School of Medicine, St. Louis, MO 63110.

We have previously shown that Complete Freund's Adjuvant (CFA)-induced peripheral inflammation results in an increase in the number of cFos immunoreactive nuclei in the pontine parabrachial nucleus (PBN) of the rat. This increase in cFos-like immunoreactivity (cFos-LI) was restricted to the Kolliker-Fuse and lateral subdivisions of the PBN, regions associated with both nociception and antinociception. Further, we have also shown that the distributions of the endogenous opioid, enkephalin, and its two putative receptors, MOR and DOR, are localized in the same regions of the PBN that show an increase in cFos-LI in response to noxious peripheral inflammation. These data suggested to us that the PBN's involvement in noxious peripheral inflammation could include local and/or general opioid circuitry. As a result, it is possible that CFA-induced noxious inflammation may alter mRNA levels for opioids and their receptors in the PBN. We have recently started a study using *in situ* hybridization to help quantify mRNA levels for opioid ligands and receptors in the PBN in response to CFA-produced noxious peripheral inflammation. Preliminary data suggest that there is an alteration in mRNA levels for the endogenous opioid ligand, enkephalin, and its putative receptors, MOR and DOR, in specific subdivisions of the lateral PBN in response to noxious peripheral inflammation.

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53.17

PHOSPHORYLATED CREB IN THE PARABRACHIAL NUCLEUS: RELATION TO PREPROENKEPHALIN mRNA EXPRESSION. O. Hermanson* and A. Blomqvist, Dept Cell Biol., University of Linköping, S-581 85 Linköping, Sweden.

The rat parabrachial nucleus (PB) contains a very large number of preproenkephalin mRNA (ppENK)-expressing neurons. We have shown that the ppENK-expression in the Kölliker-Fuse subnucleus (K-F) increases after noxious stimulation. The promoter region of the ppENK gene contains an AP-1/CRE site named ENKCRE-2, which has been shown to be crucial for ppENK transcription. It has been suggested that FOS may interact with ppENK at ENKCRE-2, but the distribution of noxious-evoked FOS in PB is not coherent with the distribution of ppENK-expressing neurons (Hermanson & Blomqvist, J. Comp. Neurol., 1996). Recent studies have instead suggested that CREB may be the primary regulator of ppENK transcription at the ENKCRE-2 site. The transcriptional ability of CREB is dependent on phosphorylation at position Ser-133. We have investigated the immunoreactivity of phosphorylated CREB (pCREB) in PB after acute noxious stimulation.

Male Sprague-Dawley rats received a brief ether anesthesia, followed by an injection of 100 µl 5% formalin in one hindpaw. Thirty minutes after the injection, the rats were killed with an overdose of sodium pentobarbital, followed within three minutes by transcardial perfusion with 4% paraformaldehyde. The brain was cut at 20-40 µm on a freezing microtome. Brain stem sections were incubated in primary antibody (rabbit anti-pCREB, UBI, 1:1000) for 24 hours, and the immunoreactivity was visualized with standard PAP procedures.

Many neurons displayed pCREB immunoreactivity in PB. Large numbers of neurons were detected in the K-F, superior, central, internal, and ventral parabrachial subnuclei. Smaller numbers of labeled neurons were detected in the dorsal and external subnuclei. The present findings show that the distribution of pCREB-expressing neurons is largely coherent with the distribution of ppENK-neurons in PB. We are currently performing double-labeling with in situ hybridization for ppENK and immunohistochemical staining for pCREB or FOS after acute noxious stimulation.

Supported by SMRC grant 7879.

53.18

FOREBRAIN ACTIVATION OF DESCENDING ANTINOCICEPTIVE SYSTEMS DEPENDS ON OPIOID AND NON - OPIOID MECHANISMS IN THE VENTRAL PERIAQUEDUCTAL GRAY. S. A. Tershner* & F. J. Helmstetter, Department of Psychology, University of Wisconsin - Milwaukee, Milwaukee, WI 53201.

The presence of certain environmental stressors activates a descending antinociceptive system which includes the amygdala, periaqueductal gray (PAG) and rostroventral medulla. We have previously shown that pretreatment with a *mu* opioid receptor antagonist into the ventral PAG attenuates tail flick (TF) inhibition produced by microinjection of a *mu* opioid agonist into the basolateral amygdala (BLA). Injections of an *epsilon* receptor antagonist into the ventral PAG was not effective. The purpose of this experiment was to further examine the role of opioid and non-opioid receptors in the ventral PAG in TF inhibition produced by *mu* receptor stimulation in the BLA. Rats maintained under constant barbiturate infusion were implanted with cannulae aimed at the BLA and ventrolateral PAG. Radiant heat TF latency was recorded at 2 minute intervals. As was previously shown, DAMGO (0.5 µg X 2) applied to the BLA produced a time dependent increase in TF latency. The administration of a *delta* opioid receptor antagonist, naltriben (.12, 1.2, 4.0 µg), or a NMDA receptor antagonist, APV (.03, .30, 3.0 µg), into the ventral PAG failed to block TF inhibition produced by amygdala stimulation. D-trp^{11}-Neurotensin, at antagonist doses (4.0 and 16.0 pmol), attenuated TF inhibition when injected into the ventral PAG. These results suggest that forebrain modulation of nociceptive responses involves *mu* opioid and neurotensin receptors located in the ventral PAG.

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53.19

***MU* OPIOIDS IN THE RVM CONTRIBUTE TO ANTINOCICEPTION FOLLOWING DAMGO STIMULATION OF THE AMYGDALA.** L. H. Poore* & F. J. Helmstetter, Department of Psychology, University of Wisconsin-Milwaukee, Milwaukee, WI 53201

Hypoalgesia produced by environmental stressors involves a descending antinociceptive system that includes the amygdala, periaqueductal gray (PAG), and rostral ventromedial medulla (RVM). We have previously shown that injections of the *mu* opioid agonist DAMGO into the basolateral nucleus of the amygdala (BLA) will increase tail flick latency in rats and this effect can be blocked by injections of lidocaine in the PAG and RVM. Furthermore, we have been able to block the effects of DAMGO with injections of the *mu* opioid antagonist CTAP in the PAG. The purpose of the present study was to determine if CTAP injections in the RVM will prevent or reverse the effects of DAMGO injections in the BLA. Rats were anesthetized by constant i.v. infusion of a barbiturate anesthetic and steel guide cannulae were positioned over the BLA and RVM. Rats were also implanted with a femoral arterial catheter for measurement of blood pressure. Injections of CTAP (.005µg/.4µl) in the RVM made 10 minutes prior to the DAMGO (2µg/µl - .25µl/side) injection in the BLA attenuated the increase in tail flick latency. Moreover this effect was not reversed by RVM injections of the same dose of CTAP made 20 minutes after the DAMGO injection in BLA. Arterial pressure remained stable throughout the session. These results suggest that *mu* opioid receptors in the RVM are involved in hypoalgesia produced by amygdala *mu* opioid receptor stimulation. Supported by NIDA grant - DA09429.

53.20

ANTINOCICEPTIVE EFFECTS OF UNILATERAL INJECTIONS OF DAMGO IN THE RAT AMYGDALA. M.S. Shin* & F.J. Helmstetter, Department of Psychology, University of Wisconsin-Milwaukee, Milwaukee, WI 53201

We have previously shown that activation of mu opioid receptors in the basolateral amygdala results in inhibition of the radiant heat tail flick (TF) reflex in barbiturate anesthetized rats [JPET 275:381]. While we have consistently found that the posterior part of the basolateral nucleus is the most effective region for antinociception produced by DAMGO, these studies typically use simultaneous bilateral injections and therefore a precise description of regional sensitivity within the amygdala has not been possible. Several recent studies have also suggested that the left versus right amygdala may not contribute equally to its various behavioral and physiological functions. In the present study rats were maintained under barbiturate anesthesia and prepared for microinjections and TF testing. After a series of baseline TF trials the mu agonist DAMGO (0.05 µg/ 0.25 µl) was infused into the right or left amygdala. TF testing continued at 2 min intervals for 40 min. While DAMGO significantly elevated TF latencies this effect was reliably larger when injections were made on the left side. In most cases, the largest effects were seen when injections were made in the posterior basolateral nucleus. These results replicate our previous mapping studies and indicate that the left and right amygdala may not be equally sensitive to DAMGO.

Supported by NIDA DA09429

PAIN MODULATION: ANATOMY AND PHYSIOLOGY—HIGHER CENTERS II

54.1

THE ELECTRIC STIMULATION OF THE CINGULUM BUNDLE ANTICIPATES THE ONSET OF AUTOTOMY INDUCED BY THE CHRONIC INFLAMMATION IN RAT. Pellicer, F.*, López-Avila, A.§ Laboratorio de Neurofisiología, Instituto Mexicano de Psiquiatría, §Facultad Mexicana de Medicina, Universidad La Salle. México D.F. Fax + 655 99 80

The evidence that the cingulum is related to emotional processing was established by Papez. Recently the cingulum cortex has been related to a neuronal network, "neuromatrix". The relevance of this concept has been focused on the possible mechanism of phantom limb and central pain. Experiments have achieved a delay or hindered autotomy, due to peripheral neuropathy or to a chronic inflammatory process. The purpose of this work was to reduce the autotomy behavior onset by electric stimulation of the cingulum bundle. Wistar rats were stereotaxically implanted in the cingulum bundle for chronic stimulation. The experimental groups were: group 1 (n=5), implanted non-stimulated control; group 2 (n=4), implanted and stimulated 10 min. daily, for 7 days (100Hz for 1 s, every 5 s); group 3 (n=4), implanted and stimulated 2h daily, for 7 days (100Hz for 1 s, every 5 s). For the induction of inflammatory process carrageenan was injected in the paw (CAR. 250 µl at 1%), 2 or 3 days after the last stimulation day. The results show that autotomy behavior appeared 20% in group 1, after 5 days of injection of CAR; while 100% in groups 2 and 3, in the same period. Further in these two groups the autotomy was more severe. We concluded that is possible to facilitate the onset and intensity of the autotomy behavior by anterior cingulum bundle stimulation, previous to a noxious regional inflammatory process. IMP project 3230 for FP.

54.2

STIMULATION OF THE CINGULUM BUNDLE AND SURROUNDING CORTICAL TISSUE: EFFECT ON TONIC PAIN IN THE RAT. P.N. Fuchs*, M. Balinsky and R. Melzack, McGill Univ., Dept. of Psychology., 1205 Dr. Penfield Ave., Montreal, PQ, Canada H3A 1B1.

Surgical lesions of the cingulum in humans decreases cancer pain and many forms of chronic pain. Similarily, a temporary block of the rat anterior cingulum bundle decreases formalin-pain and reduces autotomy following peripheral neurectomy. The present study examined changes in formalin-pain responses following stimulation of the cingulum bundle/surrounding cortical tissue (CB/CT) in the rat. Male Long-Evan rats were randomly assigned to one of four groups and the effect of stimulation the CB/CT 15-min prior to the formalin injection (Stimulation-15min, n=8; Surgery Control, n=10) and 20-min following the formalin injection (Stimulation+20min, n=10; Surgery Control, n=10) was explored. Stimulation consisted of 30 sec of 0.2 msec biphasic square-wave pulses seperated by 0.2 msec delivered at a frequency of 50 Hz and a current level of 50 µA. The stimulation remained off for 30 sec and was then repeated, until five application were delivered for a total of 2.5 min of brain stimulation over a 5-min period. Stimulation 15-min prior to the formalin injection significantly decreased pain responses during both the first (0-5 min) and second (20-60 min) period of the formalin test. Stimulation 20-min following formalin injection significantly decreased pain responses which persisted for the duration of the 35-min post-stimulation test period. The temporal pattern of analgesia following stimulation of the CB/CT is dramatically different from the short-duration analgesia following stimulation of other limbic and brainstem structures. Stimulation of the CB/CT may disrupt patterned activity in the limbic system, which is known to play an especially important role in the affective-motivational dimension of pain.

Supported by NSERC grant A7891 and the MGH Pain Centre.

54.3

DORSAL HIPPOCAMPUS FIELD CA1 PYRAMIDAL CELL NOCICEPTIVE RESPONSES AND THEIR SEPTAL MODULATION. S. Khanna*. Department of Physiology, National Uni. Singapore 119260.

Previously, we suggested that an acute noxious heat stimulus-induced depression of field CA1 pyramidal cell synaptic excitability is mediated via septal-hippocampal afferents. We have now recorded changes in field CA1 pyramidal cell neural activity to formalin injection, a model of persistent pain, and investigated the effect of medial septal region electrolytic lesion on CA1 nociceptive responses.

Extracellular recordings from field CA1 were made in urethane (1g/kg, i.p) anaesthetized male rats. In comparison to an acute noxious heat stimulus, formalin (5%, 0.05ml sc) into right hind paw induced a persistent (a) increase in period of sinusoidal rhythmic theta field activity, (b) depression of pyramidal cell synaptic excitability, and (c) decrease in extracellular activity of majority of pyramidal cells (15/20) with only few excited (5/20). In medial septal region lesioned animals, a noxious stimulus failed to evoke a depression of pyramidal cell excitability or extracellular activity. Rather, noxious stimulation excited majority of pyramidal cells studied (8/10).

The evidence suggest that (a) formalin nociception evoke a selective excitation of a small proportion of CA1 pyramidal cell, and (b) septal-hippocampal afferents are involved in a noxious stimulus-induced field CA1 pyramidal cell suppression. (Supported by ARF research grant from NUS).

54.4

The Effects of Amitriptyline on Regional Cerebral Blood Flow in Rats With Painful Peripheral Mononeuropathy. Pamela E. Paulson*, Thomas J. Morrow, Kenneth L. Casey. VAMC and Univ. of MI, Depts of Neurology, Ann Arbor, MI 48105.

Antidepressant drugs are commonly prescribed to relieve chronic pain in humans. Recently, amitriptyline has been shown to be effective in suppressing pain behaviors in an animal model of chronic pain. However, the mechanism by which amitriptyline reduces pain behaviors is unknown. We recently reported that rats exhibiting pain behaviors following chronic constriction injury to the sciatic nerve showed a significant increase in baseline (unstimulated) cerebral metabolic activation patterns compared to controls. The regions of greater activation were both somatosensory (hindlimb, somatosensory cortex and ventral posterior lateral thalamus) and limbic (anterior cingulate and retrosplenial cortices, anterior-dorsal thalamus, habenula, interpeduncular nucleus and the periventricular nucleus of the hypothalamus) regions of the brain. In the present experiment we measured amitriptyline-induced changes in baseline (unstimulated) regional cerebral blood flow in rats 12-15 days after unilateral ligation of the sciatic nerve. Regional cerebral blood flow was assessed thirty minutes after injection of amitriptyline (0.5 mg/kg, i.v.), using the technique recently developed in this lab. These experiments are in progress, results to be reported at the meeting.

This work is supported by grants from the Veteran's Administration.

54.5

EFFECTS OF OSTEOPATHIC MANIPULATIVE TREATMENT (OMT) UPON PARASPINAL MUSCLES IN SUBJECTS WITH LOW BACK PAIN AND SOMATIC DYSFUNCTION (SD): A NEUROPHYSIOLOGICAL STUDY UTILIZING MAGNETICALLY INDUCED SOMATOSENSORY EVOKED POTENTIALS (SEPs) R.A. Sugerman,*[1] Y. Zhu,[2] D.B. Hagie,[3] J.M. Jones,[3], A. Starr,[2] A. Cao.[4] Dept. of [1]Basic Sci. and [3]Osteop. Manipul. Med., COMP, Pomona, CA 91766 and [2]Dept. of Neurology, Univ. of CA-Irvine, Irvine, CA 92717, [4]California State Polytechnic University, Pomona, CA 91768.

We endeavored to determine whether SD in subjects with low back pain and muscle spasm is associated with abnormal muscle evoked potentials and whether OMT on these subjects can result in a quantifiable change in SEPs. Thirty-seven subjects were tested: 20 were classified as clinically abnormal, 12 normal, and 5 did not complete tests or were determined to be outside the physical criteria. A general health history was solicited with specific questions concerning their low back history and a pain location diagram. Subjects were examined osteopathically for positional asymmetry, low back Strain/Counter Strain tender points, level and degree of muscle spasm from which the level and degree of somatic dysfunction was determined. The specific lumbar vertebral level to be magnetically stimulated was chosen. These paraspinal muscles were stimulated at 30% of maximum output and at 3 Hz by a magnetic stimulator and SEPs were recorded by an EMG EP apparatus. Then, the subjects received OMT and were retested (within 30 minutes of the initial magnetic stimulation). Before OMT in abnormal subjects the early cortical components of their SEPs showed significantly prolonged latencies and decreased amplitudes. The principal effect of OMT on the cerebral potentials was to the latency and amplitude of the P30 parietal component. In these subjects, P30 cortical potentials were significantly decreased in their latencies and significantly increased in their amplitudes at $P < 0.05$. The latter components following the P30 were relatively less affected. Normal subjects showed no changes in SEPs. Therefore, OMT caused significant changes in SEPs in abnormal, but not normal, subjects. This work was supported by AOA Grant 93-01-375, COMP Intramural Grant 431-308, and Forty-First Medical Trustee Endowment.

54.6

SELECTIVE MODULATION OF PAIN UNPLEASANTNESS ALTERS ACTIVITY IN HUMAN CEREBRAL CORTEX. P. Rainville*, G.H. Duncan, D.D. Price, and M.C. Bushnell. Montreal Neurological Institute and Univ. Montréal, Montreal, Canada H3C 3J7 and Med. Coll. Virginia, Richmond, VA 23298.

Painful stimuli activate a number of cortical regions in humans, including primary and secondary somatosensory cortices (SI, SII), anterior cingulate cortex (ACC), and insular cortex (IC). However, the role of each area in processing the sensory and affective dimensions of pain is unknown. This study examined activation within these cortical nociceptive pathways relative to a selective alteration of the affective dimension of pain by hypnotic suggestion.

Regional cerebral blood flow was measured with a 3-dimensional high-resolution PET scanner following bolus injections of $H_2{}^{15}O$ in 4 subjects selected for their high hypnotic suggestibility. During each scan the subject's hand was immersed in either 47°C (pain) or 35°C (neutral) water. Data were collected with and without hypnotic induction, with and without suggestions of increased (↑UNP) or decreased unpleasantness (↓UNP). Statistical brain maps were derived, and directed searches were conducted of SI, SII, ACC and IC.

Subjects' ratings of unpleasantness varied according to the type of suggestion (↑UNP = 72.9, ↓UNP = 25.0, $p < 0.001$), whereas their ratings of intensity were unaffected (69.6 vs 65.0, n.s.). When 47°C and 35°C scans were compared, significant activations were observed in contralateral SI, SII, ACC, and IC both with and without hypnotic induction. However, in all regions except SI cortex, rCBF increases were more significant during ↑UNP than during ↓UNP. A direct comparison of ↑UNP and ↓UNP suggestions during 47°C stimulation revealed greater activation in IC ($p < 0.05$), with similar trends in all regions except SI.

These results suggest a differential role of various cortical regions in pain processing. Activation in insular cortex may be particularly important for generating emotional responses to pain, whereas that in SI may have the least direct relationship to unpleasantness.

Supported by the Canadian MRC.

54.7

HYPNOTIC INDUCTION AND SUGGESTION-RELATED CHANGES IN REGIONAL CEREBRAL BLOOD FLOW (rCBF) IN THE PRESENCE AND ABSENCE OF PAIN. G. H. Duncan*, P. Rainville, D.D. Price, and M.C. Bushnell. Mont. Neurol. Instit. & Univ. Mont., Montreal, Canada H3C 3J7 and Med. Coll. Virginia, Richmond, VA 23298.

Numerous studies have demonstrated the effectiveness of hypnosis in producing analgesia. However, little is known about the cerebral mechanisms of this perceptual modulation. We report the effects of hypnotic induction and hypnotic suggestions of increased or decreased pain on rCBF.

Changes in rCBF were measured using bolus injections of $H_2{}^{15}O$ and 3-D high-resolution PET. Four trained subjects received scans before and after hypnotic induction, with one hand immersed in 35°C (neutral) or 47°C (pain) water. Scans preceded by algesic or analgesic suggestions were then taken in the pain condition. Global searches were conducted using statistical brain maps.

Hypnotic induction had no effect on subjects' ratings of pain intensity (Hypnosis: 70.0, Control: 67.5, n.s.) or unpleasantness (47.5 vs 50.0, n.s.). Significant hypnotic induction-related rCBF changes included bilateral increases in occipital cortices, and decreases along the medial wall of frontal and parietal cortices. This hypnotic induction effect was less pronounced in the painful (47°C) than in the neutral (35°C) condition. In contrast, both algesic and analgesic suggestions produced significant increases in rCBF in medial and lateral frontal, and anterior insular cortices.

These findings show differential distribution of activation during hypnotic induction and suggestion. During induction, the large increases in occipital activation, reduced by pain, may reflect visualization strategies that were disrupted by the noxious stimulus. The strong frontal activation observed following hypnotic suggestions of both algesia and analgesia could underlie increased attentional effort toward or away from the pain. This mechanism could regulate the observed modulation of the pain-related cerebral activation (Rainville et al, 1996). Supported by the Canadian MRC.

54.8

CORTICAL AND SUBCORTICAL RESPONSES TO NOXIOUS HEAT DURING COUNTER-STIMULATION HYPOALGESIA IN HUMANS. K. L. Casey*, S. Minoshima, P. Svensson, T. J. Morrow and K.A. Koeppe. Neurology Research Lab., V.A. Med.Ctr., Ann Arbor, MI 48105; Div. Nuclear Medicine, Univ. Michigan., Ann Arbor, and Royal Dental College, Univ. Aarhus, Aarhus, Denmark.

A noxious somatic conditioning stimulus applied contralateral to a noxious test stimulus produces hypoalgesia of the test stimulus. To determine if this effect is mediated by brainstem suppression of spinothalamic transmission, we studied 13 right handed normal volunteers (1 female and 12 males; av.age 22 +/- 2.6 yrs.) using positron emission tomography with $H_2{}^{15}O$ to detect increases in regional cerebral blood flow (rCBF). As expected, the perceived intensity of repetitive contact heat pain on the left hand was reduced from 82.6 to 71.2 (av. of 0-100 VAS, 70=pain threshold; $p<0.05$) when the right hand was placed in painfully cold (1°C), but not in neutral (31°C), water. Statistical image subtraction analysis and standardized stereotactic registration was used to identify structures activated during noxious (50°C), as compared to innocuous (40°C), stimulation during innocuous (31°C) contralateral conditioning. These volumes of interest were used for comparison with the rCBF responses during left hand heat pain conditioned by noxious (1°C) contralateral stimulation. Of the cortical regions responsive during heat pain with innocuous conditioning, the bilateral S2, right anterior cingulate, right insular, and left premotor cortices showed reduced heat pain rCBF responses during painful cold conditioning (64-30% relative decrease; $p=0.017$-0.029 by 2 tailed t-test, except insula:$p=0.10$). In contrast, the following subcortical structures showed increased heat pain rCBF responses during painful cold conditioning: left lenticular nucleus/lateral thalamus (49%, $p=0.05$), right VPL (24%,N.S.), and dorsal midbrain (22%,N.S.). These results suggest thalamic, rather than brainstem, gating of nociceptive cortical responses during counterirritant hypoalgesia in humans. Supported by a V.A. Merit Review Grant and D.O.E. Grant DE-FG02-87-ER60561.

55.1

COLD PAIN SENSITIVITY IN ASYMPTOMATIC AND SYMPTOMATIC (NECK PAIN) FIGHTER PILOTS: DIFFERENT EFFECTS OF EXERCISE-INDUCED ANALGESIA. P. Kemppainen*[1], O. Hämäläinen[2] and M. Könönen[1]. [1]Dept. of Prosthetic Dentistry, Univ. of Helsinki and [2]Air Force Academy, Kauhava, Finland.

The effect of physical exercise on cold pain sensitivity was studied in fighter pilots. The pilots were divided in two groups: one group consisted of asymptomatic pilots (n=6), and the other group consisted of pilots (n=4) suffering from acute in-flight neck pain attacks. Different levels of exercise (50-200 W) were produced by a cycle ergometer. Cold pain sensitivity was tested by using cold pressor test. The ratings of sensory intensity and unpleasantness during cold pressor tests were evaluated by visual analogue scales. In control conditions the average sensory and unpleasantness ratings did not show any differencies between the two groups. Physical exercise increased pain thresholds in symptomatic ($p < 0,001$; ANOVA, two-way) but not in asymptomatic pilot group. During exercise the sensory intensity and unpleasantness responses to suprathreshold stimulation were attenuated ($p < 0,001$) in both of the pilot groups; this attenuation was more marked in symptomatic group ($p < 0,05$). These results show that cold pain sensitivity between symptomatic and asymptomatic pilots was modified by physical exercise in a different manner. Our present findings suggest that there could be different pain modulatory mechanisms, or mechanims act differentially, in healthy subjects than in subjects with various pain conditions.

55.2

TOLERANCE TO ISCHEMIC PAIN IS REDUCED IN INDIVIDUALS WITH DEPRESSIVE SYMPTONS. L.Piñerua-Shuhaibar, H. Suarez-Roca, D. Prieto-Rincon, A. Ferrer, E. Bonilla* and W. Maixner. INBIOMED and Inst. Invest. Clin. Univ. of Zulia, Maracaibo, POB 1151, Venezuela and Dental Res. Ctr., Univ. of North Carolina, Chapel Hill, NC 27599-7455.

Although there is an increased vulnerability to pain problems in patients with affective disorders, most experiments have paradoxically found a depressed patient to report reduced sensitivity (higher threshold) to brief phasic electrical and thermal noxious stimuli. In this study we have examined the responses to ischemic pain produced by a maximal effort tourniquet procedure, in 32 controls and 8 drug- and psychotherapy-free depressive individuals (Zung autoscale >49). Threshold was not altered in depressed patients (7.50 ± 2.00 min) vs. controls (7.48 ± 0.76 min). In contrast, tolerance was reduced in depressed patients (10.50 ± 2.44 min) vs. controls (16.63 ± 1.09 min). Sensory, affective and global verbal pain reports and cardiovascular responses during the experiments were similar for both groups. Depressed patients reported more intense post-ischemic paresthesias than controls. Anxiety levels were not related to ischemic measures of threshold and tolerance. This finding suggests that depressed patients are more sensitive to a sustained tonic painful stimulus. (Supported by FUNDACITE-Zulia)

55.3

HYPERALGESIA TO HEAT AND MECHANICAL STIMULATION IN HUMAN SKIN INDUCED BY EXPERIMENTAL TISSUE ACIDOSIS. K.H. Steen*, U. Issberner & P.W. Reeh. Dept. Dermatology, Univ. Bonn, D-53105 Bonn; Dept. Physiology I, Univ. Erlangen - Nürnberg, D-91054 Erlangen, Germany.

Tissue acidosis of human skin due to intradermal pressure infusion of acidic phosphate buffer (pH 5.2) has been shown to induce sustained graded pain and sensitization to punctuate mechanical (von Frey) stimulation (Steen et al., 1993, Neurosci Lett 154: 113) which corresponds to pH-induced nociceptor excitation and sensitization in the rat skin nerve preparation, *in vitro* (Steen et al., 1992, J Neurosci 12: 86).

Thermal thresholds (heat pain, cold and warm sensation) were examined in the forearm (n=6) using the TSA 2001 (Medoc Ltd., Ramat Yishai, Israel) with a Peltier thermode (18 mm^2). Touch and "pin prick" thresholds were determined with von Frey hairs. Infiltration of the skin with isotonic acidic phosphate buffer causing moderate pain (pH 5.2, duration: 1 h) produced reduction of heat pain thresholds by 3.5°C on average (46.3±0.9°C SEM resp. 42.8±1.8°C) significant after 15 min ($p<0.05$, Wilcoxon test). This thermal hyperalgesia was still significant after 24 hrs ($p<0.03$). In comparison to the untreated forearm, heat pain thresholds became significantly lower after 45 min of continuous infiltration (46.7±0.8°C resp. 42.1°C±0.9°C, $p<0.05$, U-test). As previously reported, the "pin prick" thresholds were significantly lowered after 30 min of low pH infusion (medians: 104.5 mN resp. 54.7 mN, $p<0.02$) and remained so for the rest of infusion period. Cold and warm threshold showed no significant differences in the pH-treated and untreated skin.

The results suggest that protons sensitize nociceptors as well for mechanical as for heat stimulation; the latter remains to be supported by primary afferent recordings. *This work was supported by DFG grant Ste 593/1-3.*

55.4

ALGOGENIC INTERACTIONS BETWEEN PROTONS AND BRADYKININ IN HUMAN SKIN. U. Issberner*, S. Haupts, P.W. Reeh & K.H. Steen. Dept. Dermatology, Univ. Bonn, D-53105 Bonn; Dept. Physiology I, Univ. Erlangen - Nürnberg, D-91054 Erlangen, Germany.

A combination of mediators mimicking the inflammatory exudate has previously been shown to potentiate the algogenic effect of low pH (Steen et al, 1996, Pain, in press). The present psychophysical studies focused on the interaction between bradykinin (BK; 10^{-7}, 10^{-6}M) and low pH (pH 6.7 & 6.1), applying various protocols of intradermal injections at 10 min intervals (0.1 ml) into normal skin through previously placed canulas (27 G) in the palmar forearms of six volunteers. In another series of protocols, the injections were performed into skin which was continuously infiltrated with a phosphate buffered electrolyte solution using a motorized syringe pump that was adjusted so as to induce around 10 resp. 20% pain magnitude on a visual analog scale in case the solution was acidified (Steen & Reeh, 1993, Neurosci Lett 154: 113-16) and no pain when the solution was neutral (control). All experiments were done double-blind, cross-over and in randomized order.

We found no more than additive effects in respect to magnitude and duration of pain induced with both concentrations of BK and of hydrogen ions. An apparent though significant reduction in tachyphylaxis found with acidic BK solution can be explained by the perfect reproducibility of the dominant pH-contribution to the pain evoked. A prolongation of the BK effect observed in the acidic skin may be due to a pH-dependent block of degradation (block of kininase I).

BK alone does not seem responsible for the faciliation of pH-induced pain. However, BK effects are known to be amplified by PGs and by 5-HT. This provides further working hypotheses. *DFG grant Ste 593/1-3*

55.5

SELECTIVE SUPPRESSION OF PAIN AND ITCH AFTER LOCAL STIMULATION OF CUTANEOUS C FIBRES

Hans-Jörgen Nilsson , Anders Levinsson and Jens Schouenborg.* Department of Physiology and Neuroscience, Lund University, Sölvegatan 19, S-223 62 Lund. Sweden.

Aim: The effect of local electrical stimulation on cutaneous C fibres, using a technique called Cutaneous Field Stimulation (CFS), on sensations of itch, cold, warmth and light touch, was analyzed in humans.

Methods: A silicon plate (60 cm^2) containing multiple needle-like electrodes (n=16) was applied either to the right volar forearm or the dorsum of the right hand of healthy subjects. The electrodes were stimulated consecutively for 25 min. (each electrode 4 Hz, 1 ms, 0.8 mA). Transdermal iontophoresis of histamine dihydrochloride (1% in distilled water, 1 mA, 30 s) was performed to evoke itch lasting approx. 30 min. A CO_2-laser was used to elicit heat pain. Von Frey monofilaments were used to determine thresholds for touch. Thermal sensations were elicited by applying a metal bar (cold: 22° C, warmth: 41° C) to the skin.

Results: CFS resulted in a pricking, slightly burning, sensation that faded rather quickly. In all subjects, a local flare reaction, lasting about 1 h, developed around the electrode sites, indicating an activation of nociceptive C fibres. Within the stimulated area, CFS induced a strong (usually complete) and long lasting (> 2 h) post-conditioning inhibition of itch (n=14). A selective block of A fibre transmission in the superficial radial nerve did not abolish this effect (n=3). The sensation of heat pain was depressed by CFS (n=3). By contrast, sensations of cold, warmth and light touch were only marginally affected by CFS (n=7).

Conclusions: CFS produces a selective and powerful inhibition of experimentally induced itch and pain. The results indicate that nociceptive C fibres have a crucial role in inducing this inhibition. *Supported by Swedish MRC proj. no. 1013 and No. 10569, and the Medical Faculty of Lund.*

55.6

A NON-SPECIFIC PAIN-RELIEVING REACTION AS A RESULT OF ADDITIONAL Na-IONS APPLICATION IN CHRONIC PAIN SYNDROMES. V.Desnizza.* Pain Research Group,Langestr. 40-42, 76530 Baden-Baden,Germany.

Modern medicine prefers to ignore that even minor injuries of different structures produce significant reactions in the extracellular matrix. Different "miracle" effects in routine and alternative medicine can be explained from the point of view of basic regulations theory (BRT) which helps to modify and use simple methods in chronic pain syndromes (CPS): usually the fact that 0,9% saline solution (SS) gives a pain-relieving effect (PRE) is ignored because the effect is short or is considered to be a placebo-effect (PE). The PRE duration is prolonged if 0,5 ml of SS is injected close to the nerve branches into the 24 paravertebral projecting points at C1-C7/L1-S1 levels 12 times. 115 patients with CPS: disc prolaps - 38;spondylosis - 29; rheumatoid arthritis - 48 were treated with SS and the results compaired with 156 patients with natural history of the disease. Pain intensity (PI) was checked by Algometer (Somedic AB),Visual Analoque Scale and Pain Questionaire.PI decreased in treated patients for 30-70% ($p<0,01$). PRE can`t be atributed to PE, as it was seen in 50-60% of cases and lasted for 6-12 months ($p<0,05$). PI correlates with the decreased microcirculation (MC) checked by laser Dopler flowmeter ($R=0,4; p<0,05$).Restored MC with SS injections removes metabolic products that irritate nerves terminals and produce pain.

55.7

DEVELOPMENT OF BEHAVIORAL SIGNS OF NEUROPATHIC PAIN FOLLOWING INJURY TO DISTAL SCIATIC NERVE BRANCHES. B.H. Lee*, E.J. Baik, E.J. Kim, S.H. Lee and C.H. Moon. Dept. of Physiology, School of Medicine, Ajou University, San 5, Wonchon-dong, Paldal-gu, Suwon 442-749, Korea.

Injury to a peripheral nerve may produce chronic pain syndrome. Recently we observed that selective injury of sciatic nerve branches can produce behavioral signs of neuropathic pain. This study was conducted to see changes in neuropathic pain syndrome after injury to the tibial and sural nerves. Male Sprague-Dawley rats were anesthetized with halothane. Neuropathic injury was induced by transecting the tibial and sural nerves, leaving the common peroneal nerve intact. Behavioral tests for mechanical and cold allodynia and spontaneous ongoing pain were performed on these rats for 20 weeks. All the rats showed severe behavioral signs of neuropathic pain. Mechanical and cold allodynia and spontaneous pain peaked at 2 weeks postoperatively and were gradually diminished over time after injury. At 20 weeks postoperatively, these rats still showed vivid cold allodynia and some rats showed mechanical allodynia and spontaneous pain. Autotomy was gradually developed on these rats. The results suggest that injury to the tibial and sural nerves induce severe neuropathic pain syndrome and this animal model may provide convenient and easy method to produce neuropathic pain. Sympathetic dependence of this model will be discussed. (Supported by KOSEF 961-0701-014-2).

55.8

LOW DOSES OF THYMULIN INJECTION IN THE HINDPAW OF RATS CAUSES LOCALIZED HYPERALGESIA. B. Safieh-Garabedian*, S.A. Kanaan, S.J. Jabbur, S.F. Atweh and N.E. Saade. Fac. of Arts and Sci. and Fac. of Med., American Univ. of Beirut, Lebanon.

We have recently demonstrated that localized inflammatory hyperalgesia induced by intraplantar injection of endotoxin in rats can be significantly reduced by intraperitoneal injection of thymulin, in high doses (Safieh-Garabedian et al., *Brain Res.* in Press). We now report that intraplantar injection of thymulin, in low doses, produces localized hyperalgesia in rats.

A group of rats were subjected to paw pressure (PP), hot plate (HP) and tail flick (TF) tests for 3 days *before* (to determine baseline values) and at 3h, 6h, 12h and 24h *after* intraplantar injection of thymulin (5ng/50µl of saline) *in the left* hindpaws. Another group of rats received the saline vehicle (50µl) injections as controls.

Following thymulin injection, a significant reduction in both PP and HP latencies appeared after 3h, peaked (40-50% reduction in nociceptive thresholds) after 6h and recovered completely after 24h. No significant effects were observed in the PP test of the opposite right paw or in the TF test and the effects of thymulin injection were antagonized by i.p. injections of either dexamethasone (0.2mg/kg) or indomethacin (4mg/kg). Saline injections had no effects.

We suggest that low doses of thymulin, known to activate immune inflammatory reactions, can produce hyperalgesia through cytokine or prostaglandin dependent mechanisms.

(Supported by grants from the *DTSabbagh Fund* and the *University Research Board*.)

55.9

THE RELATIONSHIP BETWEEN VAGINAL HYPERALGESIA AND VAGINAL TONE IN RATS. H.B. Bradshaw* and K. J. Berkley. Program in Neuroscience, Florida State University, Tallahassee, FL 32306-1051.

Vaginal hyperalgesia (VAGH) in rats, as evidenced by increased escape responses to vaginal distension stimuli, occurs as a consequence of ovariectomy, natural reproductive senescence, and experimentally-induced endometriosis. This study tested the hypothesis that VAGH is associated with an increase in vaginal tone. Adult virgin rats (n = 9) were trained to escape vaginal distension produced by inflation of latex balloons with water. The probabilities of escape responses evoked by different volumes of distension were then measured in different estrous stages before and after ovariectomy, natural reproductive senescence, or the induction of endometriosis (abdominal autotransplantation of small peices of endometrium). Vaginal tone was calculated from the pressures produced by the different distension volumes. Although VAGH was evident in all three conditions, vaginal tone failed to increase beyond levels observed in normal, estrous-cycling rats. This dissociation between escape behavior and vaginal tone is similar to earlier findings showing that escape responses in normal cycling rats do not vary predictably with the estrous variations that occur in vaginal tone. Together, the results suggest that the neural mechanisms that regulate vaginal tone differ from those that regulate vaginal behavioral sensitivity. Supported by NIH--RO1 NS 11892.

55.10

MEASURING SOCIAL INTERACTIONS TO ASSESS PAIN AND ILLNESS IN FEMALE RATS. K. J. Berkley*, M. L.Torlone and H.B. Bradshaw. Program in Neuroscience, Florida State University, Tallahassee, FL 32306-1051.

Animal models for chronic pain conditions such as endometriosis, gastrointestinal disorders, headaches, backaches and so forth are needed. They are lacking in part because we do not have a way to measure pain under conditions in which the eliciting circumstances are not obvious (i.e., during conditions of endogenous pain). Currently, the main indicators that an animal has chronic pain include large changes in its usual daily behaviors such as eating, sleeping, weight, grooming and overall activity. Such changes are likely to occur, however, only when the pain conditions are extreme. Given that humans often change their social interactions when they have endogenous pains, one way to index such pains in animals might be to assess their social behaviors. Accordingly, we developed a procedure to quantify an experimental female rat's normal social behaviors and tested in a few pilot cases how those behaviors changed under various conditions. Making use of the fact that rats in their natural environment are huddling animals who spend much of their active phase touching and grooming each other, or, depending on the female's estrous stage, mating, the procedure we developed involves measuring the percent time during a standard daily test period an experimental rat spends (a) alone, (b) in close proximity with a ovariectomized female rat or (c) in close proximity with a vasectomized male rat. The percentage pattern (i.e., % time alone, with female or with male) varied with the experimental female's reproductive condition (estrous stage; senescence; n=4), and changed under conditions of pathology (n=3). These results suggest that this method might be of value for future studies on neural mechanisms of pain and other illness behaviors associated with various disease states. Supported by NIH-RO1 NS 11892 and the Earth Trust Foundation.

55.11

Differential effects of intraoral sucrose and suckling on forepaw and hindpaw nociceptive responses in 10-day-old rats. K.Ren*, Q-q. Zhou, E.M. Blass[a] and R. Dubner. Dept. OCBS, University of Maryland Dental School, Baltimore, MD 21201; [a]Dept. Psychology, Cornell University, Ithaca, NY 14853.

Sucrose and suckling produce calming and antinociceptive effects in infant rats. This model system was adapted to study the development of endogenous pain-modulating systems. Litters of 10-day-old Sprague-Dawley rats were tested for paw withdrawal latencies (PWL) to a heated brass thermal probe (48°C) or a non-heated probe (30°C). Paw inflammation was induced by s.c. injection of 0.01 ml of complete Freund's adjuvant (CFA, 1:1). Compared to naive rats, there were significant decreases in PWLs to both 48°C and 30°C stimuli 4 h following the injection of CFA, suggesting the development of thermal hyperalgesia and mechanical allodynia. In rats that were allowed to suckle their mother and received intraoral sucrose infusion (7.5%, 0.06 ml/h) during behavioral testing, the PWLs of the forepaw to the 48°C probe were significantly increased from 13.1±2.7 to 28.7±3.4 sec (n=13, naive rats, $P<0.001$) and from 4.8±1.7 to 21.5±3.8 sec (n=8, forepaw inflamed, $p<0.001$); the PWLs of the forepaw to the 30°C probe were also increased significantly from 27.9±4.3 and 12.8±4.0 sec to 54.8±8.7 and 28.5±7.6 sec for naive ($p<0.01$) and forepaw inflamed ($p<0.05$) rats, respectively. In contrast, intraoral sucrose and suckling produced no significant effects on PWLs of the hindpaws in naive and hindpaw inflamed rats. Intraoral saline infusion did not produce significant changes in PWLs in limbs in naive 10-day-old rats. CFA-induced Fos-protein inmmunoreactivity, a marker of neuronal activation, was also significantly reduced in the spinal dorsal horn (C7-8) by infusion of sucrose and suckling in forepaw inflamed rats, suggesting that the antinociceptive effects of sucrose and suckling were mediated via descending effects at the spinal level. These results demonstrate the development of inflammatory pain and hyperalgesia in newborn rats, a differential maturation of descending control systems to forepaw and hindpaw in 10-day-old rats, and a spinal basis for suckling-induced antinociception. Supported by NIH grants DA10275 (R.D.), HD28245 (E.M.B.) and MH00524 (E.M.B.).

55.12

INTERLEUKIN-6 KNOCK-OUT (IL-6 KO) MICE EXHIBIT ENHANCED BASAL NOCICEPTION AND NEUROPATHIC PAIN-RELATED BEHAVIOR, BUT HAVE REDUCED INFLAMMATORY REACTION COMPARED TO WILD TYPE (WT) MICE. Z. Wiesenfeld-Hallin[1]*, X.-J. Xu[1], J.-X. Hao[1], S. Andell-Jonsson[2], V. Poli[3], and T. Bartfai[2]. [1]Karolinska Institute, Dept. Clin. Neurophysiol., 141 86 Huddinge, [2]Stockholm Univ., Dept. Neurochem. Neurotoxicol., 106 91 Stockholm, Sweden, [3]Inst.. Ricerche Biol. Molec., Rome, Italy

IL-6 is a multifunctional cytokine which is rapidly induced under certain pathological and inflammatory conditions. IL-6 has been implicated in nociceptive modulation in the periphery and the CNS in a complex and sometimes contradictory manner. Thus, it has been reported that peripheral administration of agents neutralizing the actions of IL-6 reduce inflammatory hyperalgesia. In contrast, it has also been suggested that local administration of IL-6 elicits antinociception and is an important mediator for the peripheral analgesic action of opioids.

We have assessed the nociceptive threshold to cutaneous mechanical and thermal stimulation, the effect of unilateral sciatic nerve section and the development of inflammation and hyperalgesia after subcutaneous carrageenan in IL-6 KO and WT mice. The IL-6 KO mice had lower response threshold to both mechanical and thermal stimulation. After nerve section female IL-6 KOs autotomized more than male IL-6 KOs and both male and female WTs. In contrast, both IL-6 KO and WT mice developed hyperalgesia to mechanical and thermal stimulation after carrageenan, but the hyperalgesia was less in IL-6 KO than in WT. The IL-6 KOs also exhibited less plasma extravasation after carrageenan than the WT. Thus, IL-6 deficiency has a complex effect, with increased basal nociceptive sensitivity, increased neuropathic pain probably involving the endocrine system and reduced peripheral inflammation.

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55.13

BEHAVIORAL RESPONSES TO NOCICEPTIVE AND NON-NOCICEPTIVE STIMULI IN GLUR2 KNOCK OUT MICE. M.W. Salter*, G.J. Keil II, Z. Jia, W.Abramow-Newerly, J.Roder. Div Neurosci, Hospital for Sick Children and Dept Physiol, Univ of Toronto, and Samuel Lunenfeld Research Instit, Mount Sinai Hospital, Toronto, Ontario.

Nociceptive (pain) and non-nociceptive neurotransmission is primarily mediated by glutamate actions at AMPA receptors. The majority of AMPA receptors are impermeant to Ca^{+2}, but channels lacking the GluR2 subunit have greatly increased Ca^{+2} permeability. Processes which involve actions of excitatory amino acids at AMPA receptors, therefore, might involve Ca^{+2}-permeable and -impermeable AMPA receptors. To investigate if GluR2 subunits might be involved in sensory neurotransmission, we have investigated the behavioral responses of mutant mice lacking the GluR2 subunit (Jia et al, *submitted*) to various peripheral stimuli. Behaviors of wild type (+/+) and heterozygous (+/-) (n=10) or homozygous (-/-) (n=9) mice were evaluated in nociceptive (cold: 4°C and hot: 55°C) and non-nociceptive (warm: 40°C) thermal (tail withdrawal), non-nociceptive mechanical (von Frey hair), and nociceptive chemical (intraplantar formalin) assays. Tail withdrawal latencies to all thermal stimuli were not significantly different for mutant mice. Behavioral responses to von Frey hairs presented in two different paradigms indicated mutant mice were significantly more sensitive to mechanical stimuli. In addition, a novel biting and scratching response was seen in mutant mice with von Frey hair strengths which did not induce any behavior in wild type mice. Lastly, mutant mice responded to intraplantar formalin (10µL, 1%) significantly different than wild type mice in terms of the number of paw shakes, and licking or lifting time. The present studies were conducted to investigate if behaviors to various peripheral stimuli are altered in mutant mice lacking the GluR2 subunit. Mutant mice responded to thermal stimuli similarly to wild type mice. The biting and scratching response in mutant mice to von Frey hair stimulation, similar to mechanical allodynia, indicates altered sensory perception or transmission of this type of peripheral stimuli. Additionally, altered responses to intraplantar formalin suggests the GluR2 subunit might be necessary for the normal development and/or expression of behavioral responses to certain types of sensory stimuli. (Supported by MRC of Canada)

55.14

OPTIMAL WEIGHTINGS OF PAIN BEHAVIORS IN THE FORMALIN TEST IN RATS. K. J. Sufka[1,2*], G. S. Watson[1], & T. J. Coderre[3]. Depts. of Psychology[1] & Pharmacology[2], The University of Mississippi, Oxford, MS 38677, Clinical Research Institute of Montreal[3], Montreal PQ H2W 1R7, Canada.

Coderre and colleagues (*Pain*, 54 (1993) 43-50) demonstrated the validity of the weighted scores technique (WST) of the formalin test by assessing the ordinality of behavioral categories associated with specific category weights. The present study sought to extend these findings by providing optimal weightings for these behavioral categories. Formalin concentration and morphine dose data from Coderre's study were submitted to Discriminant Function Analyses (DFA) and Multiple Regression Analyses (MRA). These weights are summarized below.

Study	Analyses	Category 1	Category 2	Category 3
Formalin Conc.	DFA	0.0	0.8	0.6
	MRA	0.0	0.3	0.6
Morphine Dose	DFA	0.1	0.4	0.9
	MRA	0.0	0.3	0.6

DFA and MRA weights were used to calculate composite pain scores (CPS), which were correlated with CPS derived by the WST. Formalin concentration and morphine dose were correlated with DFA-, MRA-, and WST-derived CPS. CPS intercorrelations for formalin data ranged from 0.95 to 0.98, and concentration-CPS correlations ranged from 0.77 to 0.82. CPS intercorrelations for morphine data ranged from 0.98 to 0.99, and dose-CPS correlations ranged from -0.83 to -0.85. These results suggest that formalin CPS can be accurately derived from categories 2 and 3 with weightings of 0.3 and 0.6, respectively.

55.15

Strain, Load and Frequency as Indices of Nociceptor Sensitization in the Temporomandibular Joint. Cooper*, B., Friedman, R.M. and B. Loughner, Dept. of OMFS, University of Florida, Gainesville, USA.

Recent studies have placed an increased emphasis on the contribution of the CNS to the development of allodynia and hyperalgesia. This trend has been reinforced by a failure to observe substantial sensitization of mechanical nociceptive afferents when quantified methods are used. However, minimal quantification of stimulus and response variables have made it difficult to accept these reports as definitive. In the present study, we used computer controlled joint translation and systematic evaluation of soft tissue biomechanics to examine the changes in reactivity produced by prostaglandins of the E series. Recordings were made from the trigeminal ganglion of sixteen nociceptors (10 group III , 6 group IV) that innervated the soft tissue of the temporomandibular joint (TM) of the goat. Prostaglandins (PGE_1, PGE_2) or vehicle (VEH) were introduced into the capsule (50 ul, 2.8 mM) and reactivity was reexamined at early and late post-injection intervals (5-10 and 30-60 minutes). TM nociceptors transduced jaw rotations well beyond the normal range of motion (mean transduction range: 10.5 (+/- 1.11) to 24.1 (+/- 0.69) deg). Either PGs or repeated testing of nociceptors with intense stimuli was sufficient to produce substantial (and significant) decreases in mean load and strain reactivity at the 30 minute post injection interval (-0.43 +/- 0.09 N vs -0.41 +/- 0.21 N and -8.63 +/- 1.97 % vs -6.58 +/- 1.76 % strain for PG and VEH, respectively). In contrast, PGs significantly increased the average rate of discharge over the same strain and load ranges relative to VEH treated cases (97.2 +/- 55.2 % PGE vs -1.6 +/- 15.6 % in VEH treated cases; U=3, n=7,4; p<.042). We conclude that substantial changes in response properties can be observed in mechanical nociceptors when the complete range of transduction is determined, quantified stimuli are used, and frequency rather than threshold is the index of sensitization. NIDR/DE10040

55.16

SHOCK-INDUCED HYPERALGESIA IN RATS. T.E. King*, R.L. Joynes, M.W. Meagher, J.W. Grau. Dept. of Psychology, Texas A&M University, College Station, TX 77843.

Exposure to a few moderately intense (1 mA) tailshocks has opposite effects on two measures of pain reactivity in rats. Tail-withdrawal to radiant heat is inhibited (hypoalgesia) while vocalization thresholds are lowered (hyperalgesia) to both heat and shock (King et al., *J.Exp.Psychol.: Anim. Beh. Proc.*, *22*, 1996). Prior work indicates that hyperalgesia represents an unconditioned response and enhances the acquisition of both conditioned freezing and an avoidance response to thermal pain. The present experiments further explore the behavioral properties of hyperalgesia and the underlying neural mechanisms.

We have shown that hypoalgesia is limited to the distal region of the tail (Prentice et al., *Behav. Neurosci.*, *110*, 1996). Experiment 1 shows that rats exhibit hyperalgesia irrespective of where the test stimulus is applied. Experiment 2 examined the shock conditions needed to induce hypo- & hyperalgesia. Exposure to 0.3 mA, but not 0.1 mA, tailshocks elicited both behavioral effects. Evidence indicates that the hypoalgesia is eliminated by both pentobarbital anesthesia and decerebration. Experiments 3 and 4 show that these manipulations also eliminate shock-induced hyperalgesia. Administration of a benzodiazepine (chlordiazepoxide) also attenuated the hyperalgesia. Results from additional pharmacological/physiological manipulations will be reported. Supported by MH48994 to J.W.G.

55.17

THE NEURAL MECHANISMS OF AUTOTOMY FOLLOWING BRACHIAL PLEXUS TRANSECTION IN RATS. S. D. Wang*, C. H. Cherng, S. T. Ho and J. C. Liu. Depts. of Biology & Anatomy, and Anesthesiology, National Defense Medical Center, Taipei, Taiwan, ROC.

Peripheral neurectomy in the rat usually induces an self-multilation behavior, named autotomy, directed against the denervated regions. The early reports described the autotomy and supported this behavior as an experience of parathesia and/or pain in animals akin to the deafferentation pain in humans Deafferentation pain is a chronic pain condition which mechanisms are still unknown. However, the sensory afferent and sympathetic components have been suggested as the mechanisms of deafferentation pain. In this study, we examine the sensory afferent and sympathetic nerve system with relationship to the autotomy following brachial plexus transection (BPT) in rats. Sprague-Dawley rats were undergone BPT, and then with dorsal and /or ventral rhizotomy, or with stellate ganglionectomy (StG). The autotomy behavior was observed daily for 8 weeks postoperatively. In the BPT with dorsal and ventral rhizotomy animals, autotomy behavior was significantly reduced. In BPT with StG rats, however, there is no difference of the onset time, severity and the incidence of svere autotomy in comparison with the BPT only animals. Therefore, we suggest the sensory afferent may contribute to the development of autotomy but not the sympathetic nervous system following BPT in rats. (Supported by NSC-84-2331-B016-024, ROC)

55.18

EFFECTS OF STIMULATION PARAMETERS ON THE PLASTICITY OF FLEXION WITHDRAWAL REFLEXES IN THE SPINALIZED RAT. C.L. Cleland*, J. Minette-Fore and G.F. Gebhart. Departments of Physiology and Biophysics, and Pharmacology, Univ. of Iowa, Iowa City, IA 52242

Hyperalgesia arises from both peripheral mechanisms and central plasticity. Although contributions from peripheral mechanisms and central chemical mediators have been partially clarified, the location and nature of the central plasticity remains obscure. The goal of this study was to investigate the necessary and sufficient stimulus conditions for expression of hyperreflexia in a rat model of hyperalgesia.

Rats were anesthetized with pentobarbital (45 mg/kg), their spinal cords were transected at T8-T9 and an electrode was implanted around the sciatic nerve. The following day, flexion withdrawal reflexes were evoked by stimulation of the sciatic nerve at either C (2-10 mA) or Aβ (200-400 µA) strength and measured by percutaneously recorded EMG in the knee flexor muscles. Hyperreflexia was induced by brief trains of electrical stimulation delivered to the sciatic nerve at C-fiber strength (2-20 mA).

Repetitive stimulation of the sciatic nerve induced long-lasting (>9 hours) and pronounced (>300%) increases in the magnitude of the flexion withdrawal reflex. Reflexes evoked by Aβ and C fiber stimulation were both enhanced, although the size and duration of C-fiber evoked reflexes were greater than for Aβ-fiber evoked reflexes. Systematic variation in the frequency (0.1-4 Hz), duration (250 ms-160 s) and number of stimuli (1-160) revealed that increases in duration and number monotonically enhanced the magnitude and duration of reflex enhancement, without reaching a plateau. In contrast, changes in frequency (10 stimuli) revealed an optimum (1 Hz), with greater or lesser frequencies causing less reflex enhancement.

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55.19

TEMPORAL EFFECTS OF PRIOR STIMULATION ON LATER BLOCKING OF SPINAL FIXATION IN RATS. M.M. Patterson*, T. Knewtson UHS College of Osteopathic Med., 2105 Independence Blvd., Kansas City, MO 64124-2395.

Stimulation of an anesthetized rat's hind leg for over 35 minutes with an electric stimulus can induce a long-term alteration in the spinal flexion reflex known as *spinal fixation.* The altered excitability has been shown to last for days, and is a central sensitization of the spinal reflex circuit. Recently, we reported an effect which seems contradictory to the fixation paradigm (Patterson, et.al. SNS Abstracts, 1995). Rats given a fairly low level of stimulation on day 1, followed by a fixating stimulation on day 2 or 3 showed less fixation after the second session than if they had been given only the second session. This apparent blocking of the fixation effect by the first session was seen over several stimulus parameters, and intervals.

The present studies were designed to look at the effects of longer times between sessions and of spinalization on the blocking process. In the first study, animals were anesthetized and received 40 min. of 1.5 ma. stimulation to the right hind leg. The retained flexion averaged 8.1 g. after the first session. The animals were allowed to awaken and 4 days later reanesthetized, spinalized, and given 40 min. of 2.5 ma. stimulation. Retained flexion was 5.3 g. A second group received the same treatment but with 6 days between sessions. The retained flexion was 7.6 and 6.3 g respectively. These studies show less severe blocking with the spinalization, and less with the longer time between sessions, when compared with earlier studies. The blocking effect seems to decrease with time and to be affected by spinalization.

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55.20

NOCIFENSIVE FOOT-LIFT RESPONSE TO RADIANT HEAT IN THE LAND SNAIL (*HELIX ADSPERSA*): EFFECTS OF INTER-TEST INTERVAL AND NALOXONE J.J. Quinn, R.A. Wheeler, R.J. Brennan, A.E. Baldwin, & J.T. Cannon*. *Department of Psychology and Neuroscience Program, University of Scranton, Scranton, PA 18510-4596.*

The foot-lift response to contact heat and the tail-flick response to radiant heat are two of the most widely used indices of nociception in rodents. Recently, Kavaliers and colleagues demonstrated the utility of examining the nocifensive foot-lift response to contact heat in the land snail. We have sought to establish an analog to the rodent tail-flick test in which radiant heat can be repeatedly applied to elicit a reliable withdrawal response in the land snail (*Helix adspersa).* Here, we examined the effects of test interval and naloxone on this response.

Forty-eight snails were group housed, fed *ad libitum*, and tested under red light during the dark phase of a 12/12 hour light/dark cycle. Animals (8/group) were randomly assigned to 30 min of testing at either 2 or 10 min inter-test intervals and received either: no injection , 2μL of physiological molluscan saline, or 1.0μg of naloxone in 2μL of molluscan saline. Animals were hydrated for 15 min prior to testing and injections took place after 5 min of hydration.

For behavioral testing, snails were gently restrained by a vacuum applied to the dorsal shell. They were placed on a movable sheet of transparency film which allowed for relatively unrestricted locomotion. Heat was applied through a 1.6 mm diameter hole at three locations on the caudal half of the foot: 2 mm rostral to the tip, and bilaterally 2 mm medial to the lateral edges of the foot 1 cm from the tip. The heat source was adjusted to elicit lifts with latencies of 2.5-3.5 sec.

ANOVA revealed no significant differences between uninjected and saline animals and these data were collapsed for subsequent analyses. ANOVA revealed that animals tested at 10 min intervals exhibited significantly longer latencies than 2 min animals. Animals injected with naloxone had significantly shorter latencies and this difference tended to disappear by the end of the 30 min test interval. There were no other significant main effects or interactions.

Overall, these data suggest that the foot-lift response to radiant heat may be a useful index of nociception in the land snail.

RETINAL PHYSIOLOGY

56.1

A NITRIC OXIDE DONOR AFFECTS ON-COMPONENTS OF ERG FROM CAT INNER RETINA. S. Viswanathan, L.J. Frishman* and J.G. Robson[1], College of Optometry, University of Houston, Houston, TX 77204-6052; [1]Physiological Lab, Cambridge CB2 3EG, UK.

This study examined the effect of the nitric oxide (NO) donor S-nitroso-N-acetylpenicillamine (SNAP) on the electroretinogram (ERG) of anesthetized cats. We were searching for a physiological correlate of the NO-facilitated cGMP-mediated gap junction closure that has been observed in the scotopic circuit of mammalian inner retina (Mills & Massey, 1995). ERGs were elicited by brief (< 5 msec) or 200 msec ganzfeld flashes using LEDs, and were recorded with both vitreal and intraretinal electrodes under scotopic conditions (blue flashes from darkness) and, as a control, under photopic conditions (red flashes on a steady blue background to suppress the rods). SNAP was administered intravitreally (500 μM). Under scotopic conditions SNAP selectively attenuated (but did not eliminate) the negative scotopic threshold response (STR) to weak flashes without appreciably reducing the a- and b-waves elicited by stronger stimuli. This finding is consistent with an effect on the scotopic circuit because the STR, which can be eliminated by intravitreal NMDLA (1.3-3.9 mM) and partially suppressed by TTX (3-4 μM), is known to originate from rod-driven amacrine and ganglion cells. However, the effect of SNAP was not confined to the scotopic circuit: under photopic conditions SNAP selectively attenuated a negative wave following the cone b-wave that was similar in time course to a wave from inner retina that can be blocked by NMDLA. Inner retinal contributions to the ERG at light-offset were not affected by SNAP. These findings suggest that NO may be involved in modulating photopic as well as scotopic ON circuits.

(Supported by NIH EY06671)

56.2

CONE PII COMPONENT OF MURINE ERG ISOLATED BY FLASHED BACKGROUND. A.Lyubarsky[1,2*], A.Iannaccone[2], J.Chen[3], J.Xu[3] and E.N.Pugh, Jr.[1] [1]Department of Psychology and [2]Ophthalmology, University of Pennsylvania, Philadelphia, PA 19104, [3] University of California, Los Angeles, CA 90095.

Murine retinas contain few cones ([1]) and at least one type of them absorbs at about the same peak wavelength as rods ([2]). Thus, it is impossible to segregate rod- and cone-driven components of the ERG solely by varying the wavelength of stimulation. A corneal-positive ERG component consisting mostly of b-wave and oscillatory potentials has been recorded in mouse when rod-driven components are suppressed by steady adapting lights ([3]); this signal has been attributed to cone origin. We isolated and characterized this cone b-wave component in mice with a two-flash paradigm: the first, conditioning flash photoisomerized ~1% of total rhodopsin, and saturated the rods for >3 s, as indicated by complete absence of the a-wave ([4]); a second variable intensity flash was used to test recovering cone function while rods remained in saturation. Arrestin knock-out transgenic mice, which have extremely long rod recovery times (several hours even after moderate intensity illumination) were used as functionally rodless control animals in order to obtain the parameters of the cone b-wave in conditions of complete cone dark adaptation. Cone b-waves begin to recover from saturation by 0.3 s after the conditioning flash, and reach complete recovery by 2-3 s. Work is under way characterizing the full time course of recovery of the cone PII.

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56.3

INHIBITION OF GLUTAMINE SYNTHETASE SUPPRESSES THE ELECTRORETINOGRAM B-WAVE IN THE RAT. N.L. Barnett[1], D.V. Pow[1], M.B. Plenderleith[2*] and S.R. Robinson[1]. [1]Vision, Touch and Hearing Research Centre, Department of Physiology and Pharmacology, University of Queensland, Brisbane, Australia, 4072. [2]School of Life Science, Queensland University of Technology, Brisbane, Australia, 4001.

Inhibition of retinal glutamine synthetase (GS) by methionine sulphoximine (MSO) caused a time and dose dependent suppression of the rat scotopic electroretinogram (ERG) b-wave. A-wave amplitudes were unaffected in Wistar rats (250g) injected intraocularly with 2μl of MSO (11mM, 110mM or 1.1M). ERGs were monitored for 5 hours and significant ($P < 0.05$) differences between MSO and saline-injected contralateral eyes were evident in the b-wave:a-wave ratios after 3 hours, 1.5 hours and 30 minutes respectively. The three concentrations of MSO almost totally abolished the b-wave and no recovery of the b-wave was evident even 12 hours after injection. Immunohistochemical analysis of retinas subjected to MSO (2μl, 110mM) revealed a total depletion of neuronal glutamate and an accumulation of the transmitter in the radial glial Müller cells within 1 hour. These changes in glutamate distribution following GS inhibition, an enzyme that is localised exclusively within Müller cells, arise as a consequence of the reduction of glutamine synthesis and shunting between Müller cells and neurones. The b-wave, which is thought to be generated by ON-bipolar cell depolarisation and Müller cell potassium fluxes, could be suppressed by four possible mechanisms: (i) depletion of photoreceptor glutamate suppresses tonic transmitter release in the dark, thus abolishing light stimulated changes; (ii) increased Müller cell glutamate content may reverse glutamate transport, cause glutamate efflux, elevation of extracellular glutamate and inhibition of ON-bipolar cell activation; (iii) GS inhibition and glutamate accumulation may directly compromise Müller cell function; (iv) build-up of extracellular ammonia may be toxic to bipolar cells.

Funded by The University of Queensland and Australian Research Council.

56.4

COMPUTATIONAL MODELING OF ELECTRICAL STIMULATION OF THE VERTEBRATE RETINAL GANGLION CELL R.J. Greenberg,* T.J. Velte,[2] M.S. Humayun,[3] and E. de Juan[3] Departments of Biomedical Engineering[1] and Ophthalmology[3], Johns Hopkins Univ. School of Medicine, Baltimore, MD. Howard Hughes Medical Institute[2], Harvard Medical School, Boston, MA.

We have previously shown that focal electrical stimulation of human retinas blind by Retinitis Pigmentosa and Age-Related Macular Degeneration is possible (Humayun, M.S. 1996). To better understand how the electrode avoids stimulating passing fibers from distant ganglion cells, we have developed a compartmental model for electric field stimulation of the retinal ganglion cell (RGC). This is the first model of a neuron stimulated by extracellular fields with active channels and non-schematized cell morphology. Mudpuppy RGCs were stained with intracellular injection of Neurobiotin, traced in three dimensions with the Eutectic Neuronal Tracing System, and imported into the NEURON simulation package (Hines, M., 1993). Three membrane models were applied: a linear passive model, a Hodgkin-Huxley model with passive dendrites (HH), and a completely active model (AA) with five non-linear ion channels distributed at varying densities. Stimulation was provided by an idealized monopolar point and disk electrodes located at various positions 30μm above the cells. For both HH and AA, the position of lowest cathodal threshold (propagating action potential) was over the soma. Cathodic stimuli were 58%(HH) to 73%(AA) more effective over the soma than over the axon. In the passive model, with threshold depolarization defined as 15mV, only 10% less cathodic current was required to depolarize the axon vs. the soma. Our active models demonstrate that it may be possible to electrically stimulate RGCs near their cell body at lower thresholds than at their axon, which is consistent with our clinical results. In addition, we have shown that compartmental models with active channels and realistic geometry from neuronal tracings can be achieved with reasonable computing power and should be considered in the study of extrinsically applied electrical fields.

Supported by a Jaffe Family Foundation Grant to RJG and EDJ.

56.5

GAIN CONTROL IN THE AII AMACRINE CELL OF CAT RETINA.
R.G. Smith* Dept. of Neuroscience, Univ. of PA, Phila PA 19104-6058.

At scotopic intensities, the AII cell is hypothesized to collect and amplify single-photon signals in a circuit that includes voltage-gated Na and K channels and gap junction coupling. Our simulations of the AII network suggest that it selectively amplifies single-photon signals in the presence of synaptic noise (Smith and Vardi, 1995). At low scotopic intensities, gap junction tracer coupling is modest, but at 1-2 log units above ganglion cell threshold, gap junction coupling extends to a radius of about 10 cells (Bloomfield, 1996). This raises the question of what operation the AII circuit might perform at higher intensities where photon fluctuation noise obscures single photon events. Measurements of gain at scotopic intensities show square root law behavior psychophysically and also in ganglion cells (Donner et al., 1990), which implies that noise from photon fluctuation controls gain in some manner.

To explore the consequences of AII coupling for gain control, we simulated the AII network and adjusted Na conductance to generate subthreshold "spike events" in response to 4 mV photon events. In the presence of randomly-timed photon events (20/sec), full action potentials were generated where 2 or more photon events coincided, but the amplitude of this response depended on coupling. When we modulated the gap junctions by a temporal low pass function (200-500 msec) of depolarization in the AII, action potentials were limited to a few noise peaks at the coincidence of several photon events. A faster photon arrival rate increased gap junction conductance and reduced the regenerative tendency. The result was a dynamic gain control where the number of action potentials was related to the photon fluctuation noise amplitude, suggesting square-root law behavior.
Supported by MH48168.

56.6

SPATIAL EXTENT OF THE CONTRAST GAIN CONTROL SIGNAL IN CAT RETINAL GANGLION CELLS. M.H. Rowe*, Neurobiology Program, Ohio University, Athens, OH 45701.

The existence of a contrast gain control mechanism is well established in cat retinal X- and Y-cells. Because of similarities in their properties, this mechanism has been associated with the rectifying subunits first described by Hochstein and Shapley (1976), and since the subunits extend well beyond the classical receptive field, it is sometimes assumed that the contrast gain control signal is generated over an equally extensive area. The present experiments were conducted in order to determine whether or not the contrast gain control mechanism has the same spatial extent as the subunits in cat retinal X- and Y-cells. The spatial extent of the contrast gain control mechanism was assessed by presenting a test stimulus, consisting of a drifting grating at optimal spatial and temporal frequency, within a circular aperture centered on the receptive field. The response of the cell as a function of contrast in the test stimulus was then measured both in the presence and absence of a high contrast "background" grating presented outside the aperture. When the aperture was relatively small, the background grating affected the amplitude and phase of the test responses in a manner consistent with the action of a contrast gain control. When the aperture was large enough to easily encompass the classical center/surround receptive field, the background grating had no little or no effect on either the amplitude or the phase of the test responses. Nevertheless, under these conditions, the background grating was able to elicit a vigorous subunit response in the form of a "periphery effect".

These results suggest that subunits located beyond the boundaries of the classical receptive field are not involved in contrast gain control, but rather some other aspect of Y-cell function, e.g., responses to global motion.
Supported by EY10677 and by funds from OUCOM.

56.7

INTERACTIONS BETWEEN ON AND OFF SYSTEMS AT OFF CENTRE GANGLION CELLS IN ISOLATED CAT RETINA.
D.C. West*, E.A. Read and A.M. Silllito Department of Visual Science, Institute of Ophthalmology, Bath Street, London, EC1V 9EL, UK.

We have developed a system using an in vitro eye cup preparation of cat retina that enables precise visual stimulation and response analysis combined with rapid and controllable drug delivery. In this first preliminary work we have examined the influence of blockade of the "on" system with L-AP4 on the capacity of "off" ganglion cells to provide an output that reflects the characteristics of a spatially restricted patch of drifting sinusoidal grating overlying their receptive field centre. The contrast, temporal and spatial frequency of the grating were varied. Cells were classified as X or Y using conventional visual criteria. Drugs were applied either in the perfusate or via a micropipette located adjacent to the extracellular recording electrode. We have considered the possibility that a glycinergic inhibitory mechanism driven by the "on" system (Wassle, Schafter-Trenkler, Voigt 1986 J.Neuroscience 6:594-604) may provide a significant contribution to the normal response to these stimuli in "off" centre ganglion cells.

Application of L-AP4 (1-10 uM) abolished the responses of "on" centre retinal ganglion cells and modified that of "off" centre cells. The modulation depth of the response to sinusoidal gratings (0.1-0.6 cpd, 0.5-4.0Hz) was decreased and the ability to detect stimuli that weakly activated them lost. The results seem to best support the view that a push-pull mechanism utilising an inhibitory arm driven by the "on" system is necessary for the ability of "off" centre cells to follow the full dynamic range of the stimuli used here.
Supported by the Wellcome Trust.

56.8

WITHDRAWN

56.9

STATIC AND DYNAMIC MEMBRANE PROPERTIES OF MB1 BIPOLAR CELLS FROM GOLDFISH RETINA. S. Mennerick* and G. Matthews. Dept. Neurobiology & Behavior, SUNY Stony Brook. Stony Brook, NY 11794.

To explore the electrotonic architecture of retinal bipolar cells and to extend recently employed time-resolved capacitance measurements of isolated synaptic terminals to intact cells, we developed a compartment model of isolated MB1 bipolar cells. In whole-cell recordings, current relaxations in response to 10 mV hyperpolarizing voltage steps decayed with a double-exponential time course. This suggests that the passive membrane properties of MB1 cells may be described by a two-compartment equivalent circuit with compartments corresponding to the somal/dendritic (6-10 pF) and synaptic terminal (2-4 pF) regions of the cell, linked by the axial resistance (30-60 MΩ) of the axon. Four lines of evidence validate the equivalent circuit: 1) Similar estimates of somal/dendritic and terminal capacitance were obtained whether the patch pipette was attached to the soma or to the synaptic terminal. 2) Estimates of the capacitance of the two compartments in intact cells were similar to estimates from somata and terminals that were spontaneously or experimentally isolated from parent axons. 3) The model yielded estimates of capacitance similar to values entered into a more sophisticated computer simulation of bipolar cells. 4) The model accurately reflected increases in terminal capacitance thought to represent exocytosis of synaptic vesicles. In contrast to other cells to which a two-compartment model has been applied, our results suggest precise morphological correlates in retinal MB1 bipolar cells of the equivalent circuit compartments. Additionally, the work suggests that time-resolved capacitance measurements of synaptic transmitter release may be feasible in intact tissue.
Supported by NIH grants NS07371 and EY03821.

56.10

RED-SENSITIVE CONES OF THE CYPRINID *DANIO AEQUIPINNATUS* ARE ACTIVE UNDER SCOTOPIC CONDITIONS. **Peter van Roessel, Adrian G. Palacios*, and Timothy H. Goldsmith. Department of Biology, Yale University, New Haven, USA.**

Electroretinography was used to assess the spectral sensitivity of a fresh-water cyprinid fish, *Danio aequipinnatus*. The spectral sensitivity of fully dark-adapted (>4 hours) fish was determined by measuring the magnitude of the b-wave of the electroretinogram in response dim flashes of monochromatic light of known quantal flux. The resulting scotopic spectral sensitivity curve is not consistent with rods acting alone. It could be modeled, however, using an additive combination of the spectral sensitivity curves for both rod cells and the danio's long wavelength cone (maximal sensitivity at 561 nm, Palacios et al., 1996). Changes in spectral sensitivity were observed following adaptation to increasing intensities of dim, far-red background (I<1% at λ>650 nm). Initial changes through a dynamic range of approximately 2 log units of sensitivity could be well modeled by hypothesizing a relatively increased contribution of long-wavelength cones to the rod-dominated scotopic ERG response. Still further adaptation to a red background selectively depressed the sensitivity of the long-wavelength cones, leaving a function whose peak reflects the 480 nm cone. The finding that long wavelength cones contribute to the ERG of fully dark-adapted danios suggests a plausible mechanism for dichromatic color vision in freshwater cyprinids at conditions nearing complete darkness, and accords with behavioral findings that suggest dichromatic color vision under scotopic conditions, possibly mediated by rods and red cones (Powers & Easter, 1978). Supported by NEI Grant EY00222

56.11

NEURAL NETWORK ARCHITECTURE FOR SPATIO-TEMPORAL DYNAMICS AT THE OUTER PLEXIFORM LAYER OF THE VERTEBRATE RETINA. Xianyi Yang# and Greg Maguire* #Department of Electrical and Computer Engineering, University of Houston, Houston, TX 77204-4793; @Department of Physiology, Keio University School of Medicine, Tokyo, Japan.

In the outer plexiform layer (OPL) of the vertebrate retina, there are three major types of cells making synapses: photoreceptors, horizontal cells, and bipolar cells. The photoreceptors are the only cells in the vertebrate retina that are sensitive to the light and transduce the light energy into neural activity. The horizontal cells receive sign conserving signals over a wide receptive field, and play an antagonistic role at the photoreceptor to bipolar cell pathway. There are two types of bipolar cells, the on- and off-bipolar cells, which have opposite response characteristics. The off-bipolar cells receive sign conserving signals from photoreceptors and inhibitory signals from horizontal cells directly, or indirectly though the photoreceptor terminal. Based on the neurophysiological and anatomical data, we proposed a neural network architecture for the spatio-temporal dynamics of the outer plexiform layer in the vertebrate retina. This architecture consists of transmitter processes, Hodgkin and Huxley membrane modeling (shunting equation), and a gated dipole model. The dynamics of all the OPL cells in the model responding at light-on and -off are in agreement with the physiological data. Stimulation of the model with constant center illumination and various surround illuminations also results in responses with the spatial and temporal dynamics of the physiological responses of bipolar cells. We also model the means of GABA inhibition from horizontal cell to bipolar cell. Our simulations show that GABAergic input, both direct or indirect, through the photoreceptor terminal, are possible to form the response characteristics of the bipolar cell.

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56.12

PATCH CLAMP ANALYSIS OF INTRINSIC MEMBRANE PROPERTIES OF ALPHA AND BETA CELLS IN CAT RETINAL WHOLEMOUNTS. D.W. Robinson* and L.M. Chalupa, Section of Neurobiology, Physiology and Behavior, University of California at Davis, Davis, CA 95616.

We have assessed the intrinsic spiking patterns of morphologically identified RGCs in retinal wholemounts. Current-clamp recordings were obtained at 35°C from 15 α and 59 β- RGCs anatomically identified by incorporating Lucifer yellow into the electrode solution. The intrinsic temporal properties of these neurons were assessed using a variety of sinusoidal and square wave stimuli. The resting membrane potential did not vary as a function of anatomical cell class (-55 mV) and in all cases regenerative firing was observed at rest. In response to maintained depolarizations both classes responded with maintained discharges and exhibited a linear relationship between spike output and the magnitude of injected current. Both cell classes also showed a linear relationship between the discharge rate and the amplitude of sinusoidal stimuli modulated below 10 Hz. Above 10 Hz only 1 spike per cycle was generated irrespective of the stimulus amplitude. The tuning curves of α and β -RGCs did not differ in any systematic manner and exhibited a peak at around 15 Hz. In all cases, the peak spike rate obtained to temporal modulation was higher than that observed to maintained depolarizations at an equivalent amplitude. As the stimulus frequency was increased above 10 Hz, the ability of both cell classes to follow the stimulus decreased quite markedly. Nevertheless, even at the highest stimulus frequencies, the spikes were tightly time-locked to the phase of the stimulus cycle. Many of the temporal properties examined mirror those obtained from psychophysical experiments, suggesting that RGCs shape retinal output to a greater degree than was previously thought. Supported by NIH grant EY-03991 to LMC

56.13

NEURAL MECHANISMS TUNE THE *LIMULUS* EYE TO ITS VISUAL ENVIRONMENT

F.A. Dodge*, C.L. Passaglia, and R.B. Barlow
Marine Biology Laboratory, Woods Hole, MA. Syracuse University & SUNY Health Science Center, Syracuse, NY.

How are the integrative mechanisms of the *Limulus* eye adapted for encoding information about potential mates? We measured the spatial and temporal properties of the eye by drifting sinusoidal and squarewave gratings on a monitor viewed by an animal located in a glass-sided seawater tank.

We found that both the spatial and temporal transfer functions of the eye have bandpass characteristics which tune the eye for specific stimuli. Lateral inhibition attenuates retinal sensitivity to low spatial frequencies and the eye's optics cutoff sensitivity to high spatial frequencies yielding maximal sensitivity to crab-sized objects (30°) at the sighting distance measured behaviorally. Lateral inhibition also suppresses responses to large spatial changes in illumination as a crab orients. Regarding the eye's temporal response properties, self inhibition attenuates retinal responses to slow modulations of light intensity and the finite duration of quantal events reduces responses to high temporal frequencies yielding maximal retinal sensitivity in the range of 1-5Hz. This range includes the temporal components of animal motion (1-3Hz) and natural light fluctuations (2-5Hz) that highlight reflective crab-sized objects. In sum, the lateral eye is both spatially and temporally tuned for seeing moving crab-sized objects in a dynamic visual environment.

Supported by NSF (BNS9421359) and NIH (MH49741 & EY00667).

56.14

BLUE-SENSITIVE PHOTORECEPTORS THAT MEDIATE FEEDING BEHAVIOR IN *MANDUCA* ARE LOCATED IN THE VENTRAL RETINA OF THE COMPOUND EYE. R. H. White*, J. Stockslager and R. R. Bennett, Biology Department, University of Massachusetts/Boston, Boston, MA 02125-3393

The spectral sensitivity of flower visitation in the nocturnal sphingid moth *Manduca sexta* indicates that feeding behavior is mediated mainly by blue-sensitive receptors. Distribution across the retina of the 3 spectral classes of receptors (green, blue, UV) was mapped by measuring ERG spectral sensitivity in localized regions, and by the light-dependent ultrastructural responses of individual receptors. The contribution of the 3 receptor types to ERGs was estimated by matching sensitivity curves with the summed absorption spectra of the 3 corresponding rhodopsins. Blue-sensitive receptors, mixed with green and UV cells, were found only in the ventral half of the retina. The dorsal half contained only green and UV receptors. The ventral localization of blue receptors is appropriate for guiding a moth that hovers above flowers while feeding.

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56.15

RELATIONSHIPS BETWEEN FEEDING STRATEGY AND RETINA STRUCTURE AND PHYSIOLOGY IN SHOREBIRDS L.-M. Rojas, T. Cabana, P. Lachapelle* and R. McNeil. Sc. Biologiques, Université de Montréal, C.P. 6128, Succ. Centre-Ville, Montréal, Canada H3C 3J7, and Ophthalmology, McGill University, Montréal, Canada H3H 1P3.

Depending on the sensory modality most often solicited in their food foraging, aquatic birds can be classified as tactile or visual. Visual birds are generally active during daytime, whereas tactile birds can combine both diurnal and nocturnal activity. Electroretinograms (ERGs) were recorded on shorebirds having different foraging behaviors: *Charadrius wilsonia* is recognized as being visual, *Limnodromus griceus* as tactile, *Catoptrophorus semipalmatus* as visual during daytime but tactile at night, and *Himantopus mexicanus* as visual and tactile during the day and visual under moonlight conditions. Their eyes were then prepared for histology. The luminance-response function under scotopic condition revealed that *H. mexicanus* and *C. wilsonia* have a higher retinal sensitivity than *C. semipalmatus* and *L. griceus*. For instance, at -2 Log-unit, the average amplitude in μV is 322.9 ± 44.0; 259.5 ± 47.7; 160.4 ± 28.3; 94.8 ± 13.0, respectively, taking into account the size of the pupil relative to the eye. These results are in accordance with their cones to rods ratio: 1:1.12; 1:1.56; 1:0.75; 1:0.97, respectively. Thus, these findings suggest the existence of a correlation between the structure and function of the retina and the foraging behavior of these birds. However, under photopic condition no significant difference was found in the ERG response between the four species. We conclude that visual capability is one, but not the sole, factor influencing their feeding strategy. (Supported by NSERC of Canada and CONOCIT of Venezuela)

56.16

EFFECTS OF DEFOCUS-INDUCED MYOPIA AND HYPEROPIA ON SEROTONERGIC AMACRINE CELLS IN CHICK RETINA. S.T.Wong[1], A.J.Bakelaar[1], A.K.Ball[2], J.S.Frank[1]*, J.G.Sivak[1], School of Optometry, University of Waterloo, Waterloo, Ont., Canada N2L 3G1[1]; Department of Biomedical Sciences, McMaster University, Hamilton, Ont., Canada L8N 3Z5.[2]

Avian animal models are often used in vision research since the visual capabilities of birds are highly developed. Myopia and hyperopia can be readily produced in young chicks using goggles with rigid contact lenses. Previous studies in our laboratory have shown that concave lenses produce enlarged myopic eyes, while convex lenses produce hyperopic eyes with smaller dimensions (Irving 1992). Other studies suggest that abnormal myopic eye growth is mediated by local retinal control (Troilo et al. 1987, Wallman et al. 1987) and that amacrine cells are involved (Wallman 1991). We examined how serotonergic amacrine cells are affected in ametropias produced by defocusing lenses.

Newly-hatched broiler chicks were unilaterally exposed to visual defocus ranging from -20 to +15 diopters (D). Retinoscopy and ultrasonography determined refractive states and axial lengths. After sacrifice, retinal wholemounts were processed for serotonin fluorescent immunohistochemistry and viewed by confocal microscopy. Prior to treatment, there were no differences between both eyes. After 5-7 days, each treated eye showed a refractive error nearly equal to the sign and magnitude of the defocusing lens. Myopic and hyperopic eyes respectively showed significantly larger or smaller dimensions compared to control eyes. There were no significant differences in serotonergic cell densities for moderate hyperopia (+15D) and moderate myopia (-10D). At high myopia (-20D), there were significantly higher serotonergic cell densities in treated eyes compared to control eyes. These results indicate that serotonergic amacrine cells may play a role in ametropia and eye growth mechanisms.

Supported by Natural Sciences and Engineering Research Council of Canada.

56.17

RETINYL ESTER HYDROLYSIS IN THE EYE: SUBSTRATE SPECIFICITY. A.T.C. Tsin*, J.A. Ray, N.L. Mata. Division of Life Sciences, The University of Texas at San Antonio, San Antonio, Texas 78249.

Hydrolysis of 11-*cis* and all-*trans* retinyl esters in the eye is catalyzed by microsomal hydrolases in the retinal pigment epithelium (RPE). In the present study, we have examined the hydrolysis of 11-*cis* and all-*trans* retinyl esters with fatty acids of various acyl-chain lengths and degree of saturation (i.e., all-*trans* and 11-*cis* retinyl stearate, palmitate, myristate, oleate, and linoleate). All-*trans* and 11-*cis* retinyl palmitate (A*t*RP and 11*c*RP) were hydrolyzed at faster rates than all other retinyl esters. Of the 18-carbon species examined, unsaturated (vs. saturated) 11-*cis* retinyl ester were hydrolyzed at higher rates, whereas these differences were not observed in the hydrolysis of all-*trans* retinyl esters. Employing substrates of two different radiolabels (i.e. [^{3}H]11*c*RP and [^{14}C]A*t*RP, [^{14}C]triolein, or [^{14}C]cholesteryl oleate), we also obtained experimental evidence showing that the hydrolysis of [^{3}H]11*c*RP was not affected by the presence of either all-*trans* RP or esterase substrates. However, similar competition experiments showed that the presence of cholesteryl oleate, triolein, or 11*c*RP significantly reduced the hydrolysis of A*t*RP. Based on results from these experiments, we suggest two catalytic sites for retinyl palmitate hydrolysis: a higher activity/high specificity site for the 11-*cis* isomer and a lower activity/non-specific site for the all-*trans* isomer. As the visual cycle in the RPE includes only the hydrolysis of 11-*cis* retinyl ester (but not the all-*trans* isomer), our finding that 11-*cis* retinyl ester hydrolysis is associated with high activity as well as high specificity is consistent with its role in the rapid supply of visual chromophore during bleaching and regeneration of visual pigments. Supported by grants from the NIH (NEI and MBRS) and The San Antonio Area Foundation.

56.18

LIPOCALIN TYPE PROSTAGLANDIN D SYNTHASE IN RETINAL PIGMENT EPITHELIUM (RPE) IS SECRETED INTO INTERPHOTORECEPTOR MATRIX (IPM). Gordon W.C. [1], Marcheselli V.L. [1], Beuckmann C.T. [2], Urade Y. [2], Hayaishi O. [2], Bazan NG* [1] LSU Neuroscience Center and LSU Eye Center, New Orleans, LA[1]; Osaka Bioscience Institute, Osaka, Japan[2].

PGDS, glutathione-independent prostaglandin D synthase, a 26 kDa glycoprotein, forms prostaglandin D2 in brain, and occurs in retina. It has been sequenced, cloned, and found to be identical to cerebrospinal fluid β-trace. Homology searches show PGDS to be a lipocalin (secretory hydrophobic transporter), but PGDS is the only member displaying enzymatic activity, suggesting dual function. This study determines retinal PGDS distribution. Enzymatic activity of PGDS was determined on retinal protein fractions. Northern and Western blots of RPE, IPM, and retina were run. Immunohistological PGDS localization in retina was determined. *Xenopus* and Sprague-Dawley (albino), Long Evans (pigmented), and Royal College of Surgeons (RCS) rats were used. PGDS activity for retina was 4.4 ± 0.6 nmol/(min•mg protein); Northern blots were positive for PGDS expression: *in situ* hybridizations showed PGDS mRNA only in RPE; a strong immunopositive signal occured in RPE, a weaker signal extended proximally to the outer limiting membrane. PGDS occurred in all retinas examined, but RCS rat RPE had less overall labeling and some dim cells. *Xenopus* PGDS expression was 3-fold greater in RPE than retina. IPM protein levels of PGDS were highest (>300-fold); PGDS is a soluble protein, secreted into IPM by RPE. COX-2 was used to show distribution of an intracellularly secreted enzyme; protein levels and distribution were compared with PGDS. COX-2 was a major RPE and retinal membrane component, but not in IPM. Strong PGDS activity was immunologically localized to RPE cells and IPM, but its mRNA occured only in RPE. Thus, PGDS is synthesized in, and then secreted from, the RPE in high concentrations, suggesting a dual function, an RPE synthesizing enzyme and an IPM transporter molecule (lipocalin). This may be an example of the gene sharing concept introduced by J. Piatigorsky (1989). NIH/NEI EY05121

56.19

THE 5' FLANKING REGION OF ZEBRAFISH INTERPHOTORECEPTOR RETINOID-BINDING PROTEIN (IRBP) DRIVES REPORTER GENE EXPRESSION IN PRIMARY CHICK RETINAL CELL CULTURES L.L. Cunningham*[1,2], J.M. Nickerson[3], F. Gonzalez-Fernandez[1,2], J. Peoples[3], J.H. Boatright[3] Graduate Program in Neuroscience[1] and Department of Ophthalmology[2], University of Virginia, Charlottesville, VA; The Emory Eye Center[3], Atlanta, GA

Interphotoreceptor retinoid-binding protein (IRBP) is a vitamin-A binding protein that is thought to function in the visual cycle by transporting retinoids between retinal photoreceptors and the overlying retinal pigment epithelium. Expression of IRBP is restricted to retinal photoreceptors and cells of the pineal gland. Expression of IRBP mRNA is regulated in a light-dependent manner in zebrafish (Rajendran et al., submitted). In order to study the *cis* elements that contribute to the regulation of IRBP expression, we cloned IRBP as well as 3 kb of its 5' flanking sequence from zebrafish. 797 bp of 5' flanking sequence were cloned upstream of the gene encoding chloramphenicol acetyltransferase (CAT). This plasmid was used to transfect (via calcium phosphate precipitation) primary retinal cell cultures prepared from E8-E11 chicken embryos. Four days after transfection, cells were harvested and assayed for CAT activity, which was considered an index of promoter activity. Results indicate that the 5' flanking region of zebrafish IRBP can drive reporter gene expression in the chicken retinal cell culture system. This system will be useful in determining the *cis* elements contributing to the tissue-specific and light-dependent regulation of IRBP expression. *Supported by NIH 5T32GMO8328-04, NIH R01EY09378, NIHEY09412, Research to Prevent Blindness (Olga Keith Weiss Award to J.M.N.), and a grant-in-aid from Fight for Sight (J.H.B.)*

56.20

RESPONSES OF CAT SCN-PROJECTING GANGLION CELLS

M. Pu*and P. Sterling Department of Neuroscience, University of Pennsylvania, Philadelphia, PA 19104

Retinal ganglion cells projecting to suprachiasmatic nucleus exhibit several distinct dendritic morphologies, but the spontaneous and evoked firing patterns are unknown. We injected fluorescent beads into the SCN and two days later placed the retina *in vitro* where we recorded extracellularly from retrogradely labeled neurons. Spontaneous firing was vigorous (>20 spikes/s), highly regular over periods up to 5 hours. A spot centered on the dendritic field evoked a transient burst followed by steady, brisk firing that was sustained indefinitely. Spatial summation occurred to spots from 0.1-1.5 mm diameter. At stimulus off, the cell returned to spontaneous level. Cells responded over mesopic and photopic intensities. One cell whose spectral responses were plotted responded from 450 to 676 nm with a peak around 510 nm.

(supported by grant NS34324)

AUDITORY SYSTEMS: CENTRAL ANATOMY—HINDBRAIN

57.1

A COMPARISON OF THE CROSSED AND UNCROSSED, GABA-IMMUNO-REACTIVE PROJECTIONS OF THE CAT DORSAL NUCLEUS OF THE LATERAL LEMNISCUS A. Shneiderman*, D.A. Stanforth, and R.L. Saint Marie, Neuroanatomy Dept., House Ear Institute, Los Angeles, CA 90057.

The dorsal nucleus of the lateral lemniscus (DNLL) provides separate crossed (60%) and uncrossed (40%) inhibitory (GABAergic) projections to the inferior colliculus (IC). Immunocytochemical studies have shown that DNLL neurons exhibit a range of somatic GABA concentrations. We examined the laterality of DNLL projections and compared this with their somatic GABA concentration, position within DNLL, and prevalence of GABA-immunoreactive (GABA-IR) perisomatic puncta. Laterality was determined by retrograde transport of HRP from large injections confined to one IC. GABA-IR was determined from 1.5 µm plastic sections which contained retrogradely labeled neurons in the DNLL opposite to the injected IC. DNLL neurons with crossed and uncrossed projections differed in several ways. Those with crossed projections (47-59% of the total were retrogradely labeled) tended to be lightly-to-moderately GABA-IR, although some examples of negatively and darkly stained neurons were occasionally found. They were more prevalent in the lateral part of DNLL and in areas dense with GABA-IR neuropil. Their somata were frequently covered with GABA-IR perisomatic puncta. DNLL neurons which did not transport HRP from the contralateral IC (41-53% of the total) were presumed to contribute uncrossed projections to the uninjected IC. These tended to be moderately-to-strongly GABA-IR and were more often found medially in DNLL, predominately among the lemniscal fiber fascicles in areas generally lacking GABA-IR neuropil. They had few GABA-IR perisomatic puncta. The results indicate that DNLL neurons differ in their somatic GABA concentrations, position within DNLL, and their afferent inputs, depending on the laterality of their efferent projections. This suggests that the two types of projections may contribute different kinds of information to the IC.

Supported by NIH/NIDCD grant RO1-DC00726.

57.2

DEVELOPMENT OF GABAERGIC CIRCUITS IN THE AUDITORY BRAINSTEM OF THE FERRET. Craig K. Henkel* and Judy K. Brunso-Bechtold. Department of Neurobiology and Anatomy, Bowman Gray School of Medicine, Wake Forest University, Winston-Salem, North Carolina 27157-1010

Inhibitory actions of gamma-aminobutyric acid (GABA) at synapses in the central auditory system are supported by a variety of evidence for several levels of the adult brainstem and midbrain. It is of considerable interest then to determine whether GABA is differentially regulated among various nuclei, circuits or cell types during development. Thus, we undertook immunohistochemical studies with standard methods using antibodies to GABA (Wenthold *et al.*, '87) to describe the distribution of GABAergic cells and axons in the ferret auditory brainstem. At birth and in the adult, GABAergic neurons and axons were found generally in comparable regions of the cochlear nuclei, superior olivary complex, nuclei of the lateral lemniscus, and inferior colliculus. There were no obvious transient populations of GABAergic neurons during postnatal development. However, some of the most immunopositive neurons in the auditory brainstem at birth circumscribed the medial half of the lateral superior olivary nucleus. While principal neurons in the lateral superior olivary nuclei in the adult material were only ever moderately immunopositive, their distribution was extended from medial to lateral and across the core of the nucleus by the time of onset of hearing at approximately 30 days of age. The ventral cochlear nucleus and central nucleus of the inferior colliculus also demonstrated a comparable gradient. Thus, results support the hypotheses that GABAergic neurons involved in a variety of ascending, descending and local circuits are not disparate in their temporal appearance, that GABAergic neurons in different hierarchical levels appear synchronously, and that inhibitory processes in tonotopically ordered nuclei develop in a topographic sequence. NIDCD grants DC00335 and DC00813

57.3

EVIDENCE THAT DISTINCT POPULATIONS OF GABA NEURONS ARE EXPRESSED IN THE SUPERIOR OLIVARY COMPLEX. SA Jenkins and DD Simmons*, Dept. of Physiological Science, Program in Neuroscience, and Brain Research Institute, UCLA, Los Angeles, CA 90095-1527.

Efferent projections from the brainstem to the cochlea play an important role in the modulation of auditory nerve activity. The modulatory actions of cochlear efferent neurons are believed to be mediated, in part, by GABA neurons in the superior olivary complex (SOC). The purpose of this study is to define the expression of GABA neurons within the SOC of the adult hamster brainstem using immunocytochemical and in situ hybridization techniques.

Our results indicate that there may be distinct populations of cells containing GABA. A postembedding immunocytochemical technique was used to identify GABA containing neurons in 1.5 μm plastic sections through the SOC. Three categories of labeling occurred: dark, moderate and unlabeled. Greater than 70% of darkly labeled cell bodies were in the lateral superior olive (LSO) and the superior paraolivary nucleus (SPN). The majority of moderately labeled cell bodies were in periolivary regions.

Preliminary investigations have been done on the expression of the two isoforms of glutamic acid decarboxylase (GAD), the synthetic enzyme of GABA. Within the SOC, the cRNA probe for GAD67 preferentially labels cells in the LSO while the probe for GAD65 preferentially labels cells in periolivary regions. Although less precise, immunoreactivity to the GAD67 protein mostly paralleled the GAD67 transcript expression.

Combined, these data raise the possibility of different functional roles for GABA within neurons of the SOC. It is possible that these distinctions relate to the projection of the neuron.

(Supported by grants from the NIDCD and the UCLA Academic Senate)

57.4

GLYCINE TRANSPORTER MOLECULES (GLYT1 AND GLYT2) IN THE DEVELOPING AUDITORY BRAINSTEM OF RATS. E. Friauf*, C. Aragón, C. Gimenez and F. Zafra. Zentrum der Physiologie, Universität Frankfurt, Germany; Centro de Biología Molecular, Universidad Autónoma Madrid, Spain.

The synaptic action of many neurotransmitters is terminated (and possibly modulated) by high-affinity transporters which remove the transmitter molecules from the synaptic cleft. Two different glycine transporters (GLYT1 and GLYT2) have been cloned as yet. Here we have used immunocytochemistry to analyze the spatial and temporal distribution of GLYT1 and GLYT2 in auditory brainstem nuclei of postnatal rats, which are particularly rich in glycinergic synapses.

In adult rats, GLYT2 and GLYT1 immunoreactivity (ir) are present in all auditory brainstem nuclei up to the level of the inferior colliculus. GLYT2-ir is more intense than GLYT1-ir, and neuronal somata within the cochlear nuclear complex (except for octopus cells) and the superior olivary complex are most intensely decorated with GLYT2-ir puncta. Neurons in the MNTB, known to be heavily glycinergic, form the only cell population displaying a cytoplasmic staining for GLYT2. As the labeling pattern is very reminiscent of that found for glycine receptor subunits, these results comply with a strict co-localization of GLYT2 (presynaptic) and the inhibitory glycine receptor (postsynaptic).

At postnatal day (P) 1, weak amounts of GLYT-ir can already be seen in most auditory brainstem nuclei, with the labeling for GLYT2 appearing earlier and being heavier at a given age than that for GLYT1. Signal intensity increases rapidly during the first postnatal week, and at P8, GLYT2-ir is already very intense in all auditory brainstem nuclei. Both transporter molecules appear to be expressed most heavily around P12, i.e. around hearing onset, and labeling declines until adult levels are reached around P21. Our results show that GLYT1 and GLYT2 are abundant in the developing auditory system before the onset of hearing, suggesting that they also play a role during early ontogeny, when synapse maturation occurs. Supported by a DFG grant (SFB 269/B5).

57.5

CHANGES IN GEPHYRIN IMMUNOLABELING IN THE AUDITORY BRAIN STEM OF MICE WITH MUTATIONS IN GLYCINE RECEPTOR SUBUNITS. J.M. Bonneau and R.A. Altschuler*, Kresge Hearing Research Institute, University of Michigan, Ann Arbor 48109-0506

Two strains of mice have nonlethal mutations in genes coding for glycine receptor (GlyR) subunits: α1 in the spasmodic (spd) and β in the spastic (spa). As glycine is an important inhibitory neurotransmitter it is useful to see the effects of these mutations on glycinergic synapses. Gephyrin is an essential element in the GlyR/cytoskeletal complex and binds to a specific 49 amino acid sequence of the β subunit. Its localization identifies and defines the extent of the GlyR complex. We therefore examined spd and spa mutants for changes in the immunolocalization of gephyrin in the auditory brain stem. All of the mice exhibited perisomatic labeling in numerous cell types in cochlear nucleus and superior olivary complex but the most dramatic observations in this study were the changes in the medial nucleus of the trapezoid body (MNTB) in the spa/spa and spa/+ mice. MNTB neurons of the spa mutants had a reduction in perisomatic placement of gephyrin and an increase in numbers and size of densely labeled gephyrin sequestering regions. The decrease in labeled postsynaptic GlyR complexes and the increase in intracellular gephyrin accumulation indicate that a mutation in the gene encoding the β subunit of the GlyR in mice alters the receptor complex in the auditory brain stem. The mutation in the β subunit having its most pronounced effect in the MNTB is particularly interesting in light of recent findings of elevated levels of expression of the β subunit of the GlyR in MNTB neurons over other neurons in SOC regions in normal rats.

(supported by NIH, NIDCD grant R01DC00383-09A2)

57.6

ULTRASTRUCTURAL LOCALIZATION OF GLUTAMATE RECEPTOR SUBUNIT IMMUNOREACTIVITY IN THE GERBIL LATERAL SUPERIOR OLIVE (LSO) I.R.Schwartz* and P.R.Eager, Dept. of Surgery/Otolaryngology, Yale Univ. Sch. of Med., New Haven, CT 06520

Vibratome sections of aldehyde fixed brains of young adults were incubated with antibodies (Ab) recognizing the glutamate receptor subunits GluR1, 2/3, or 4 (Chemicon), processed and sections of the LSO examined electron microscopically.

In accord with our light microscopic findings, GluR4 had the greatest concentration of immunoreactivity (IR) throughout the somatic and dendritic cytoplasm and at postsynaptic specializations. Very heavy patches of label were seen associated with some stacks of endoplasmic reticulum (ER). Asymmetric postsynaptic densities were heavily labeled over the entire length of the convex specialization. A second pattern of postsynaptic localization, less heavy than the first, was also seen at some terminals. Both patterns were observed in a single dendrite, the first associated with terminals containing large round synaptic vesicles, the second with a terminal with smaller pleiomorphic synaptic vesicles. GluR1 IR was also found throughout the somatic and dendritic cytoplasm but usually in somewhat lesser amounts. It was concentrated in small patches again in association with smaller clusters of ER. Postsynaptic IR was sometimes similar to that seen with GluR4, but was usually thinner and more spread. GluR2/3 IR was generally sparse in the cytoplasm of LSO somata and dendrites, but was seen in association with ER. IR was found at postsynaptic regions, but differed from the GluR4 pattern. It usually occupied less than the entire synaptic apposition. It was often punctate and associated with a cluster of labeled ribosomes immediately beneath the labeled density. With all three Abs some presynaptic IR was observed, occasionally within or near the synaptic junction, but sometimes at the sides of the terminal away from the synapse. All three Abs also showed IR in glial processes. This was most prominent with GluR4 where fine glial processes around synaptic terminals were often IR.

While postembedding immunocytochemical analysis of serial sections will be required to prove the exact distribution of each GluR at individual synapses, the present results suggest that in some cases they are not coincident and thus that the receptors in LSO neurons, in a single dendrite or even at a single synaptic terminal, may have several different combinations of subunits.

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57.7

AMINO ACIDS IN RAT COCHLEAR NUCLEUS SLICES. **L. Zheng[1]*, D. A. Godfrey[1], T. G. Godfrey [1]and H. J. Waller[2]. Depts. of [1]Otolaryngology and [2]Neurological Surgery, Medical College of Ohio, Toledo, OH**

Using high performance liquid chromatography (HPLC), amino acids were measured in samples microdissected from freeze-dried sections of rat cochlear nucleus slices. Slices were cut with a tissue chopper or vibratome, then incubated in artificial cerebrospinal fluid (ACSF). Data are shown for the fusiform soma layer of the dorsal cochlear nucleus (DCN), for incubated chopped slices (Inc-Chop), incubated vibratome slices (Inc-Vib), and incubated vibratome slices exposed to 50 mM K^+ for 3 (3 min K^+) or 10 min (10 min K^+). These results are compared to those for chopped nonincubated slices and to in vivo data. Average concentrations (mmol/Kg dry wt) are presented for aspartate (Asp), glutamate (Glu), glutamine (Gln), glycine (Gly), γ-aminobutyrate (GABA), taurine (Tau), and serine (Ser).

	Asp	Glu	Gln	Gly	GABA	Tau	Ser
In Vivo	14	41	19	25	9	11	5
Noninc	11	28	11	17	5	5	5
Inc-Chop	6	12	4	23	3	3	6
Inc-Vib	4	15	6	18	3	4	3
3 min K^+	5	17	9	21	4	5	4
10 min K^+	6	20	12	24	5	6	5

Most amino acids had similar concentrations in sections from near the surface or near the middle of a slice and in sections from slices cut with the chopper or vibratome. Compared with the in vivo data, there was a large loss of Asp, Glu, Gln, GABA and Tau in incubated slices, which was independent of incubation time between 4 and 12 hr. The concentrations of these amino acids were decreased even without incubation, suggesting rapid loss from the cut slices. In slices exposed to 50 mM K^+ for 3 or 10 min, Gln increased by 100% or more in all 3 DCN layers, while Ser increased by more than 60%. (Supported by NIH grant DC00172).

57.8

DENSITOMETRIC EVALUATION OF MARKERS FOR CHOLINERGIC TRANSMISSION IN RAT SUPERIOR OLIVARY COMPLEX. W. Yao* and D.A. Godfrey. Dept. of Otolaryngology, Medical College of Ohio, Toledo, Ohio 43699.

The superior olivary complex (SOC) gives origin to the olivocochlear bundle (OCB), for which acetylcholine is a neurotransmitter. However, little else is known about cholinergic elements in the SOC. Using NIH image 1.59, labeling densities in the lateral superior olive (LSO, *l*ateral, *i*ntermediate and *m*edial limbs), ventral nucleus of the trapezoid body (VNTB), superior paraolivary nucleus (SPN), medial nucleus of the trapezoid body (MNTB), and a middle zone (MZ, including the medial superior olive) were measured for acetylcholinesterase (AChE) histochemistry, choline acetyltransferase (ChAT) immunohistochemistry, muscarinic receptor subtype 2 (m2) immunohistochemistry, general muscarinic receptor (M35) immunohistochemistry and N-methylscopolamine (NMS) muscarinic receptor binding. The mean densities (4 rats), after subtracting the spinal trigeminal tract density as a background, are expressed as percentages of an average for cochlear nucleus (SOC/CN)%:

	LSO-*l*	LSO-*i*	LSO-*m*	VNTB	MZ	SPN	MNTB	Mean
AChE	53	57	81	114	52	61	40	65
ChAT	96	80	99	136	47	61	93	88
m2	76	67	82	137	64	73	63	80
M35	29	16	24	93	22	19	57	37
NMS	28	28	36	74	31	47	31	39

In general, the markers of cholinergic transmission are less prominent in the SOC than in the CN. VNTB contained the highest densities for all markers, in some cases higher than in the CN, suggesting relatively more cholinergic mechanisms there. In the LSO, AChE showed more labeling in the medial limb than in the lateral and intermediate limbs. Across the SOC, there are high correlations between AChE and m2 (r=0.94), m2 and NMS (r=0.92), and AChE and NMS (r=0.87). Other correlations among markers are moderate (r=0.59-0.80). (NIH grant DC 00172)

57.9

NEUROTRANSMITTER CHEMISTRY IN THE COCHLEAR NUCLEUS OF THE MOUNTAIN BEAVER. D.A. Godfrey[1]*, N.L. Mikesell[1], T.G. Godfrey[1], and J.A. Kaltenbach[2], Departments of Otolaryngology of [1]Medical College of Ohio, Toledo, OH, and [2]Wayne State University, Detroit, MI

Amino acids and enzymes of acetylcholine metabolism were mapped in the mountain beaver cochlear nucleus (CN) using microdissection of freeze-dried tissue sections, high performance liquid chromatography (HPLC) assay of amino acid concentrations, and radiometric assays of choline acetyltransferase (ChAT) and acetylcholinesterase (AChE) activities. Averaged results for two animals, which had similar chemistries, are presented for the amino acids aspartate (Asp), glutamate (Glu), glutamine (Gln), γ-aminobutyrate (GABA), glycine (Gly), and taurine (Tau), all in mmol/kg dry wt, and for ChAT, in μmol/kg dry wt/min, and AChE, in mmol/kg dry wt/min, for granular (Gran), dorsal (DCN), posteroventral (PVCN), and anteroventral (AVCN) regions.

	Asp	Glu	Gln	GABA	Gly	Tau	ChAT	AChE
Gran	10.6	42.5	43.8	5.7	9.5	18.0	13.0	84.7
DCN	9.1	42.2	45.9	10.4	17.2	15.4	7.1	43.9
PVCN	9.1	19.0	22.2	4.7	7.4	5.0	35.4	24.3
AVCN	10.9	19.2	22.6	6.1	9.1	5.2	45.7	42.1

The magnitudes of most amino acid concentrations resemble those measured with the same methods in rat CN, but Gln is much higher and Gly much lower. Although AChE activities are mostly similar, ChAT activities are strikingly lower than in rat CN. The value in the granular region is about 5% of that in rat and 8% of that in cat. Unlike in rat and cat, but in accord with its rather homogeneous histological appearance, the DCN contained few superficial-to-deep chemical gradients.
(Supported by NIH grant DC00172 and departmental funds.)

57.10

DEVELOPMENTAL EXPRESSION OF A HIGH THRESHOLD K^+ CHANNEL IN THE CENTRAL AUDITORY SYSTEM OF RATS
W. Li*, Y.-H. Liu, T.M. Perney, CMBN, Rutgers, Newark, NJ 07102.

The Kv3.1 K^+ channel gene encodes for a delayed rectifier-like channel with an unusually high activation threshold and very rapid deactivation kinetics. Computer simulations have predicted that the presence of the Kv3.1 channel would enhance a neuron's ability to follow high frequency synaptic input. Kv3.1 channels are highly expressed in several auditory nuclei including the ventral cochlear nucleus (VCN), the medial nucleus of the trapezoid body (MNTB) and inferior colliculus (IC). These data suggest a role for Kv3.1 channels in auditory temporal processing.

We used *in situ* hybridization and immunohistochemistry to examine the temporal exprssion pattern of Kv3.1 expression in the developing auditory system. Our *in situ* results showed that up until PD7, there was very low levels of Kv3.1 mRNA in the VCN, MNTB and IC. Between PD10 and PD14, there was a considerable jump in expression levels in the VCN. Significant increments in Kv3.1 message in the MNTB and IC were not observed until PD12 and PD14, respectively. Developmental changes in Kv3.1 immuno-reactivity followed a similar time course as that of the Kv3.1 message. Interestingly, the pattern of neuropil versus somatic staining was somewhat different from that of adult and varied among VCN, MNTB and IC.

Our data suggests that changes in Kv3.1 expression levels may contribute to the developmental changes in temporal acuity observed in auditory neurons. In addition, the large increase in Kv3.1 expression near the time of hearing onset coupled with the serial maturation pattern suggests that neuronal activity may play a role in regulating Kv3.1 expression levels.
(Supported by a Rutgers Research Council Grant and PHS R29 DC02728-01)

57.11

AN ORGANOTYPIC SLICE CULTURE OF DEVELOPING AUDITORY BRAINSTEM NUCLEI. C. Lohmann* and E. Friauf. Zentrum der Physiologie, Universität Frankfurt, D-60590 Frankfurt, Germany.

Nuclei in the mammalian auditory brainstem provide a suitable model to investigate the development of topographically organized *excitatory* and *inhibitory* projections. Excitatory (glutamatergic) and inhibitory (glycinergic) inputs, originating from neurons in the cochlear nuclear complex (CN) and the medial nucleus of the trapezoid body (MNTB), respectively, converge on neurons in the lateral superior olive (LSO) in a precisely tuned fashion. We have established an organotypic slice culture of the LSO, MNTB, and CN to be able to perform critical experiments which are not feasible *in vivo*. Ventral brainstem slices from rats between postnatal day 1-6 were maintained in a CO_2 incubator on porous membranes with serum-containing medium (Stoppini et al. 1991, J Neurosci Meth 37:173-182) for up to one week. Interestingly, neuron survival in the LSO (in contrast to CN and MNTB) required an elevated extracellular K^+ concentration, indicating that a depolarized membrane potential is crucial for their health. Organotypicity could be demonstrated after one week in culture: 1) Nissl staining revealed a well-preserved cytoarchitecture of the above auditory nuclei. 2) The topography of the internuclear connections, as shown by biocytin labeling, was indistinguishable from that in situ: for example, neurons located in *lateral* aspects of the MNTB projected into *lateral* aspects of the LSO. 3) Principal neurons in the LSO could be easily identified by their typical somadendritic morphology. Furthermore, axons of some CN neurons, which had been cut during the slicing procedure, regenerated in culture and terminated properly in the LSO. We conclude that auditory brainstem nuclei of neonatal rats can be kept in organotypic culture for at least one week provided that depolarization-induced processes can exert their positive influence on LSO neurons. Supported by the DFG (SFB 269/B5).

57.12

DYNORPHIN-LIKE IMMUNOREACTIVITY IN THE CHICK AUDITORY BRAIN STEM. R.A. Code*, Dept. of Biology, Texas Woman's University, Denton, TX 76204-3799.

Dynorphin-like immunoreactivity (DYN-I) was studied in brain stem auditory nuclei of the domestic chick using an antiserum to dynorphin-B and standard immunohistochemical procedures. In the cochlear nucleus magnocellularis (NM) of young posthatch chicks, DYN-I can be localized to both nerve cell bodies and terminals. Although few in number, DYN-I cell bodies are usually found in medial regions of NM. Many non-DYN-I nerve cell bodies in NM, however, are ringed by DYN-I structures that are especially prominent in lateral regions of NM. These structures, presumably nerve terminals, have a distinct calyceal, rather than punctate morphology, reminiscent of auditory nerve terminals, the end-bulbs of Held. In the cochlear nucleus angularis (NA), there was little, if any, DYN-I. The dendritic fields of neurons in the nucleus laminaris (NL) contained DYN-I as did an occasional cell body in lateral NL. A few small DYN-I cell bodies were observed in the superior olivary nucleus (SO).

This is the first report of DYN-I in the avian auditory brain stem. The data suggest that dynorphin may be a neuromodulator within end-bulbs of Held and may be expressed in neurons of several auditory nuclei.

The DYN-B antiserum was kindly provided by Dr. Stanley Watson, University of Michigan's Mental Health Research Institute. This work was supported by research grant number 1 R29 DC02633 from the National Institute on Deafness and Other Communication Disorders, NIH.

57.13

DISSOCIATED CELL CULTURES OF CHICK BRAIN STEM AUDITORY NUCLEI. N.J. Hack, K.M. Charters, T.N. Parks and S.B. Kater*, Department of Neurobiology and Anatomy, University of Utah, Salt Lake City, UT 84132.

The nucleus magnocellularis (NM) and nucleus laminaris (NL) of the avian brainstem auditory system are each composed of a single type of large principal neuron with highly specialized shape. These neurons show distinct morphological transformations during development and substantial change following deafferentation. The mechanisms underlying these changes may be best understood by partitioning the complexity of the events seen in vivo. In the absence of previous reports of cell culture of these nuclei, we are now defining methods for maintaining these neurons in vitro in order to obtain an experimental system compatible with high-resolution analysis of changes in dendritic morphology, calcium homeostasis, and gene expression. Papain dissociation and mechanical trituration of brainstem slices containing the NM and NL from embryonic chicks yields a mixed population of cells in culture. Such neurons can survive and display outgrowth for at least 5 days in vitro. Calretinin (CR) staining provided firm evidence that both neurons from the NM and NL survive well in vitro and can display strikingly similar morphologies to those seen in vivo. The embryonic stage of the donor from which neurons were derived is an important determinant of the number and viability of CR positive cells surviving in culture. Directly after plating on poly-L-lysine coated glass, many large CR-positive cells retain significant architecture, exclude trypan blue, and maintain calcium homeostasis. With subsequent days in vitro an array of CR-positive neuronal morphologies are displayed. Our system allows us to follow individual neurons over several days in culture. Some neurons retract their processes while some generate new processes reminiscent of several stages of normal developmental dendritic remodelling in vivo.
Supported by development funds from the University of Utah.

57.14

SYNAPTIC REARRANGEMENT IN THE BARN OWL AUDITORY BRAINSTEM DURING THE SENSITIVE PERIOD. M.F. Kubke, L.L. Rigby, L. Basu, C.E. Carr and [1]A. Moiseff*. Univ. Maryland, Dept. Zool., College Park, MD 20742 and [1]Univ. Connecticut, Dept. Physiol. & Neurobiol., Storrs, CT 06269.

Barn owls use interaural time differences (ITDs) as a cue for sound localization. The auditory nerve (AN) projects to the nucleus magnocellularis (NM) which in turn projects bilaterally to nucleus laminaris (NL) where ITDs are computed. In the first 2 months posthatch, the owl's head grows, increasing the interaural distance and thus the range of ITDs experienced. These changes are accompanied by changes in excitatory and inhibitory connections in the auditory brainstem and by changes in the auditory brainstem response (ABR).

Synapses were visualized with an antibody against synaptic vesicle protein SV2 (gift of K. Buckley), while GABAergic synapses with an antibody against GAD (GAD-1440). Neurons were also labeled with antibodies against calbindin and calretinin.

In very young birds, ABRS are highly variable and not well differentiated. SV2 and GAD, however, delineate connections in the auditory brainstem and calretinin labels the axons of the time coding pathway. At 2-3 weeks posthatch, the shape of the ABR becomes predictable. At this time, there are dramatic changes in the maturation of the synaptic structures in the auditory brainstem. AN terminals in NM are fairly mature, and by P21 are indistinguishable from the adult. In NL, there is a large increase in the density of GAD terminals that peaks at P14 and drops by P21. Similarly, calretinin appears in NL cell bodies by P21. These morphological changes are temporally correlated with the appearance of synchronous ABR's in the young barn owl.
Supported by NIH DCD000436 to CEC and NIH DCD00277 to AM.

57.15

SYNAPSES IN THE AUDITORY BRAINSTEM OF THE BARN OWL FORM DURING THE PERIOD OF EMBRYONIC CELL DEATH. D.P. Massoglia*, M.F. Kubke, L.L. Rigby and C.E. Carr. University of Maryland, Dept. Zoology, College Park, MD 20742.

In the barn owl, the auditory nerve (AN) projects to the intensity coding nucleus angularis (NA), and to the phase coding nucleus magnocellularis (NM). NM projects bilaterally to nucleus laminaris (NL) where ITDs are computed. Synaptogenesis in these auditory brainstem nuclei was described at the light and electron microscopic level the period of cell death (E17-E25). Neurons and synapses were visualized with antibodies against MAP2 and synaptic vesicle protein (SV2; kindly provided by K. Buckley).

At the onset of cell death (E17), synapses are formed between AN and NA and between NM and NL, but not between AN and NM. The NM input to NL is confined to the distal dendrites of NL neurons, and forms a band of terminals on the edges of NL, appearing to recapitulate the pattern found in chickens. By E21, NM receives AN inputs, NL dendrites have begun to retract and NM terminals have moved further into NL. Thus synaptogenesis and reorganization of the inputs to NL overlaps with the period of cell death.

Early in the cell death period, NL neurons receive both their appropriate inputs from NM and reach the NL projection area in the inferior colliculus core. NL neurons do not, however, receive primary sensory (AN) input via NM until later in the cell death period. This appearance of sensory input coincides with the movement of NM terminals into NL.

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57.16

DEVELOPMENT OF BRAINSTEM AUDITORY NUCLEI IN AN ECHOLOCATING BAT. R. Boatright and E. Covey*, Dept. of Psychology, University of Washington, Seattle, WA 98195.

Echolocating bats are able to navigate and forage in complete darkness based on acoustic information from echoes of the ultrasonic pulses that they emit. Because many species of bats do not begin to echolocate until several weeks after birth, we might expect to see a correlation between the onset of echolocation behavior and the maturation of those nuclei that are important for the echolocation process. We investigated the pre- and postnatal development of auditory structures in the big brown bat, *Eptesicus fuscus*, in terms of gross cytoarchitectural changes and in terms of cell density and distribution. The results show that all brain structures undergo substantial growth between birth and adulthood; however, the growth rates for different nuclei are not uniform. For example, at birth the medial nucleus of the trapezoid body (MNTB) is 78% of its adult size, indicating a substantial amount of prenatal growth. Other nuclei that are relatively mature at birth (64-71% of adult size) are the lateral superior olive (LSO), the cochlear nucleus (CN) and the inferior colliculus (IC). Nuclei that double their size between birth and adulthood include the medial superior olive (MSO), the superior paraolivary nucleus (SPN) and the nuclei of the lateral lemniscus (NLL). The medial geniculate body (MGB) is the least developed auditory nucleus at birth, with only 31% of its growth occurring prenatally. Amount of growth in each nucleus is roughly correlated with changes in cell density. In the LSO, CN and MSO, density decreases by over 50% prenatally, but by 20% or less postnatally. In contrast, cell density in the MGB decreases by only 9% prenatally, but by 28% postnatally. If decreases in cell density are in part due to increased formation of neuropil and synaptic connections, it is possible that the delayed development of the MGB is associated with postnatal learning and establishment of echolocation behavior.

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57.17

MORPHOLOGY OF NEURONS CULTURED FROM SUBDIVISIONS OF THE COCHLEAR NUCLEUS. J.L. Fitzakerley* and L. Schweitzer. Department of Anatomical Sciences and Neurobiology, University of Louisville School of Medicine, Louisville, KY, 40206.

In the adult animal, neurons of the cochlear nuclear complex (CN) have been classified on the basis of their morphology, and cells with particular characteristics have been localized to specific CN subdivisions. It is not clear, however, whether cultured CN neurons exhibit the variety of somato dendritic morphologies observed among CN cells *in vivo*. The objectives of this study were: 1) to describe the morphology of cultured CN neurons using standard anatomical techniques, and 2) to compare cultures obtained from different subdivisions of CN. Low density, primary cell cultures were prepared from the CN of 7 postnatal day C57B6 mice and maintained in serum-free media. Cultures were obtained from the anterior or posterior regions or from the whole nucleus by dividing some CN at the nerve root. Cultures were fixed after 4 days *in vitro*, and MAP2-positive cells were identified using immunohistochemical techniques. CN neurons were successfully cultured from all three regions, although fewer cells were consistently obtained from the anterior region. Soma size was similar among all 3 groups, however, neurons obtained from the posterior region tended to have nuclei that occupied less of the cytoplasm. When plated at the same density, fewer neurons cultured from the posterior CN extended processes as compared to whole CN preparations. Neurons cultured from the different CN regions also exhibited differences in dendritic structure that may be due to differences in the density of the surviving neurons. The goal of these experiments is to establish cultures of CN subdivisions that provide equivalent environments in which to study the differentiation of CN neurons.

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57.18

AXONS FROM ANTEROVENTRAL COCHLEAR NUCLEUS (AVCN) THAT TERMINATE IN MEDIAL SUPERIOR OLIVE (MSO) OF CAT: OBSERVATIONS RELATED TO DELAY LINES. D. L. Oliver* and G. E. Beckius, Department of Anatomy, University of Connecticut Health Center, Farmington, CT 06030-3405.

A delay line composed of axons was proposed as the mechanism for computation of interaural time difference (ITD) in MSO. We hypothesize rostrocaudal differences in MSO innervation may be related to a map of ITD. In two cats, anterior AVCN was injected with biotinylated dextran where neurons had BFs of 1.4-1.5 kHz. Complete 3-D reconstructions of axons were made in serial 100 μm-thick sections. Axons were traced from the injection site, through the superior olive, and to the contralateral lateral lemniscus. Axons terminated in bilaterally symmetrical, single, horizontal laminae. Due to their similar injection sites, these axons may have overlapping response areas. In the contralateral MSO, rostral neurons always receive inputs from axons that travel in the rostral trapezoid body and take a short, direct path from the cochlear nucleus. Innervating middle MSO are axons that either follow a short path through the middle trapezoid body or take a longer path via the rostral MSO (the Jeffress-type delay line). In the caudal MSO, axons either follow a short path through the caudal trapezoid body, take a longer path via middle MSO, or take the longest path via rostral MSO. Thus on the contralateral side, rostral MSO neurons may receive well-synchronized, phase-locked inputs that follow a uniformly short path-length; successively more caudal neurons may receive less synchronized inputs because of more variable path-lengths. On the ipsilateral side of MSO, the path-lengths follow a different pattern. Axons that innervate the middle MSO follow the shortest path from the cochlear nucleus. Axons to the rostral MSO may follow a longer route (the Jeffress-type delay line). Neuron in the caudal MSO receive variable-length inputs and may be less synchronized. Thus, our results support the Jeffress model and suggest that differential path lengths may contribute to small ITDs in rostral MSO and larger ITDs caudally. It also appears that the combination of different path lengths to middle and caudal MSO may provide less synchronized inputs to those areas.

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57.19

BILATERAL DESCENDING INFERIOR COLLICULUS PROJECTIONS TO THE VENTRAL NUCLEI OF THE TRAPEZOID BODY IN THE GERBIL.

N. Kuwabara*[1], M.A. Notestine[2] and J.M. Zook[2]. [1]*Dept. of Anat. Sci. and Neurobiol., Univ. of Louisville Sch. of Med., Louisville, KY 40292.* [2]*Dept. of Biol. Sci. and OUCOM, Ohio Univ., Athens, OH 45701.*

The ventral nucleus of the trapezoid body (VNTB) receives both ascending and descending afferent projections and contributes both ascending and descending efferent projections, suggesting that it is the major intersection of the ascending and descending auditory systems in the superior olivary complex (SOC). In this study descending projections of the inferior colliculus (IC) were studied with the objective of examining the relationship of the afferent and efferent projections of the VNTB. Intracellular and extracellular labelings with Biocytin and Neurobiotin were combined with HRP retrograde tract-tracing technique in gerbil brainstem preparations. Biocytin was injected in the IC either ipsilateral to or contralateral to the HRP-injected ventral cochlear nucleus (VCN). In some cases axons that project to the VNTB cells were intracellularly labeled with Neurobiotin in braintem tissue slices taken from gerbils with HRP-injected CN.

A sizable projection from the IC to the contralateral VNTB was found as well as the traditional projection from the IC to the ipsilateral VNTB. The axon terminals of IC cells within the contralateral VNTB were associated with HRP-labeled cells that, in turn, project to the VCN ipsilateral to the VNTB (contralateral to the originating IC). In the case where both Biocytin and HRP were injected in the same side, a minor descending projection from the IC was seen only in the ipsilateral VNTB, where they were associated with HRP-labeled cells. These results show that a majority of IC descending information is relayed to the contralateral VCN by way of both the ipsilateral and contraleteral VNTB's. (Supported by NIH DC01303, DC02397, OUCOM and Univ. of Louisville Sch. of Med.)

57.20

AXON TERMINALS ON OLIVOCOCHLEAR NEURONS. J.C.Adams* ENT Dept., Mass. Eye & Ear Infirmary Boston, MA 02114.

Olivocochlear (OC) neurons were retrogradely labeled by HRP injections of cat cochleas. The retrogradely labeled cells were made fluorescent using the transported HRP to biotinylate the tissue and then applying FITC-labeled streptavidin. Nerve endings apposed to the labeled cells were immunostained with antibodies to synaptophysin, GAD, or substance P. Immunoreactive sites were labeled with CY-5 labeled secondary antisera so the positive cells and terminals could been viewed in the same tissue. Few immunostained nerve terminals were found apposed to any olivocochlear cell body or dendrite. Medial and lateral OC cells were indistinguishable by their innnervation pattern as seen by synaptophysin and GAD immunostaining. Substance P positive terminals were associated with only lateral OC cells. This suggests an innervation to lateral cells from the inferior colliculus which is not shared by medial cells. These results indicate that OC cells are sparsely innervated. This property may be partly responsible for the chopper sound-evoked discharge patterns that characterize medial OC cells, as well as other sparsely innervated cells of the cochlear nucleus.

Supported by a PHS grant DC 00269.

57.21

AFFERENT AND INTRINSIC PROJECTIONS TO THE DORSAL NUCLEUS OF THE LATERAL LEMNISCUS. J.M. Zook*, B. Schanbacher and N. Kuwabara, Biol. Sci., Coll. Osteo. Med., Ohio U., Athens, OH 45701.

Recent anatomical and physiological data suggest that the dorsal nucleus of the lateral lemniscus (DNLL) represents much more than a simple relay station passing ascending auditory information on to the inferior colliculus (IC). One striking anatomical feature is the ordering of DNLL cells and dendrites into a complex 3-D pattern of nested spheres: each spherical layer representing a part of the tonotopic projection of the DNLL to the IC (Willard & Ryugo,'83; Merchan et al.,'94). In the gerbil, only the ventral DNLL shows such a spherical pattern; dorsally cells and dendrites show a simpler pattern, extended parallel or perpendicular to the surrounding lemniscal fibers.

We have been examining the pattern of ascending and intrinsic axon terminals within these two distinct areas using intra-axonal injections of Lucifer Yellow and biocytin in a tissue slice preparation of the gerbil brainstem. In the ventral, spherically organized DNLL terminal branches tend to follow restricted paths along a single curving cytodendritic layer. Multiple branches coming off a single main axon often ramify within separate regions of the same cytodendritic shell. Some intracellularly labeled cells in the ventral DNLL showed locally terminating, intrinsic axon branches limited to single cytodendritic layers, but more commonly, intrinsic axons ramified across many different layers. In the dorsal DNLL, the distribution of ascending and intrinsic axons is less clearly organized, with some tendency for axons to terminate in horizontal bands. At least in the ventral part of the gerbil DNLL, the distribution of afferent input appears to be dictated by this region's striking cytodendritic organization. (Supported by DC02397 and OUCOM).

EXERCISE AND THERAPY

58.1

THE INFLUENCE OF CONTRALATERAL AFFERENT STIMULATION ON QUADRICEPS FEMORIS (QF) MAXIMAL VOLUNTARY ISOMETRIC CONTRACTION (MVIC) - L.E. Tremblay*, Chris Sulway and David Frake, Physiotherapy Program, Univ. of Ottawa, Canada.

Past research shows a decrease in muscular force generated during a bilateral lower extremity contraction. Our investigations have been to decide whether this is central, postural or mechanical in origin. This study was undertaken to examine whether a stimulus to the contralateral (contra) lower extremity has an influence on the ipsilateral (ipsi) QF MVIC, in the hope that the stimulus response would explain the decrease in force. Forty five volunteer subjects (male, 27 ± 5 years) were obtained for the study. The following forms of contra stimulation were administered: MVIC, tetanizing electrical stimulation (TES), QF M-response, QF H-reflex, QF patellar reflex, vibratory (on the patellar tendon) and cutaneous stimulus. The stimulation was applied using standardized protocol to the femoral nerve, the inguinal skin surface or the patellar tendon. The ipsilateral QF MVIC was measured with a KIN-COM 550 H at 0° per sec. angular velocity, fixed at an angle of 90°, using standardized protocol. Futhermore an interpolated twitch technique (ITT) was used on the IPSI QF during bilateral contraction to evaluate motor unit activation. This technique aims to induce an M-response during MVIC. EMG recordings of both QF were monitored on a few subjects for subjective comparison. Among the healthy subjects, contra-stimulation produced the following ipsi inhibitory effects ($P<0.05$): MVIC - 5 ± 1.2%, TES - 12.2 ± 1.8%, QF H-reflex - 11.6 ± 2.6%, QF patellar reflex - 17.5 ± 3.3%, QF M-response, vibration and cutaneous stimulus - no significant effect. By using the ITT, we can exclude postural and mechanical sources as possible origins for the deficit. The inhibition can however be explained by peripheral afferents either within a spinal cord loop or a longer sensori-motor cortex loop. Further research is needed to decide the exact mechanism of the central inhibition.

58.2

ELECTRICAL ACTIVATION OF CUTANEOUS AFFERENTS FOR REDUCTION OF SPASTICITY: EFFECT OF STIMULUS FREQUENCY

Brian D. Schmit*, Julius P.A. Dewald, Joseph D. Given and W. Zev Rymer
Sensory Motor Performance Program, Rehab. Institute of Chicago, and Dept. of Physical Medicine and Rehabilitation, Northwestern University Medical School, Chicago, Illinois 60611

Electrical activation of cutaneous afferents with a stimulus frequency in the range of 1-100 Hz reduces spasticity in stroke subjects. The results of the current study provide evidence for an expanded range of frequencies effective for spasticity reduction and follow from previous results demonstrating spasticity reduction by activation of cutaneous afferents at 20 Hz. The effects of a wider range of stimulus frequencies will be used to optimize the stimulus parameters for spasticity reduction.

Spasticity was quantified by examining the stretch reflex response to constant velocity ramp flexion and extension of the elbow joint. Elbow torque, position, and velocity were measured along with surface EMG's of the biceps, lateral head of the triceps and brachioradialis. Stimulus electrodes were placed on the skin over the biceps muscle. The EMG's were measured using a custom amplifier with an artifact suppression circuit to ascertain that the stimulus was below motor threshold at all elbow positions. Trials were initiated in the extension position and ramped to the flexion position held for 10 seconds and then ramped back to extension. Sixty seconds were allowed between trials. The angular velocity was selected to produce a stretch reflex. The experimental protocol consisted of 20 prestimulus ramp stretches followed by fifteen during stimulation and thirty post stimulus trials. A stimulus intensity between sensory and motor thresholds was applied at a frequency of 1, 20 or 100 Hz.

We observed reductions in spasticity during stimulation and throughout the 30 minutes following stimulation in the frequency range of 1-100 Hz. The efficacy of cutaneous afferent stimulation over a wide range of stimulus frequencies may provide additional information for identifying the physiologic mechanism responsible for the lasting effects of cutaneous stimulation. These data will be used to develop the framework for the use of electrical stimulation to reduce spasticity in stroke and spinal cord injury using surface skin electrodes.

This work is supported by NIH grants T32-HD07418 and NS-19331-11 and NIDRR grant H133B30024

58.3

NERVE CUFF RECORDED RESPONSES FROM CUTANEOUS NERVES IN MAN, EVOKED BY SLIPPAGE EVENTS ON THE SKIN. R. R. Riso*and P.J. Slot, Center for Sensory-Motor Interaction, Aalborg Univ. Fredrik Bajersvej 7D, Aalborg, 9220-Denmark

We are studying means to extract control signals from mechanoreceptor afferents within the digital skin in spinal injured, individuals. Such signals are needed for closed-loop control in FES that can restore grasp function. It has been demonstrated that an artificial reflex can be set up for use with FES grasp: This entails detecting the afferent activity evoked by slippage of a grasped object and using this activity to increase the grip strength to arrest the slippage (Lickeal et al., IEEE Ann. Meeting, Amsterdam, 1996). To optimize the slippage based control strategy we wish to know the effect of friction of the grasped surface and the effect of the slip velocity. ENG was recorded using a tripolar nerve cuff implanted chronically around the digital nerve subserving the lateral border of the index finger in a C6 subject. A servo-controlled mechanical stimulator was used to move a contactor across the digital skin. The ENG was filtered (1k-5kHz), and sampled (12.8kHz). Vertical and lateral positions of the contactor, and the normal and sheer forces were also recorded (400Hz). We studied 3 frictional surfaces: sandpaper, suede and silk, and investigated velocities of sliding from 2.5 to 25mm/s. Results showed that the ENG increased in a linear fashion with increasing velocity for each different surface. The effect of different frictional surfaces was that the ENG decreased for lower friction. Close examination of the sliding events revealed that the ENG sliding was mainly driven by skin stretch immediately before the onset of the sliding and then again immediately after in a cyclical slip-catch-slip sequence. The effect of the increase in ENG with increased sliding velocity is useful because it suggests that an adaptive FES controller could be developed so that increased sliding velocity would be detectable in the ENG.

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58.4

MUSCLE AFFERENT ACTIVITY RECORDED DURING PASSIVE EXTENSION-FLEXION OF RABBIT'S FOOT. K. Mosallaie*, R R. Riso and T. Sinkjaer., Aalborg Univ., Fredrik Bajersvej 7D., 9220, Aalborg, Denmark

Damage to the spinal cord such as traumatic injuries results in a permanent loss of motor function and sensation due to the disruption of communication at the lesion site. However, the peripheral motor and sensory nerves and muscles retain some functionality below the lesion. FES can be used to restore function in paralyzed patients, however, lack of feedback results in imprecise and inefficient movements. Natural sensors, which are already present in the body, may provide information for feedback control in an FES system. The objective of this study was to study muscle afferent activity during passive motion of the ankle joint. The nerve signals were recorded from peroneal and tibial nerve using cuff electrodes in a reduced rabbit model. The experimental apparatus consist of a computer controlled position servo system to rotate the animal's ankle in dorsi-flexion/plantar-flexion. Neural activity was recorded simultaneously from tibial and peroneal nerves during passive trapezoidal motion. Eexcursions of 10, 20 and 30 (deg) and rates of 5, 10, 20, and 30 (deg/sec) along with different starting positions were investigated. Descending branches of the tibial and peroneal nerves and the sural nerve were transected to eliminate cutaneous input. Also lidocaine was infused into the joint capsules of the ankle and knee which did not significantly diminish the responses. The evoked activity included a mixed components from both primary and secondary muscle afferent endings. Evoked activity from both nerves increased when the corresponding muscles were stretched. Nerve activity decreased abruptly when the muscles were shortened. However, the maximum peak was nearly independent of the rotation rate and was mostly dependent on the excursion magnitude. For either nerve, the activity increased with increasing velocity of rotation. We also observed silent periods, close to the neutral ankle position, where there was no significant neural activity from either of the extensor or flexor muscle afferents. We conclude that the evoked activity mainly originates from muscle afferents with both position and velocity sensitivities.

The Danish National Reseasrch Foundation

58.5

CONDITIONING HUMAN LONGER LATENCY REFLEX RESPONSES IN THE BICEPS BRACHII. S.L. WOLF*, R.L. SEGAL, P.A. CATLIN, R.L. SLAVEN, J.K. TAFFS, H.C. TREES, E.F. VICKERS. Div. Phys. Ther., Emory Univ. Sch. of Med., Dept. Rehab. Med., Atlanta, GA 30322.

Conditioning of the biceps brachii spinal stretch reflex (SSR) produces concurrent changes of the same direction in longer latency reflex responses (LLRR, Wolf et al. 1995; Wolf and Segal, 1996). The purposes of this study were to determine if the magnitude of the biceps brachii LLRR in able-bodied subjects could be reduced with direct operant conditioning, and to examine the relationship of changes in the conditioned biceps brachii LLRR with changes in the biceps brachii SSR, and the LLRR and SSR of a synergist muscle, the brachioradialis. Fourteen subjects participated in six baseline sessions. Then, all subjects were randomly assigned to either control (n=6) or training groups (n=8). The control group received eight more baseline sessions, while the training group received eight sessions with feedback of the biceps brachii LLRR magnitude in an attempt to downtrain the response. The training group significantly reduced the magnitude of the LLRR compared to mean baseline values by 57% in the biceps brachii and by 68% in the nontargeted brachioradialis. The SSR of the biceps brachii and brachioradialis did not change significantly. Training effect specificity occurred since changes were only seen in the response targeted by operant conditioning. Because response specificity had not been observed previously when conditioning SSR responses, different sites within the nervous system may be altered for each training effect.

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58.6

EFFECTS OF TWO TRAINING PROCEDURES ON PROPRIOCEPTIVE SENSITIVITY. D.K. Fry-Welch* and C.J. Worringham. Physical Therapy Dept., University of Michigan-Flint, Flint, MI 48502.

Although many studies examine proprioception, very few studies have examined training-induced changes in proprioceptive sensitivity. Thirty subjects, age 19.5-40 years with no known upper extremity neurologic or orthopedic abnormalities, participated in pre- and post-treatment testing sessions. The proprioceptive comparison (PC) test required subjects to determine which elbow was more flexed without vision and the proprioceptive matching (PM) test required subjects to match the non-dominant elbow position with the dominant elbow position without vision. Ten subjects received training on distinguishing elbow angles similar to the PC test. Ten subjects received training on matching elbow positions similar to the PM test. Ten control subjects received no training.

The PM and PC trained groups both improved performance significantly on the post-test similar to their training (p=0.028 and p=0.028 respectively). The PM trained group also improved significantly on the PC test (p=0.020). The control group did not improve on either test. The results suggest that proprioceptive sensitivity can be improved with training in normal adults. The finding that the PM trained group improved on both tests, whereas the PC trained group did not may be explained either by: 1) the error correction component in the feedback for the PM, but not PC, trained group; or 2) the graded motor output inherent in the PM but not the PC training.

Supported by the Foundation for Physical Therapy.

58.7

PROPRIOCEPTIVE COORDINATION OF AXIAL MOVEMENT. P. Cordo, V. Gurfinkel, S. Verschueren, T. Smith and J. J. Collins*
R. S. Dow Neurological Sciences Institute, Portland, OR 97209

To understand how the CNS coordinates axial movement, sitting up from a supine position was examined in normal human subjects. Sitting up is a complex movement, because body mass distribution favors lifting of the legs rather than the trunk during activation of hip flexors. Due to the sequential nature of sitting up, proprioception might play an important role in the coordination of this movement.

Twelve subjects—lying on a large force plate—sat up from a supine position with the arms across the chest. Movement time was constrained to 2 s. Recordings were made of whole-body kinematics, support-surface forces and the EMG activity of neck, trunk and leg muscles. In addition to standard trials, subjects performed sit-ups with the legs strapped to the force plate (i.e., making sitting up easier), with the legs hanging off the end of the table (i.e., making sitting up more difficult), and during vibration of the thoracic back extensor muscles.

We found that sitting up is carried out in two distinct and sequential phases. In the first phase, the upper body curls in a sequence of neck, upper-trunk and lower-trunk flexions, thereby shifting the upper-body center of mass towards the hips. In the second phase, hip flexor activation rotates the pelvis towards the feet, thereby lifting the trunk into a sitting position. Strapping the legs to the force plate eliminates the sequential properties of sitting up, resulting in one synchronized movement of the neck and trunk. Hanging the legs from the force plate delays sitting up and adds a rapid knee extension to the sequence. Vibration of the thoracic paraspinalis muscles delays or can even prevent sitting up, thereby demonstrating proprioceptive coordination.

58.8

SINGLE-LEG BALANCING FOLLOWING TREATMENT ON A BAPS$_{sx}$ BOARD AND A THERASTEP$_{sx}$ EXERCISER. B.R. Etnyre* and J.A. O'Kroy. Human Performance and Health Sciences Department, Rice University, Houston, TX 77005.

A common treatment used for improving lower extremity proprioception is by exercising on a BAPS$_{sx}$ board. A foam half-cylinder called Therastep$_{sx}$ recently has been introduced to perform a similar function to the BAPS$_{sx}$ board at considerably less cost. The purpose of this study was to compare the effects of exercise with each instrument on a single-leg balancing task. Thirty-one subjects (mean age=19.6, S.D.=.85) participated. Each subject performed a block of three 20 sec trials by balancing on one leg on a force plate (AMTI) prior to any exercise. The subjects then exercised for one minute on one instrument followed by another block of trials on the force plate. After the second set of trials the subjects exercised on the other instrument for one minute before performing a third block of trials. The BAPS$_{sx}$ board exercise was performed by rotating the ankle in circles. The Therastep$_{sx}$ exercise was performed by balancing on one leg on the instrument. Approximately half the subjects exercised on the BAPPS board first and the others used the Therastep first. The excursion of the Center of Pressure (COP) on the force plate was measured in the X (lateral) and Y (fore-aft) planes. The deviations were integrated for each trial and average force was normalized to body weight. The percent of COP time within a 2 cm radius was also calculated for each trial. The trials following the Therastep$_{sx}$ had the least deviation in the X and Y directions (3.46 cm and 0.75 cm, respectively) compared with the trials following the BAPS$_{sx}$ exercise (3.47 cm and 0.79 cm) and the pre-exercise trials (4.0 cm and 0.78 cm). Average percent of time of the COP within a 2 cm radius was greatest following the Therastep$_{sx}$ exercise (87.0%) compared with the post-BAPS$_{sx}$ board balancing (84.9%) and the pre-exercise means (81.2%). However, no significant differences were revealed for any of the variables between the pre-exercise, post-BAPS$_{sx}$ and post-Therastep$_{sx}$ balancing measures. It was concluded that if these exercise instruments improved proprioception it was not measured by a single-leg balancing task.

58.9

EFFECTS OF FATIGUE ON VOLUNTARY ELBOW FLEXION MOVEMENTS. H. Jiang, G. L. Gottlieb, D. M. Corcos.* University of Illinois at Chicago, Rush Medical Center and NeuroMuscular Research Center, Boston University,

Few studies have been conducted on how the control of limb movement is affected when muscles are fatigued. These studies suggest that fatigue leads to only small changes in movement kinematics which are accompanied by small increases in the duration of the agonist EMG. We hypothesized that one possible reason for observing only small changes in movement kinematics and EMG is that only small forces were generated in the particular movements studied. As such we designed an experiment which compared fatigued and unfatigued movements performed against an inertial load over two distances (20° and 60°). Considerably larger torques are generated for the longer distance.

Consistent with previous results, there were small changes in both EMG and kinematics for movements over the short distance in the fatigued condition. In contrast, for the long movement, peak movement velocity was reduced by 25% and peak inertial torque was reduced by 37%. This reduction in movement velocity and peak inertial torque was associated with a decrease in agonist EMG, a prolongation of the agonist burst and a delay in the activation of the antagonist muscles. The results suggest that even when muscles are fatigued to the point that they can only produce 50% of their maximum unfatigued voluntary contraction, when force requirements are low, movement is minimally affected by fatigue but when high force levels are required, movement may be significantly slowed. The muscle activation patterns are modified by fatigue in a manner that may represent a central adaptation to changes in peripheral, neuromuscular properties.

This work was supported by NIH grants AR 33189, NS 28176, NS 01508 and NS 23593.

58.10

MYOELECTRIC CHANGES DURING MUSCLE FATIGUE AND RECOVERY. M. B. Iyer*[1,2], J. F. Andrews[2,3] and T. W. Findley[1,2]. [1]Dept. of Physical Medicine and Rehabilitation, UMDNJ-New Jersey Medical School, Newark, NJ; [2]Kessler Institute for Rehabilitation, Dept. of Research, West Orange, NJ; [3]Dept. of Elec. and Computer Engg., NJIT, Newark, NJ.

This study utilizes time- and frequency-domain analyses to examine the changes in myoelectric activity during fatigue and recovery following a sustained maximal isometric contraction. Muscle fiber conduction velocity (MFCV), power spectrum median frequency of the electromyogram (EMG), and the coherence function between EMG signals and force were studied in healthy human subjects. EMG signals were recorded from the abductor digiti minimi muscle. The experimental protocol was as follows: 1) the pre-fatigue phase consisting of brief isometric contractions in the range of 20% to 30% of maximal voluntary contraction (MVC); 2) the fatigue phase involving a sustained isometric contraction at 100% of MVC until the force output decreased to 50%; and 3) the recovery phase comprising a series of short contractions (range 20% to 30% of MVC) at brief intervals starting 30 seconds after termination of the fatigue phase. During the pre-fatigue and recovery phases, subjects produced either steady or nearly sinusoidal isometric contractions. The median frequency (MF) was computed from the auto-power spectrum of the EMG signal. The MFCV was computed by cross-correlation of surface EMG signals. Both MFCV and MF values decreased with fatigue. This study showed a rapid return to pre-fatigue values both in MFCV and MF. In contrast to the literature, no overshoot of these values was observed in the recovery phase. Studies are in progress to determine whether this finding is possibly due to the measurements being obtained during submaximal contractions under aerobic conditions.

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58.11

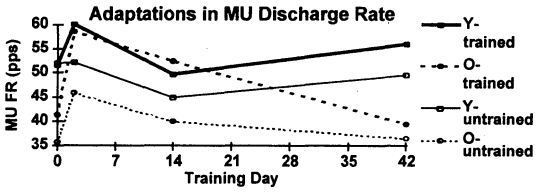
CROSS-TRANSFER PHENOMENON: FACILITATED BY ELECTRICAL STIMULATION? J.Lambert*, T.Hortobagyi, K.Scott, J.Hill, C.Webb. *Department of Physical Therapy and Biomechanics Laboratory. East Carolina University, Greenville, NC, 27858.*

The purpose of the study is to determine if voluntary eccentric strength training elicits a greater cross-transfer of strength than passive electrically stimulated training. Sedentary females, matched according to maximum strength were randomly assigned to voluntary (VOL, n=8) or electromyostimulation (EMS, n=8) trained groups. Subjects of both groups trained 3-4 sessions/week for 6 weeks with a progressive training protocol of isokinetic eccentric contractions of the knee extensors. EMS-evoked contractions of the vastus lateralis and vastus medialis muscles were elicited at the maximum tolerable intensity. Matched subjects in the VOL and EMS groups trained at the same schedule with identical contractile intensities as determined by the maximal force that could be electrically evoked for the EMS subject. The contralateral leg was not exercised. The two trained groups and a control (CON, n=6) group, that did not receive training, were tested 3 times (2 times prior to training and 1 time following the 6 weeks of training). Pre to post training increases in volitional eccentric strength were observed for both legs of the VOL and EMS trained groups ($p<.05$), but not the CON group. There was no significant difference between the VOL and EMS groups for increased strength of trained legs (VOL 54%, EMS 36%), or the untrained legs (VOL 23%, EMS 34%). These results indicate that the EMS-evoked eccentric training is equivalent to voluntary training in eliciting a cross-transfer of strength, suggesting that the responsible neural adaptations may not require volitional effort.

58.12

Adaptations in Human Motor Unit Discharge Behaviour to Strength Training C. Patten*, G. Kamen. Motor Control Laboratory, Dept. of Exercise Science, University of Massachusetts, Amherst, MA 01003

Six young (mean: 23 yrs) and six older (mean: 76 yrs) adults performed a six week isometric strengthening program of non-dominant hand fifth finger abduction. To monitor for neural adaptations to strength training, motor unit (MU) signals were obtained from the abductor digiti minimi (ADM) muscle using a quadrifilar needle electrode. Force and MU signals were measured under maximal voluntary force conditions at baseline, 2, 14, and 42 days of training. To monitor for cross transfer effects, force and MU signals were also obtained from the untrained hand. MU signals were identified using custom written spike recognition software.



A transient increase in MU discharge rate (MUFR) accompanied significant increases in force (mean: 12%) following a single session of strength training. Overall, strength increases averaged 25% in all subjects. Notably, both groups demonstrated dissociation between MUFR and force levels, however, the pattern of adaptation in MU discharge behaviour differed markedly between young and older subjects.

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58.13

AGING EFFECTS ON MOTOR SKILL LEARNING AS AN EARLY NEUROMUSCULAR ADAPTATION DURING CONCENTRIC AND ECCENTRIC DORSIFLEXOR STRENGTH TRAINING. [1] D. Connelly, [3] H. Carnahan, [1,2] A.A. Vandervoort, and [1,2] J.T. Inglis*. [1] Faculty of Kinesiology, [2] Department of Physical Therapy, University of Western Ontario, London, Ontario; [3] Department of Kinesiology, University of Waterloo, Waterloo, Ontario, Canada.

Torque values and integrated electromyography (iEMG) increase dramatically at the onset of resisted exercise and can be used to quantify neuromuscular adaptation. A 'learning effect' also contributes to this early increased force and iEMG production. The purposes of the study were: 1) to investigate the effects of age on motor learning during concentric and eccentric dorsiflexor (DF) isokinetic strength training and 2) to chart the time course of skill acquisition in older versus young subjects. Twenty women, 15 older ($\bar{x} = 74.7 \pm 3.8$ years) and 5 young ($\bar{x} = 24.2 \pm 1.6$ years), volunteered for a two-week, 3 days/week, strength training program. They were tested in a sitting position on a KinCom isokinetic dynamometer at 30, 90 and 180°/sec through 40° of DF movement. Criterion curves of lever arm angle patterns were cross-correlated against subject-generated angle patterns. Correlations for pre-test angle patterns for older and young women were significantly different ($p<0.01$). A significant difference was found between the pre- and post-test movement patterns for older ($p<0.01$) but not young subjects. Post-test DF movement patterns did not differ with age. The major training effect was observed at 180°/sec velocity, after several days of training ($p<0.05$). Only older subjects improved significantly in DF movement patterns over time. At the onset, young subjects performed at a higher level and maintained this level throughout the training. These results suggest that the 'learning effect' was gradual over the course of training. The decrease of several factors: muscle force production, control of muscle shortening and lengthening, and proprioceptive feedback may have contributed to the age differences.

Supported by NSERC and PFC-RCL funding.

58.14

LEARNING TO COORDINATE CONTACT FORCE AND BALANCE GOALS IN MULTIJOINT, STANDING PULLS. J. L. Patton[1]* & W. A. Lee[2]. [1]Bioengin. [2]Phys. Ther. & Inst. Neurosci, Northwestern U., Chicago, IL 60611.

Subjects making "bounce" pulls must learn to organize motion for two goals: produce a brief manual force and maintain balance. Skilled subjects could easily achieve the task's dual goals if their center of mass (CM) motion resembles a simple model of an inverted pendulum driven by constant ankle torque and a unidirectional, elastic spring. In contrast, they might learn a more complex organization and separately control each goal. We tested these contrasting hypotheses by comparing model predictions to subject data.

Ten standing subjects practiced (for 5 days) brief, bounce pulls in the horizontal direction to target forces (20-80% of maximum), while force plate, pulling force, and body kinematics were recorded. Subjects developed posterior momentum, then briefly pulled and reversed their motion, and finally they recovered their balance. An 8-segment link determined CM motion. We compared the effects of practice and pulling force on: the overall error of a regression model fit to CM motion, the difference between initial and final posture, the difference between pre-impact and rebound positions and speeds, and changes in ankle torque patterns. All should decrease with practice if subjects learn the model.

The regression model's error was small, varied with pulling force, and did not reduce with practice. Subjects CM at the end of movement was anterior to its initial position; this *anterior shift* was greater after practice. Rebound position was not significantly different than pre-impact position, and rebound speed was not significantly lower than pre-impact speed. These results suggested that practice effects occurred mostly in balance recovery, and that damping alone cannot explain the observed behavior. Ankle torque patterns were smoothly decreased during balance recovery, especially after practice. A modified model using these ankle torque profiles worked better than the original model for low and mid range force levels. However, it did not fully explain the anterior CM shift at high forces, suggesting that subjects also learn to capitalize on complex kinematics that the simple model neglects.

The differential effects of practice on CM motion during the force production and balance recovery phases suggest that subjects learn to control the dynamics of those phases independently. That is, the CNS learns a more complex organization after practice. A two-DF model with at least one varying parameter will be needed to fully model skill acquisition in this task. (Supported by NSF IBN-9319601)

58.15

HEAD AND TRUNK STABILIZATION DURING MULTIJOINT STANDING PULLS. W.A. Lee*[1,2] & J.W. Steege[1]. [1]Prog. Physical Therapy & [2]Inst. Neuroscience, Northwestern Univ., Chicago, IL, 60611.

It is often asserted that pitch plane head rotations are minimized during voluntary movements to facilitate sensory inputs for motor control, but little is known about how angular head stabilization (HS) changes as a novel, whole-body motor skill is learned. This study analyzed pitch plane HS and its relationship to angular and linear trunk movements during the learning of a multijoint pulling task. Ten healthy adults stood on a force platform, grasping a handle which was attached to a load cell. On each of 5 days, they made 120 abrupt pulls to target forces equal to 20, 40 and 80 percent of their theoretical maximum. They were told to keep their feet stationary, but could otherwise move their bodies freely. Kinetic, kinematic, and pulling force data were recorded.

Subjects adopted a general pattern of head rotations that persisted across force and practice levels; they rotated their heads forward during the Pulling phase (from the onset of the pulling force to the peak pulling force, F_{pk}), reversed the direction of head rotation at F_{pk}, and then rotated their heads backward during the Release phase (from F_{pk} to the offset of pulling force). Angular trunk movement patterns were more variable. After practice, there was significantly less head rotation during the Pulling phase, indicating that overall HS had improved. Significant decreases in peak angular trunk velocity during both the Pulling and Release phases indicated that overall angular trunk stabilization also improved with practice. During 80% pulls, peak linear (AP) velocities of the neck also decreased with practice in 7 subjects, suggesting that these subjects may have learned to stabilize their heads via an avoidance mechanism in which they minimized both linear and angular trunk movements. During 40% pulls, in contrast, linear trunk perturbations of the head (peak AP neck velocities) increased with practice in 9 subjects; head movements nevertheless decreased in 5 of those subjects, suggesting that they may have improved HS by changing predictive control of their neck muscles. The results were inconclusive for 20% pulls: the magnitudes of head and trunk motion were small and showed no consistent practice effects. (Supported by NSF ISN9319601.)

58.16

PHASING AND FUNCTION OF BIARTICULAR THIGH MUSCLES DURING BACKWARD PEDALING. L.H. Ting*[1,2], S.A. Kautz[1,3], D.A. Brown[1,3], F.E. Zajac[1,2,3] [1]Rehabilitation R&D Center, VA Palo Alto HCS (153), CA, 94304, Departments of [2]Mechanical Engineering and [3]Functional Restoration, Stanford University, CA, 94305

Computer simulations of pedaling have shown that a simple control scheme using only three pairs of reciprocating functional muscle groups is sufficient to control pedaling. Predicted performance using this scheme closely matches maximum startup pedaling experimental data (Raasch et al., J. Biomech, in press). Furthermore, simulations of a variety of pedaling tasks were achieved. In smooth, steady-state forward pedaling, rectus femoris (RF) and hamstring muscles (HAM) can act as an alternating pair, with RF activity centered around top-dead-center (TDC), where the leg moves anteriorly, and with HAM activity centered through bottom-dead-center (BDC), where the leg moves posteriorly. In backward pedaling simulations, the phasing of this muscle pair was reversed to accommodate smooth movement through TDC and BDC, leading to the hypothesis that RF activity at BDC facilitates anterior leg movement and HAM activity at TDC facilitates posterior leg movement (Raasch, Ph.D. Thesis, 1995).

In this study, EMG data from subjects pedaling forwards and backwards smoothly at 60 rpm were compared to the model. RF was active through TDC during forward pedaling, and through BDC during backward pedaling. Similarly, HAM was active through BDC during forward pedaling, and through TDC during backward pedaling. This is consistent with the hypothesis that RF is used during anterior movement of the leg and HAM during posterior movement. However, RF and HAM activity extended beyond the predicted regions around TDC and BDC, and the phasing of these muscles was not strictly reversed between forward and backward pedaling. This suggests that there may be additional functionality of RF and HAM during pedaling which is not modeled. RF timing was closely correlated with extensor activity. Its function in both extension and anterior movements of the leg could account for the observed phase shifts. A modified model with dual function of RF is presented.

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58.17

RECRUITMENT OF MOTONEURONES BY TRANSCRANIAL MAGNETIC STIMULATION IN SPINAL CORD INJURY. H.C. Smith[1], N.J. Davey[1], D.W. Maskill[1], G. Savic[2], P.H. Ellaway*[1]. [1]Dept of Physiology, Charing Cross & Westminster Medical School, London, W6 8RF, UK. [2]National Spinal Injuries Centre, Stoke Mandeville Hospital, Aylesbury, HP21 8AL, UK.

The stimulus threshold for compound motor evoked potentials (cMEPs) in response to transcranial magnetic stimulation (TMS) is higher in patients with incomplete spinal cord injury (Davey *et al* 1995, Abst Soc Neurosci 21:172.7). We have now used the cMEP response as an index to investigate motoneurone recruitment.

Surface electromyographic responses in thenar muscles to TMS were studied in 10 incomplete neck injury patients (ages 18-74 years, post-injury time 3-28 months) and 10 control subjects (ages 22-48 years) whilst maintaining a mean (± SE) isometric contraction force of 15 ± 1.6% maximum voluntary contraction (MVC). The motor level of the injury in the patients (range C4-C7) was rostral to the level at which the nerves innervating the thenar muscles leave the spinal cord (C8/T1). Mean threshold stimulus strength (T) to TMS (round coil, centred over vertex), expressed as percentage of maximum stimulator output (%MSO), for evoking cMEPs in thenar muscles was 46 ± 3%MSO in the patients and 31 ± 2%MSO in the controls. Threshold for biceps brachii, a muscle innervated more rostrally, was no different in the patients (49 ± 4%MSO) from the controls (53 ± 4%MSO). Mean cMEP area at 1.5T, expressed as a percentage of the area at threshold, was greater in controls (2115 ± 454%) than in patients (912 ± 318%). However, the level of voluntary EMG produced at 50% MVC expressed relative to threshold cMEP area was equivalent in controls (917 ± 195%) and patients (926 ± 307%). We conclude that TMS at 1.5T recruits a smaller proportion of available motoneurones in the patients.

Our results suggest that the motoneurone recruitment curve to TMS is less steep in spinal cord injury and this may result from a rewiring of excitatory or inhibitory synaptic inputs in the cortex or spinal cord. *Supported by the Wellcome Trust.*

58.18

FACILITATION OF CORTICOSPINAL PATHWAYS WITH INCREASING VOLUNTARY CONTRACTION IN SPINAL CORD INJURY. N.J. Davey*[1], H.C. Smith[1], D.W. Maskill[1], G. Savic[2], P.H. Ellaway[1]. [1]Dept of Physiology, Charing Cross & Westminster Medical School, London, W6 8RF, UK. [2]National Spinal Injuries Centre, Stoke Mandeville Hospital, Aylesbury, HP21 8AL, UK.

In incomplete spinal cord injury patients the stimulus threshold for compound motor evoked potentials (cMEPs), produced in response to transcranial magnetic stimulation (TMS), is higher than in controls (Davey *et al* 1995, Abst Soc Neurosci 21:172.7). We have now used the size of the cMEP response as an index of corticospinal excitability during a voluntary increase in isometric contraction force.

Surface electromyographic responses of thenar muscles to threshold TMS were recorded in 10 incomplete neck injury patients (ages 18-74 years, post-injury time 3-28 months) and 10 control subjects (ages 22-48 years). The motor level of injury in the patients (range C4-C7) was rostral to the level at which the nerves innervating the thenar muscles leave the spinal cord (C8/T1). Mean (± SE) maximum voluntary contraction (MVC) was assessed as 49 ± 13N in the patients and 73 ± 6N in the controls. A ring attached to a force transducer was placed over the interphalangeal joint of the thumb and subjects were instructed to pull to a particular percentage of their MVC. Mean cMEP areas, expressed as a percentage of maximum, in patients were 5 ± 1% in relaxed muscle, 45 ± 10% at 10% MVC, 56 ± 7% at 20% MVC, 73 ± 9% at 50% MVC, and in controls 6 ± 3% in relaxed muscle, 73 ± 5% at 10% MVC, 83 ± 3% at 20% MVC, 82 ± 6% at 50% MVC. Evidently facilitation of the cMEP to TMS plateaus at a higher percentage of MVC in spinal cord injury.

Spinal cord trauma may indiscriminately have blocked axons of corticospinal neurones that could have been recruited at any level of voluntary effort. This could mean that an equivalent level of depolarisation of the motoneurone pool, or corticospinal neurones, is reached at a higher percentage of MVC in the patients than in the controls. *Supported by the Wellcome Trust.*

MOTOR SYSTEMS AND SENSORIMOTOR INTEGRATION: CIRCUITRY AND PATTERN GENERATION I

59.1

EXISTING STOMATOGASTRIC NEURON MODELS PREDICT SOME, BUT NOT ALL, RESPONSES TO RHYTHMIC INPUT. A.L. Weaver, R.A. DiCaprio* & S.L. Hooper. Biol. Sci., Ohio U., Athens, OH 45701

Prior work has shown that when trains of hyperpolarizing current pulses are injected into Lateral Pyloric (LP) neurons isolated from the pyloric network, but still receiving inputs from anterior ganglia (i.e., the stomatogastric nerve is intact), the neuron's rebound delay depends strongly on pulse amplitude but relatively little on train frequency, and changes rapidly (in 3-5 sec) when pulse amplitude is altered. Alternatively, robust rebound activity of similarly isolated Pyloric (PY) neurons slowly develops (over ~10 sec) during a train, and depends strongly on train frequency and duty cycle.

We implemented the LP (Buchholtz et al.) and PY (Harris-Warrick et al.) neuron models in GENESIS. The LP model reproduces our physiological data; rebound delay after single hyperpolarizing pulses decreases as pulse amplitude increases, and rebound delay decreases only slightly following later pulses. In the absence of I_h, rebound after single pulses or during pulse trains is extremely small both in amplitude and duration. The PY model, which lack I_h or other currents with long time constants, was derived from conductance measurements of PY neurons isolated from anterior influences. This model requires injecting depolarizing current after the hyperpolarizing pulses to display rebound firing, and thus far we have been unable to mimic in it the slowly developing, robust PY neuron rebound activity observed physiologically with anterior inputs intact.

However, increasing I_h time constants in the LP model 3-fold results in the model exhibiting slowly developing PY neuron type rebound responses. These results thus suggest that anterior influences may induce functional expression of extremely slow conductances in PY neurons. [Research supported by OU, NSF, and HSFP.]

59.2

DUTY CYCLE, SPIKES/BURST, AND INTRABURST AND OVERALL SPIKE FREQUENCY AS PYLORIC CYCLE FREQUENCY CHANGES. S.L. Hooper* & J.B. Thuma. Biol. Sci., Ohio Univ., Athens, OH 45701

We have been investigating the activity of the lobster (*Panulirus interruptus*) pyloric network as cycle frequency changes. We have shown that some pyloric pattern elements maintain phase considerably better than others, and have begun analyzing pyloric network duty cycle (burst length/period) and spike firing characteristics as cycle frequency changes.

The table shows pyloric neuron duty cycles at 1Hz cycle frequency, and the slope of the duty cycle vs. frequency line (positive: burst length changes too little to maintain duty cycle; negative: burst length changes too much). Intraburst spike frequency, spikes/burst, and overall spike frequency (5 spikes/burst x 2 bursts/sec = 10Hz overall spike frequency) were measured for the IC, LP, PD, and VD neurons. PD, LP, and IC neuron intraburst spike frequencies decreased as cycle frequency increased; VD intraburst spike frequency remained approximately constant. Spikes/burst decreased by ~2-fold for all neurons as cycle frequency doubled, and overall spike frequency remained roughly constant.

	IC	LP	PD	PY	VD
Duty Cycle	0.20±0.06	0.22±0.05	0.25±0.04	0.38±0.09	0.34±0.09
Slope	-0.04±0.04	0.00±0.05	0.09±0.03	-0.19±0.06	-0.05±0.06

Coupled with our results on pyloric muscle response to changing stimulus input, these data suggest that cycle frequency changes should induce dramatic changes in muscle contraction amplitude, particularly the phasic component. The relatively constant overall spike frequency observed here is interesting because two of these muscles have a summated contraction component that is likely sensitive to this parameter, and hence this component may be maintained as cycle frequency changes. [Research supported by OU, NSF, and HSFP.]

59.3

MOTOR PATTERN EXPRESSION OF A LATERAL PYLORIC CONSTRICTOR MUSCLE. T.A. Ellis, A.S. Donath, L.G. Morris, J.B. Thuma* & S.L. Hooper. Biol. Sci., Ohio Univ., Athens, OH 45701

Pyloric constrictor muscle p1 of the lobster, *Panulirus interruptus*, is innervated by the lateral pyloric (LP) neuron, and is involved in the early phase of pyloric constriction. We are examining this muscle's response to physiologically relevant neural inputs to determine its contributions to pyloric movements. Stomachs were slit open mid-ventrally and pinned dorsal surface up. The p1 muscle was left attached to its ossicles, and surrounding muscles cut so only p1 muscle contractions were observed. A nerve containing the LP axon was stimulated using trains of trains (0.5-2 sec periods) that mimic LP neuron activity (60Hz, 20% duty cycle) observed *in vitro*. p1 muscle contractions (as revealed by ossicle movements) were recorded with a CCD camera and S-VHS VCR at 60 frames per second and analyzed using a digital video analysis system.

Preliminary experiments show that p1 contractions are small with little or no facilitation. They relax slowly and temporally summate; total contraction amplitude is thus a sum of phasic and tonic components. At a 2 sec period (400msec burst duration, 24 spikes), p1 contractions are 80% phasic with a total amplitude of 0.6 mm (8% of muscle length). At a 1 sec period (200msec, 12 spikes), p1 contractions are 37% phasic with a total amplitude of 0.35 mm; at a 0.5 sec period (100msec, 6 spikes) p1 contractions are only 20% phasic and 0.15 mm in total amplitude.

p1 contractions are thus similar to dorsal dilator muscle (cpv1b) contractions in that both these muscles show a graded transition between rhythmic (faithful follower) and tonic (sustained) contractions as cycle period changes. These data thus provide further evidence that pyloric movements may qualitatively change as pyloric network frequency changes, and that pyloric movement cannot be easily deduced from pyloric neural output. [Research supported by OU, NSF, and HSFP.]

59.4

THE PYLORIC DILATOR MUSCLES SHOW VERY DIFFERENT CONTRACTION AMPLITUDE, FACILITATION, AND SUMMATION. L.G. Morris* & S.L. Hooper. Biol. Sci., Ohio Univ., Athens, OH 45701

The dorsal (cpv1b) and ventral (cpv2b) dilator muscles of the lobster, *Panulirus interruptus*, are innervated by the Pyloric Dilator (PD) neurons and are thought to open the cardiopyloric valve of the stomach. We are examining the response of these muscles to physiologically relevant neural input to determine the movements of this region. Isotonic contractions were elicited by stimulating the nerve that contains the PD neuron axons using trains of trains that mimic *in vitro* PD neuron activity. Resulting contractions in these muscles were observed separately.

We have shown that cpv1b muscle contractions are small (0.2 mm maximum, 2% of muscle length), relax slowly, and hence show significant summation. Total contraction amplitude is therefore the sum of phasic and tonic components, and at 2Hz cycle frequency an average of 80% of cpv1b's total contraction amplitude is tonic. Total contraction amplitude of cpv1b shows relatively little facilitation (up to 3.5 fold).

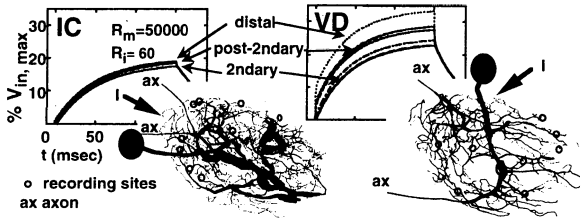
Although initial contractions of muscle cpv2b are often undetectable, they show remarkable facilitation. In trains in which the initial contraction is measurable, 3,000-fold increases in contraction amplitude can be observed. Cpv2b contractions can become very large (2 mm maximum, 10% of muscle length) yet relax quickly and show little summation (at 2Hz, an average of 9.5% of total contraction amplitude is tonic).

The large (10-fold) difference in contraction amplitude of these two putative synergist muscles suggests they may play different functional roles in generating stomach movements. Furthermore, our results show that a single rhythmic neural input can simultaneously induce two qualitatively different activity patterns (one rhythmic contractions, the other a sustained contraction) in different muscles. [Research supported by OU, NSF, and HSFP.]

59.5

COMPUTATION STYLES IN IDENTIFIED NEURONS FROM THE CRAB STOMATOGASTRIC GANGLION. **D.K. Hartline*, K. Graubard, A.E. Wilensky and G. Orr, U. of Hawaii, Honolulu, HI 96822 and U. of Washington, Seattle, WA 98195**

Intermixture of input and output synapses along neurites, as occurs in stomatogastric neurons, opens a possibility for "local" integration of inputs in relative isolation from other functional regions of a cell. The rate of attenuation of electrical signals spreading from a synaptic input to more distant neurites is a key factor in this. VD and IC neurons present very different morphologies. We compared signal attenuation in passive compartmental models of VD and IC to assess potential differences in computational capabilities. Serial optical sections of Lucifer yellow filled cells were made with a confocal microscope for 3D reconstruction with imaging software. For current injected in distal neurites of either cell type, attenuation of voltage responses was five-fold in secondary neurites and distal neurites "down stream" of them. In VD but not IC, attenuation was less in neurites having a secondary neurite in common with the injection site, and still less in those having a post-secondary neurite in common. The results suggest that local computation, while possible in both types, may be more regionally limited in IC than in VD cells (Human Frontier Science Program; NIH NS15697).



59.6

SYNCHRONOUS BURSTING CAN ARISE FROM NON-OSCILLATORY CELLS CONNECTED WITH MUTUAL EXCITATION. P.F.Rowat* and A.I.Selverston Biology Department, U.C.San Diego, La Jolla, CA 92093–0357

Mutual excitation between two neurons that are quiescent in isolation (do not burst), is not thought to cause bursting behavior: it merely raises the level of excitation. On the contrary, we show, by modelling, that two quiescent cells can be induced to burst in synchrony by mutual excitatory synaptic connections. The underlying dynamical mechanism is a combination of the escape and release mechanisms believed to underly bursting when two quiescent cells are connected with reciprocal inhibition. The mechanism can be realized physiologically in two different ways: either the presynaptic threshold for graded synaptic transmission in each cell is at a different membrane potential, or the cells are identical but each has a different steady current either from experimentally injected currents or from different amounts of postsynaptic current from other cells. In either case, a characteristic feature of the mechanism is that bursts in one cell begin slightly before, and terminate slightly after, bursts in the other cell: one cell's bursts *contain* the other's.

We propose that this mechanism underlies oscillations in two small networks in the lobster. In *Homarus gammarus*, oscillations of the commissural pyloric oscillator in the stomatogastric nervous system of the lobster have been shown by Cardi and Nagy (1994) to be driven by two mutual excitatory cells, CP and LE; neither cell has been shown to be an endogenous oscillator, and the CP bursts contain the LE bursts. In *Homarus americanus*, the large and small cells in the cardiac ganglion are connected with reciprocal excitation and the large cell bursts contain the small cell bursts (Hartline 1979).

Finally, we note this mechanism could be present in cortex, since mutual excitation and synchronous oscillations are both widespread there.

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59.7

ANALYSIS OF THE STOMATOGASTRIC PYLORIC NETWORK USING THE HYBRID NETWORK METHOD.

G.Le Masson* and M. Moulins. Lab. de Neurobiologie et Physiol. Comp. URA CNRS 1126 and Université Bordeaux I, Arcachon 33120, FRANCE.

We use the "hybrid network" method to study systematically the individual impact of cellular and synaptic parameters on the overall activity of a simple neural network, the stomatogastric pyloric network of Crustacean. Multi-compartmental models of the three major types of interacting neurons (PD-LP-PY) were constructed, and used to replace their previously photo-inactivated biological equivalents in the pyloric circuit *in vitro*. We then examined the effect of linear changes in synaptic efficacy and in the maximal conductances of four intrinsic currents (ICa, IKCa, Ih and IA) expressed by each cell, on three major parameters of the pyloric pattern; pace makers cycle period, LP and PY bursts phases.

We have identified several combinations of cellular and synaptic parameters that provides the entire network with notable emergent properties such as a strong ouput pattern stability. We have also found target parameters that are crucial in the regulation of the pyloric period or for the maintenance of correct phasing within each cycle. The flexibility associated with these parameters has been compare to the target effects of known modulatory inputs to this network.

Finally we have studied the stability of the hybrid network in various modulatory environments. Our results suggest the existence of "stabilizing" modulatory influences, capable of constraining the network in highly stable functionnal configurations.

59.8

DYNAMIC INTERACTIONS BETWEEN MOTOR PATTERN GENERATING NETWORKS IN THE STOMATOGASTRIC GANGLION OF INTACT LOBSTER. S. Clemens (§), P. Meyrand and J. Simmers*. Laboratoire de Neurobiologie et Physiologie Comparées, Univ. Bordeaux I & CNRS, 33120 Arcachon, France. clemens@lnpc.u-bordeaux.fr

Rhythmic movements of the gastric mill and pyloric regions of the crustacean foregut are controlled by two stomatogastric neuronal networks which have been intensively studied *in vitro*. By using EMG electrode recordings from the European lobster, *Homarus gammarus*, we have monitored simultaneously the motor activity of pyloric and gastric mill muscles for up to three months in intact and freely behaving animals. Both pyloric and gastric mill networks are almost continuously active *in vivo*, regardless of the presence of food. In an unfed animal, the pyloric network expresses the typical threephasic pattern seen *in vitro*, but at slower cycle periods of 2.5-3.5s (instead of ca. 1-1.5s). Gastric mill activity occurs at mean cycle periods of 20-50s but may suddenly stop for up to tens of minutes, then restart without any apparent behavioral reason. When conjointly active, the two networks express a strict coupling that involves a significant prolongation (up to 300%) of pyloric PY motoneuron bursts after onset of each gastric LG/MG burst. During feeding, this gastro-pyloric interaction disappears as the two networks now express accelerated rhythmic motor patterns (pyloric period: 1.2-1.5s; gastric period: 10-15s). The return to control levels of network activity occurs progressively over the following two to three days (depending on the type of food originally presented), and is associated with a gradual reappearance of the gastro-pyloric interaction. Our data suggest that this interaction appears only during a "free run" mode of foregut activity, and that it is not simply a consequence of activity-dependent changes from within the two networks, but rather is specified by extrinsic modulatory influences.

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59.9

STABILIZING CONTROL OF A MOTOR PATTERN GENERATING NETWORK VIA INTERACTING HIGHER ORDER MODULATORY INTERNEURONS. S. Faumont, J. Simmers and P.Meyrand* Lab. de Neurobiol. et Physiol. Comp., Univ. Bordeaux I & CNRS, Place Peyneau, 33120 Arcachon, France.

Recent results have shown that neural networks are far from being fixed entities, but can be dynamically specified by modulatory neurons to satisfy particular behavioral demands (Meyrand et al.(1991), Nature 351, 60). While this lability is required for the initial configuration of a functional network from a pool of neurons, both short and long-lasting stability is necessary for its ensuing operation. How can such network stability be achieved? In the stomatogastric nervous system of the lobster, *Homarus gammarus*, bursting in an identified modulatory interneuron (PS) causes a massive restructuring of stomatogastric circuitry, followed immediately by a robust and long-lasting (for tens of minutes) activation of the gastric mill network when the interneuron falls silent. This delayed gastric activation is caused by PS itself, partly through a specific modulatory action on the gastric mill network, and partly via recruitment of another higher order interneuron (CG) also known to modulate the gastric network. PS not only elicits monosynaptic epsps in CG, but its repetitive burst firing is also able to induce a progressive build-up of slow plateau-like depolarisations that may eventually sustain elevated CG firing for several tens of seconds after a sequence of PS bursting. In parallel with this indirect excitatory action via CG, PS directly and longlastingly activates a gastric network element, Interneuron 1, which in turn provides strong feedback inhibition to CG. Rebound from gastric-timed Int 1 inhibition triggers active membrane properties and intense firing in CG, thereby reinforcing the latter's excitatory drive back to the motor network. Our results suggest that through a combination of longlasting actions on the cellular properties of two coupled neurons, one a modulatory input and the other a component of the motor network itself, PS is able to prime the gastric network to express selfsustaining rhythmicity that far outlasts the interneuron's own discharge.

59.10

MATHEMATICAL MODELS OF THE CRAYFISH SWIMMERET SYSTEM. F.K. Skinner* and B. Mulloney. Neurobiology, Physiology, and Behavior, Univ. Calif., Davis CA 95616-8755.

Swimmerets are paired, jointed limbs on many segments of the abdomen that can move rhythmically to propel the crayfish forward in the water. Locomotion in segmented animals requires well-coordinated movements of different limbs, but the neural basis of this coordination is not well-understood anywhere, and the swimmeret system poses problems of coordination clearly. Each swimmeret is driven by a modular neural circuit located in the segmental ganglion that innervates it. A separate coordinating circuit organizes the activity of these modules in different segments. Axons of coordinating interneurons project from each ganglion to both anterior and posterior neighbors, so this circuit is bidirectional.

From initial alternative hypotheses about the synaptic organization of this coordinating circuit, we developed alternative models of the crayfish swimmeret system. Each model includes a chain of modular local circuits of nonspiking interneurons that drive the motor neurons of each limb, linked to modules in neighboring segments by coordinating interneurons. Different patterns of connections made by these coordinating interneurons defined the different models. Local interneurons were described by Morris-Lecar equations that included graded currents from reciprocal inhibitory synapses. Coordinating interneurons were also driven by these local interneurons, "fired" when the presynaptic cell was depolarized, and caused synaptic currents in their target neurons. Comparing the performance of each model with experimental results suggests specific features of the real coordinating circuit needed to produce normal swimmeret coordination.

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59.11

'BUILDING BLOCKS' OF INSECT WALKING PATTERN GENERATION: IONIC BASIS OF CENTRAL RHYTHMIC SYNAPTIC DRIVE TO LEG MOTONEURONS. A. Büschges* and R. Haas. FB Biologie, Univ. of Kaiserslautern, 67653 Kaiserslautern, Germany.

During walking and searching movements of an insect leg, the activity of the leg motoneuron (MN) pools of each individual leg joint is generated by the interaction between signals from central rhythm generating (CRG) sources for each leg joint and peripheral signals as well as coordinating signals from other leg joints and legs (Bässler, 1993, Biol. Cybern. 69, 305). We recorded from leg MNs during walking and searching movements in a 'semi-intact' walking leg preparation of the stick insect middle leg. The recordings revealed that actual shaping of motoneuronal activity in the movement cycle is mediated by cyclic alterations of the motoneurons' membrane potential around its value at rest. As an example, in MNs driving the leg muscles of the femur-tibia (FT-) joint, e.g. extensor MNs, the activity was generated mainly by depolarizing synaptic signals during swing, i.e. the return stroke, and hyperpolarizing synaptic inputs during stance, i.e. the power stroke. The reverse was the case for flexor MNs.

We were interested in the nature of the synaptic drive from CRGs on the MN pools of the individual leg joints. We investigated this by pharmacologically activating CRGs in the isolated mesothoracic ganglion. Rhythmicity was induced by bath application of the muscarinic agonist pilocarpine with concentrations of 1-3x10^{-4} M. First, we focused on the MNs of the FT-joint, i.e. FETi, SETi and Flexor MNs. During rhythmicity MNs of both pools received slight tonic depolarization, but mainly rhythmic hyperpolarizing synaptic inputs, which appear in antiphase for both MN pools. DCC and SEVC recordings revealed the reversal potential of these inhibitory inputs between -80 to -85mV (FETi, n=10). After injection of TEA (4mM) through the intracellular electrode inhibition in FETi was significantly decreased by about 85% (n=3). Thus, it appears that potassium ions contribute to cyclic inhibition of motoneurons by CRGs during locomotor behavior. Currently we are investigating the ionic basis of central rhythmic synaptic drive for MNs supplying the other leg joints.

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59.12

TESTING THE CELLULAR REDUCTION HYPOTHESIS IN MODEL NEURAL CIRCUITS. H.J. Chiel* and R.D.Beer. Departments of Biology and Computer Engineering and Science, Case Western Reserve University, Cleveland, OH 44106.

A central hypothesis of cellular neurobiology, the cellular reduction hypothesis, is that one can explain the function of a neural circuit by analyzing the properties of its individual nerve cells and their interconnections. However, work on small, experimentally tractable nervous systems has shown that neural circuits have extensive recurrent connections, can functionally reorganize in response to neuromodulators, and have complex interactions with the periphery. These studies make it increasingly difficult to ascribe particular functions to individual neurons, and raise questions about the most useful way to decompose and understand neural circuits.

To address this question, we have begun to analyze model neural circuits capable of controlling locomotion that were generated using genetic algorithms, or hand-designed to be modular. Although these circuits are far simpler than those found in animals, they allow us to analyze highly recurrent circuits that are tractable to mathematical analysis without being limited by missing data, and that have many properties similar to those found in biological nervous systems. We have developed a set of tools that allow us to analyze these circuits as an experimentalist would do: we can examine their properties in isolation, their connectivity, and their function within the context of the circuit as a whole.

Our preliminary results indicate that (1) although one can readily distinguish motor neurons from interneurons in a modular circuit, all neurons play a role in pattern generation in a highly recurrent circuit, (2) in a recurrent circuit, motor neurons and interneurons play overlapping and time varying roles in producing the walking pattern, and (3) this dynamic redundancy provides recurrent circuits with a great deal of robustness in response to lesions of individual connections between neurons. We are also using the tools of dynamical systems analysis to complement the neurobiological analysis of these circuits.

Supported by Santa Fe Institute and ONR grant N00014-90-J-1545.

59.13

PHASE-SENSITIVITY AND ENTRAINMENT IN BURSTING NEURONS: A SIMULATION STUDY R. J. Butera, S. S. Demir, A. A. DeFranceschi, J. W. Clark*, and J. H. Byrne†. Dept. of Elec. & Comp. Eng., Rice U., Houston, TX 77251 and †Dept. of Neurobiology & Anatomy, U. of Tex. Med. Sch., Houston, TX 77225.

Biological oscillators frequently exhibit a phasic dependency of the effect of perturbations. Although the parametric aspects of phase sensitivity and entrainment have been analyzed extensively, little is known about the underlying biophysical mechanisms. We used a model of neuron R15 in *Aplysia* to study the mechanisms determining the phase-response curve (PRC) of the cell in response to both extrinsic current pulses and modeled synaptic input. We also compared entrainment predictions from PRCs with those from actual simulations.

The PRCs of the model exhibited minimal dependence on stimulus amplitude, and a strong dependence on stimulus duration. Phase-plane analysis of the effect of transient current pulses provided several important insights into the relationship between the PRC and the underlying dynamics of the model. First, there was a strong correlation between pre-stimulus concentration of Ca^{2+} and the post-stimulus phase of the oscillation. Second, the existence of a stable fixed point in the presence of a hyperpolarizing stimulus was a sufficient criteria for the model to exhibit phase-independent resetting if the pulse was sufficiently long in duration. Third, the system nullclines of the two slow variables provided well-defined limits on the ability of a hyperpolarizing input to perturb the oscillation. These limits were related to the magnitude and duration of the input. Furthermore, the predictions of phase-locked 1:m entrainment from PRCs were valid, even when the duration of the periodically applied pulses were a significant portion of the control limit cycle.

These results demonstrate that experimentally applied hyperpolarizing current pulses are sufficient to determine the shape of the PRC in response to an inhibitory synaptic input, provided that the duration of the pulse is similar to that of the synaptic current. These findings also show that when utilizing hyperpolarizing stimuli, the PRCs alone are sufficient to predict the dynamic behavior of the model in response to transient and periodic input (see adjacent paper by Canavier et al.).

Supported by grants from the ONR and the NIMH and a Whitaker Foundation Graduate Fellowship in Biomedical Engineering (RJB).

59.14

ANIMATION OF A CENTRAL PATTERN GENERATION NETWORK: A NEW APPROACH FOR VISUALIZING NEURON ACTIVITY AND INTERACTIONS IN REALISTIC COMPUTATIONAL MODELS. N. Koshiya*, J.C. Smith, and W.B. Marks, Laboratory of Neural Control, NINDS, NIH, Bethesda, MD 20892.

We have developed methods for animating simulations from "realistic" computational models of CPGs which allow dynamic visualization of neuron activity and interactions in a computer-generated movie format. These methods were applied to animate simulations from our models of the mammalian brainstem respiratory network (Smith, J.C. *Soc. Neurosci. Abs. 20*, 1994) which consist of seven major types of interacting interneurons in the ventrolateral medulla. To generate the animations, we used *Mathematica* to construct a virtual elastic surface on which the different types of neurons were arrayed. Neuron activity is represented by upward surface motions described by a Gaussian-like function where the amplitude of the motion scales with the instantaneous neuronal spiking frequency. Functional synaptic interactions between neurons (excitatory or inhibitory) are represented as colors (red and blue, respectively) which flow in "synaptic traffic lanes" on the surface from presynaptic to postsynaptic neurons. The color intensities in these lanes change with the strength of synaptic activation allowing the activation patterns to be visualized dynamically. Changes in excitatory and inhibitory postsynaptic conductances are also separately color encoded, where the color intensity scales with the summated inhibitory or excitatory conductance. These conductance color changes are superimposed on the surface motions, providing a visual correlation of the regulation of neuron activity by synaptic inputs. Images are computed every 62.5 msec during the rhythmic respiratory cycle (2 - 3 sec period) and are compiled as a QuickTime movie/video including sound generated from the membrane potential of a selected model neuron. These animations are a tool for visualizing the complex dynamics of central pattern generation networks.

59.15

A SPIKE TRAIN ANALYSIS FOR DETECTING THE DEPENDENCE OF SPIKE GENERATION OF ONE NEURON ON THE DOUBLET SPIKE FIRING OF ANOTHER. **D. C. Tam*.** Center for Network Neuroscience and Dept. of Biological Sciences, University of North Texas, Denton, TX 76203.

A spike train analysis technique is introduced to determine how consecutive spike firings in one neuron contribute to the generation of spikes in another neuron (typical in temporal summation). In particular, we examine the dependence of spike firing in a given neuron with respect to the doublet firing in another neuron. We extended the analysis of conventional cross-correlation techniques to include the contribution of spike firing of more than a single preceding spike by computing the conditional probability of the next spike firing in a reference neuron given that the conditional neuron has fired two consecutive spikes (a doublet) prior to its firing. The conditional probability (or the joint probability) of spike firing is estimated by the statistics obtained from the conditional (or joint) distribution of the pre-interspike intervals (pre-ISIs) and post-cross intervals (post-CIs) relative to the reference spike. The characteristic features (such as peaks and valleys) revealed by the conditional probability density function provide information about the specific time intervals in which excitatory or inhibitory couplings between this neuron pair are correlated with the consecutive firing of a doublet in the conditional neuron. Thus, the contribution of spike firing from more than one spike can be revealed so that the temporal summation of consecutive spike firings can be deduced. The results of this analysis can lead to a better understanding of the influence of spike generation of one neuron on the burst-firing in another neuron. (Supported by ONR N00014-94-1-0686)

59.16

A CROSS INTERVAL SPIKE TRAIN ANALYSIS TECHNIQUE (PRE-CI/ISID/POST-CI) FOR REVEALING THE COUPLING RELATIONSHIPS BETWEEN THE FIRING PATTERNS OF NEURON-PAIRS. **M. A. Fitzurka*† and D. C. Tam.‡** †Dept. of Physics, Catholic University of America, Washington DC, 20064 and ‡Center for Network Neuroscience, Dept. of Biological Sciences, University of North Texas, Denton, TX 76203.

A cross interval spike train analysis technique was developed to detect the correlation between pairs of simultaneously recorded spike trains. This new method, *pre-CI/ISID/post-CI analysis*, shows the relationship between trends in the firing of one neuron (the reference) and the firing in another (or compared) neuron. This trend, or ISID, is defined as the difference between consecutive ISIs in the reference spike train ($\Delta\tau_n = \tau_n - \tau_{n-1}$). How this trend, $\Delta\tau_n$, is affected by the cross-interval preceding it (the pre-CI, $\tau'_{n-2,-1}$) is revealed by the distribution of points in a *pre-CI/ISID* phase plane. Additionally, how that trend, $\Delta\tau_n$, affects the next subsequent CI (the post-CI, $\tau'_{n,+1}$) is seen in the location of the points in an *ISID/post-CI* phase plane. In this way, the CIs (with respect to a compared neuron) leading to and from a given trend (in a reference neuron) can be compared. Whether or not these plots are symmetric reveals whether or not the couplings between the neurons preceding and succeeding a local trend are similar. This analysis was applied to simulated spike trains (where the processes generating firing patterns are known) to demonstrate its ability to detect correlation trends. Spike trains recorded experimentally from cultured neuronal networks were analyzed as well to reveal specific correlation trends found between pairs of interconnected neurons in biological networks. (Supported by ONR N00014-94-1-0686).

59.17

CROSS-CORRELATION ANALYSIS OF SPONTANEOUS ACTIVITY IN THE HIPPOCAMPUS OF THE ANESTHETIZED RAT. I. Espinosa*, J. Quiza and A. Sierra. Lab. of Cybernetics, Physics Dept., Sch. of Sciences, Mexico National University, Mexico, DF 04510.

In urethane anesthetized rats we made a series of tungsten microelectrodes excursions across the hippocampus with the object of simultaneously recording spontaneous activity in small ensembles of neurons and later inferred functional connectivity using cross-correlation analysis. In eight experiments we recorded with one, two or four single microelectrodes that were guided towards pyramidal borders in CA1, CA3 and granular layer of DG and positioned the array where more than one unit could be discriminated per microelectrode. At the end of some of the experiments the microelectrode trajectory was verified by visual inspection of brain slices. The spikes were carefully sorted and a preliminary cross-correlation analysis shows signs of shared input and direct excitatory connections among the neurons recorded. Units in the same microelectrode have excitatory connections and units in different microelectrodes show histograms with shared input characteristics. Wiring diagrams have been obtained for some neuron ensembles.

Partially supported by DGAPA UNAM IN100593.

59.18

EXPERIMENT AND THEORY OF THE ROLE OF SPONTANEOUS TRANSMITTER RELEASE IN CULTURED NEURAL NETWORKS P.E. Latham*, B.J. Richmond, and P.G. Nelson. Lab. of Developmental Neurobiology, NICHD, and Lab. of Neuropsychology, NIMH, Bethesda, MD 20892.

In long term (several hour) recordings from networks of cultured mouse spinal cord neurons, we have observed activity patterns that include random spiking, bursting, and transitions between these states. In an attempt to understand this behavior we have developed a reduced model which lumps neurons into two classes, excitatory and inhibitory, each of which can be described by its average firing rate. Comparison of our model with experiments indicates that: i) the rate of spontaneous transmitter release plays a crucial role in the observed state changes; and ii) the variety of behavior observed in experiments is explained by a relatively simple picture involving the interaction of excitatory and inhibitory cell populations. In addition, preliminary results indicate that network dynamics is extremely sensitive to system parameters (e.g., probability of transmitter release, pattern of connectivity), and small changes produce either epileptic activity or the complete absence of firing. This dependence is so sensitive that a long-term feedback mechanism regulating some of these system parameters is required to keep the network in the range of activity observed in experiments. Support: IRP/NICHD and IRP/NIMH.

59.19

COMPUTATION OF HIGH SAFETY-FACTOR IMPULSE PROPAGATION AT AXONAL BRANCH POINTS. M.D. Goldfinger*. Dept. Physiology & Biophysics, Wright State University, Dayton, OH, 45435.

The purpose of this work was to reexamine the paradoxical theoretical result of low-safety factor impulse propagation at an axonal branch point (eg,1).

Differential equations were solved numerically using trapezoidal integration (2). For constant-current stimulation of finite-length single-diameter passive fibers, numerical solutions (using $\Delta t = 50.$ nanosec and $\Delta x/\lambda = 0.0165$) reconstructed analytical solutions (3) with negligible errors ($\leq 0.06\%$). Numerical solutions also reconstructed the speeding of voltage transients with increasing electrotonic length (3). Using the H&H equations (4), the computed membrane action potential ($\partial V/\partial x = 0$) was compared with that computed with 8 other numerical methods (incl. explicit, implicit, predictor-corrector); all computed waveforms superimposed, differing by not more than 1.6mV. For branch point passive properties, computed solutions reconstructed constant-current-elicited voltage transients as well as the distribution of axial current at the branch point as recorded from an exact analog compartmental circuit (5).

Solutions were computed for a single impulse propagating from a 1.μm-diameter axon into two daughter branches ($\Delta t = 12.5$ nanosec and $\Delta x \equiv 0.0165 \cdot \lambda_{parent}$). Local conduction velocity -CV(x)- changed in the vicinity of the branch point; the eventual CV(x) in the daughters was predictable from parental the pre-branch local CV(x) and the daughter diameter. No impulse failure occurred despite daughter diameters as large as 10. μm. These CV(x) patterns were reconstructed with 1.μm-diameter daughters by proportionately reducing the daughters' axial resistivity.

Supported by NSF BCS-9315856 and Wright State University.

References: (1) Goldstein & Rall,1974, Biophys.J.*14*:731, (2) Goldfinger et al.,1992, Biol.Cybern.*66*:399; (3) Jack et al., 1975, *Electric Current Flow in Excitable Cells*; (4) Hodgkin & Huxley, 1952, J. Physiology *117*:500; (5) Goldfinger & Moradmand, 1995, Soc.Neurosci.Abst *21*:1090.

COMPARATIVE NEUROANATOMY: LOWER VERTEBRATES

60.1

ZEBRIN II EXPRESSION IN SEA LAMPREYS (*PETROMYZON MARINUS*), M. J. Lannoo* and R. Hawkes. Muncie Center for Medical Education, Indiana University School of Medicine, MB 209, Ball State University, Muncie, IN 47306 and The University of Calgary School of Medicine, Calgary, Alberta, CANADA T2N 4N1.

Zebrin II is a 36 kD polypeptide expressed by Purkinje cells in the cerebellum of elasmobranchs, teleosts, birds, and mammals, and by acousticolateralis pyramidal cells in developing teleosts. Cloning studies suggest that zebrin II is the respiratory isozyme aldolase C. In agnathans, Larsell described a cell type in the corpus cerebellum of adult lampreys that he termed the "primitive Purkinje cell". To determine whether these primitive Purkinje cells express zebrin II, ammocete larvae and adult sea lampreys were obtained and fixed by transcardial perfusion. Their brains were frozen sectioned and reacted with mab anti-zebrin II according to standard procedures. Four adults and two ammocetes were examined. No mab anti-zebrin II immunoreactivity was detected in adults. In ammocetes, however, acousticolateralis Purkinje cells consistently and robustly label. This zebrin II expression appears to be similar to that which we described previously for pyramidal cells in ostariophysan teleosts. Three possibilities exist for the lack of zebrin II expression in primitive Purkinje cells: 1) these cells are not Purkinje cells; 2) zebrin II$^-$ cells are present phylogenetically before zebrin II$^+$ cells; or 3) primitive Purkinje cells are equivalent to embryonic zebrin II$^+$ Purkinje cells but at a stage in their development that precedes the expression of zebrin II.

Supported by NIH grant NS30702-01 (MJL) and the Medical Research Council of Canada (RH.).

60.2

THE DISTRIBUTION OF ZEBRIN II IMMUNOPOSITIVITY IN THE CEREBELLAR CORPUS OF STINGRAYS. R.L.Puzdrowski*, Dept. Anatomy and Neurosciences, Univ. Texas Med. Br., Galveston, TX 77550.

Anti-Zebrin II is an antibody directed against a 36kDa aldolase epitope expressed by Purkinje cells. Two patterns of Zebrin II labeling have been described in the cerebellar corpus of vertebrates. In the mammalian cerebellar corpus, the Zebrin II antibody labels longitudinal zones of Purkinje cells. In teleosts, all purkinje cells of the corpus are Zebrin II immunopositive. To determine which of these patterns represents the primitive condition for jawed vertebrates the distribution of Zebrin II immunopositivity was examined in two species of elasmobranch rays, Dasyatis sabina and Dasyatis americana. Rays were anesthetized with MS222 and transcardially perfused with elasmobranch Ringer's followed by 4% paraformaldehyde in 0.1M Phosphate buffer. The brains were removed and sectioned at 35μm. The sections were blocked in normal horse serum and incubated for 24-72hrs with monoclonal anti-zebrin II (culture fluid 1:50-1:1000). Sections were incubated in biotinylated anti-mouse IgG, followed by avidin-biotin-peroxidase complex, and reacted for HRP using DAB as the chromagen. In stingrays, anti-Zebrin II labels all Purkinje cells of the corpus. No banding pattern or compartmentation was observed. Purkinje cell projections to the deep cerebellar nucleus, and anterior, descending, and posterior octaval nuclei also were revealed. Western blot analysis demonstrates that the Zebrin II antibody recognizes a polypeptide antigen of the same molecular weight in stingrays, teleosts, and mammals. Based on these results it is concluded that a Zebrin II staining pattern, in which all Purkinje cells of the cerebellar corpus are immunopositive is the primitive condition for jawed vertebrates. Zebrin II antibodies were kindly provided by R. Hawkes. Supported by NSF IBN-9421393.

60.3

AFFERENT AND EFFERENT CONNECTIONS OF THE OPTIC TECTUM IN THE SENEGAL BICHIR *Polypterus senegalus* [Osteichthys: Cladistia] P. H. Holmes* and R.G. Northcutt. Neurobiology Unit, Scripps Institution of Oceanography, and Dept. of Neuroscience, School of Medicine, University of Calif., San Diego, La Jolla, Ca 92093-0201.

Non-retinal afferents and efferent connections of the optic tectum were determined by injecting horseradish peroxidase or biotinylated dextrans into the optic tectum. Analysis of efferents also involved examination of degenerating fibers after tectal lesions. The tectum receives input from the ipsilateral caudal entopeduncular nucleus, the anterior, ventromedial, and possibly ventrolateral thalamic nuclei. Other sources of afferents include the central pretectal nucleus, ipsilateral nucleus supracommissuralis, ipsilateral torus semicircularis, bilateral lateral tegmental nucleus, and the ipsilateral locus coeruleus. Medullar afferents include all divisions of the reticular formation, contralateral dorsal and medial octavolateral nuclei, and the contralateral descending trigeminal nucleus. Ascending efferents project to the pretectum, the anterior, intermediate, ventromedial, ventrolateral, central posterior, and dorsal posterior thalamic nuclei. Efferents cross in the postoptic decussation to innervate the contralateral central pretectal nucleus, nucleus supracommissuralis, and nucleus anterior and intermedius of the thalamus. Descending efferents project to the ipsilateral torus lateralis, the contralateral torus semicircularis, ipsilateral tegmentum, and nuclei of the posterior tubercle. After crossing in the caudal mesencephalon, efferents descend and project bilaterally to the reticular formation. (Supported in part by a Markey Fellowship to P.H.H.)

60.4

AFFERENT AND EFFERENT CONNECTIONS OF THE TELENCEPHALIC DORSOCENTRAL REGION IN A WEAK ELECTRIC FISH, *GYMNOTUS CARAPO*. S.A L. Corrêa*, K. Grant and A Hoffmann. Lab. of Neurophysiology, University of São Paulo, Ribeirão Preto SP 14049-900 Brazil.

This is the first attempt to investigate possible connections between the dorsocentral (DC) area of the telencephalon and other regions of the central nervous system in *Gymnotus carapo*. To this end electrophoretic injections of biotinylated dextran amine (BDA) were applied to the different frontal planes of the DC. This resulted in retrograde labeling of the area ventralis pars centralis (Vc), pars dorsalis (Vd) and pars ventralis posterior, nucleus entopeduncularis, area dorsalis pars lateralis (DL) and pars lateralis (PGl), pars medialis (PGm) and pars rostralis (PGr) of the preglomerular complex. Anterograde labeling was detected in Vd, Vc, DL, PGl, PGm, pretectal nucleus, magnocellular tegmental nucleus, optic tectum (TeO) and dorsal torus semicircularis (TSd). We observed that the DC is heavily interconnected with the preglomerular complex, wich is involved in the processing of multisensory information (auditory, gustatory, visual and electrosensory). The PGl receives electrosensory information via the TSd and the nucleus electrosensorius (nE) and projects toward caudal planes of the DC, while fibers from rostral and medial DC planes reach the PGl, TSd and TeO, structures that in gymntiformes are related to the processing of electrosensory information. Thus, in gymnotiform fish, electrosensory information may be conducted from the nucleus electrosensorius to the dorsal telencephalon via the PGl..

This study was supported by the Brazilian agencies CNPq, CAPES, FAEPA and French CNRS.

60.5

IMMUNOCYTOCHEMICAL LOCALIZATION OF cGnRH-II and sGnRH in the Nervous System of Zebrafish, *Danio rerio*. S.P. Matz* and R.G. Northcutt. Department of Neurosciences. U.C. San Diego, La Jolla, CA 92093

The distribution of gonadotropin-releasing hormone (GnRH) was examined in zebrafish using antisera to salmon GnRH (sGnRH) and chicken GnRH-II (cGnRH-II). sGnRH-immunoreactive (ir) cell bodies were localized in the olfactory bulb, ventral telencephalon, preoptic area and posterior tubercle. cGnRH-II-ir cell bodies were localized in posterior tubercle only.

GnRH-ir fibers are widely distributed in the CNS. Fibers immunoreactive to sGnRH are found in the olfactory bulb, telencephalon, diencephalon, optic tectum, midbrain and spinal cord. Furthermore, sGnRH-ir fibers are observed in the olfactory and optic nerves. Fibers in the optic nerve appear to be efferent fibers since terminal-like swellings are observed in the retina and no GnRH cell bodies are observed in the retina. We could not determine whether fibers in olfactory nerve were efferent axons or dendrites emanating from GnRH cells in the olfactory bulb. cGnRH-II-ir fibers were localized in the same areas as sGnRH the density of staining was much less, and the fibers were thinner.

The results of this study suggest that zebrafish are immunoreactive to sGnRH and cGnRH-II. Although there is overlap in the distribution of these two antibodies, the reactivity to sGnRH is much more widely distributed.

60.6

EVOLUTION OF COGNITION IN TELEOSTS: CYTOARCHITECTURE OF THE HYPERTROPHIED DORSAL TELENCEPHALON OF SQUIRRELFISH, L.S.Demski* and J.A.Beaver. Division of Natural Sciences, New College, Univ. of South Florida, Sarasota, FL 34243

Squirrelfish are common coral reef fishes with enlarged eyes and visual pathways and highly sensitive hearing, a unique combination for teleosts. They are also unusual in having an exaggerated development of certain parts of the area dorsalis telencephali (Dc, pars centralis; Dd, pars dorsalis; Dl, pars lateralis); regions most likely related to the sensory enhancements. Toward a functional analysis of the areas, we describe their cellular components using Golgi, Nissl, and plastic preparations of Holocentrus rufus. The Dc is a spherical expansion (radius of ca.1.2-1.4 mm) with a midline fusion; it contains scattered large multipolar neurons (ca. 22-25μm, soma; 10-12.5 μm nucleus) with spiny dendrites extending spherically (at least up to 400 μm) to overlap those of many other similar cells as well as fibers of the lateral forebrain bundle. A thickened mantle (ca. 0.5mm deep) or "cortex-like" zone, most likely related to Dd and/or Dl of other teleosts, overlies Dc and is seperated from it by a capsule of myelinated fibers. The mantle zone has cords of small (ca. 7.5-10μm, soma; 5μm, nucleus) and medium (ca. 11-12.5μm, soma; 7.5μm, nucleus) sized cells separated by radially projecting axons and glial fibers. The larger cells have a denser cytoplasm. Both types have "lateral" dendrites (at least up to at least 125μm in the larger and 75μm in the smaller cells) that branch from spherical cell bodies and lie in a plane concentric with the outer pial surface and hence perpendicular to the radial fibers; other dendrites extend more ventrally in an elliptical pattern between the lateral ones. The largest dendrites are spiny and overlap those of cells in adjacent columns. The cells often occur in clusters of two or more with somal membranes in close apposition. Axons of all cell types originate from the soma or basal dendrites. Supported by grants from NSF and Florsheim Endowment to New College.

60.7

CONNECTIONS OF LATERAL LINE NEUROMASTS AND OTOLITHIC ENDORGANS OF THE INNER EAR IN A CLUPEID. C.A. McCormick*. Dept. Biology, Oberlin College, Oberlin OH 44074

Connections of nerves associated with the inner ear and lateral line were studied in the gizzard shad, Dorosoma cepedianum using horseradish peroxidase. Labeling was carried out in exsanguinated specimens (see McCormick and Braford, 1994). Additional specimens were used to determine the cytoarchitecture of the octavolateralis area, the relationship of the swimbladder to the inner ear, and the presence or absence of trunk neuromasts. As reported in other Clupeiformes, the utricle and cephalic lateral line canals are associated with the swimbladder, and the utricle contains three sensory maculae. The utricle has a major bilateral projection to the dorsomedial zone of the descending octaval nucleus; this zone is located medial to nuc. medialis and ventral to the cerebellar crest. Other utricular fibers terminate in ventral regions of the descending octaval nuc. Most saccular and lagenar fibers project ipsilaterally to a dorsal region of the descending octaval nuc. that is lateral, not medial as in other teleosts, to the utricular terminal zone. A few fibers also supply the dorsomedial zone, bilaterally in the case of the saccule. Other connections of the otolithic endorgans include the anterior octaval nuc., nuc. magnocellularis, and the eminentia granularis. Most anterior and posterior lateral line fibers terminate in ventral and dorsal areas within nuc. medialis, respectively. Both head and trunk neuromasts provide light input to the dorsomedial zone of the descending nuc. and possibly also to the more lateral saccular/lagenar zone; connections to nuc. magnocellularis and the eminentia granularis are also present. The descending octaval nuc. in the shad therefore has an expansive dorsomedial zone like that of otophysans, but the input to this zone is dominated by utricular rather than saccular input. The results of this study will be presented in an evolutionary context. Supported by NSF IBN-9309977.

60.8

IDENTIFICATION AND LOCALIZATION OF NEUROHYPOPHYSIAL PEPTIDES IN THE BRAIN OF A CAECILIAN AMPHIBIAN. S.K. Boyd[1]*, C. Hilscher-Conklin[1], J.M. Conlon[2]. [1]Biological Sci., Univ. of Notre Dame, Notre Dame, IN 46556; [2]Regulatory Peptide Center, Biomedical Sci., Creighton Univ. Sch. of Med., Omaha, NE 68178.

We used HPLC to isolate and purify brain and pituitary peptides from the caecilian *Typhlonectes natans* which belongs to the little-recognized amphibian order, Gymnophiona. Based on elution profiles, immunoreactivity and mass spectrometry, this caecilian possesses arginine vasotocin (AVT). Using immunocytochemistry, AVT cells were located in the preoptic area, hypothalamus, amygdala, ventral thalamus, trigeminal nucleus and spinal cord. AVT fiber distribution was extensive. Caecilian brains also contained a putative mesotocin, based on elution profile and immunoreactivity. MT-immunoreactive cells were present in the preoptic area, hypothalamus and amygdala only. Fiber distribution was more restricted than that of AVT. Neurohypophysial peptides of this caecilian species, therefore, are similar to those of anuran and urodele species, despite caecilians being evolutionarily separated by at least 200 million years from the other two orders.

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60.9

QUANTITATIVE THREE-DIMENSIONAL CHARACTERIZATION OF DENDRITIC TREES IN AMPHIBIAN FOREBRAIN. C. Mohr*[ab] S.K. Boyd[a], S. Raghavachari[b] and J.A. Glazier[b]. University of Notre Dame, Departments of Biology[a] and Physics[b], Notre Dame, Indiana 46556, USA.

Three-dimensional description of neuron anatomy is difficult. Current practices often depend on unrelated and subjective criteria (eg, shape of dendrite tree or soma, position of soma) to classify neurons. We have developed an unambiguous method for categorizing neurons, using a quantitative analysis of their dendrite structure. Golgi-stained neurons in the telencephalon of the South African clawed frog (*Xenopus laevis*) were drawn in their entirety using a computer-supported tracing system (NTS5, Eutectics) which automatically performs 3-D reconstruction. We quantified neuronal shapes using 1) the mean opening angle of the dendrites, 2) vector length (quantifies directionality of projection), and 3) neuronal asphericity (describes dendritic tree shape). Vector length is the vector sum of individual branch segment vectors and neuronal asphericity was calculated using the invariants of the inertia tensor. We then performed cluster analysis with the Ward's minimum variance statistic. We distinguished four non-overlapping groups of neurons with statistically significant separation. This method differs substantially from 2-D description of neurons. It describes a restricted set of neuron types in quantitative, objective fashion. General application of this method will allow comparison of neuron types both within and across species.

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60.10

ORGANIZATION OF THE CAUDAL RHOMBENCEPHALIC ALAR PLATE IN THE URODELE AMPHIBIAN *PLEURODELES WALTL*: PRESENCE OF DORSAL COLUMN AND LATERAL CERVICAL NUCLEI A. González*, A. Muñoz, M. Muñoz, O. Marín, H. Vara, C. Sánchez-Camacho, and †H.J. ten Donkelaar. Dept. Cell Biology, Univ. Complutense, Madrid, Spain, and †Dept. Anat. & Embryol., Univ. Nijmegen, The Netherlands.

The cytoarchitecture, chemoarchitecture, and fiber connections of the caudal rhombencephalic alar plate were studied in the ribbed newt, *Pleurodeles waltl*. A cytoarchitectonic analysis revealed that the caudal medullary alar plate consists of poorly segregated inner and outer cell layers in which different cell populations could be (immuno)histochemically distinguished. The inner cell layer is formed dorsomedially by the nucleus of the solitary tract, characterized by the presence of tyroxine hydroxylase and calbindin D-28k positive neurons, and ventrolaterally by the nucleus of the descending trigeminal tract, identified by the presence of calbindin, NADPH-diaphorase and nitric oxide synthase positive neurons with dorsolaterally directed dendrites intermingled wih the fibers of the descending trigeminal tract.The outer cell layer at the obex level forms the dorsal column nucleus (DCN), whereas the ventrolateral part of this cell layer forms a lateral cervical nucleus (LCN). With anterograde and retrograde tracing the DCN and LCN were further delineated. Labeling of ascending dorsal root afferents showed that the dorsal column and the DCN are somatotopically arranged: lumbar primary afferents terminate on medial DCN neurons, whereas cervical primary afferents terminate on lateral DCN neurons. The LCN is densely innervated by the dorsolateral funiculus. Retrograde tracing showed extensive, predominantly contralateral, projections of both the DCN and LCN to the torus semicircularis and the ventral thalamus through the medial lemniscus. These data show that in urodeles, in addition to the direct ascending spinal projections, a dorsal column-medial lemnical projection via the DCN as well as the spinocervicothalamic system via the LCN would be involved in the transmission of somatosensory information to various supraspinal centers.

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60.11

DEVELOPMENT OF AFFERENT CONNECTIONS TO THE STRIATUM AND THE NUCLEUS ACCUMBENS IN THE ANURAN AMPHIBIAN *Xenopus laevis* WITH EMPHASIS ON THE DOPAMINERGIC PROJECTIONS. O. Marín*†, W.J.A.J. Smeets‡, M. Muñoz†, A. Muñoz†, C. Sánchez-Camacho†, H. Vara† and A. González†. †Dept. Cell Biology, Univ. Complutense, Madrid, Spain. ‡Graduate Sch. Neurosci., Dept. Anatomy & Embryology, Vrije Univ., Amsterdam, The Netherlands.

Recently, we have presented data about the afferent connections of the basal forebrain of adult anuran and urodele amphibians. To broaden our insight into the organization of the basal ganglia (BG) of amphibians, we have now studied the development of the inputs to the striatum and the nucleus accumbens, using biotinylated or Texas Red-conjugated dextran amines as retrograde tracers. In double labeling experiments, antibodies against tyrosine hydroxylase were used as marker of catecholaminergic structures. Already at *late embryonic stages*, the basal telencephalon received inputs from cells located in the amygdala, the suprachiasmatic nucleus, the raphe nucleus and the rhombencephalic reticular formation. At these stages, the rostral part of the posterior tubercle seems to be the only source of the dopaminergic input to the basal forebrain. During *premetamorphosis* not only a differentiation between afferents to the striatum and the nucleus accumbens could be made, but also new sources of inputs were detected in the pontomesencephalic reticular formation and the nucleus of the solitary tract. During *prometamorphic stages* a gradually increase occurs in the number of cells that project to the basal telencephalon. At the begining of the *metamorphic climax* the organization of the BG afferents resembles largely the pattern observed in juveniles and adults. Remarkably, during larval stages the cells that contribute to the dopaminergic innervation of the basal forebrain show a rostrocaudal gradient in time of apparence. Moreover, the dopaminergic fibers reach the striatum earlier that the nucleus accumbens, which is in agreement with the development of the telencephalon and the dopaminergic cell groups.

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60.12

OBSERVATIONS ON HABENULAR AFFERENTS IN RANID FROGS. T.J. Neary*. Department of Biomedical Sciences, Creighton University School of Medicine, Omaha, NE 68178.

Following applications of wheat germ agglutinin-horseradish peroxidase in the habenula of bullfrogs, R. catesbeiana, intensely labelled cells were seen bilaterally, with a slight ipsilateral preponderance, in the magnocellular preoptic nucleus and in the superficial ventral thalamic nucleus (Vs, nomenclature of Neary and Northcutt [1983]). Levine [1980] and others have demonstrated that the Vs (rostral visual nucleus in his nomenclature) receives a strong input from the retina. Together, these findings suggest that one or both of the habenular nuclei in ranid frogs may be strongly influenced by visual stimuli. The Vs lies lateral to the magnocellular preoptic nucleus and labelled cells were present as a "bridge" between the two populations. This suggests that the Vs may be derived from the preoptic area and not from the ventral thalamus, as hypothesized by Neary and Northcutt [1983].

Self-supported.

60.13

RHOMBOMERE IDENTIFICATION IN A DEVELOPING REPTILE. M.B. Pritz*. Sect. of Neurol. Surg., Indiana U. Sch. of Med., Indianapolis, IN 46202-5124.

Subdivisions of the hindbrain were investigated in Alligator mississippiensis embryos. Embryos were examined between Ferguson stages 8 and 14.5 (8 to 17 days after egg laying). Brains were dissected free from non-neural tissue, divided at the isthmus, and examined with a dissecting microscope from both the internal (ependymal) and external surfaces. Afterwards, hindbrains were embedded in gelatin, sectioned on a sliding microtome, and stained with cresylviolet or with antibodies to acetylated tubulin or vimentin or processed for peanut agglutinin histochemistry. Rhombomeres were visable at the earliest stage available, stage 8, became less distinct by stage 12.5, and disappeared by stage 14. Whole mount, unstained hindbrains accurately identified the presence and absence of rhombomeres which were confirmed by light microscopic analysis of sectioned tissue stained with cresylviolet, with antibodies to vimentin and acetylated tubulin, and by peanut agglutinin histochemistry. These observations in Alligator identify rhombomeres whose morphology is similar to those neuroepithelial subdivisions described in other vertebrates. Taken together, these data suggest that transient hindbrain segmentation is a feature common to all developing vertebrate brains.

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60.14

ULTRASTRUCTURAL ORGANIZATION OF NUCLEUS ROTUNDUS IN A REPTILE. D. M. Schroeder* and M. B. Pritz. Indiana University School of Medicine., Medical Sciences, Bloomington, IN 47405 and Section of Neurological Surgery, Indianapolis, IN 46202.

Nucleus rotundus is a prominent nucleus in the dorsal thalamus of reptiles. It receives retinal input via the optic tectum and projects to a well defined area of the telencephalon, the dorsal ventricular ridge. The present study examined nucleus rotundus in one species of reptiles, *Caiman crocodilus*, at the ultrastructural level.

Within the core area of nucleus rotundus, medium sized neurons are scattered among rich neuropil. A number of these neurons are clustered in groups and many are juxtaposed in such close proximity that their membranes appear to fuse. Within the neuropil there are many dendritic profiles densely surrounded with boutons and forming synaptic aggregations. This type of neuropil organization in *Caiman* is a distinguishing feature of dorsal thalamic nuclei of mammals. Most of these boutons contain spherical vesicles and only occasionally are boutons present with pleomorphic vesicles. As previously reported (Pritz, 1995), local circuit neurons are not present in nucleus rotundus of this species; thus the synaptic aggregations must consist primarily of the dendrites of the relay cells and the synaptic contacts from its afferents. Numerous unmyelinated axons are found throughout.

60.15

DISTRIBUTION OF CRF IMMUNOREACTIVE STAINING IN THE BRAIN OF *Anolis carolinensis*. Gary R. Ten Eyck*, Amy-Lacy Adams, John M. Matter, Chris A. Lowry and Cliff H. Summers. Dept. of Biology, University of South Dakota, Vermillion, SD 57069.

Immunoreactive staining to CRF was widely distributed in the CNS of the lizard *Anolis carolinensis*. Brains were frozen sectioned (-20°C) at 20 µm, then fixed with paraformaldehyde on slides. Polyclonal antibody against ovine CRF (Peninsula Laboratories), was applied (1:500) to the frozen sections and made visual by a reaction to an avidin-biotin 2° antibody with DAB. Staining was particularly heavy in medial (hippocampal) cortex and hypothalamus. Hypothalamic CRFir was evident as two vertical bands, parallel to each other and to the third ventricle, spanning from the nucleus accumbens to the posterior hypothalamus. The strongest staining of these bands was in the paraventricular nucleus. Some diffuse CRFir staining was evident in all hypothalamic nuclei. Extrahypothalamic CRFir staining occurred in medial cortex, septum, paleostriatum (amygdala), anterior dorsal ventricular ridge, nucleus accumbens, optic tectum, cerebellum, periaqueductal grey, and raphe nuclei. Anoline central CRFir distribution is similar to that seen in mammals. Hypothalamic, hippocampal, amygdalar, periaqueductal and raphe immunoreactivity for CRF is coincident with immune staining for serotonin and glucocorticoid receptors, suggesting a role in regulation of stress.
Supported by NSF grant OSR-9108773.

60.16

NADPH-DIAPHORASE NEURONS IN THE SUBSTANTIA NIGRA IN TURTLES. C. Kaeppner*, W. Iaccino, and A. S. Powers. Dept. of Psychology, St. John's University, Jamaica, NY 11439.

Neurons positive for NADPH-diaphorase (NADPH-d), a marker for nitric oxide, are seen in the midbrain tegmentum of turtles, in the region of substantia nigra. The substantia nigra of mammals, however, does not contain NADPH-d. The present study investigated whether the NADPH-d-positive cells in turtles are catecholaminergic and whether they project to the telencephalon. For immunohistochemistry, painted turtles (Chrysemys picta) were perfused intracardially with saline followed by 4% paraformaldehyde. Frozen sections (40µm) were incubated with an antibody against tyrosine hydroxylase (TH) and stained for immunofluorescence. TH-positive cells in the midbrain were photographed, and the tissue was subsequently stained for NADPH-d, using a standard histochemical technique. Again the tissue was photographed. Comparison of the two sets of photographs showed that virtually all of the TH-positive neurons in the substantia nigra contained NADPH-d. For tract tracing, Fluoro-Gold (FG) injections were made in the striatum of turtles. After 60 days survival, the animals were perfused with saline and 4% paraformaldehyde. The location of FG-positive cells in the midbrain was mapped and photographed. Then the tissue was stained for NADPH-d and re-photographed. Cells in the substantia nigra were double-labelled with FG and NADPH-d. These results show that the NADPH-d-positive cells of the midbrain tegmentum in turtles are the catecholamine-positive cells that project to the striatum, the equivalent of substantia nigra, pars compacta in mammals. Interestingly, this region contains NADPH-d in turtles but not in mammals. Interpretation of this finding awaits further study of the role of nitric oxide in the basal ganglia.
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60.17

CO-LOCALIZATION OF VASOTOCIN AND GALANIN IN THE BRAIN OF THE TURTLE *Chrysemys picta*. R. Goldberg*[1], J.Haldar[2], and A.S. Powers[1]. Depts. Of Psychol.[1] and Biol. Sci.[2], St. John's University, Jamaica, NY 11439.

Vasotocin (VT) is a peptide found in the brains of nonmammals. It shares a common structure with mammalian vasopressin (VP) but differs by one amino acid. Galanin (GAL) is a peptide found in both mammals and nonmammals. GAL and VP colocalize in the hypothalamus of many mammals. This study investigated the location of VT and GAL in the brain of the painted turtle *Chrysemys picta*. Preliminary experiments confirmed that our VP antibody stained the same cells as a VT antibody. Turtles were injected with colchicine (135µl in saline) and allowed to survive 3 days. They were perfused intracardially with saline followed by 4% formaldehyde. Brain sections (40µm) were incubated with an antibody against mammalian VP (made in guinea pig) and an antibody against human GAL (made in rabbit). They were washed and then incubated for 1 hr in a solution containing secondary antibodies, one against rabbit conjugated to rhodamine and the other against guinea pig conjugated to fluorescein. As has been shown before (e.g., Fernandez-Llebrez, et al.,1988; Smeets et al., 1990), VT-positive cells were found mainly in the supraoptic nucleus (SON), the paraventricular nucleus (PVN), and the bed nucleus of the stria terminalis. Also as has been shown before (Jiminez, et al.,1994), GAL-positive cells were found only in a few restricted locations within the hypothalamus, but GAL-positive fibers were widely distributed throughout hypothalamic and extrahypothalamic areas. Some but not all cells of the SON and PVN were positive for both VT and GAL. Dense fibers positive for both were seen in the hypophysis. These findings are important because they show conservation over evolution and suggest that those functions mediated by GAL may be the same in reptiles as they are in mammals.
Supported by NIH grant # NS-31785 to ASP.

LEARNING AND MEMORY: PHARMACOLOGY I

61.1

DIFFERENTIAL EFFECTS OF ACUTELY ADMINISTERED ETHANOL ON SPATIAL AND NON-SPATIAL COGNITIVE PROCESSING. P.E. Simson, D.B. Matthews, & P.J. Best*. Miami University, Center for Neuroscience Research and Dept. of Psychology, Oxford OH, 45056.

Acute administration of ethanol selectively impairs spatial memory (Matthews, Simson & Best, 1995) and spatial learning (Vandergriff, Matthews, Simson & Best, 1995) in a dose-dependent manner. This impairment is remarkably similar to that found following lesions to the hippocampal system (O'Keefe & Nadel, 1978; Jarrard, 1986). Lesions to the hippocampal system also often facilitate learning/performance on non-spatial learning and memory tasks (M'Harzi, Jarrard, Willig, Palacios & Delacour, 1991; White & McDonald, 1993; Matthews & Best, 1995; White, Matthews & Best, 1995). However, it has yet to be determined if acute administration of ethanol can similarly facilitate non-spatial cognitive processing.

In the first experiment, we investigated the effect of acutely administered ethanol on spatial memory in a task that does not require behavioral generalization by the animal during testing. In the second experiment, we investigated the effect of ethanol on non-spatial cognitive processing.

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61.2

THE EFFECTS OF ETOH ON SPATIAL AND NON-SPATIAL WORKING MEMORY IN THE RAT. A.M. White*, P.E. Simson and P.J. Best. Dept. of Psychology and Center for Neuroscience Research, Miami Univ., Oxford, OH 45056.

In rodents, the acute administration of ETOH leads to a pattern of learning and memory impairments similar to those that follow hippocampal system damage (Matthews et al., 1995). Jarrard et al. (1987) report that hippocampal lesions impair performance on a spatial, but not non-spatial, delayed match-to-sample task on a radial-arm maze. The purpose of the present experiments is to assess the effects of ETOH on a similar set of tasks. Subjects were trained on either a spatial or a non-spatial delayed match-to-sample task on a six-arm radial-arm maze. Ten trials were conducted during each day of training, the first of which was a forced trial. In the spatial task, subjects were required to remember the spatial location of the food reward received during the forced trial and to return to that location during nine subsequent free-choice trials. The spatial location of the food reward changed across days. In the non-spatial task, five of the six maze-arms contained salient intramaze cue inserts. Subjects were required to remember which cue insert was located on the maze-arm visited during the forced trial, and to enter the arm containing that cue during the nine subsequent free-choice trials. The spatial location of the cue changed across trials and the cue signalling the goal arm changed across days. Ethanol (0.0 g/kg, 0.75 g/kg, 1.5 g/kg & 2.0 g/kg) caused a dose dependent impairment in performance on the spatial task.
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61.3

INHIBITION OF CHOLESTEROL SYNTHESIS BLOCKS ASSOCIATIVE LEARNING. W.T.O'Brien *[1,3], G.Xu [2,3], G.Salen [2,3] & R.J. Servatius [1,3]. [1] Depts. of Neuroscience (Graduate School of Biomedical Sciences), and [2] Medicine, New Jersey Medical School, Newark, New Jersey and [3] DVA Med.Cen. East Orange, N.J. 07018

Administration of BM15.766 (BM) blocks the last enzymatic step of cholesterol (CHOL) biosynthesis leading to a decrease in brain, liver and plasma CHOL and an increase in the immediate precursor, 7-dehydrocholesterol. This biochemical profile resembles that of children with Smith-Lemli-Opitz (SLO) syndrome, a congential disorder associated with severe mental retardation. Using the classically conditioned eyeblink response (CCER) paradigm, we assessed new motor learning in just weaned rats fed BM (n=3) by gavage for 4 mos, rats given exongeous CHOL during the last two months of BM treatment (n=3), and non-treated controls (n=5). For conditioning, a 600 ms white noise conditioned stimulus coterminated with a 100 ms periorbital shock. The rats were given 300 trials. Treatment with BM or BM+CHOL did not alter either the spontaneous blink rate or sensory reactivity to the to-be-conditioned stimulus. The BM treated rats failed to learn the CCER after 300 trials. The BM+CHOL treated rats exhibited only a slight learning impairment, evident in the first 20 trials. Chronic treatment with a cholesterol synthesis inhibitor blocked acquisition of the CCER, while exogenous CHOL may be useful as a counter treatment. Just-weaned rats treated with CHOl-synthesis inhibitors may be appropriate for use as an animal model for Smith-Lemili-Opitz. Supported by GSBS-UMDNJ and DVA Med. Research Funds and PHS grants HL17818, HL18094, DK26756.

61.4

APOPTOSIS IN RAT FETAL HIPPOCAMPUS AND PHEOCHROMOCYTOMA (PC 12) CELLS IS INDUCED BY CHOLINE DEFICIENCY. M. Holmes-McNary, R. Loy*, M-H. Mar, and S. Zeisel. Department of Nutrition, University of North Carolina, Chapel Hill, NC, 27599-7400 and *Department of Neurology, University of Rochester, Rochester, NY, 14620.

Choline deficiency (CD) causes DNA damage, many biochemical changes and cell death in hepatocytes. We now know that cell death in these hepatocytes is due to an apoptotic process. In order to understand this process and the generality of the effects of CD, we investigated the effects of CD on the rat pheochromocytoma (PC12) neuronal cell line, which express neuronal-like properties, and in whole rat brain. PC12 cells were plated in choline-free DMEM/F12 supplemented with 15% serum for 2 days, then cultured in serum-free N2 media with 70μM choline chloride (N2-CS) or without choline chloride (N2-CD) for 2 days. Cell viability, by exclusion of trypan blue, was 86% for cells in N2-CS and 73% for cells in N2-CD. Cells maintained in N2-CD showed an increase in DNA strand breaks (TUNEL method). Classical apoptotic bodies were detected in N2-CD cultured cells (25%) compared to cells in N2-CS (10%). Cleavage of DNA into 200 bp internucleosomal fragment sizes in PC-12 cells was studied by gel electrophoresis. We used an *in-vivo* model to determine the dietary effects of CD on the neuronal cell types in fetal rat brain. Pregnant rats were fed a CS or a CD diet for 6 days and embryonic day 18 fetal brain slices were analyzed for DNA strand breaks (TUNEL method). In fetal rat hippocampus CD caused DNA strand breaks and apoptosis in neuronal-type cells like the response of PC-12 cells. These results suggest that CD induces apoptotic cell death in neuronal-like cells and fetal rat brain.

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61.5

DEVELOPMENTAL CHANGES IN HIPPOCAMPAL STAINING FOR ACETYLCHOLINESTERASE FOLLOWING PRENATAL CHOLINE TREATMENT. R. Loy, D.D. Heyer and K. Maguire-Zeiss*. Department of Neurology, University of Rochester, 435 E. Henrietta Road, Rochester, NY 14620.

Choline supplementation *in utero* results in a long-lasting facilitation of spatial memory and a variety of correlated brain changes, including enhanced hippocampal LTP, elevated levels of hippocampal NGF, an increase in the size of diagonal band neurons and their content of mRNA for low affinity neurotrophin receptor, and alterations in phospholipase D, choline acetyltransferase and acetylcholinesterase (AChE) activities. Behavioral improvement in memory capacity is apparent early postnatally, as are decreases in AChE activity. To determine if these changes could be localized anatomically, male rats were treated *in utero* from embryonic day 12-17 (300 mg/kg/day choline chloride, *p.o.* to the dam) with supplemental choline or control sacharine solution and raised to either postnatal day 16 or 90, then anesthetized and perfused with formalin. Frozen, coronal brain sections were stained histochemically for AChE. Staining in the hippocampus and dentate gyrus was significantly reduced in laminae corresponding to septal terminal fields in choine-treated rats compared to controls at postnatal day 16. There also appeared to be fewer AChE-positive interneurons, particularly in the temporal dentate hilus and CA3. By contrast, at postnatal day 90, there was no longer a significant difference between choline-treated and controls, either in the density or the pattern of staining. Thus, choline-induced changes in cholinergic activity may develop early but show compensation later in life. Supported by AG09525.

61.6

PRENATAL CHOLINE AVAILABILITY ALTERS THE POSTNATAL DEVELOPMENT OF ACETYLCHOLINESTERASE (AChE) AND CHOLINE ACETYLTRANSFERASE (ChAT) ACTIVITIES IN RAT HIPPOCAMPUS. J.M. Cermak*, T. Holler and J.K. Blusztajn. Boston Univ. Sch. of Med., Boston, MA 02118.

Choline supplementation during embryonic days (E) 12-17 permanently enhances performance on visuospatial memory tasks in rats. In order to characterize the neurochemical mechanisms that may mediate this effect, we investigated the development [postnatal days (P) 1, 3, 7, 17, 27, 35, 90] of ChAT and AChE activities in the rat hippocampus, cortex, and striatum as a function of prenatal choline availability. During E11 through E17 Sprague-Dawley pregnant rats received a choline-deficient, control, or choline-supplemented diet. The average choline intake was 0, 1.3, and 4.6 mmol/kg/day, respectively. Small, but significant changes in hippocampal ChAT activity were observed on P17 and P27 only. ChAT activity in hippocampal homogenates was higher in choline-deficient and lower in choline-supplemented male rats compared to controls ($p<0.05$). AChE activity in hippocampal homogenates was higher (by 10-30%) in choline-deficient and lower (by 10-15%) in choline-supplemented rats compared to controls on P7, 17, 27 and 35 ($p<0.001$). Prenatal choline availability had no effect on striatal and cortical AChE and ChAT activities. The reductions in hippocampal AChE activity in choline-supplemented rats are consistent with the notion that decreased degradation of hippocampal acetylcholine contributes to the enhanced memory performance in these animals. (Supported by AG09525)

61.7

PRENATAL AND PRE+POSTNATAL CHOLINE SUPPLEMENTATION REDUCES THE THRESHOLD TO INDUCE LONG-TERM POTENTIATION IN HIPPOCAMPAL SLICES FROM ADULT RATS. J.P. Jones*[1], W.H. Meck[1], C.L. Williams[1], & H.S. Swartzwelder[1,2,3], Departments of Psychology: Experimental[1], and Psychiatry[2], Duke University, Durham, NC 27708 and Veterans Administration Medical Center[3], Durham, NC 27710.

Perinatal choline supplementation has been found to increase the capacity and precision of visuospatial memory throughout adulthood, and to reduce the detrimental effects of normal aging on memory. In this study we investigated possible changes in the induction of long-term potentiation (LTP) in hippocampal slices from adult male rats (6-8 mo) following a combination of both pre- and postnatal choline supplementation, choline deficiency, or control procedures (ED 9 – PD 30). Our data indicate that the threshold for initiation of LTP, as well as LTP magnitude following theta-burst stimulation trains in area CA1 were enhanced in hippocampal slices from choline supplemented rats compared to controls and deficients. We also investigated the effects of prenatal choline supplementation, choline deficiency, or control procedures (ED 12 – 17) in the initiation of LTP in older adult male rats (12 mo). These data also showed a significant reduction in the threshold for inducing LTP even with the reduced time period of treatment, coupled with possible age-related alterations in hippocampal LTP. Taken together, these electrophysiological data support the hypothesis that choline availability to the developing fetus during periods of neurogenesis and synaptogenesis enhances the subsequent initiation of hippocampal LTP. These alterations in synaptic plasticity may underlie the observed differences in memory capacity between control and perinatally choline-supplemented rats.

(Supported by AG09525)

61.8

MODULATION OF LEARNING AND NEURONAL MEMBRANE COMPOSITION IN THE RAT BY ESSENTIAL FATTY ACID PREPARATION: TIME COURSE ANALYSIS. Shlomo Yehuda[1]*, Sharon Rabinovitz[1] and David I. Mostofsky[2] [1]Psychopharmacology Laboratory, Department of Psychology, Bar Ilan University, Ramat Gan, Israel 52900, and [2]Department of Psychology, Boston University, Boston, MA 02215, USA.

Previous studies have shown that chronic administration of SR-3 (a 1:4 mixture of α-linolenic and linoleic acid) affects spatial learning, thermoregulation, pain threshold and protection from seizures. The mode of action is unknown. One possible explanation is that the preparation induces changes in the fatty acids profile and in the cholesterol level in the neuronal membrane.

This study used 15 independent groups of rats (n=12) which were given either saline, mineral oil (vehicle) or SR-3 (25 mg/kg) for 0, 1, 2, 3 or 4 weeks. The learning capacity was measured in the Morris Water tank and the fatty acids profile and the cholesterol level were examined by the GC method in synaptosomes obtained from the frontal cortex of the rats.

SR-3 improved the learning capacity and induced major changes in the neuronal membrane composition, such as an increase in the total level of fatty acids, an increase in the level of essential fatty acids and a decrease in the cholesterol level. Those changes occurred after 3 weeks of treatment. The biochemical variables can predict the behavioral variables but not vice versa. The changes in the neuronal membrane may result in a modification of the membrane fluidity, which may, in turn, enhance cognitive and neuropharmacological effects.

Supported by Ginzburg Chair and The Farber Center.

61.9

EFFECTS OF MAGNESIUM ON LEARNING AND MEMORY: A CELLULAR AND BEHAVIORAL STUDY, H. Lu*, G. Wang[1] and F. Wu. Lab. of Neurobiology, Nanjing Univ. Nanjing 210093, China, Biology Department, Nanjing Normal Univ.[1] Nanjing 210024, China

Many works have revealed that magnesium to be involved in long-term potentiation(LTP) in the hippocampus, a form of synaptic plasticity which is believed to be involved in learning and memory. However, there is little direct evidence showing the effects of magnesium on learning and memory. Using Y-maze discrimination task and one trial step-through passive avoidance task respectively, we found the discrimination learning capability and the retention of passive avoidance behavior of mice were significantly attenuated after the treatment of $MgCl_2$ for two weeks. The plasma and hippocampal magnesium levels increased as a result of magnesium exposure. In parallel with this, the post-synaptic density(PSD) in both of the hippocampal CA3 area and cerebral sensorimotor cortex became significantly thinner and the length of synaptic active zone in the cerebral sensorimotor cortex was obviously shortened. Furthermore, the hippocampal synaptosome of mice with long term magnesium treatment showed greatly reduced uptake of $^{45}Ca^{2+}$. These findings suggest that high levels of magnesium induced learning and memory disorders which are associated with the changes in PSD and the length of synaptic active zone and decreased calcium influx into the hippocampal nerve terminals. (Supported by Excellent Young Teacher Project of Jiangsu Provincial Education Committee, China)

LEARNING AND MEMORY: PHARMACOLOGY II

62.1

NITRIC OXIDE SYNTHASE INHIBITION IMPAIRS DELAYED RECALL IN MONKEYS, SPATIAL LEARNING IN RATS, AND INDUCES CONDITIONED TASTE AVERSION. W.J. Jackson*, M.A. Prendergast, A.V. Terry, Jr., and J.J. Buccafusco. Medical College of Georgia, Alzheimer's Research Center, Dept. Physiol. Endo.., Dept. Pharm. Tox., and Dept. Veterans Affairs Med. Ctr., Augusta, GA 30912.

The free radical gas nitric oxide is synthesized by NO synthase (NOS) in brain regions associated with learning and the formation of memory and appears to function as a neuromodulator of these processes. We examined the effects of peripheral administration of the NOS inhibitor L-Name on delayed recall in 7 mature macaques, and on spatial navigation learning in adult rats. In monkeys, doses of L-Name including 1, 5, 25, and 50 mg/kg each produced impairment of delayed matching-to-sample accuracy. The task impairment produced by the 25 mg/kg dose of L-Name was blocked by concomitant administration of an equi-mol dose of L-arginine. For the 50 mg/kg dose only, task impairment occurred concomitantly with significant gastrointestinal disturbance and lethargy. In rats, L-Name (5, 20, and 50 mg/kg) impaired acquisition of cued spatial learning in the Morris water maze, although even in the presence of L-Name, the animals eventually learned the task. To examine the aversive properties of L-Name administration in rats, a two-bottle conditioned taste aversion (CTA) trial was employed with water-deprived rats. The low dose of L-Name (5mg/kg) did not induce a CTA to the novel 20% sucrose solution employed. Both the 20 mg/kg and 50 mg/kg did, however, induce CTA following an initial pairing of the sucrose taste and drug administration. Consumption in both groups was decreased by 50-60% during repeated exposures to the solution. Both the spatial learning deficits and CTA were blocked by co-administration of L-arginine. These data indicate that L-Name produces deficits in the cognitive performance of both monkeys and rats that are associated with attenuated biosynthesis of NO. At higher doses (>20-25mg/kg), however, inhibition of task performance may occur along with drug-induced malaise, possibly related to peristaltic dysregulation of gastrointestinal musculature. Supported by the VA Medical Research Service

62.2

INVOLVEMENT OF THE NITRIC OXIDE/CYCLIC GMP SYSTEM IN OBJECT RECOGNITION MEMORY OF THE RAT. J. Prickaerts, J. de Vente, W. Raaijmakers and H. W. M. Steinbusch*. European Graduate School for Neuroscience in Brain and Behavior, University of Maastricht, P.O. Box 616, 6200 MD Maastricht, The Netherlands

Recently, several studies have suggested a possible role for the nitric oxide (NO)/cGMP system in memory processes. Thus, NO is a retrograde messenger which increases cGMP levels by activating soluble guanylyl cyclase. The effects of 7-nitro indazol (7-NI), a NO synthase inhibitor, and zaprinast (ZAP), a cGMP-selective phosphodiesterase inhibitor, were evaluated on recognition memory of rats in the object recognition task. This task is based on the differential exploration between a new and a familiar object. Two doses of 7-NI (10 and 30 mg/kg) and ZAP (3 and 10 mg/kg) were used. The substances were administered i.p. immediately after the exposure to two identical objects, i.e., at the start of the retention interval. After a retention interval of 1 h, saline-treated rats spent more time exploring the new object which demonstrates that they recognized the familiar one. Both doses of 7-NI impaired the discrimination between the two objects after the 1 h interval. After a 4 h interval, saline-treated rats did not discriminate between the objects. The highest dose of ZAP facilitated object recognition after the 4 h interval. Further, this dose of ZAP (10 mg/kg) reversed the recognition deficit of 7-NI (10 mg/kg) at the 1 h interval. Interference with the NO/cGMP system can also induce cardiovascular effects which may influence memory performance. The highest dose of 7-NI slightly increased mean arterial blood pressure (MAP) 1 h after its administration. Four hours after administration of ZAP (10 mg/kg), MAP was also slightly increased, yet not 1 hour after ZAP administration. However, these effects on blood pressure seem not to be related to or explain the differential effects on object recognition memory. These results therefore indicate that the NO/cGMP system is involved in memory processes of object recognition independently of its cardiovascular role.

62.3

INHIBITION OF NEURONAL NITRIC OXIDE SYNTHASE IMPAIRS LEARNING OF RATS IN A 14-UNIT T-MAZE. R.C. Meyer*, E.L. Spangler, N. Patel, and D.K. Ingram. Gerontology Research Center, NIH/NIA, Baltimore, MD 21224.

Nitric oxide (NO), a putative retrograde messenger of glutamatergic synaptic transmission, has been suggested to play a role in establishing LTP (Schuman & Madison, Science, 254:1503, 1991). We have shown previously that the NO synthase (NOS) inhibitor N^G-nitro-L-arginine (NARG) produces dose-dependent learning impairment in rats in a 14-unit T-maze, and that this impairment is attenuated by sodium nitroprusside (SNP), a NO generator (Ingram, Ann. NYAS, 717:16, 1994). However, it is possible that hypertension produced by NARG contributed to the impaired performance. In two new experiments, we have investigated 7-nitro-indazole (7-NI), a NOS inhibitor that is selective for neuronal NOS (nNOS) and does not produce hypertension (Moore et al. Brit. J. Pharmacol. 108:296, 1993). In addition, we tested the NO donor molsidomine (MOL) which is much longer acting than SNP (Vertesi et al. J. Cardio. Pharmacol. 17:3, 1991). Subjects were 3-4 mo old male Fischer-344 rats. Rats were trained to avoid a shock in a straight runway, and 24 hr later received training in the 14-unit T-maze. **Experiment 1**: 30 min prior to maze training, rats were given i.p. injections of either 7-NI (25, 50, or 65 mg/kg) or saline. The 7-NI produced significant learning impairment in a dose-dependent manner. **Experiment 2**: 30 min prior to maze training, rats were given i.p. injections of either 7-NI (70 mg/kg), 7-NI (70 mg/kg) plus MOL (2 or 4 mg/kg), or saline. Both doses of MOL significantly attenuated the learning deficit induced by 7-NI. These results suggest an involvement of NO in memory formation, and would support the hypothesis that NO acts as a retrograde messenger to enable synaptic strengthening during learning.

62.4

INTRA-SEPTAL INFUSIONS OF GLUCOSE DO NOT REVERSE BUT POTENTIATE INHIBITORY AVOIDANCE DEFICITS WHEN CO-INFUSED WITH THE GABA AGONIST MUSCIMOL. M.B.Parent* & P.E.Gold. Dept. of Psychology, University of Virginia, Charlottesville, VA 22903.

Systemic infusions of glucose, as well as direct brain injections into the medial septum, hippocampus, and amygdala enhance retention and reverse memory deficits produced by aging, neural pathologies, and some drugs. However, in examining interactions between glucose and the GABA agonist muscimol, we noted that intra-septal infusions of a high dose of glucose did not reverse and instead appeared to potentiate the memory-impairing effects of intra-septal muscimol infusions. Because hyperglycemia increases GABA release in some brain regions (e.g., substantia nigra, Amoroso, et al., 1990), we hypothesized that glucose may potentiate the impairing effects of muscimol by increasing endogenous GABA release in the medial septum. We predicted that a dose of muscimol that has no effect on memory should impair when co-infused with a high dose of glucose. Different groups of rats were given an intra-septal infusion of vehicle, glucose (33 nmol), muscimol (3 nmol), or glucose co-infused with muscimol 15 min prior to one trial inhibitory avoidance training (1 mA, 1 sec). On a retention test 24 hr later, the retention performance of rats given infusions of muscimol or glucose alone was not impaired. However, infusions of muscimol with glucose produced a robust deficit. Although glucose typically reverses memory deficits, these results indicate that glucose potentiates the memory-impairing effects of a GABA agonist, perhaps by increasing endogenous GABA levels. Supported by NIA (AG07648, AG05658) & NINDS (NS32914).

62.5

EFFECT OF GLUCOSE ON MEMORY IN HUMANS: DOSE-DEPENDENT FACILITATIVE ACTION ON LONG-TERM MEMORY. C. Messier*, A. Desrochers and M. Gravel. School of Psychology, University of Ottawa, (Ont) Canada K1N 6N5.

The memory-improving effect of glucose has been demonstrated in rodents and humans. In rodents, this facilitative effect has been observed for two doses: 100 mg/kg and 3 g/kg. In the present experiment, we examined the effect of water, saccharin and 7 doses of glucose (10,100,300,500,800,1000,2000mg/kg) on the performance of young women. The memory task consisted in the immediate recall of 8 lists of 20 words presented on a computer screen at a rate of one word/2 sec. The lists were presented randomly and were equivalent for imagery index, subjective and objective frequency, word lenght and syllable count. Subjects arrived fasted in the morning and their baseline memory performance was tested using the first two lists. They then ingested the solution and were tested using the remaining 6 lists. The word recall rate was computed for the first 5 words of each list (primacy effect), the last 5 words (recency effect) and the remaining 10 words in the middle of each list. The results showed that for the 2 pre-treatment lists, subjects better recalled the first 5 words and the last 5 words compared to the remaining 10 words. In the water and saccharin groups, the primacy effect gradually disappeared from list 3 to 8. The ingestion of glucose (100, 1000 and 2000 mg/kg) prevented this disappearance while the other doses of glucose did not. The results show that the same 2 optimal doses are effective in humans and that glucose appears to modulate long-term memory processes. Supported by a Natural Sciences and Engineering Research Council grant to C.M. and by the U. of Ottawa.

62.6

INTRA-HIPPOCAMPAL PERFUSIONS OF GLUCOSE ENHANCE SPONTANEOUS ALTERNATION SCORES AND HIPPOCAMPAL ACETYLCHOLINE RELEASE. S.N.Pal, M.E.Ragozzino, K.E.Unick, A.C.Durham, M.R.Stefani & P.E.Gold*. Department of Psychology, University of Virginia, Charlottesville, VA 22903.

Recent evidence indicates that hippocampal acetylcholine (ACh) output increases while rats are tested in a spontaneous alternation task. Systemic injections of glucose improve alternation scores and augment the accompanying increase in ACh output. The present experiment determined whether direct administration of glucose into the hippocampus would improve alternation scores and would, at the same time, augment the increase in ACh release in the hippocampus assessed during behavioral testing. Guide cannulae for microdialysis probes were implanted unilaterally in the hippocampus. At the time of testing, a microdialysis probe was inserted and 10 dialysate samples (20 µl in 12 min) were collected for analysis for ACh content by HPLC-EC detection. The first five samples provided baseline measurements. For glucose treatment, the dialyzing solution was changed from aCSF to 6.6 mM glucose in aCSF for two samples, beginning 12 min prior to spontaneous alternation testing in a plus maze. In the absence of administration of extra glucose, ACh output increased during spontaneous alternation testing. Unilateral delivery of glucose into the hippocampus through the microdialysis probe improved spontaneous alternation scores and also augmented the extent of increase in ACh output seen during behavioral testing. As observed before, administration of glucose did not result in changes in ACh output in control (resting) rats. Some rats were prepared with bilateral probes to determine whether the rise in ACh output after glucose administration was bilateral or only ipsilateral to the site of infusion. Importantly, unilateral glucose administration did not affect behavior-induced ACh release in the contralateral hippocampus. These findings suggest that increases in extracellular glucose availability augment ACh release in a manner correlated with regulation of memory processing. *Supported by NIA (AG07648) and NINDS (NS32914).*

62.7

GLUCOSE VS. AMPA RECEPTOR MODULATORS: COMPARISONS BETWEEN FACILITATORY EFFECTS ON MEMORY AND LTP INDUCTION IN THE FREELY MOVING RAT. U. Staubli*, F. Xu & J. Scafidi. Center for Neural Science, New York University, 4 Washington Place, New York, NY 10003.

Considerable evidence from this and other laboratories indicates that a class of allosteric AMPA receptor modulators (AMPAkines), which cross the blood-brain barrier and facilitate glutamatergic transmission and hippocampal LTP induction, are highly effective at promoting the encoding and retention of memory when given before learning. This behavioral facilitation is dose-dependent and observed across a broad range of paradigms, with the threshold for synaptic facilitation corresponding to that for memory enhancement. A different strategy for memory modulation is suggested by the work of others indicating that systemic glucose injections, given before or shortly after training, are capable of enhancing some forms of learning. The objective of the present experiment was to (1) replicate the glucose-mediated memory enhancement, using paradigms identical to those in which memory is routinely facilitated by AMPAkine treatment, and (2) determine possible interactions between effective glucose dosages and LTP induction in area CA1 *in vivo*. Well trained animals (n=22) were tested on multiple days with each of three concentrations of glucose (100, 200 and 300 mg/kg) or saline, given 30 min before or 30 sec after the sample phase in spatial and olfactory working memory paradigms. Glucose given post-sampling failed to promote retention in either task, whereas pre-sampling glucose did produce an effect, but only in the spatial task: animals injected 30 min pre-sampling at 200 mg/kg, but not 100 or 300 mg/kg, made significantly more correct choices in an 8-arm radial maze before entering any of the 5 arms already sampled 3 hours earlier; the total # of re-entry errors to task completion was however not reduced by glucose. Despite its facilitatory action on correct choices before 1st error, glucose at 200 mg/kg given 30 min before LTP induction in awake rats failed to produce more or longer-lasting potentiation than saline injections.

(supported in part by Whitehall F93-17, Whitehead, and Cortex Pharmaceuticals, Inc.).

62.8

DEFICITS IN SPONTANEOUS ALTERNATION PERFORMANCE FOLLOWING INFUSION OF MORPHINE INTO THE MEDIAL SEPTUM ARE REVERSED BY INFUSION OF GLUCOSE INTO THE AMYGDALA. E. C. McNay* & P. E. Gold, Dept. of Psychology and Neuroscience Graduate Program, University of Virginia, Charlottesville, VA 22903.

The amygdala and the septohippocampal system appear to mediate functionally distinct memory systems. Previous work in our laboratory has shown that morphine infusions into the medial septal cause deficits in both spatial and aversive learning while morphine infusions into the amygdala cause a deficit in aversive learning but have no effect on spatial learning. In these cases, deficits caused by morphine can be reversed by co-infusion of glucose into the same brain area. In this study, we investigated whether deficits caused by infusion of morphine into one brain area could be reversed by infusion of glucose into a separate area. Three guide cannulae, aimed at the medial septum and bilateral amygdala, were implanted in rats at least one week prior to behavioral testing. Thirty minutes prior to test on a Y-maze spontaneous alternation task, the rats received an infusion into the medial septum of either morphine (4.0 nmol) or aCSF and simultaneous infusion of glucose (18.3 nmol) or aCSF bilaterally into the amygdala. In comparison with both unoperated and control animals, rats receiving septal morphine were impaired. This impairment was fully reversed by infusions of glucose into the amygdala. These results suggest that glucose injections into the amygdala can modulate spatial, as well as aversive, learning, demonstrating functional interactions between memory systems.

Supported by NIA (AG07648) and NINDS (NS32914).

62.9

SEXUAL STEROIDES HORMONES EFFECTS ON ALTERATION OF MEMORY CAUSED BY OZONE IN RATS. S.Rivas-Arancibia*, S. Schneider-Rivas; A. Lechuga-Guerrero; G. Borgonio-Pérez and A. Fuentes. Departamento de Fisiologia. Facultad de Medicina. Universidad Nacional Autonoma de Mexico.

A free radicals hypothesis for neurodegeneration implicates that the oxidative stress as a contributing factor to aging alterations and the neurodegenerative diseases. The exposure to ozone increased production of free radicals which causes oxidative stress. The effect of the ozone concentration 0.3 ppm for 4 hours, cause long-term memory deterioration when the animals were submitted to passive avoidance conditioning. This work analyses the role of the sexual hormones and their effect on the alteration of memory in animals exposed to ozone. They were individually housed with free access to food and water and divided in six groups and each group received one of the following treatment: 1) without treatment (control), 2) ozone exposure; 3)Testosterone enanthate (20 mg, i.m) five minutes before the ozone exposure, 4) Testosterone (20 mg, i.m) five minutes after the ozone exposure, 5) estradiol valeriate (0.8 mg, i.m) five minutes before the ozone exposure, 6) estradiol valeriate (0.8 mg, i.m) five minutes after the ozone exposure. All group were submited on a one-trial passive avoidance conditioning. Long-term-memory results indicate that the control goup is significantly different from group exposed to ozone and estradiol. The testosterone groups didn't show statistically significant difference with control group and the ozone group is significantly different from the estradiol group. This results suggest that long-term memory deterioration caused by ozone exposure can be bloked by the use of testosterone applied before or after exposure to ozone. The estradiol increase the alteration of long term-memory provoked by ozone exposure.

62.10

DHEAS ENHANCES SPATIAL MEMORY AND HIPPOCAMPAL PRIMED BURST POTENTIATION D.M. Diamond[1,2]*, B.J. Branch[1], K. Coleman-Mesches[1], M.H. Mesches[1] and M. Fleshner[3]. Dept. of Pharmacology[1], Univ. Colorado. Health Sci. Ctr. and VAMC[2], Denver, Dept. of Psychology[3], Univ. Colorado., Boulder

Dehydroepiandrosterone sulfate (DHEAS) serves a protective role in a broad range of physiological systems. Work in the past decade has shown that the brain synthesizes DHEAS *de novo*, with high levels of the steroid taken up by the hippocampus (*J Steroid Biochem Mol Biol,* 37:395, 1990). We recently reported that DHEAS enhanced primed burst (PB) potentiation, a form of LTP (*Neurosci Lett*, 202:204, 1996). The focus of this study was to test the hypothesis that DHEAS would enhance hippocampal-dependent spatial memory.

Male adult rats were administered DHEAS in their drinking water (range 50-400 µg/ml) for 5 days prior to, and during, water maze training (4 trials/day). Subjects receiving lower doses (50 & 100 µg/ml), but not the higher doses (200 & 400 µg/ml), exhibited a significant enhancement of memory. We then recorded PB potentiation in two of the groups of animals which were run in the water maze (50 & 400 µg/ml). Both groups developed significantly greater PB potentiation than the control group. These findings indicate that DHEAS can enhance cognitive and electrophysiological measures of hippocampal function.

Supported by PHS T32-AA07464, the VA and ONR

62.11

EFFECTS OF DHEAS ON HIPPOCAMPAL ELECTROPHYSIOLOGY *IN VITRO*. M.H. Meschesl[1]*, C.J. Frazier[1,2], G.M. Rose[1,2,3] and D.M. Diamond[1,3] Dept. of Pharmacology[1] and Neurosci. Training Program[2], Univ. Colorado Health Sci. Ctr. and VAMC[3], Denver, CO

Dehydroepiandrosterone sulfate (DHEAS) is the most abundant adrenal steroid produced by people, is synthesized *de novo* in the brain and can improve memory. We recently reported that DHEAS produced an inverted-U enhancement of hippocampal primed-burst (PB) potentiation, a form of LTP, *in vivo* (Neurosci. Lett., 202:204-9, 1996). Here, we have begun to investigate the mechanisms by which DHEAS affects hippocampal electrophysiology, plasticity and memory.

We have studied the effects of DHEAS on CA1 in hippocampal slices from male rats. Preliminary findings indicate that DHEAS reduces both feedforward and feedback inhibition to increase the excitability of CA1, which may be related to the antagonism of $GABA_A$ receptors by this neurosteroid (Prog. Neurobiol., 38:379, 1992). A 10 min application of DHEAS (10-100 μM) significantly increased the amplitude of the CA1 population spike for at least 30 minutes. We will present work designed to characterize the possible relationship between this effect and the DHEAS-induced enhancement of hippocampal PB potentiation and spatial memory described in the adjacent poster.

Supported by PHS T32-AA07464, AG10755, the VA and ONR

62.12

PREVENTION BY APAMIN AND CHARYBDOTOXIN OF AMNESIA INDUCED BY K^+-CHANNEL OPENERS IN MICE. A. Bartolini, C. Ghelardini, N. Galeotti, ^A. Quattrone and ^S. Capaccioli Depts of Pharmacology and ^Pathology, I-50134 Florence, Italy, SPON: Eur. Neurosci. Association*

Etcheberrigaray et al. (1993) reported that 113-pS tetraethylammonium (TEA)-sensitive K^+-channel was functionally absent in fibroblasts from Alzheimer disease (AD) patients and in human fibroblasts treated with β-amyloid, the main constituent of neurite plaques in AD (Etcheberrigaray et al., 1994). More recently, Bartolini et al. (1995) observed that mice treated with the K^+-channel openers, pinacidil (2.5-25 µg per mouse i.c.v.) and minoxidil (10 µg per mouse i.c.v.), injected immediately after the training session caused a reduction of memory retention of 31-66% and 68% respectively. K^+-channel regulation appears, therefore, to be one of the critical steps in memory storage. The aim of the present study was to investigate the effects of two K^+-channel blockers, apamin and charybdotoxin, on amnesia induced by K^+-channel openers. The experimental procedure consisted of a mouse passive avoidance test in which a painless punishment (fall into cold water, 10°C) was used. In these experimental conditions, apamin and charybdotoxin, at the respective doses of 1 ng and 1 µg per mouse i.c.v. administered 20 min before the test, completely prevented minoxidil and pinacidil induced amnesia without improving cognition in unamnesic mice. Doses of the two K^+-channel blockers 10-time lower were not able to protect mice against minoxidil and pinacidil amnesia whereas doses 10 times higher produced convulsions and death. Apamin and charybdotoxin, at the dose active in the passive-avoidance test, did not modify the pain threshold, as revealed by the hot-plate and 0.6 % acetic acid writhing tests, and were devoid of behavioural side effects as shown by the animex and rota-rod tests. These data show that apamin and charybdotoxin are endowed with antiamnesic properties in this animal model.

This work was supported by grants from MURST.

62.13

ENHANCEMENT OF SPONTANEOUS ALTERNATION SCORES IN THE RAT BY INTRA-SEPTAL INJECTION OF THE K-ATP CHANNEL BLOCKER GLIBENCLAMIDE. M.R. Stefani*, G.M. Nicholson and P.E. Gold. Neurosci. Grad. Program & Dept. of Psych., U. of Virginia, Charlottesville, VA 22903.

Peripheral and central administration of D-glucose enhance memory on a variety of tasks and attenuate the memory-impairing effects of the opioid agonist morphine. The mechanism(s) by which glucose enhances memory is not known. One possible mechanism is via modulation of the ATP-sensitive postassium channel (K-ATP), a channel believed to contribute to the presynaptic regulation of neurotransmitter release by glucose and opiates. The present studies examined the ability of glibenclamide (GLIB), a K-ATP blocker, to increase spontaneous alternation (SA) scores and to attenuate a morphine-induced impairment in alternation scores. Morphine (4 nmol), injected into the rat medial septal area (MSA) 30 min prior to behavioral testing, produced a significant decrease in alternation scores assessed in a Y maze. Glibenclamide (10 nmol), administered concurrently with morphine, blocked the morphine-induced impairment. In a second experiment, rats were tested for SA performance in a plus maze following infusion of GLIB or glucose into the MSA. Both GLIB (10 nmol) and glucose (20 nmol) significantly enhanced SA scores. The results of these studies suggest that the K-ATP blocker glibenclamide is capable of producing effects on spontaneous alternation scores similar to those observed for glucose, including reversal of a morphine-induced decrease in SA scores on the Y maze and increase in SA scores on the plus maze, and suggest a mechanism by which glucose may modulate behavior. *Supported by NIA (AG07648), NINDS (NS32914) and NIMH (MH11057).*

62.14

REINFORCING PROPERTIES OF CHLORPHENIRAMINE IN GOLDFISH R. Mattioli, C. A. Nelson, J.P. Huston* and Spieler, R. Oceanographic Ctr, Nova Southeastern Univ., Dania, FL 33004, USA.

Many H_1 histamine receptor blockers, including chlorpheniramine (CPA), have been used to treat allergic reactions in humans. Recently, however, it was suggested that CPA can have a reinforcing effect in baboons. Moreover, evidence exists that the histaminergic tuberomammilary system plays an important role in reinforcing processes in rats. In teleost fish, histaminergic neuronal cell bodies occur only in the posterior part of the basal hypothalamus and it was suggested that these neurons are homologous to the tuberomammilary E groups in rats. The aim of the present study was to investigate whether CPA has a reinforcing effect in goldfish. For this purpose we used a conditioned place preference method that consisted of 1) establishing the less preferred of two compartments for each fish in a 10 min test; 2) confining each fish, 24 h later, in the less preferred compartment for 25 min, after it was injected i.p. either with CPA (1mg/kg, n=15) or with vehicle (V, n=14) and 3) reexamining behavioral preference, 24 h after injection and confinement.

In the first 10 min test, no differences between vehicle and CPA groups were obtained for either compartment. In the second test, the CPA group increased the time spent in the compartment in which they received the substance. They changed the compartment preference and spend more time in the previously less preferred compartment than did the V group (p=0.037, V X CPA, Mann-Whitney U test).

These results indicate that CPA has a reinforcing effect in goldfish. Considering that the only known histaminergic system in teleost fish is in the basal hypothalamus, and that this system appears to be homologous to the tuberomammilary system of mammals, we suggest that the tuberomammilary histaminergic system may be mediating the reinforcing effect of H_1 receptor blockers in other vertebrates as well.

Funding Source: FAPESP proc. 95/4337-0

62.15

H_3 RECEPTOR LIGANDS MODULATE MEMORY PROCESSES IN MICE. W. A. Stutts, E. L. Orr, H. Lal*, and M.J. Forster. Dept. of Pharmacology, Dept. of Anatomy and Cell Biology, University of North Texas Health Science Center, Fort Worth, TX 76107

Previous investigations suggest that histamine (HA) is involved in modulation of memory of rats and mice for avoidance tasks. The current study examined this hypothesis by testing mice for memory: (1) following H_3 receptor ligands thought to modulate HA release presynaptically, or (2) following agents inhibiting HA biosynthesis or degradation. It was expected that an increase in neuronal HA would result in facilitation of memory processing whereas a decrease in HA release would disrupt memory processing. Each drug's dose effect on memory consolidation and memory retrieval was evaluated using one trial inhibitory (passive) avoidance training. A dose dependent facilitation of memory consolidation was obtained with the H_3 antagonist thioperamide whereas imetit, an H_3 agonist, produced a dose dependent disruption. On the other hand, the specific enzyme inhibiting compounds alpha-fluoromethylhistidine, a neuronal HA depleter and metoprine, a histamine-methyl transferase inhibitor showed no effect. This lack of effect of the enzyme inhibitors on memory processing suggests that an intact physiological signaling system via the H_3 autoreceptor is necessary for effective modulation of HA at levels appropriate for memory modulation.

62.16

THE ROLE OF THE HISTAMINERGIC NEURON SYSTEM IN REWARD AND MEMORY PROCESSES. J.P. Huston , Ch. Frisch, C. Privou, P.K. Zimmermann and R.U. Hasenöhrl. Inst. Physiol. Psychology, Univ. Düsseldorf, D-40225 Düsseldorf, Germany. (Spon: EBBS)

Experiments performed with lesions of the histamine (HA) neurons of the nucleus tuberomammillaris (TM) provided evidence for an involvement of this nucleus in reinforcement and memory. Uni- or bilateral DC- and excitotoxic lesions resulted in a lateralized increase in self-stimulation behavior and in a facilitation of mnemonic processes in adult and aged rats in diverse learning tasks. To investigate whether the observed effects are HA-mediated we performed a series of studies with systemic and intracranial injection of specific HA antagonists. First, we blocked HA transmission at H1 or H2 receptor sites with chlorpheniramine or ranitidine in brain structures known to be involved in reward and memory and assessed the effects with either an inhibitory avoidance task (up-hill test), a place-preference paradigm or intracranial self-stimulation. The intracranial injection of the H1 antagonist chlorpheniramine into the nucleus basalis mangocellularis induced a conditioned place preference and facilitated inhibitory avoidance learning when administered post-trial. The H2 receptor antagonist ranitidine was not reinforcing in the place preference test. In a intracranial self-stimulation study the H1 receptor blockade in the nucleus pedunculo pontinus decreased lateral hypothalamic self-stimulation, whereas an enhancement in self-stimulation behavior followed the H1 receptor blockade in the nucleus accumbens. The chronic intraperitoneal administration of chlorpheniramine improved the performance of aged rats in the Morris water maze and in the step-through avoidance task. *Supported by DFG.*

62.17

COGNITION ENHANCING EFFECTS OF S 17092, A POTENT AND LONG ACTING INHIBITOR OF POST-PROLINE CLEAVING ENZYME (PPCE)

J. Lepagnol, C. Lebrun, P. Morain*, G. de Nanteuil, V. Heidet. Institut de Recherches Servier, 125 Chemin de Ronde, 78290 Croissy sur Seine, France.

S 17092 (1-[phenylcyclopropyl carbonyl]-2-[thiazolidinyl carbonyl]-octahydro-1-H-indole) is a selective inhibitor of PPCE, the main enzyme responsible for the catabolism of some peptides involved in cognitive function (substance P, vasopressin). S 17092 is a potent inhibitor of PPCE in the rat cerebral cortex *in vitro* (IC50 = 1.3 μM) and *in vivo* (ID50 = 0.5 mg/kg i.p. and 10 mg/kg p.o.). The *in vivo* effect is of long duration (> 9 hours) and results from a high penetration of the compound in the brain. The inhibitory effects are similar after acute and chronic (2 weeks) treatments. *Via* reduced degradation of substance P, S 17092 inhibits the scopolamine-induced amnesia in the passive avoidance test in the rat (ED50 = 0.3 mg/kg i.p. and 1 mg/kg p.o.). In the test of social memory in the rat (olfactive episodic memory), S 17092 (10 mg/kg p.o.) prevents the time-dependent spontaneous forgetting and this effect is similar to that of vasopressin. On the contrary, SP is devoid of any effect. Chronic treatment with S 17092 (10 mg/kg p.o, 2 weeks) antagonises the age-associated memory deficits in the test of spontaneous alternation in the mouse.

In view of these results, S 17092 seems to be a good candidate for treatment of cognitive deficit associated with cerebral aging and with neurodegenerative diseases in which deficits in SP and AVP levels have been described.

LEARNING AND MEMORY: PHARMACOLOGY III

63.1

N-METHYL-D-ASPARTATE (NMDA) RECEPTOR BLOCKADE DIFFERENTIALLY MODULATES FETAL AND NEONATAL CONDITIONED TASTE AVERSIONS (CTA): BRAIN KETAMINE CONTENT.

G.A. Mickley*[1], S.A. Balogh[1], K. Neimanis[1], P. Goulis[1], J. Hug[1], K. Sauchak[1] and B.K.Yamamoto[2], [1]Departments of Psychology and Chemistry, Baldwin-Wallace College, Berea, OH 44017-2088. [2]Department of Psychiatry, Case Western Reserve University, Cleveland, OH 44016.

NMDA receptor blockade can either potentiate the formation of a CTA (in E18 rat fetuses) or impair CTA learning (in P0 rat pups)(Mickley et al. *Dev. Brain Res.* 85, 119-127, 1995; Mickley et al. *Soc Neurosci. Abstr.* 21/2, 1233, 1995). In these studies, ketamine was administered in different doses and routes of administration. Therefore, we attempted to see if brain levels of ketamine could offer a parsimonious explanation for the disparate results of the two experiments. We injected dams bearing E18 fetuses with 100 mg/kg ketamine, i.p. (the dose that produced CTA potentiation). Neonatal (P0) rats received 0.1, 1.0 or 10mg/kg doses of ketamine, i.p. (doses that produced CTA amnesia). Thirty minutes later, rats were decapitated and the cerebral hemispheres quickly dissected. Brains were analyzed for ketamine content using high pressure liquid chromatography (HPLC). Fetal brain tissue contained high levels of ketamine (14.4±1.6μg/g) while significantly lower levels (<6μg/g) were detected in the neonatal brain. These data contrast with those from adult rats studies (Welzl et al., *Psychobiol.* 18, 43-47, 1990) suggesting that high doses of ketamine (e.g., 25mg/kg, i.p.) cause CTA amnesia while low doses (e.g., 6-12 mg/kg, i.p.) do not. NMDA receptor blockade may differentially modulate learning in the adult and developing rat.

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63.2

GABAERGIC AND CHOLINERGIC INTERACTIONS IN THE STRIATUM ON INHIBITORY AVOIDANCE.

S. E. Cruz-Morales* and R. A. Prado-Alcalá. Master in Psychology, ENEP-Iztacala and Fac. Med., UNAM. Tlalnepantla, Edo. Mex. 54030, México.

The systemic administration of cholinergic blocker scopolamine induces amnesia, which can be reversed by systemic injection of GABA antagonists. To evaluate whether similar effects can be obtained in the dorsal striatum, rats were trained in inhibitory avoidance with 1.0 mA and then injected into the striatum with scopolamine (30.0 μg), picrotoxin (1.0 μg), bicuculline (1.0 μg), or the combination of scopolamine and picrotoxin (SCP+PX) or bicuculline (SCP+BI). Retention of the task was measured 24 hr later. The administration of SCP, PX and BI induced amnesia, and contrary to the effects of systemic treatment, SCP+PX and SCP+BI also produced amnesia. These results indicate that GABA antagonists exert their protective effects againsts SCP-induced amnesia in a cerebral region outside the dorsal striatum.

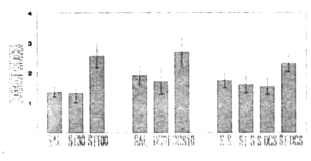
Supported by DGAPA-UNAM.

63.3

ENHANCEMENT OF INTERMEDIATE-TERM MEMORY BY AN ALPHA-1 AGONIST (ST 587) AND A PARTIAL AGONIST AT THE GLYCINE SITE OF NMDA RECEPTOR (D-CYCLOSERINE)

R. Pussinen and J. Sirviö*, A.I. Virtanen Institute, University of Kuopio, P.O. Box 1627, FIN-70211 Kuopio, Finland

In order to study the role of norepinephrine in memory, the present study investigated whether the activation of alpha-1 adrenergic receptors and NMDA receptors can influence intermediate-term memory. Therefore, the effects of ST 587 (30 or 100 μg/kg, s.c.), a putative alpha-1 agonist, and D-cycloserine (DCS, 1 or 10 mg/kg, s.c.), a partial agonist of the glycine site at the N-methyl-D-aspartate (NMDA) receptor on the retention of the radial arm task using the delayed non-matching to sample (baited arm) task was investigated in rats. The results indicated that ST 587 (100 μg/kg) or DCS (10 mg/kg) when administered singly 45 minutes before a sampling phase increased the number of correct choices during a retention phase (4 hours after a sampling phase) (Figure). In addition, the combination of the subthreshold doses of the drugs (50 μg/kg ST 587 + 5 mg/kg DCS) had a same kind of influence on the retention of this task (8 hours after a sampling phase). These data suggest that the activation of alpha-1 adrenoceptors and NMDA receptors both of which are known to be involved in the regulation of intracellular calcium levels facilitates synergistically hippocampus-dependent type of memory. *This study was supported by the Academy of Finland.*



63.4

REVERSAL OF LEARNING IMPAIRMENT IN DBA/2 MICE BY COGNITIVE ENHANCERS AND GLUTAMATERGIC AGENTS.

Ying Lu* and Jeanne M. Wehner, Institute for Behavioral Genetics, University of Colorado, Boulder, CO 80309

Previous studies demonstrated that DBA/2J mice perform poorly on a contextual fear-conditioning task. The present study demonstrates that acute treatment with oxiracetam (10-1000 mg/kg) or aniracetam (10-100 mg/kg), administered prior to training and test sessions, reversed the contextual learning impairment in a dose-dependent manner without affecting auditory cue conditioning. These effects were inhibited by the AMPA receptor antagonists, NBQX and GYKI-52466, which did not alter the contextual and auditory freezing when administered alone. Performance in contextual but not auditory conditioning was also enhanced by the NMDA antagonists, (+)-MK-801 (0.1-0.3 μg/kg), CPP (0.01-0.3 mg/kg), and (+)-HA-966 (0.1-3 mg/kg). The inactive stereoisomers, (-)-MK-801 and (-)-HA-966, were without effect. The combined administration of 30 mg/kg oxiracetam with 1 μg/kg (+)-MK-801 produced an additive response. These results suggest that DBA/2 mice may be learning impaired due to altered function of both AMPA and NMDA receptors. (MH-48663 and AA-00141)

63.5

EFFECTS OF THE NMDA RECEPTOR ANTAGONIST MEMANTINE ON LEARNING AND MEMORY IN HUMANS, M.M. Schugens*, R. Egerter, I. Daum, P.A. Löschmann and T. Klockgether. Institute of Medical Psychology and Behavioral Neurobiology and Department of Neurology, University of Tübingen, Germany

The effects of NMDA receptor antagonists on learning and memory have been widely examined in animals. The present study aimed to investigate the influence of the noncompetitive NMDA receptor antagonist Memantine on human learning and memory.

Sixteen healthy volunteers participated in a double-blind placebo controlled study. Subjects were randomly assigned to receive either 30 mg Memantine or placebo p.o. Four hours after drug intake a battery of neuropsychological tests was administered.

The two groups did not differ significantly on affect-arousal ratings or in performance on attention, fluency, or short-term memory tests. Apart from a slight impairment in immediate word list recall, the Memantine group did not show consistent memory deficits. Subjects receiving Memantine did, however, display a significant retardation in the acquisition of eyeblink conditioning and fewer CRs in total compared to the Placebo group. These effects may be related to the action of Memantine in the cerebellum.

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63.6

DISTAL CUES, BUT NOT THEIR SPATIAL ORGANIZATION OR ROOM GEOMETRY, ARE CRUCIAL FOR WORKING MEMORY PERFORMANCE IN THE RADIAL MAZE. M.L. Shapiro*, G. Tirado-Santiago, N.Y. Zayas-Monge, L. Zozula. Dept. Psychology, McGill Univ., Montreal, Quebec, Canada.

Spatial learning but not working memory (WM) performance in the radial maze is impaired in rats given NMDA receptor antagonists at doses that also block primed-burst potentiation. The impairment is not due to procedural learning deficits, because pre-trained rats given NMDA antagonists perform the WM task normally in a familiar room but fail to learn the same task in an unfamiliar room. Because the same procedures and movements are required to solve the task in both rooms, learning about the stimuli in the new room must be impaired by the drug.

To identify the stimuli that are required for good WM performance in well-trained rats, an 8-arm radial maze was surrounded by black curtains that could be altered to change the shape of the room from square to circular. Rats were trained to perform the standard WM task while the curtains defined a square room, with each "wall" holding a prominent distal cue. After reaching criterion performance, the rats were given extensive additional training in this stable cue configuration and then were given probe tests with: (1) the same cues in a new configuration; (2) the same cue configuration in a circular room shape; (3) both a new room shape and a new cue configuration; (4) with the distal cues removed; (5) with an entirely new cue set in the room; and (6) in an unfamiliar room. For each probe test, the rats were brought to the testing room in an opaque container via a novel route. The probe tests were designed to resemble those that influence hippocampal place fields.

Altering the stimulus configuration, the room shape, or both slightly increased the time the rats needed to complete the task, but had no effect on WM performance. Removing or replacing the cues with new stimuli impaired WM performance slightly, whereas WM performance in the new room was profoundly impaired. The results suggest that: (1) good WM performance in the radial maze requires rats to encode the content of the environment, perhaps including its geometry, and (2) after this content is encoded, the spatial relationships among stimuli is not crucial for WM. Supported by NSERC Canada.

63.7

IMPAIRMENT OF ASSOCIATIVE PROCESSES BY EITHER AN NMDA AGONIST OR AN NMDA ANTAGONIST, H. Dai*, J. Gui and R. J. Carey. Psychiatry, SUNY Health Science Center and Research and Development Service 151, VA Medical Center, Syracuse, NY 13210.

Habituation is a basic learning process. In rodents, habituation is reliably observed as a reduction in exploratory behavior in novel environment following a brief pre-exposure to the environment. In as much as NMDA receptors are known to be critically involved in learning processes, we examined the impact of pharmacological alteration of the NMDA receptors upon the habituation process. In a series of experiments, groups of rats were given pretreatments of either the NMDA antagonist dizocilpine (MK-801) or the NMDA agonist cycloserine before their first exposure to a novel environment. The animals were given a 10 min exposure to a novel open-field environment and subsequently re-tested in the same environment 1 or 3 days later to test for habituation. The animals which received the 10 min familiarization test reliably exhibited a decrease in exploratory locomotion on the second test as compared with their first test or with rats which were tested in the environment for the first time. Neither MK-801 (0.1 mg/kg) nor cycloserine (3.0 mg/kg) treated rats differed from saline treated animals in locomotor behavior in the familiarization test. In the test for habituation in which all animals were treated with saline, rats previously treated either with MK-801 or cycloserine failed to exhibit habituation. Thus, either antagonism or activation of the NMDA receptors effectively blocked habituation at dose levels which did not modify spontaneous exploratory behavior. This blockade of habituation was not a state dependency phenomenon in that MK-801 either given after the familiarization test or given before the habituation test to saline animals did not impair habituation. These findings argue against the conceptualization in drug development that if antagonism of a receptor impairs association then pharmacological activation of the same receptor can enhance learning. In the case of the NMDA receptor, it would appear that any pharmacological occupancy of the NMDA receptor may diminish the availability of the receptor to participate in association processes.

63.8

EFFECTS OF NEONATAL MK-801 TREATMENT ON NON-SPATIAL LEARNING. G. Griesbach*& A. Amsel. Dept. of Psychology & Inst. for Neuroscience, University of Texas, Austin, TX 78712.

Neural changes in the hippocampus, that result from NMDA receptor/channel intervention, are known to have a deleterious influence on learned behavior. Neonatal MK-801 treatment, over a critical period of development in laboratory rats, has been shown to cause a long-lasting learning impairment, particularly on the Morris Water Maze, the prototypical spatial learning apparatus. The present experiment studied the effects of neonatal treatment with MK-801, a non-competitive NMDA antagonist, on patterned single alternation (PSA), a form of non-spatial learning that has been related in several of our experiments to hippocampal function. In PSA, rewarded (R) and nonrewarded (N) trials are alternated on a straight-runway apparatus, and learning depends on a discrimination based on carryover memorial cues from the preceding R and N trials. Neonatal rats were injected daily from postnatal days (PND) 7 through 19 with MK-801 and trained on PSA at PND 22. Training consisted of 10 sessions of 40 trials each, 3 sessions per day for 3 days and one session on the 4th day. The intertrial interval was 60s. Rats injected with MK-801 were significantly impaired in PSA when compared to rats injected with the saline vehicle. Because PSA is impaired in the MK-801-treated rats, the implication from this experiment, as in many others in our laboratory, is that NMDA receptor blockade during hippocampal development has deleterious consequences not only on spatial learning, but also on learning that is clearly non-spatial in nature. See, e.g., Amsel, 1993, Hippocampus, 3, 251-256. Supported by NIAAA grant AA07052.

63.9

ATTENUATED MK801-INDUCED IMPAIRMENT OF RADIAL MAZE PERFORMANCE IN RATS: A POSSIBLE MODEL PREDICTING EFFICACY IN ALZHEIMER'S DISEASE. A. Bartolomeo, H. Morris, J. Moyer and C.A. Boast*. Wyeth-Ayerst Research, Princeton, NJ 08543.

Glutamate has been implicated in learning and memory processes. In particular, blockade of NMDA receptors impairs memory in a variety of animal studies. The non-competitive NMDA antagonist, MK801, has been used for this purpose. Decreases in glutamate functioning have been reported in Alzheimer's Disease (AD) patients. Suggestions have been made that agents which can increase glutamate release, or balance the loss of excitatory input by reducing inhibitory input in key brain regions, may have utility in treating AD. Specifically, modulators of serotonin have been proposed as potential AD therapies, based on their ability to affect glutamate neurotransmission. We have studied a number of agents in rats (ritanserin, ondansetron, buspirone, WAY-100635, WAY-100802, arecoline) for their potential to attenuate a radial maze memory impairment caused by MK801 (0.1 mg/kg ip). Of these agents, the 5HT-1A antagonists, WAY-100635 and WAY-100802, significantly decreased the MK801 impairment. The 5HT-2 antagonist, ritanserin, the 5HT-3 antagonist, ondansetron, and the muscarinic agonist, arecoline, attenuated the MK801 impairment. The majority of animals treated with the 5HT-1A partial agonist, buspirone, in combination with MK801, were unable to complete the task. Since it has been demonstrated that 5HT-1A antagonists can increase glutamate release and also that they can block the inhibitory effects of serotonin in the hippocampus, we speculate that these mechanisms contribute to the beneficial effects observed in this model. The attenuation of the MK801-induced radial maze impairment may be a suitable model to evaluate potential drugs for the treatment of AD.

63.10

EFFECTS OF MK-801 ON SPATIAL MEMORY IN HOMING AND NONHOMING PIGEON BREEDS, E. F. Meehan and A. Wieraszko*. The College of Staten Island/CUNY, Staten Island, NY 10314.

Homing pigeon breeds, the product of artificial selection on the basis of navigational and spatial ability, differ from nonhoming breeds in hippocampal size and distribution of NMDA dependent receptors. The effects of dizocilpine maleate (MK-801, 0.1 mg/kg ip), a noncompetitive NMDA antagonist, on spatial reference memory (RM) were compared between the 2 breeds in a radial arm maze task. MK-801 disrupted the acquisition of RM in the nonhoming group, but not the homing group, which was equivalent to the 2 saline only control groups. In agreement with previous findings with mammals, working memory (WM) was not affected by MK-801. This behavioral dissociation, coupled with differences in NMDA dependent long-term potentiation (LTP) between breeds, suggests an exceptional opportunity to investigate the role and function of the dorsomedial telencephalon region in spatial RM, via anatomical, neurochemical, and behavioral comparisons between homing and nonhoming pigeon breeds.

63.11

ROLE OF NUCLEUS ACCUMBENS IN MK-801 INDUCED IMPAIRMENT OF REACTIVITY TO SPATIAL AND NON SPATIAL CHANGE IN MICE.
A. Usiello, P. Roullet, M. Ammassari-Teule, E. Passino and A. Mele*. Dip. Genetica e Biologia Molecolare, Università di Roma "La Sapienza" and Ist. di Psicobiologia e Psicofarmacologia, C.N.R., Rome, Italy.
Nucleus accumbens is part of a larger limbic-striato-pallidal system mediating adaptive and learning strategies. Dopaminergic pathways from the ventral tegmental area and glutamatergic inputs from limbic structures like the amygdala and the hippocampus are the major afferents to this nucleus. We have recently shown, in mice, that systemic injections of MK-801, a specific non competitive antagonist of glutamatergic NMDA receptors, induced performance deficits in a non associative task specifically designed to test the encoding of spatial information. Basically, this task consists in placing the subjects in an open field containing five objects and examining their reactivity to the displacement (spatial change) or the substitution (non spatial change) of some of these objects. Purpose of the present study was to assess the role of the nucleus accumbens in mediating the MK-801 impairing effects on the response to spatial change. Two experiments were carried out. First, systemic injections of MK-801 were performed in mice with ibotenic lesions of the nucleus accumbens. Second, MK-801was injected bilaterally into the nucleus accumbens. The results showed that ibotenic lesions of the nucleus accumbens, by itself, paradoxically improved the reactivity of mice to spatial change but did not block the impairing effect of systemic MK-801injections. Intra-accumbens injections of MK-801, however, abolished the reactivity to spatial change in a dose-dependent fashion.No treatment nor the lesion affected the reactivity to non spatial change. Taken together these results suggest that the nucleus accumbens is involved in the response to spatial change but does not represent the only target site mediating the impairing effect of MK-801 systemic injections.

63.12

MK-801 ATTENUATES THE MEMORY ENHANCING EFFECT OF INTRA-DORSAL STRIATAL INFUSIONS OF PLATELET-ACTIVATING FACTOR.
L.A. Teather[1,2]*, M.G. Packard[1] & N.G. Bazan[2], Department of Psychology, University of New Orleans and [2]LSUMC Neuroscience Center of Excellence, New Orleans, Louisiana
Recent evidence indicates that post-training injections of the bioactive lipid platelet-activating factor (PAF) *enhances*, and the PAF antagonist BN 52021 *impairs*, memory processes in the rodent hippocampus, entorhinal cortex, amygdala, and dorsal striatum (i.e. caudate-putamen). The mechanism by which PAF enhances memory is unknown. PAF increases glutamate release from presynaptic hippocampal terminals *in vitro*, and it has been proposed that PAF acts as a retrograde messenger in hippocampal long-term potentiation. The dorsal striatum receives sensory information via glutamatergic input from the cortex and thalamus, and the cortico-striatal pathway has been shown to exhibit various forms of use-dependent synaptic plasticity. In the present experiment we examined possible interactions between PAF and the glutamate NMDA receptor using the non-competitive receptor antagonist MK-801. Rats received an 8-trial training session in a striatal-dependent cued water maze task, in which a visible escape platform was located in a different quadrant of the maze on each trial. Following trial 8, rats were given an intra-dorsal striatal injection of a PAF agonist (mc-PAF; 1.0 µg/0.5 µl) or saline, in addition to a peripheral (IP) injection of MK-801 (0.025 mg/kg) or saline. Retention was tested 24 hours later, and latency to mount the escape platform was used as a measure of memory for the previous day's training. Rats given intra-striatal PAF had lower escape latencies on the retention test, indicating a memory enhancing effect of PAF. Concurrent IP injections of MK-801 blocked the memory enhancing effect of intra-striatal mc-PAF. Importantly, the 0.025 mg/kg dose of MK-801 did not affect retention when administered alone. The finding that a subeffective dose of MK-801 blocks the memory enhancing effect of dorsal striatal PAF infusions suggests that an interaction between PAF and glutamatergic systems may mediate the formation of striatal basal-memory processes. NS23002 (NGB)

63.13

THE NON-COMPETITIVE NMDA RECEPTOR ANTAGONIST MK-801 POTENTIATES MORPHINE-INDUCED IMPAIRMENT OF MEMORY CONSOLIDATION IN MICE: INVOLVEMENT OF DOPAMINERGIC MECHANISMS.
V. Cestari*, C. Rossi-Arnaud[1], C. Castellano. Istituto di Psicobiologia e Psicofarmacologia (C.N.R.) Via Reno 1, 00198 Roma, Italy - [1]Facoltà di Psicologia, Università degli Studi di Roma "La Sapienza", Via dei Marsi 78, 00185 Roma, Italy.
Four sets of experiments were carried out with CD1 mice. The animals were posttraining injected intraperitoneally with the drugs used and tested in a one-trial inhibitory avoidance task. In a first series of experiments posttraining administration of morphine or of the non-competitive NMDA receptor antagonist MK-801 impaired memory consolidation. In the second set of experiments the memory consolidation impairment exerted by MK-801 was potentiated by the administration of the D1 dopamine (DA) receptor antagonist SCH 23390 or by that of the D2 DA receptor antagonist (-)-sulpiride. In the third set of experiments the administration of a per se non effective dose of MK-801 potentiated the memory impairment exerted by morphine. In the fourth series of experiments per se non effective doses of the D1 DA receptor agonist SKF 38393 or of the D2 DA receptor agonist LY 171555 antagonized the impairing effect on memory consolidation exerted by a combination of MK-801 and morphine, suggesting the involvement of dopaminergic mechanisms.

63.14

THE INITIAL PHASE OF LEARNING OF CLASSICAL FEAR CONDITIONING IN GOLDFISH IS SELECTIVELY INHIBITED BY THE NMDA RECEPTOR ANTAGONIST MK-801.
X. Xu*. Department of Psychology, Grand Valley State University, Allendale, MI 49401.
Previous experiments showed that administration of intracranial MK-801 blocks learning of classical fear conditioning in goldfish. The impairment of learning was decreased when fish received limited pretraining, suggesting that only the early phase of conditioning may be sensitive to disruption by MK-801. The present studies investigated the possibility that classical conditioning in goldfish consists of two successive phases and only the initial phase is critically dependent on NMDA receptor function. A series of experiments showed that the anterograde amnestic effect of MK-801 is decreased when fish received pretraining consisting of six or more conditioning trials and eliminated when fish received 12 pretraining trials. MK-801-sensitive learning is inferred to be completed within 12 trials. The neural mechanism of the learning which occurs during the later trials is manifestly insensitive of MK-801. Furthermore, MK-801 impaired learning when fish received pretraining consisting of six pseudoconditioning trials, indicating that MK-801-sensitive learning is associative, or dependent upon the contiguity of the CS and US. The effects of MK-801 on classical fear conditioning in goldfish resemble the effects of atropine on maze learning in rats. The similarities suggest that learning in instrumental and classical conditioning tasks may consist of two successive phases which are mediated by different neural mechanisms. (Supported in part by GVSU research grant-in-aid)

63.15

MICROINJECTION OF GABA-A RECEPTOR AGONIST MUSCIMOL REVEALS FUNCTIONAL SPECIALIZATION WITHIN THE HIPPOCAMPUS OF RATS
J.B. Mao* and J.K. Robinson, Dept. of Psychology, S.U.N.Y. at Stony Brook, Stony Brook, NY, 11794-2500.
Evidence from lesion studies has suggested that the dorsal extent of hippocampus may be especially critical to hippocampal involvement in spatial learning and memory, sensory-attentional processing, or both. This functional specialization is also predicted by distinct patterns of cortico-hippocampal connectivity. Inhibitory GABAergic interneurons are widespread throughout the hippocampus, and are likely to participate in hippocampal information processing. To evaluate the hypothesis that a functional specialization exists within the hippocampus, the present study compared the effects of muscimol microinjected into either dorsal (DH) or ventral (VH) regions of hippocampus on performance of an operant, spatial delayed nonmatching-to-position (DNMTP) task that simultaneously assessed visual stimulus detection and working memory parameters. Rats were extensively pretrained on DNMTP, then cannulated. Following retraining, muscimol (.003-0.2 µg/0.3 µL into DH; 0.2-1.0 µg/0.5 µL into VH) was administered. In VH, no reliable effects on nonmatching choice accuracy, stimulus detection, or procedural parameters were detected at any dose of muscimol. In contrast, a significant dose-dependent increase in errors of omission to sample-phase visual stimuli was observed following muscimol injection into DH. The smallest concentration of muscimol that was effective in DH was two hundred times weaker than the maximum administered into VH, suggesting a pronounced dissociation of sensitivity to GABA-receptor agonism between these two regions. These results indicate that the contribution of the hippocampus to sensory-attentional processing may vary along the septo-temporal axis. Supported by NIMH grant 1RO3MH55290-01

63.16

BACLOFEN INFUSED IN RAT HIPPOCAMPAL FORMATION IMPAIRS ACQUISITION AND RETENTION OF SPATIAL LEARNING.
M.P. Arolfo, H.F. Carrer*, M.A. Zanudio and O.A. Ramirez. Depto. de Farmacología, Fac. Cs. Químicas, U.N.C., Córdoba, Argentina.

Recent studies show that baclofen (a selective GABA-B agonist), impairs different kinds of learning (McNamara and Skelton, Pharm. Biochem. Behav. 1996; Ramirez et al., unpublished results).

Neuroanatomically, GABA-B receptors can be subclassified according to their signal transduction mechanisms. GABA-B receptors are known to be located pre- and post-synaptically and can be identified electrophysiologically (Bowery, TIPS 1989; Dutar and Nicoll, Neuron, 1988). It has been demonstrated that baclofen modulates, in a biphasic manner, the mechanisms underlying the long term potentiation (LTP). The aim of the present study was to investigate a differential action of baclofen on spatial learning. Male Wistar rats (8-10 weeks of age) were implanted with cannulae aimed bilaterally to the hippocampal formation. Baclofen (20mM, 2mM) or sterilized saline, was microinfused, 1 h before each session (3 trials/session, 1 session/day) along 4 days. Our results show that baclofen 20mM and 2mM impaired the acquisition and retention of spatial information. Supported by grants from SECyT and CONICOR Argentina to O.A. Ramirez.

63.17

FAILURE OF SENSITIZATION IN GOLDFISH TREATED WITH $GABA_B$ AGONIST BACLOFEN. A. C. Kalyva[1], E. G. Antzoulatos[1], P. Angelogianni*[2] and M. M. Nikoletseas[1]. [1]Dept. of Psychol., Deree, The American College of Greece, A. Paraskevi, GR-15342; [2]Dept. of Experimental Physiol., Sch. Medicine, U of Athens, GR- 11527, Greece.

A dramatic suppression of respiratory activity occurs when goldfish (*Carassius auratus*) is presented with novel stimuli. Muscle potentials from the opercular region show a decrease in amplitude and an increase in duration. This branchial suppression response (BSR) habituates, if the stimulus is weak, and is subject to heterosynaptic facilitation (is sensitized), if a strong stimulus is presented. In the present study two groups of fish of four subjects each were used in a sensitization experiment. The experimental group received baclofen (16 hrs in 25 mg/lt aqueous solution up to one hr before the experiment). The weak stimulus was a flashing light (2 s duration, aprox 19 x 10^6 lumens intensity, 6 Hz flashing rate). The sensitizing stimulus was a 5 ms duration constant current shock of 50 mA delivered subcutaneously through teflon coated silver electrodes. The BSR was recorded through a tungsten electrode inserted in the operculum and was displayed in a computerscope. Five minutes after the first presentation of the light stimulus, the shock stimulus was presented five times at 5 min intervals. The weak stimulus was presented again five min after the last shock. Results showed sensitization of the BSR in the undrugged group, as indexed by a wider, lower amplitude muscle potential ($p < 0.05$). The baclofen group failed to show sensitization. Muscle potentials in the latter group remained narrow, and of high amplitude, indicating a diminished alarm reaction to the light stimulus. In general, baclofen treated animals showed low responsiveness to stimuli. The data are discussed in terms of the role of inhibitory systems in the development of sensitization. (Supported by Deree College funds)

63.18

GABA INFLUENCES ON THE CONDITIONED BRANCHIAL DEFENSIVE REFLEX IN THE GOLDFISH. E. G. Antzoulatos, A. C. Kalyva and M. M. Nikolciseas*. Dept. of Psychology. Deree, The American College of Greece, A. Paraskevi, GR-15342, Greece.

The branchial defensive reflex (BDR), characterized by muscle potentials of decreased amplitude and increased duration, is elicited when goldfish (*Carassius auratus*) are presented with novel stimuli. We report here the conditioning of BDR and the effects of baclofen, a $GABA_B$ agonist, on the conditioned BDR. An undrugged group (UG) and a baclofen-treated (BG) group (20hrs in a 25 mg/lt aqueous solution) were presented with the conditional stimulus (CS), a flashing light (5 Hz, 5 x 10^6 lumens, 2 s) followed by the unconditional stimulus (UCS), a 50 mA DC, 5 ms shock with an interstimulus interval (ISI) of 8 s. The third group (PG) was presented with unpaired stimuli in random order to control for pseudoconditioning. The experiment consisted of 25 trials with 5 min intertrial interval. In the UG group a conditioned BDR developed in less than ten trials. CR latency gradually shortened and in CS alone test trials CR duration exceeded the 8 s ISI. In the PG group the original BDR was sensitized but remained weak compared to the UG group. Baclofen had an inhibitory effect on the development of the conditioned BDR; occasionally the CR showed increased amplitude and decreased duration of muscle potential. (Supported by American College of Greece funds).

63.19

SYSTEMIC AND NUCLEUS BASALIS INFUSIONS OF N-METHYL-D-ASPARTATE ENHANCE MEMORY IN A DOUBLE Y-MAZE. K. I. Mason*, P. E. Mallet, K. Jhamandas, R. J. Boegman and R. J. Beninger. Departments of Psychiatry, Psychology, and Pharmacology & Toxicology, Queen's University, Kingston, Ontario, Canada.

N-methyl-D-aspartate (NMDA) receptors have long been implicated in learning and memory. There are many findings which demonstrate that the administration of NMDA receptor antagonists impairs memory. Few studies, however, have investigated the role of NMDA agonists in mnemonic function. Hence, the present study examined the effects of systemic and intra-nucleus basalis magnocellularis (nbm) injections of NMDA on memory. Wistar rats were trained in a two-component double Y-maze task consisting of a spatial discrimination and delayed alternation. In the first experiment, rats (n=12) received saline (0.9%), NMDA (0.2, 2.0 and 10.0 mg/kg), physostigmine (0.1 mg/kg), or no injection, in a counterbalanced order. In the second experiment, rats (n=19) were surgically implanted with bilateral cannulae in the nbm prior to maze training. Once trained, animals received bilateral intra-nbm injections (0.5 µL) of saline (0.9%), NMDA (0.05, 0.075, and 0.10 µg/side), the benzodiazepine receptor partial inverse agonist N-methyl-β-carboline-3-carboxamide (FG 7142; 0.2 µg/side), or no injection, in a counterbalanced order. During testing, delays of 0-, 30- and 60-seconds were introduced. Systemic physostigmine improved choice accuracy in both tasks; systemic NMDA, intra-nbm FG 7142, and intra-nbm NMDA produced an improvement in the delayed alternation task only. These results support the notion that NMDA receptors in general, and the nbm in particular, are involved in cognitive processes mediating memory (supported by OMHF).

63.20

SPATIAL LEARNING IN THE ABSENCE OF NMDA RECEPTOR-DEPENDENT LONG TERM POTENTIATION: REPLICATION WITH CGS19755. D.P. Cain* and D. Saucier. Dept. Psychology and Graduate Program in Neuroscience, Univ. of Western Ontario, London, Ontario N6A 5C2 CANADA.

NMDA receptor-dependent LTP in the hippocampus is thought to be required for normal spatial learning in the water maze. We evaluated the ability of rats given CGS19755, a competitive antagonist of NMDA receptors, to acquire the water maze task. CGS19755 (4.0 mg/kg i.p.) completely blocked LTP in the dentate gyrus evoked by stimulation of the perforant path, measured at 1 and 24 hrs. Several days later the same rats received the same regimen of electrical stimulation in the absence of any treatment. Significant LTP of both the EPSP and population spike was measured at both 1 and 24 hrs. The same rats were then made familiar with the general task requirements by nonspatial pretraining using a standard protocol (3 trials/day, 4 days, curtains around pool, hidden platform moved after every trial; Morris, 1989). They were then given CGS19755 (4.0 mg/kg) and trained to find the hidden platform in the maze pool. Naive CGS rats were trained similarly, and had a severe deficit in finding the platform. The nonspatially pretrained CGS rats acquired the task as rapidly as controls as judged by measures of platform search time, platform quadrant dwell, and percent direct swims. These results replicate our earlier findings with the NMDA receptor antagonist NPC17742, and demonstrate that rapid spatial learning can occur in the absence of NMDA receptor-dependent LTP in the dentate gyrus. Supported by a grant from N.S.E.R.C.

63.21

MODULATION OF DIVIDED ATTENTION BY INFUSIONS OF BENZODIAZEPINE RECEPTOR AGONISTS AND INVERSE AGONISTS INTO THE BASAL FOREBRAIN. L.A. Holley Miner*, A.J. Hanje and M. Sarter. Department of Psychology & Neuroscience Program, The Ohio State University, Columbus, OH 43210.

Infusions of benzodiazepine receptor (BZR) agonists or inverse agonists into the basal forebrain were previously demonstrated to produce dissociable effects on sustained attention (Psychopharmacol 120:99-108). The present experiment examined the effects of such infusions on divided attention. Divided attention was assessed by using a crossmodal divided attention paradigm developed for rats.(Psychopharmacol 155:213-220). The effects of infusions of the BZR agonist chlordiazepoxide (CDP; 10 - 60 µg/0.5 µl/hemisphere) or the partial inverse agonist FG 7142 (0.2 - 6.0 µg/0.5 µl/hemisphere) into the basal forebrain were assessed. Infusions of CDP increased response latencies while decreasing response accuracy in the bimodal component of this task, suggesting the lack of a speed-accuracy tradeoff which was previously observed following systemic administration of CDP. Small doses of FG 7142 increased both accuracy and response latencies during the condition of modality uncertainty. These findings suggest that bidirectional modulation of basal forebrain GABAergic transmission mediates dissociable effects on divided attention.

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63.22

BRETAZENIL DOES NOT IMPAIR SHORT-TERM MEMORY OF RATS AT DOSES EFFECTIVE AGAINST ETHANOL WITHDRAWAL. Y. Egilmez, C.J. Wallis, J. Grewal, H. Lal and M. Martin*. Department of Pharmacology and SAINT, University of North Texas Health Science Center, Fort Worth, TX 76107.

Bretazenil is a partial benzodiazepine receptor agonist with potent anxiolytic and anticonvulsant effects. In the present experiments, we studied the effects of bretazenil on short-term memory and motor performance of rats at doses that are effective to alleviate ethanol-withdrawal symptoms. Male Long Evans rats were trained to find a hidden platform in a Morris Water Maze (1.8 m in diameter and 60 cm high, filled with opacified water and maintained at 24 °C). Following the acquisition, rats were given a nutritionally balanced liquid diet with or without (control) ethanol (4.5%) for 10 days. Twelve hours following the removal of the ethanol/control diet, rats were tested for their performance at the water maze on three consecutive test days. Each day, the platform was placed in a different location, and the effect of bretazenil (0.08, 0.32 and 1.25 mg/kg) was tested at 15, 30 and 60 min. following an information trial on measures of "latency to reach the platform", "path length" and "speed". There was no significant difference between ethanol and control groups in any of the measures used. Bretazenil did not significantly effect any of the performance in control group while decreasing speed in ethanol group at the lowest dose. These findings suggest that bretazenil does not impair short-term memory performance in rats at doses effective against withdrawal. (Supported by NIAAA #AA09567).

63.23

LEARNING DEFICITS INDUCED BY PONTINE TEGMENTUM LESIONS ARTIFACT OF ANXIETY? F.Leri* and K.B.J. Franklin. Department of Psychology, McGill University, Montreal, Canada, H3A-1B1.

Normal animals were trained on a delayed non-matching to position task (DNMP) in a T-maze. Subsequent bilateral NMDA lesions to the pontine tegmentum (PTg), including the pedunculopontine nucleus, did not alter performance. During acquisition, the first trial was forced, however, when the first trial was made a free choice, the performance of the PTg group dropped, suggesting PTg lesions prevented generalization to a new condition. Naive animals with NMDA lesions of the PTg or sham lesions, were tested for their capacity to acquire DNMP with free sample choice. The sham lesioned subjects improved but, even after 15 days of training, PTg lesioned animals did not. We noted that PTg lesioned rats failed to eat the food reinforcement, and did not habituate to handling, suggesting that the failure to perform DNMP might be due to fear. DNMP performance of these PTg-lesioned animals significantly improved when they were injected with diazepam (1mg/kg) before testing. The performance of controls was not altered.

When naive animals were tested for acquisition of DNMP under diapezam, only the PTg group improved with training. Sham-lesioned rats acquired DNMP when diazepam was discontinued, but the performance of the PTg group deteriorated. These results suggest that PTg lesions did not directly affect learning or memory, but induced a state of high anxiety which influenced performance on the DNMP task. This conclusion is supported by the finding that bilateral NMDA lesions to the PTg produced high levels of anxiety as measured by performance on an elevated + maze. The anxiety scores were not reduced with 7 daily exposures to the apparatus, but were significantly attenuated by diazepam (1& 2 mg/kg).

Supported by the *Natural Science and Engineering Research Council of Canada* and the program *Formation de Chercheurs et L'Aide à la Recherche du Quebec.*

BIOLOGICAL RHYTHMS AND SLEEP: SLEEP II

64.1

SLEEP DEPRIVATION BY GENTLE HANDLING DOES NOT AFFECT GALANIN mRNA EXPRESSION IN THE RAT ANTERIOR HYPOTHALAMUS J.Toppila, L.Alanko, M.Asikainen, I.Tobler, D.Stenberg* and T.Porkka-Heiskanen. Dept. of Physiology, Inst. of Biomedicine, FIN-00014, Univ. of Helsinki, Finland and Inst. of Pharmacology, Univ. of Zürich, Switzerland.

Galanin (GAL) is widely expressed in CNS and colocalized with neurotransmitters which take part in sleep regulation. Experimental lesions of the anterior hypothalamic area stongly inhibit sleep indicating a role of this brain area in the regulation of sleep. We found earlier that 24 h REM sleep deprivation (REMSD) with the platform method increased GAL mRNA in the periventricular nucleus (PeN) and medial preoptic area (MPA). We now studied the effect of total sleep deprivation (SD) on GAL mRNA expression in the same nuclei. SD was obtained by gentle handling i.e. by introducing or removing objects in the rat cage. Six hours of SD during the light phase is known to cause a strong rebound of EEG slow wave activity in the rat. Brains of male rats (kept in L:D 12:12, lights on at 0900 h) were collected immediately after 6 h SD (starting at lights on), or 12 h SD (dark phase). N=6 in each group. Unhandled animals (N=6+6) kept in their home cages served as controls. In situ hybridization was made from 20 μm cryosections through the anterior hypothalamus using ^{35}S-labelled 48-mer oligonucleotide probe for GAL. Slides were dipped in photographic emulsion, and developed after exposure. The number of cells expressing GAL mRNA counted under dark field photomicroscopy in corresponding sections did not change significantly after 6 or 12 h SD in PeN or MPA in contrast to 24 h of REMSD. 12 h of SD may be too short to induce GAL expression in the anterior hypothalamus. It is also possible that the loss of REM sleep in the prolonged REMSD causes the increase, and 12 h of SD does not enhance REM pressure enough to enhance galanin mRNA as after 24 h REMSD.

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64.2

CHANGES IN GLUTAMATE DECARBOXYLASE (GAD) mRNA LEVELS ASSOCIATED WITH THE PLATFORM TECHNIQUE OF REM SLEEP DEPRIVATION.

L.Ramanathan*, R.W. McCarley and P.Shiromani. *VA-Harvard Medical School, West Roxbury, MA 02132.*

Emerging evidence shows that gamma-aminobutyric acid (GABA) plays an important role in sleep. In the hypothalamus, sleep-active cells are thought to be GABA and inhibitory on wake-active cells, while in the dorsal raphae, GABA cells show Fos expression with cholinergically-induced REM sleep. Increased GABA release has been found with REM sleep in the dorsal raphae and with non-REM sleep in the posterior hypothalamus. The present study investigated changes in the expression of GAD mRNA using the platform technique of REM sleep deprivation. Rats were placed on either large platforms (LP) or small platforms (SP) for 1 and 5 days, while control animals were kept in dry cages throughout the experimental period.

Total RNA was extracted from six different brain regions (cerebellum, cortex, striatum, hippocampus, pons and hypothalamus) electophoresed on 0.8% agarose gels, transferred in 10X SSC by overnight capillary blotting to zeta probe membrane and probed with [32P]-UTP labelled GAD by in vitro transcription. Preliminary assessment of Northern blots indicate no changes in the levels of GAD mRNAs in any of the brain regions.

We and others have previously shown that sleep and REM-sleep deprivation induces differential expression of various messages including c-fos, preproenkephalin, tyrosine hydroxyfase and IL-1. We have now extended these observations to include GABA.

Supported by DVA Research and NS30140

64.3

PROLONGED REM SLEEP DEPRIVATION ELEVATES TYROSINE HYDROXYLASE mRNA IN LOCUS COERULEUS.

R.Basheer*, M.Magner, K.Ryan, A.Segall, R.W.McCarley and P.Shiromani. *Dept Psychiatry, Harvard Medical School- VA Medical Center, West Roxbury, MA 02132.*

The rat LC consists of densely packed norepinephrine neurons. Electrophysiological studies have shown that LC neurons stop firing during REM sleep. With REM sleep deprivation these neurons should continue to discharge, resulting in an increased turnover and synthesis of the neurotransmitter and its synthesizing enzyme TH. To study this phenomenon, rats were deprived of REM sleep using the platform technique and the levels of TH mRNA were assessed by *in situ* hybridization. We also measured two of its known transcriptional activators, pCREB and c-fos, using immunohistochemistry. There was a significant increase in TH mRNA after 3- and 5- days on small platform (REM sleep deprived) whereas animals from 1day (small or large platform) or 5 days (large platform) did not show any significant change relative to dry cage controls. c-Fos protein was not detected in LC in any of the above conditions whereas pCREB protein was detected in all rats.

supported by DVA Research and NS30140

64.4

HYPOTHALAMIC GHRH MRNA AND TNFα MRNA LEVELS ARE HIGHER DURING THE DAY THAN NIGHT. S. Bredow, F. Obál Jr., N. Guha-Thakurta, P. Taishi and J.M. Krueger*. Dept. Physiol. & Biophys., Univ. Tennessee, Memphis, Memphis, TN 38163, USA.

Growth hormone releasing hormone (GHRH) and tumor necrosis factor α (TNFα) are involved in physiological sleep regulation (reviewed Krueger *et al.* 1995). Rats sleep about twice as much during daylight hours than nighttime hours. It is thus posited that levels of GHRH mRNA and TNFα mRNA are higher during the day than during the night in areas of the brain involved in sleep regulation. Male Sprague-Dawley rats (n=48, 320-350g) on a 12:12 light/dark cycle were sacrificed by decapitation at 4 hr intervals (8 animals/time point) starting 1 hr after light onset (08:00). The brains were quickly removed and dissected into cerebellum (CB), brain stem (BS), cortex (CT), hippocampus (HC) and hypothalamus (HT). Total RNA was extracted and analyzed for GHRH, TNFα and β-actin mRNAs by reverse transcriptase-polymerase chain reaction (RT-PCR). HT and CB levels of GHRH mRNA were higher during the light period than during the dark phase ($P<0.05$). The GHRH mRNA signal did not vary in the HC, CT or BS. HT β-actin levels did not vary significantly across the day. HT and HC TNFα mRNA levels were higher during the day than the night, but did not vary in the other brain areas examined. Since the HT is involved in sleep regulation, the data suggest that higher levels of GHRH and TNFα mRNAs in the HT could be associated with the greater amounts of sleep occurring during the day.

Krueger *et al.* (1995), Advances Neuroimmunol. 5, 171.

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64.5

THE TNF 55-KD RECEPTOR AND THE IL1 TYPE I RECEPTOR ARE INVOLVED IN PHYSIOLOGICAL SLEEP REGULATION. J. Fang*, L. Kapás, Y. Wang and J. M. Krueger. Dept. of Physiology & Biophysics,Univ. of Tennessee, Memphis, TN 38163.

Interleukin 1 (IL1) and tumor necroses factor (TNF) are the best characterized sleep-promoting cytokines. Recently, we [1] observed that the IL1 type I receptor and the TNF 55 kD receptor are involved in IL1β and TNFα induced sleep, respectively. However, it was unknown whether these receptors are involved in physiological sleep regulation. In the present experiment we determined the sleep patterns in TNF 55 kD receptor knockout (TNFR-KO), IL1 type I receptor knockout (IL1R-KO), and B6x129-F2 (F2) mice. Both types of knockout mice have a B6x129 background.

Methods. Three groups of mice (TNFR-KO, n=14; IL1R-KO, n=7; and B6x129-F2, n=13) were implanted with EEG and EMG electrodes. After surgery mice were kept in recording cages in a thermoneutral environmental chamber with ambient temperature at 30+/-1 °C and with a 12:12 h light-dark cycle (light on at 5 a.m.) throughout the experiment. Food and water were freely available. Experiments started 10 days after surgery. Sleep was recorded for 1 to 3 days in different experiments. Sleep data were visually scored in 10 second epochs and analyzed with two way repeated ANOVA and Student-Newman-Keuls test (SNK).

Results. 1) TNFR-KO mice had significantly less non-rapid eye movement sleep (NREMS, [F(1,25)=6.69, p<0.02]) and rapid eye movement sleep (REMS, [F(1,25)=13.09, p<0.002]) compared to F2 controls, primarily due to the decrease in sleep during the light period in TNFR-KO mice. 2) IL1R-KO mice had significant decreases in NREMS during the dark period (SNK: q(2,11)=3.90, p<0.05) and significant increases in REMS during the light period (SNK: q(2,11)=3.79, p<0.05) compared to F2 controls.

Discussion. Both IL1 and TNF promote NREMS in several species. Current data showed that the absence of the IL1 type I receptor or the TNF 55kD receptor gene results in alterations of physiological sleep. These data support the idea that IL1 and TNF are involved in physiological sleep regulation and suggest that endogenous IL1 and TNF may promote sleep at different times of day.

1. *Fang, J., Renegar, K. B., Kapás, L., Wang,Y., and Krueger, J. M.* The IL1 type I receptor and the TNF 55-kD receptor are involved in sleep regulation. J. Sleep Res. (in press).

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64.6

NERVE GROWTH FACTOR ENHANCES SLEEP IN RABBITS.
S. Takahashi*, S. Gala, L. Kapás, and J. M. Krueger. Department of Physiology and Biophysics, University of Tennessee, Memphis, TN 38163

It is hypothesized that nerve growth factor (NGF) is involved in the regulation of sleep. NGF is a well-characterized neurotrophic factor that elicits rapid-eye-movement sleep (REMS) after 1 µg microinjection into the pons in cats[1]. Further, a specific immunotoxin for NGF receptor-positive cholinergic basal forebrain neurons inhibits REMS, suppresses electroencephalogram (EEG) power and transiently impairs the circadian distribution of non-rapid-eye-movement sleep (NREMS) in rats[2]. The aim of the present study was to determine the effects of intracerebroventricular (icv) injection of NGF on sleep and brain temperature (Tbr) in rabbits. Male New Zealand White rabbits (3.5-5.5 kg) were implanted with EEG electrodes, a brain thermistor and an icv guide cannula. The animals were kept on a 12:12 h light-dark cycle (light on at 0600) at 21±1°C. Rabbits received recombinant human β-NGF (R&D Systems) icv [0.01 µg (n=5), 0.1 µg (n=6), 1.0 µg (n=8) or 10 µg (n=4)]. On a separate control day, the same rabbits were given 25 µl pyrogen free saline icv. Injections were between 8:45 and 9:15 a.m. EEG and Tbr were recorded for 23 h after injections. Two-way ANOVA for repeated measure was used for statistical analyses.

The highest two doses of NGF significantly increased both NREMS and REMS across the 23-h recording period. REMS was enhanced dose-dependently, i.e. 16 % (0.01 µg), 43 % (0.1 µg), 56 % (1.0 µg) or 65 % (10 µg) increases above control. Tbr was not affected by any dose of NGF. These results suggest that NGF is involved in both REMS and NREMS regulation.

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1.Yamy J, et al. Neuroscience 66:9-13, 1995. 2. Kapás L, et al. Brain Res. in press.

64.7

THE EFFECTS OF CCK-8NS AND CCK-4 ON SLEEP, SLOW WAVE ACTIVITY OF THE EEG AND BRAIN TEMPERATURE IN RATS.
Hee-Yoon Chang and Levente Kapás*. Department of Physiology and Biophysics, University of Tennessee, Memphis, TN 38163.

Systemic injection of 10-50 µg/kg cholecystokinin-octapeptide sulfate ester (CCK-8SE) increases both non-rapid-eye-movement sleep (NREMS) and EEG slow wave activity (SWA) and causes hypothermia (1). In the present study we tested whether non-sulfated CCK-8 (CCK-8NS) and CCK-tetrapeptide (CCK-4) have sleep-promoting and hypothermic effects in rats. These two peptides have similar affinities to CCK-B receptors as CCK-8SE, but their affinity to CCK-A receptors is 600 and 50,000 times lower, respectively.

Male Sprague-Dawley rats (290-390 g), were implanted with chronic electrodes for cortical EEG, EMG and brain temperature (Tbr) recordings. After a habituation period of 7-14 days the animals were injected intraperitoneally at dark onset with isotonic NaCl on one (control) day and with CCK-8NS (10 or 50 µg/kg, n = 7 for both) or CCK-4 (10 or 50 µg/kg, n = 8 and n = 7, respectively) on another, test day.

After 50 µg /kg CCK-8NS, there was a slight tendency towards an increase in NREMS in the first 2 h after the injection and later, both NREMS and rapid-eye-movement sleep (REMS) decreased in h 7-8. The increase and decrease in NREMS were accompanied with tendencies toward increased and decreased SWA, respectively. Tbr was not affected. 10 µg/kg CCK-8NS caused similar but slighter effects than the high dose. The low dose of CCK-4 did not affect sleep, SWA, and Tbr; 50 µg/kg CCK-4 did not affect NREMS and Tbr, but there was a slight tendency towards increased REMS and decreased SWA for 1-2 h after the injection. These results suggest that the activation of CCK-B receptors is not sufficient to elicit sleep and hypothermic responses.

1. Kapás et al., Brain Res. 1988 438: 155-164. Supported, in part, by NIH (NS-30514).

64.8

REDUCTION OF NON-RAPID EYE-MOVEMENT SLEEP (NREMS) IN HUMAN GROWTH HORMONE (hGH) TRANSGENIC MICE. J. Zhang, F. Obál, Jr., J. Fang, B. J. Collins[†]*, and J. M. Krueger. Dept. of Physiol. and Biophysics, Univ. of Tenn., Memphis, TN 38163; [†]Sect. Endocrinol., Univ. of Illinois, Chicago, IL 60612.

Transgenic mice, bearing a 4.8 kilobase (kb) sequence of upstream rat tyrosine hydroxylase (TH) promoter sequences linked to an hGH reporter, exhibited a dwarf phenotype. Previous studies suggest that the expression of hGH at sites of TH synthesis in the brain exerts negative feedback on GH-releasing hormone (GHRH)-producing neurons of the hypothalamus. Hypothalamic GHRH has been shown to be involved in physiological sleep and to form part of the homeostatic sleep process. The present study was designed to determine whether the reduction of GHRH observed in the arcuate nucleus of THhGH transgenic mice would be reflected in altered sleep patten.

Male hGH transgenic mice were selected from offsprings of two male hGH transgenic breeders by polymerase chain reaction (PCR) which targeted a 645 basepair (bp) fragment of the TH-hGH transgenic gene. Animals were implanted with electroencephalogram (EEG) recording electrodes over the frontal and parietal cortices and were allowed one week to recover from surgery. Sleep-wake activity was recorded for 3 consecutive days in both control (n = 9) and hGH transgenic (n = 9) mice which were maintained in a 12:12-hour light-dark cycle. The states of vigilance [NREMS, rapid eye-movement sleep (REMS), and wakefulness] were scored visually in 10 seconds intervals. The duration of states of vigilance was expressed as percent of recording time every two hours.

Two-way analysis of variance (ANOVA) for repeated measures revealed that the mean duration of NREMS for every two hours was significantly reduced in hGH transgenic mice over the 24 hour recording period ($p < 0.01$). But the mean duration of REMS in transgenic mice was not significantly different from that in control mice. Therefore, the ectopic expression of GH significantly suppressed the NREMS in transgenic mice possibly via the inhibition of endogenous GHRH secretion.

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64.9

Pharmacological and EM-immunocytochemical evidence for recurrent axon collaterals mediating feedback inhibition to NOS+, cholinergic neurons of the laterodorsal tegmental (LDT) nucleus. C.S. Leonard*, J. Rhee, G.E. Stutzmann, and C. Aoki, Center for Neural Science, NYU, New York, 10003.

Increased activity among NOS+, cholinergic neurons of the LDT contributes to the induction of the EEG-desynchronized states of *waking* and *REM* sleep by releasing Ach and possibly nitric oxide in the thalamus, basal forebrain and reticular formation. Recent evidence suggests LDT activity is under inhibitory feedback control since LDT neurons have recurrent axon collaterals (Surkis et al. 1996, Neurosci., In Press.) and are hyperpolarized by Ach. We now show that *endogenous* Ach hyperpolarizes LDT neurons and demonstrate by EM that local axon collaterals form synapses with NOS+ dendrites in the LDT. Intracellular recordings from a guinea pig brain slice indicated that eserine, a cholinesterase inhibitor, hyperpolarized and decreased R_{input} of LDT neurons like exogenous Ach. Atropine abolished the eserine effect and depolarized cells beyond their resting potential indicating significant baseline Ach release. Recorded neurons were then biocytin-injected, and studied by EM following NOS immunocytochemistry. Results indicate that NOS+ terminals from injected cells made symmetric type synaptic contacts with NOS+ and NOS- dendrites. Injected cells also received synaptic contact from both NOS+ and NOS- terminals. In addition, axonal terminals from injected cells were observed in direct apposition to unlabeled axonal terminals. These data support the hypothesis that the activity of LDT cholinergic neurons is under direct inhibitory feedback control. The presence of axo-axonic interactions raises the possibility that local release of Ach and NO may also have presynaptic actions upon synaptic afferents to the LDT. Supported by NIH NS27881 (C.S.L.) & NSF RCD 92-53750 (C.A.).

64.10

REVERSAL OF GAMMA-HYDROXYBUTYRATE-INDUCED FACILITATION OF PARADOXICAL SLEEP BY NCS-382.
V. Girodias, I. Beaulieu, H. H. Webster, M. Schmitt, J.J Bourguignon, R. Godbout*. Département de psychiatrie, Université de Montréal, Montréal (Québec) H4J 1C5.

Gamma-hydroxybutyrate (GHB) facilitates paradoxical sleep (PS) in the rat when administered in low doses at the begining of the subjective night (*Soc.Neurosci.Abs.* 1995; 21: 450.). We analysed the effect of the newly synthesized GHB receptor antagonist NCS-382 in this model. GHB (10 or 160 mg/kg i.p.) or saline were given with or without NCS-382 co-treatment in six rats at 8:00 (lights on at 6:00 for 12h); EEG, EOG and EMG were recorded individually for four hours thereafter. Each rat was exposed in random order to each treatment level. GHB 10 mg/kg decreased PS latency (24.0 ± 4.8 min. vs 53.5 ± 10.2 min.; $p < .02$). Latency to slow wave sleep (SWS) and durations of SWS and PS were not affected by GHB at any dose. The co-administration of NCS-382 totally blocked the effect of GHB on PS latency. These results suggest that PS facilitation by low doses of GHB may be due to a selective activation of high affinity GHB receptors.

Supported by CRSNG and CNRS

64.11

EFFECTS OF MICROINJECTIONS OF α1 AND α2 ADRENOCEPTOR AGONISTS INTO THE AMYGDALA ON SLEEP-WAKE STATES, L.D. Sanford*, S.H. Fuchino, D.E. Tidikis, R.J. Ross and A.R. Morrison. Univ. of Penna. Sch. of Vet. Med. and Med. and Phila. VA Med. Center, Phila., PA 19104.

Noradrenaline, originating in the locus coeruleus (LC), has long been implicated in the regulation of sleep-wake states. LC projects to the amygdala, which has been demonstrated to have a role in modulating arousal and behavioral state. Virtually every region of the amygdala has a modest concentration of noradrenergic fibers, with the densest concentration of fibers being in the central nucleus of the amygdala. We examined the effect of microinjections into the amygdala of an α1 adrenoceptor agonist, methoxamine, and an α2 adrenoceptor agonist, clonidine, on sleep and wakefulness. Rats (90 day old, male, Sprague-Dawley) were implanted with electrodes for determining behavioral state and with guide cannulae for bilateral microinjections into the amygdala. Rats were injected during non-rapid eye movement sleep with methoxamine (1.0 -10.0 µg / 0.4 µl normal saline; N=6) clonidine (0.01 - 0.05 µg / 0.2 µl normal saline; N=5) or multiple microinjections of normal saline alone (0.2 µl; N=4). Each rat received 5 microinjections, normal saline or drug, with 7 day intervals between injections. Neither multiple normal saline microinjections nor clonidine, at the doses tested, significantly affected any sleep measure that we examined. Methoxamine (2.5 - 10µg) decreased time spent asleep during the first 3 hours post-injection ($p < .05$). Moreover, time spent asleep remained suppressed one week following the last microinjection of methoxamine, suggesting the possibility of a prolonged alteration in sleep. The results indicate that an α1 adrenergic mechanism in the amygdala can influence amygdaloid modulation of behavioral state. Support: MH42903, NS35281,Dept. of Vet. Affairs and Merck Summer Fellowship.

64.12

DIFFERENTIAL EFFECTS OF PARADOXICAL SLEEP DEPRIVATION ON α AND β ADRENERGIC RECEPTORS IN RAT BRAIN. **J.N. Nobrega*, D.C. Hipólide, R. Raymond and S. Tufik. Clarke Institute of Psychiatry, 250 College St., Toronto, Canada, and Psychobiology Dept., Universidade Federal de São Paulo, Brazil.**

Previous examinations of adrenergic receptors after paradoxical sleep deprivation (PSD) have produced conflicting results. In the present study comprehensive autoradiographic analyses were used to address the possibility that PSD induces differential regional effects on specific adrenergic receptor subtypes. Ten rats were sleep deprived for 96 hr using a multiple platform technique, and then sacrificed along with 10 home cage controls. Beta adrenergic receptors were labeled with 150 pM [^{125}I]iodo-pindolol in the presence of 50 nM CGP-20712A to mask β_1 sites, or 70 nM ICI-11855 to mask β_2 sites. Non specific binding was defined in the presence of 200 µM isoproterenol. Alpha$_2$ sites were labeled with 5 nM [^{3}H]UK-14,304 in the presence or absence of 10 µM phentolamine. Quantitative analyses indicated that β_1 binding was significantly reduced in 12 of 69 brain regions examined; β_2 binding was reduced in 25 of 75 regions examined. Increased [^{125}I]iodopindolol binding was not observed in any brain region. In contrast to the widespread decreases in β adrenergic binding, [^{3}H]UK-14,304 binding to α_2 sites was not altered in any of the 89 brain regions examined, including locus coeruleus. These results indicate that PSD induces different effects on α and β adrenergic receptors. The observed effects on β receptors are consistent with the hypothesis that pradoxical sleep serves to counteract the downregulation of noradrenergic receptors which normally occurs during waking (Siegel and Rogawsky, *Brain Res.* 1988, 13, 213-233). (Supported by CNPq and AFIP, Brazil).

64.13

PERFUSION OF A 5-HT$_{1A}$ ANTAGONIST IN THE DORSAL RAPHE NUCLEUS (DRN) INCREASES SEROTONIN (5-HT) RELEASE IN DRN AND DECREASES REM SLEEP. Alvhild Alette Bjorkum*, Robert E. Strecker, Tarja Porkka-Heiskanen[1] and Robert W. McCarley. Dept. of Psychiatry, Harvard Medical School, Brockton VA Med. Ctr., Brockton, MA 02401, USA, and [1]Institute of Biomedicine, University of Helsinki, Finland.

The serotonergic system is involved in modulation of sleep and waking. Our laboratory has recently shown that microdialysis perfusion of the selective 5-HT$_{1A}$ receptor agonist 8-OH-DPAT in the DRN decreases 5-HT release and increases REM sleep in the freely moving cat. Conversely, we now demonstrate that DRN perfusion of antagonists at the 5-HT$_{1A}$ receptor produces an increase in extracellular 5-HT levels and a decrease in REM sleep. Microdialysis was performed in freely moving cats implanted with electrodes for the recording of behavioral state and with cannula used to quide the microdialysis probe to the DRN. Behavioral state and extracellular 5-HT levels were simultaneously assessed in a 3h control period, and during the subsequent addition of p-MPPI, a potent and selective 5-HT$_{1A}$ antagonist, to the artificial CSF perfusate. Perfusion of 5-HT$_{1A}$ antagonist in concentrations of 100 nM (n=3) and 300 nM (n=2) produced a 2 and 7 fold increase in extracellular 5-HT respectively. The highest dose of the antagonist also produced a 50% decrease in REM sleep. These effects are presumptively caused by the blocking of 5-HT$_{1A}$ autoreceptor-mediated negative feedback, which consequently increases serotonergic neural activity. This 5-HT$_{1A}$ antagonist-induced increase in 5-HT neuronal activity presumably produces an increase in 5-HT release in brainstem target areas involved in the regulation of REM sleep, such as the cholinergic neurons of the LDT\PPT and the medial pontine reticular formation. Support: MH39683; Dept. Vet. Affairs; Fulbright.

64.14

INTRACEREBRAL PERFUSION OF VITAMIN B12 AGGRAVATES CATAPLEXY IN NARCOLEPTIC CANINES. K. Honda[1,2], S. Nishino[1], D. M. Damm[1], S. Inoué[2], W. C. Dement[1]* and E. Mignot[1] [1]Stanford Sleep Research Center, Stanford University School of Medicine, CA., [2]Tokyo Medical and Dental University, Tokyo.

Vitamin B12 supplementation (methylcobalamin, methyl-B12) is known to improve circadian rhythm sleep disorders in human patients (1). It has recently been reported that intracerebroventricular (ICV) infusion of methyl-B12 significantly increased rapid-eye-movement (REM) and non-REM sleep in rats (2). The mode of action of methyl-B12 on sleep/circadian rhythms is not known, but an enhancement of the synthesis of central acetylcholine by transmethylation may be involved (3). Furthermore, a significantly lower level of vitamin B12 in the CSF has been reported in Alzheimer's disease, a disorder characterized by degeneration of cholinergic neurons (4).

Cataplexy, an abnormal manifestation of REM sleep atonia, is one of the most disabling symptoms of narcolepsy. Experimental evidence in the canine model suggests that central cholinergic systems are critically involved in the regulation of cataplexy (5). In this study, we tested the effects of central administration of methyl-B12 in narcoleptic Dobermans, and found that ICV perfusion of methyl-B12, (10-5 - 10-2 M) using a microdialysis probe, dose-dependently aggravated cataplexy in narcoleptic dogs. In contrast, cyano-B12, an analog of vitamin B12 which does not have methyl donor ability, had little effect on cataplexy. The hypothesis that the transmethylation by methyl-B12 is involved in the effect on cataplexy is, however, not bolstered by the fact that S-adenosylmethionine (10-5 - 10-2 M), an endogenous methyl donor, induced only minor modifications of cataplexy. Although the mode of action of methyl-B12 should be further studied, our current results suggest that methyl-B12 may be an important modulator of normal and abnormal REM sleep. (Supported by NIH grants NS27710 and NS23724)

(1) Kamgar-Parisi, B. et al. Sleep, 1983, 6; 257-264., Okawa M. et al. Sleep, 1990, 13; 15-23. (2) Honda K. and Inoué S. Int. J. Biometeorol., 1991 35; 114. (3) Nadeau A. and Roberge A. Internat. J. Vit. Nutr. Res., 1988, 58; 402-406. (4) Ikeda T. et al. Acta. Psychiatr. Scand., 1990, 82; 327-329. (5) Nishino S. et al. J. Neurosci., 1995, 15; 4806-4814.

64.15

MODEL OF HIPPOCAMPAL NEURONS INCORPORATING A CATIONIC NON-SELECTIVE CHANNEL AND A CELLULAR Ca^{2+} STORE. Teresa Ree Chay* and Young Seek Lee Dept. Biol. Sci., Univ. Pittsburgh, Pittsburgh, PA 15260 USA and Dept. Biochem. Hangyang Univ., Ansan, Korea.

We have studied the origin of autorhythmicity in hippocampal neurons by constructing a minimal one-compartmental mathematical model. The plasma membrane of our model contains **i)** a cationic non-selective channel (NSC) which is activated by a depletion of luminal Ca^{2+} in the intracellular Ca^{2+} stores and **ii)** a Ca^{2+} channel that is activated by voltage and inactivated by intracellular Ca^{2+} ions. In addition, the plasma membrane contains **iii)** an AMPA-receptor and **iv)** an AMPA-sensitive Na^+ channel; **v)** a GABA-receptor and **vi)** a GABA-sensitive K^+ channel. Activity of a Ca^{2+}-releasing channel (CRC) in the Ca^{2+} store is gated by intracellular Ca^{2+} ions. In this model, the luminal calcium concentration oscillates slowly, and this slow oscillation induces the electrical bursting via the NSC. The spikes are due to the Ca^{2+} channel, which is controlled by compartmentalized Ca^{2+} ions underneath the plasma membrane. Neurotransmitters such ACh, NE, 5-HT, and carbachol can switch a silent cell to bursting and a bursting cell to spiking by raising the activity of the CRC (via the GTP-binding proteins). This model is incorporated into a network of neurons arranged in a ring structure. The network generates synchronized reentrant rhythms including chaos.

64.16

Effects on the electroencephalogram induced by the changes in cerebrospinal-fluid Ca^{2+}-concentration: cellular mechanisms. Alessandro Formenti*, Elda Arrigoni, Mauro Mancia. Inst. of Human Physiol. II. Univ. of Milan, I-20133 Italy

It is well-known that in the pathological states accompanied by hypercalcemia, among neurological symptoms, a state ranging from drowsiness to lethargy accompanied by low frequency electroencephalographic (EEG) activity may be present. Sleep and wakefulness EEG rhythms are driven by the thalamo-cortical relay neurons that fire with single spikes during wakefulness (relay mode), and oscillate and synchronize their firing, producing bursts of action potentials, during slow-wave-sleep (oscillatory mode). The transition from the single spike to the rhythmic burst discharge is caused by a resting membrane hyperpolarization and low voltage-activated (LVA) calcium channels deinactivation. On these bases it was hypothesized that the fluctuation of extracellular Ca^{2+} concentration, especially in certain diseases, may represent a common mechanism that causes the modification of the sleep-wake cycles, changing the membrane potential and hence the discharge mode of the thalamic pacemaker and cortical neurons.

In vivo EEG recordings and *in vitro* whole-cell current and voltage-clamp were carried out using Wistar rats. A $CaCl_2$ solution (100 mM, 5 to 20 µl) perfused into the third ventricle tripled the EEG amplitude and induced a decrease in the EEG frequency at all the doses tested. The neuronal discharge, induced by depolarizing current stimuli during current-clamp recordings on single cultured thalamic neurons, changed from the single spike mode to the burst mode, when the extracellular Ca^{2+} concentration rose from 1 to 5 mM. Current-to-voltage relationship of LVA Ca^{2+} channels, obtained from voltage-clamped thalamic neurons, showed an increase in the peak current amplitude and a shift of the current activation to positive voltage values at increasing extracellular Ca^{2+} concentrations (0.5 to 5 mM).

In conclusion the increase in the extracellular Ca^{2+} concentration induces a strong membrane hyperpolarization and a consequent recovery of LVA Ca^{2+} channels from inactivation, favouring the transition of the neuronal firing from single spikes to burst mode. This condition is supposed to occur in many pathological states in which Ca^{2+} metabolism is involved.

64.17

NEUROTENSIN MICRO-INJECTIONS INTO THE BASAL FOREBRAIN PROMOTE CORTICAL ACTIVATION ASSOCIATED WITH THE STATES OF WAKE AND PS IN THE RAT.

E.G. Cape, A. Alonso, A. Beaudet and B.E. Jones*. Montreal Neurol. Inst., McGill Univ., Montreal, Quebec H3A 2B4, Canada.

Neurotensin has been shown to be contained in afferent fibers and to bind through high affinity receptors to cholinergic neurons in the basal forebrain. In vitro application of Neurotensin has been shown to produce a slow depolarization and rhythmic bursting in these cells. To examine the effect of this modulation upon EEG and state of the animal, Neurotensin was injected into the region of cholinergic basalis neurons using a remotely controlled microinjection device in chronically implanted, freely moving rats. As compared to Ringer, Neurotensin micro-injections (500 - 1500 pmoles) produced a decrease in delta activity (1-3 Hz) and an increase in both high frequency gamma activity (30-60 Hz) and rhythmic theta activity (4-8 Hz). This EEG pattern was associated with a behaviorally quiet, waking state that alternated with paradoxical sleep (PS). Preliminary results indicate that the effects of Neurotensin can be attenuated by peripheral administration of the neurotensin receptor antagonist, SR48692, or the muscarinic cholinergic antagonist, atropine. These results suggest that Neurotensin, which promotes rhythmic bursting in cholinergic neurons, can enhance gamma and theta cortical activity associated with the states of Wake and PS. (Supported by the Canadian MRC.)

64.18

CHANGES IN EXTRACELLULAR BASAL FOREBRAIN ADENOSINE LEVELS DURING SPONTANEOUS SLEEP-WAKING AND PROLONGED WAKEFULNESS.

Tarja Porkka-Heiskanen*, Robert E. Strecker, Alvhild Alette Bjørkum, and Robert W. McCarley. Dept. of Psychiatry, Harvard Medical School, Brockton VA Med. Ctr., Brockton, MA 02401, USA and [1]Institute of Biomedicine, University of Helsinki, Finland.

Adenosine (AD) is an inhibitory neuromodulator of the CNS that is thought to have a role in sleep regulation. Systemic treatment of AD and AD agonists produces increased sleep and decreased wakefulness, whereas AD antagonists, such as caffeine, induce arousal. However, it is not known whether AD levels undergo spontaneous changes during the sleep-wake cycle. Our development of a highly sensitive microbore high performance liquid chromatography (HPLC) assay for AD allowed detection of AD levels from in vivo brain microdialysis samples. Male cats were chronically prepared with electrodes to record EEG, EOG, EMG, and PGO spike activity. Guide cannulae were also implanted above the substantia innominata region of the basal forebrain, the VA/VL of the thalamus, and the cingulate cortex. Microdialysis probes were lowered through the guide cannula into the target areas and perfused with artificial CSF at a rate of 1.5 µl/min. Samples were analyzed by a microbore HPLC assay with UV detection (detection limit < 50 fmol/sample). In the basal forebrain, AD levels were higher during waking (100%) than sleep (82.5 ± 3.2%). No difference was found between waking and REM-sleep (95.3 ± 5.3%). Similar state-dependent changes were found in the thalamus and the cortex. During 6h of sleep deprivation, produced by gentle handling, AD levels in BF stayed at the level of normal waking, or increased slightly. AD levels declined sharply during the rebound sleep that followed deprivation. These data show that AD levels change according to vigilance state, and support the hypothesis that high CNS levels of AD promote drowsiness and the transition from wakefulness to sleep. Supported by NIMH grant MH39683; Dept. Vet. Affairs; Fulbright Fellowship to AAB.

64.19

VASOPRESSIN AND CIRCADIAN RHYTHMS IN SIMULATED SHIFT-WORK,

C. H. Wideman*, H. M. Murphy and G. R. Nadzam. Departments of Biology and Psychology, John Carroll University, Cleveland, OH 44118.

The role of vasopressin (VP) in circadian rhythms was examined using VP-containing, Long-Evans (LE) and VP-deficient, Brattleboro (DI) rats. Animals were maintained in individual cages while telemetered body temperature (BT), heart rate (HR), and activity (AC) data were collected. Subjects were exposed to a 12h/12h light/dark cycle (photic zeitgeber) and had one feeding period commencing at the second hour of the dark cycle (nonphotic zeitgeber). A simulation of shift-work was accomplished by introducing an 8h phase advancement (shift to a component in the cycle when the rat is normally inactive) for 5 days followed by a return to the original cycle (weekend) for 2 days. The alternation between shift-work and weekends was continued for 3 weeks. Feeding was shifted in synchrony with the light/dark cycle. When transferred from the habituation to the shift paradigm, both strains of animals evidenced a disruption of their circadian rhythm patterns. Immediately following the shift, the rats had an increase in BT, HR, and AC, which was followed by a precipitous drop in the three physiological variables in the "new active period". During the shift period, DI rats adapted more rapidly, readily, and completely than LE rats. When returned to the weekend schedule, DI rats adapted by the second day; whereas, LE rats had irregularities in rhythms of BT and HR. Results demonstrate that VP is significantly involved in circadian rhythm disruptions noted in a simulated shift-work paradigm. (Research supported by a Focused Giving Grant from Johnson & Johnson.)

64.20

STIMULATION OF ADENOSINERGIC A1 RECEPTORS ENHANCE nonREM SLEEP SLOW WAVE ACTIVITY IN NEONATAL RATS.

[1,2]R. N. Morrissette*, [2]I. A. Yun, [2]D. L. Stevenson and [2]H. C. Heller. [1]Interdepartmental Program in Neurosciences, UCLA, Los Angeles, CA 90025; [2]Department of Biological Sciences, Stanford University, Stanford, CA 94305.

The non-rapid eye movement (nonREM) sleep electroencephalogram is characterized by high amplitude, low frequency slow wave activity (SWA). NonREM SWA is a measure of the intensity of slow waves (0.75-4.0 Hz), and serves as an indicator of sleep depth within nonREM sleep. In contrast to adult rats, where sleep deprivation promotes a compensatory increase in nonREM SWA, we have found that a compensatory increase in nonREM SWA following sleep deprivation is not present in neonatal rats until postnatal day 24 (P24). Therefore, in order to test whether rats younger than P24 have the ability to enhance nonREM SWA, we gave intraperitoneal (IP) injections of the adenosinergic A1 receptor agonist, n^6-cyclopentyladenosine (CPA) to male Long-Evans rats aged P18 to P24. Systemic injections of CPA have been shown to increase nonREM SWA in adult rats.

CPA (1.0 mg/kg) significantly enhances nonREM SWA in rats as young as P18. This finding suggests that nonREM SWA can be enhanced in neonatal rats younger than P24 even though increased nonREM SWA is not seen in recovery from sleep deprivation.

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BIOLOGICAL RHYTHMS AND SLEEP: CIRCADIAN RHYTHMS I

65.1

MELATONIN RECEPTORS IN CHICK ASTROCYTES *IN VIVO* AND *IN VITRO*.

Vincent M. Cassone*, Melissa G. Whitfield Rucker, Thomas Leath, Arjun Natesan, Laura Schneider and Evelyn Tiffany-Castiglioni. Departments of Biology and Veterinary Anatomy, Texas A&M University, College Station, TX 77843-3258.

Recently, we (Reppert et al., 1995, Neuron 15: 1003-1015) have successfully cloned messages encoding two melatonin receptors from a cDNA library derived from chick brain. *In situ* hybridization using radiolabeled riboprobes of these two receptor mRNA's reveals dramatically different distributions. One receptor, the Mel1a receptor, accurately reflects the distribution of most *in vitro* binding of the melatonin agonist 2-[^{125}I]iodomelatonin (IMEL) in visual system centers of the avian brain. The other, the Mel1c receptor, is found primarily within non-neuronal elements of the chick nervous system. However, it was not possible to identify the cell-types expressing this hybridization definitively. We have employed two approaches to this question.

First, primary astrocytes from E17 chicks were isolated and cultured in DMEM with 10% FBS for 26 days at 38°C. Cells were harvested and frozen at -80°C until assay. Cell membranes were extracted, total protein was assayed, and equal aliquots of membrane were assayed for 2-[^{125}I]iodomelatonin (IMEL) binding. Specific, reversible and high affinity IMEL binding was observed in these cell membranes. Second, employing digoxigenin-labeled riboprobes for the Mel1a and Mel1c melatonin receptors, we observe *in situ* hybridization for Mel1a receptor in neurons and Mel1c hybridization in astrocytes. The area of hybridization within astrocytes is greater during the day than during the night. These data are consistent with previous *in vivo* studies in our laboratory indicating a daily change in astrocytic fiber length such that fibers are longer during the day than during the night. Exogenous melatonin administration during the day, furthermore, decreases astrocytic fiber length to night-time values. The data are consistent with the hypothesis that melatonin affects astrocyte function via the Mel1c, but not the Mel1a, melatonin receptor. **Supported by grants from the AFOSR to VMC.**

65.2

TRANSCRIPTIONAL REGULATION OF THE NOCTURNIN GENE BY A RETINAL CIRCADIAN CLOCK.

C.B. Green, D.L. Osterbur and J.C. Besharse*, Department of Anatomy & Cell Biology, University of Kansas Medical Center, Kansas City, KS 66160.

We have recently identified a rhythmic retinal mRNA in *Xenopus laevis* that encodes a protein that we have named nocturnin. It contains a leucine zipper-like dimerization motif and sequence similarity to a yeast transcription factor named CCR4. It is expressed specifically in photoreceptor cells, the same cells that contain an endogenous circadian clock. We have identified sequences with high similarity in both human and mouse that are expressed in brain and retina.

Because the molecular mechanisms by which circadian clocks regulate gene transcription are unknown, we began an investigation of nocturnin gene regulation. Nuclear run-on analyses, performed on nuclei isolated from retinas at different times of day in both cyclic light and constant darkness verified that rhythmic nocturnin mRNA levels were the result of circadian control of transcription of this gene. Characterization of the nocturnin gene structure revealed that the gene was composed of 3 exons and 2 introns and spanned about 10 kilobases (kb). Differential polyadenylation of the primary transcript results in 2 nocturnin messages, both containing identical open reading frames. Both messages show identical time courses of expression; the mRNA levels are low (undetectable) during the day, rise sharply in early night, and return rapidly to undetectable levels by mid-night. The transcription start site was identified by both primer extension and by 5'-RACE and a consensus TATA box was observed 30 base pairs upstream of this start site. Characterization of the nocturnin promoter provides the means to identify promoter elements and trans-acting factors involved in circadian control of gene transcription. Funded by NIH grant EY02414 (JCB).

65.3

Subjective Day Phase Shifts in the Mollusc *Aplysia californica*: The Role of a Transcription Factor? D. Whitmore, R. Day and G.D. Block*. Univ. of Virginia, Center for Biological Timing, Charlottesville, Va 22903.

Subjective day phase shifts are a common, but relatively unexplored feature of circadian pacemakers compared to light-induced phase shifts. Activity-dependent subjective day phase shifts can be produced in the rodent (Mrosovsky,1988), and a number of treatments phase shift the pacemaker in mammalian SCN brain slice preparations (Gillette et al, 1988). Eskin and colleagues have described a subjective day phase shifting pathway in the mollusc *Aplysia* which is activated by the neurotransmitter serotonin (5HT). 5HT activates a cAMP-dependent signal transduction cascade in which increased cAMP leads to membrane hyperpolarization and a subsequent phase shift which requires protein synthesis. We examined levels of the *Aplysia* transcription factor CCAAT enhancer binding protein (ApC/EBP) in response to these subjective day phase shifting treatments. Using quantitative RT-PCR we show that 5HT, forskolin, hyperpolarization and low calcium all increase the amount of C/EBP mRNA in the retina. The pairing of light and 5HT in the late subjective night produces large phase shifts and superinduces C/EBP mRNA levels. A protein of predicted molecular weight and isoelectric point shows corresponding increases in response to these treatments on two-dimensional SDS protein gels. This correlational evidence argues for a role of ApC/EBP in 5HT mediated phase shifting in *Aplysia*. (NIH NS15264).

65.4

REGULATION OF THE DROSOPHILA PROTEIN TIMELESS INDICATES A MECHANISM FOR RESETTING THE CIRCADIAN CLOCK BY LIGHT, Melissa Hunter-Ensor, Andrea Ousley*, Amita Sehgal. Dept. of Neuroscience and Ctr for Sleep and Respiratory Neurobiology, University of PA Medical School, Phila., PA 19104.

In *Drosophila melanogaster*, our understanding of the generation of circadian rhythms has until recently depended on the first circadian rhythm gene, *period* (*per*). With the identification of *timeless*, a new clock gene that disrupts locomotor as well as molecular rhythms, we are now fitting *tim* into what had previously been a *per*- based model of the clock. We recently raised an antibody against a bacterially expressed fragment of TIM, and examined the spatial and temporal regulation of TIM expression. Like *per*, *tim* mRNA cycles in a wild type background with peak levels observed early in the evening. TIM protein levels also cycle in nuclei, rising late at night while PER levels are also rising. Peak levels of TIM lag behind the *tim* mRNA peak by several hours. TIM is expressed in those cells that have been shown to express PER. TIM is not observed in the photoreceptor nuclei of per^0 flies, suggesting that PER is required for the nuclear localization of TIM. TIM staining is not observed in tim^0 flies. The phase of TIM cycling suggested that TIM levels were reduced at the end of the night, considerably earlier than the decrease in PER levels. We found that pulses of light at different times of day result in the lowering of TIM levels. This was assayed by immunocytochemistry as well as by western blot analysis. We suggest that the mechanism for TIM's response to light is post-transcriptional, as we observed no significant changes in *tim* or *per* RNA in response to light treatment. These findings suggest a mechanism for resetting the circadian clock by light.

A.S. supported by NIH, American Cancer Society grants; MHE behavioral training grant at U. of PA; A.O. NIH postdoc fellowship

65.5

NEUROPEPTIDE Y ADVANCES THE CIRCADIAN RHYTHM OF THE RAT SUPRACHIASMATIC NUCLEUS IN VITRO BY AN INTERACTION WITH A Y2 RECEPTOR. V.K. Gribkoff*[1], R.L. Pieschl[1], T.A. Wisialowski[1], A.N. van den Pol[2] and E.D. Yocca[1]. [1]CNS Drug Discovery, Bristol-Myers Squibb Pharmaceutical Research Institute, 5 Research Parkway, Wallingford, CT 06492 and [2]Sect. Neurosurgery, Yale University, New Haven, CT 06510.

The mammalian hypothalamic suprachiasmatic nucleus (SCN) generates circadian neuronal discharge rhythms underlying some behavioral cycles. While cells within the SCN have an intrinsic circadian rhythmicity, the precise timing is susceptible to modulation by factors such as the timing of light-dark cycles. Several neuromodulators have been implicated in the mediation of this plasticity; these include melatonin, serotonin and neuropeptide Y (NPY). NPY originates in the retinoreceptive cells of the intergeniculate nucleus of the thalamus; these cells innervate the SCN via the geniculohypothalamic tract, where they may act as a link between visual cues and circadian rhythms. Previous evidence suggests that NPY microinjected into the SCN in vivo or in vitro can shift the phase of the SCN circadian rhythm. In the present experiments we have used the multi-unit recording technique to examine the effects of NPY and receptor-specific NPY analogs on circadian discharge rhythms in the rat SCN in vitro.

SCN slices were perfused on day 1 with 1.0 μM NPY, 1.0 μM [Leu^{31},Pro^{34}]-NPY (a Y1 agonist), 1.0 μM NPY fragment 13-36 (a Y2 agonist) or vehicle from circadian time (CT) 7.5-8.5. Electrical activity was recorded for 36-48 hr., and the peak of the discharge rhythm on day 2 was calculated. NPY and NPY fragment 13-36 significantly advanced the peak of the SCN rhythm relative to vehicle and [Leu^{31}, Pro^{34}]-NPY (ANOVA and Fisher's test, $p<0.05$). The time for the peak for each treatment in CT was 5.74±0.22 vehicle; 5.56±0.28 [Leu^{31}, Pro^{34}]-NPY; 4.62±0.35 NPY; 4.59±0.25 NPY fragment 13-36. These data demonstrate that NPY exerts its effects on the circadian electrical activity rhythm in the SCN by activation of the Y2 receptor, and further demonstrate the utility of the multi-unit recording method.

65.6

EVIDENCE THAT CIRCADIAN AND VISUAL SIGNALS FROM THE SCN AND THE LGN ARE INTEGRATED ON NEUROENDOCRINE DOPAMINE CELLS IN THE RAT HYPOTHALAMUS. T.L. Horvath*. Yale University, Dept. of Ob/Gyn, New Haven CT, 06520.

Prolactin has been implicated to play a major role in light- or time-shift-induced physiological alterations in activities of mammals including humans. The key role of hypothalamic dopamine and the circadian visual system (SCN and IGL) in the regulation of prolactin secretion have been recognized. However, the signaling pathway via circadian and visual signals reach the lactotrophs in the anterior pituitary is ill-defined. We hypothesized that direct connections may exist between the SCN or the intergeniculate leaflet (IGL) of the LGN and neuroendocrine dopamine cells of the hypothalamus. Experimental: The combination of anterograde (PHA-L) and retrograde (fluorogold) tracing techniques and immunocytochemistry employing ABC and PAP techniques and different color chromogens was used. PHA-L was injected into either the SCN or the IGL, in parallel with intraperitoneal administration of fluorogold to label neuroendocrine (median eminence-projective) cells. In the anteroventral periventricular nucleus, medial preoptic area, periventricular and arcuate nuclei, retrogradely labeled tyrosine hydroxylase-immunoreactive (putative dopaminergic) cells were frequently found to be targeted by PHA-L labeled SCN and IGL efferents. These cells also received a massive VIP and NPY innervation, neuropeptides that are characteristics of SCN and IGL efferents, respectively. Electron microscopic examination confirmed the putative connections to be synaptic. Conclusion: These experiments provided the first evidence that the SCN and IGL have direct efferents onto neuroendocrine dopamine cells which may underlie the time- and light-dependent activity of prolactin secretion. Furthermore, it is suggested that the IGL may not only subserve the SCN in supporting circadian activities, but, can also convey non-circadian visual signals directly to neuroendocrine cells. (Supported by the Brown-Coxe Fellowship for T.L.H.)

65.7

PROMINENT ROLE OF SLOWLY INACTIVATING SODIUM CURRENT IN SPONTANEOUS FIRING AND MEMBRANE POTENTIAL OSCILLATIONS IN RAT SUPRACHIASMATIC NUCLEUS. C.M.A.Pennartz* and A.M.S.Geurtsen. Netherlands Inst. Brain Res., Grad. Sch. Neurosciences Amsterdam, Amsterdam, the Netherlands.

The biological clock is located in the suprachiasmaticus nucleus (SCN). Neurons in this nucleus exhibit a circadian rhythm in spontaneous firing rate. To investigate the physiological basis of this rhythm, it is of paramount importance to unravel the ionic mechanisms governing spontaneous firing in SCN. Firing patterns were studied by whole-cell patch-clamp recording in transversal slices (250 μm) using Kgluconate-filled pipettes with resistances of 4-8 MΩ. Biocytin staining of the recorded neurons revealed a diversity of morphology, including monopolar and bipolar cell types.

In all cells, spontaneous or evoked action potentials were consistently preceded by depolarizing ramps (spike prepotentials). These ramps were shown to be Na^+ dependent and sensitive to tetrodotoxin (0.5-1 μM). The Na^+ current underlying depolarizing ramps was activated well below firing threshold (-70 to -60 mV) and activated and inactivated more slowly than the fast Na^+ current. However, inactivation proceeded much more rapidly than has been observed for the persistent Na^+ current in neocortex. When fast action potentials were suppressed by lidocaine (0.5-1.0 mM), we observed damped oscillations in membrane potential. These autorhythmic oscillations were shown to involve the slowly inactivating Na^+ current and a TEA-sensitive K^+ current. Nimodipine (2-8 μM) failed to affect the oscillations. Our observations strongly implicate the slowly inactivating Na^+ current in ionic mechanisms governing spontaneous firing in suprachiasmatic nucleus neurons. In addition, the Na^+ current mediating spontaneous firing in SCN presents novel kinetic characteristics and may be unique to neurons of the biological clock.

65.8

INTERGENICULATE LEAFLET (IGL) AND VENTRAL LATERAL GENICULATE (VLG) PROJECTIONS IN THE RAT. R.Y. Moore*, M.M. Moga And R. Weis. Department of Psychiatry and Center for Neuroscience, University of Pittsburgh, Pittsburgh, PA 15261.

The mammalian lateral geniculate nucleus has two subdivisions that derive from ventral thalamus, the IGL and VLG. The IGL is now recognized to be a component of the circadian timing system whereas the VLG has long been considered involved in visual reflex and oculomotor integration. In this study, we analyzed IGL and VLG projections in the rat using anterograde (PHAL) and retrograde (HRP) transport.

The IGL and VLG have a pattern of partially separate and partially overlapping efferent projections. Both project along a dorsal pathway over the surface of the thalamus and a medial pathway to innervate the zona incerta, dorsal hypothalamic area and midline thalamus. IGL, but not VLG, axons terminate in the paraventricular thalamic nucleus. Both project through a caudal pathway to the lateral posterior nucleus, pretectal area, superior colliculus, periaqueductal gray and posterior hypothalamic area. The VLG projects selectively to the lateral tegmental field of the midbrain, interpeduncular nucleus, pontine gray, median raphe, parabrachial area, Kolliker-Fuse nucleus and inferior olivary complex. The IGL projects selectively through the geniculohypothalamic tract to the suprachiasmatic nucleus (SCN), retrochiasmatic area, subparaventricular zone, anterior hypothalamic area, medial preoptic area and organum vasculosum lamina terminalis. Thus, the IGL projects to the circadian pacemaker (SCN) and areas to which it projects whereas the VLG projects predominantly to visual and motor control areas. Supported by NS 16304.

65.9

A CIRCADIAN RHYTHM IN LUMINESCENCE IN SUPRACHIASMATIC NUCLEUS CULTURES FROM A *FOS/LUCIFERASE* TRANSGENIC MOUSE. Michael E. Geusz*, Steve A. Kay, Colin Fletcher, Gene D. Block, Neal G. Copeland, Nancy A. Jenkins, and Richard N. Day. NSF Center for Biological Timing, University of Virginia, Charlottesville, VA 22903 and Mammalian Genetics Laboratory, ABL-Basic Research Program, NCI-Frederick Cancer Research and Development Center 21702.

Entrainment of the circadian pacemaker in the suprachiasmatic nucleus (SCN) to light/dark cycles may involve the immediate-early gene *c-fos*. Light pulses applied during the subjective night phase shift the pacemaker and induce expression of c-*fos* in the SCN. To examine pacemaker control of c-*fos*, we generated a transgenic mouse expressing the human c-*fos* promoter coupled to the firefly luciferase gene. SCN sections from these mice were maintained in a Hepes-buffered medium with luciferin. Continuous luminescence recordings were made with either a luminometer or a photon-counting module. Sections containing the SCN, but not tissue from other regions, showed near-24 h luminescence rhythms lasting up to five cycles with an average period of 22.9 ±0.76 h (s. e. m., n=6) as analyzed by periodogram. In control experiments, SCN explants from a second transgenic mouse expressing the luciferase gene driven by the cytomegalovirus promoter demonstrated high-level luminescence that was not circadian. (n=5). Brain slice cultures from the *fos/luciferase* mouse were also imaged to determine the distribution of the transgene's expression. Luminescence was detected in the supraoptic and paraventricular nuclei of the hypothalamus as well as in the SCN. These studies demonstrate that rhythmic expression of the c-*fos* gene promoter in the SCN can be monitored non-invasively in real time in living tissue. *Supported by the NSF Center for Biological Timing and the National Cancer Institute, DHHS, under contract with ABL.*

65.10

INDIVIDUAL CELLS IN CULTURED MOUSE SCN EXPLANTS EXPRESS CIRCADIAN RHYTHMS IN FIRING RATE FOR WEEKS ON A MULTIMICROELECTRODE PLATE

Erik D. Herzog*, Michael E. Geusz, Sat Bir S. Khalsa and Gene D. Block. NSF Center for Biological Timing, Univ. of Virginia, Charlottesville

Recent evidence strongly suggests that the mammalian suprachiasmatic nucleus (SCN) is comprised of multiple circadian oscillators. However, it is unknown which circadian properties are intrinsic to individual cells or to cellular interactions. As an initial step towards characterizing circadian properties in the SCN, we record extracellular spikes from cultured explants.

From neonatal C57BL mice (postnatal days 2-6), we grew 300 µm sections of individual or paired SCN onto an array of 64, embedded microelectrodes. The multimicroelectrode plate (MMEP) offers a sterile and stable environment for long-term recording of extracellular action potentials. Over the next 2-3 weeks, the explants spread to cover approximately twice their area while retaining the gross appearance of the SCN *in vivo*. After as little as one week (earliest time tested) or as long as 7 weeks (latest time tested) in culture, SCN neurons exhibited spontaneous action potential activity on as many as all 64 electrodes. We discriminated the spikes on as many as 8 electrodes simultaneously and often saw synchronous bursting across several electrodes. In some, but not all cases, spike activity was rhythmic. In cases where we recorded from the same cell for at least 3 days, periods averaged 23.9 hours (± 0.7 s.d.) and ranged from 22.4 to 25.0 h (n= 13 cells, 6 explants). The longest recording to date was terminated after 25 days while impulse activity continued. *Supported by: NSF Center for Biological Timing*

65.11

RESTORATION OF A DIURNAL CORTICOSTERONE RHYTHM IN CRH DEFICIENT MICE BY A CONSTANT INFUSION OF CRH. S. Weninger[1,2], L. Muglia, L. Jacobson, D. S. Bae[2], C. Luedke, and J. A. Majzoub*. Division of Endocrinology, Childrens Hospital, [1]Program in Neuroscience, Harvard Medical School, [2]Howard Hughes Medical Institute, Boston, MA 02115.

The hypothalamic-pituitary-adrenal (HPA) axis of most mammals exhibits circadian rhythmicity. The molecular basis of this rhythm is not known, although the hypothalamus and pituitary, but not the adrenal, are required for its generation. Recently, a line of CRH knockout (KO) mice has been generated in our laboratory. To determine the role of CRH in the circadian rhythm of the HPA axis, we have monitored morning (9AM) and evening (6PM) levels of corticosterone (cort) and ACTH in CRH KO and wild-type (WT) mice. Mice were maintained on a 12:12 light:dark cycle with lights on at 7AM. Although both genotypes have similar basal AM levels of cort (2µg/dL, vs. 2.5µg/dL in WT and KO respectively), the CRH KO animals lack the PM rise in cort seen in WT mice (12µg/dL). To determine if the rhythm in ACTH is also absent in CRH KO animals, ACTH levels were measured in adrenalectomized WT and CRH KO animals. Again, CRH KO animals had no detectable ACTH diurnal rhythm, whereas levels in WT animals rose 2-fold in PM vs. AM. To test the hypothesis that constant CRH replacement can restore the circadian cort rhythm, we chronically infused CRH using Alzet osmotic minipumps at doses of 0.1, 0.25, 0.5, or 1.0µg/day. All but the lowest dose tested were able to restore the diurnal rhythm, measured at 3 and 6 days following pump implantation ($p<.002$ at 2 highest doses measured after 6 days). These data demonstrate that CRH is required for normal diurnal variation in ACTH and cort levels. Since CRH is believed to be necessary for ACTH synthesis, we postulate that tonic CRH may act to maintain corticotroph ACTH stores, and that other secretagogues, possibly such as vasopressin, actually drive the circadian rhythm in ACTH release. Supported by NIH, HHMI, NARSAD and Burroughs Wellcome.

65.12

GENOTYPIC DIFFERENCES IN THE ONTOGENY OF CIRCADIAN RHYTHMICITY IN HONEY BEES. D. Moore[1]*, T. Giray[2], and G.E. Robinson[2]. [1]Dept. of Biological Sciences, East Tenn. State Univ., Box 70703, Johnson City, TN 37614. [2]Dept. of Entomology, Univ. of Illinois, Urbana, IL 61801.

A major factor contributing to the division of labor within the honey bee colony is age polyethism, in which the behavioral tasks performed by individual worker bees gradually change with age. Genotypic differences exist for the rates at which workers advance through this behavioral development which begins with a progression of in-hive duties and ends with the achievement of forager status. As a first step in assessing the possible role of circadian rhythmicity within the age-related development of behavior, we monitored locomotor activity in newly emerged, individually isolated worker bees under constant conditions (DD, 25°C). "Fast" and "slow" genotype bees were taken from different single-patriline colonies in the field. Prior screening established that slow colony bees required significantly more days to became foragers than did fast colony bees.

All bees, regardless of genotype, were arrhythmic immediately after emergence. However, fast bees developed a circadian rhythm of locomotor activity significantly sooner than did the slow bees: 5.6 ± 0.6 days *vs.* 9.0 ± 0.6 days ($p < 0.001$). The free-running period was significantly shorter ($p < 0.01$) in the fast bees (23.3 ± 0.3 h, N = 22) than in the slow bees (24.2 ± 0.2 h, N = 33). The circadian period and the time to onset of rhythmicity (a once-in-a-lifetime event, occurring from 3 to 16 or more days post-emergence) were significantly correlated ($p < 0.01$). Furthermore, a significant difference ($p < 0.001$) between the two genotypes was also observed for yet another temporal pattern, hour-to-hour locomotor activity. Our results demonstrate: (a) the existence of an ontogeny of circadian rhythmicity in this species; (b) genotypic differences for the development of rhythmicity; and (c) three vastly different temporal processes that tend to vary with behavioral development time. *(Supported by NSF grant # IBN 9211386 to GER)*

NEUROETHOLOGY: SONGBIRDS I

66.1

A NEW SOURCE OF AUDITORY INPUT TO THE SONGBIRD ANTERIOR FOREBRAIN PATHWAY. **G.E.Vates*, D.S. Vicario, & F. Nottebohm. The Rockefeller University, New York, NY 10021.**

Learned vocalizations in songbirds are produced by a specialized vocal control pathway in the forebrain that includes HVC and its projection to RA. HVC also projects to the anterior forebrain pathway, which is necessary for vocal learning during development but seems to play little role in adult vocal production. Song learning depends on auditory information both to memorize the tutor's song and to guide sensorimotor practice that leads to song imitation. Auditory input is thought to enter the vocal system at the level of HVC.

We have now recorded single and multi-unit activity in one anterior forebrain nucleus, LMAN, in response to auditory stimuli (bird's own song played forward and in reverse, conspecific songs, long calls, and synthetic sounds) in awake adult male zebra finches. Responses were seen to all stimuli, but were strongest for the bird's own song. Auditory responses showed a pattern of early bursts (latency 15-25ms) and tonic elevation of activity that long outlasted the stimulus. Because the response latency was the same or less than that seen in HVC, we tested HVC's contribution to these responses by lesioning HVC either reversibly with lidocaine or permanently by bilateral suction. Auditory responses were still seen in LMAN in the complete absence of HVC.

These results demonstrate the existence of a short-latency auditory input to LMAN that does not depend on HVC. We are now working to identify its anatomical basis. LMAN appears to be a convergence point where 3 different sources of information can be combined: 1) the HVC-X-DLM-LMAN pathway carrying auditory and/or motor signals; 2) the recently demonstrated RA-DLM-LMAN projection carrying an "efferent copy" of motor commands; and 3) an undescribed pathway carrying short-latency auditory input that has not been processed in the vocal motor pathway. (MH40900, MH53542)

66.2

INTERHEMISPHERIC COORDINATION OF PREMOTOR NEURAL ACTIVITY DURING SINGING BY ZEBRA FINCHES. E. T. Vu*[1], M. F. Schmidt[2], and M. E. Mazurek[2]. [1]Division of Neurobiology, Barrow Neurological Institute, Phoenix, AZ 85013 & [2]Division of Biology, Caltech, Pasadena, CA 91125.

Unilaterally stimulating the forebrain nucleus HVC during singing alters the temporal pattern of the song of zebra finches, suggesting that neurons in HVC participate directly in specifying the song temporal structure (Vu et al., *J. Neurosci.* 14: 6924, 1994). Electrical stimulation of one HVC (on either side) would not have led directly to perturbation of neural activity in the contralateral HVC because each HVC does not project directly to the contralateral HVC. However, if the patterns of neural activity in both HVC's contribute to the song temporal pattern, then the rapidly altered song pattern following unilateral HVC stimulation suggests that the activity pattern in the contralateral HVC was also altered by the electrical stimulus.

To confirm this prediction, we implanted chronic microelectrodes in both HVC's of adult male zebra finches. We then recorded multi-unit neural activity in one HVC during singing and occasionally applied electrical stimuli to the contralateral HVC. In 4 out of 4 animals, the pattern of activity in HVC was altered every time the song pattern was altered by stimulation in the contralateral HVC. Following the brief stimulus, neural activity in the unstimulated HVC was first suppressed and then resumed in synchrony with the new song pattern. The neural suppression was not always immediate: elevated neural activity sometimes persisted for 10-70 ms after the end of the stimulus before being suppressed.

These results strongly suggest that the output of song premotor areas in the forebrain is continuously monitored and that an active mechanism exists for resynchronizing the outputs from the two hemispheres whenever a mismatch in their timing signals occurs. This would require feedback pathways to forebrain song control nuclei from lower stations of the motor pathway.

Supported by NINDS Award #NS08915 to ETV.

66.3

BIRDS' EXPERIENCE OF THEIR OWN SONGS SHAPES THE AUDITORY SELECTIVITY IN THE ANTERIOR FOREBRAIN OF JUVENILE ZEBRA FINCHES M.M.Solis* and A.J.Doupe, Neuroscience Graduate Program, Departments of Physiology and Psyehiatry, UCSF, San Francisco, CA 94143.

Auditory experience of both tutor and bird's own plastic song is crucial to song learning. The anterior forebrain pathway (AFP) may process this auditory information, since it is required for normal song acquisition, and contains auditory neurons throughout song development. AFP neurons also become selective for bird's own song during song learning, preferring it to conspecific song and to the reversed version of bird's own song (Doupe 1996). By an intermediate stage of learning (60d), many AFP neurons are already song and order selective, but often respond equally well to both the juvenile song and tutor song. These response properties could reflect experience of both of these stimuli, or might simply result from acoustic similarity between them.

In order to minimize the similarity that usually develops between a bird's own song and that of its tutor, juvenile male birds with highly abnormal song were produced by sectioning the tracheosyringeal (ts) nerve bilaterally before the onset of vocalization. At 60d, these birds sang temporally patterned series of harmonic stacks. Single units were recorded in two AFP nuclei, LMAN and X, of these 60d birds. The stimuli presented included the bird's ts cut plastic song (BOS), tutor, and conspecific songs. Some neurons were both song and order selective for BOS, responding more to it than to all other song stimuli. Despite obvious acoustic differences between BOS and tutor song, other neurons still responded equally to these two stimuli. Some of these neurons preferred tutor over conspecific song, yet lacked order selectivity for tutor song; others were untuned, having similar responses to all song stimuli.

The existence of song and order selectivity to the unnatural ts cut songs demonstrates that a bird's experience of its own vocalizations can shape the selectivity of neurons in the AFP, as is true for HVc (Volman 1993). Neurons tuned in this way could provide information to the bird about its developing vocalizations during sensorimotor learning. Responses to tutor song may reflect the broad selectivity of neurons in younger birds, residual similarity between BOS and tutor song, or the creation of selectivity in the AFP by both stimuli. (Supported by a NSF Predoctoral Fellowship and McKnight Endowment Fund)

66.4

SPECTRAL AND TEMPORAL SENSITIVITY OF AUDITORY NEURONS OF NUCLEUS HVC IN THE ADULT MALE ZEBRA FINCH. F.E. Theunissen* and A.J.Doupe, Depts of Psychiatry and Physiology, UCSF, San Francisco, CA 94143.

Auditory neurons in nucleus HVc have been shown to respond selectively to the bird's own song (BOS) in contrast to other complex auditory stimuli such as conspecific songs or the BOS played in reverse. This selectivity emerges during development and song learning, and it has been postulated that these highly selective auditory neurons are part of the sensory feedback pathway required in the learning task. To begin to elucidate the potential role of these neurons in song recognition, we systematically investigated their tuning to the quality of the BOS along spectral and temporal dimensions.

To do so, we synthesized artificial versions of the BOS with different degrees of spectral and temporal degradation. We decomposed the waveform of the original song into a complete set of 120 band-passed signals (120 signals of 70 Hz bandwidth between 500 and 8000 Hz). Each band-passed signal was further described by a carrier frequency modulated by a low frequency amplitude envelope and an instantaneous phase. The spectral quality of the song was degraded by averaging the amplitude envelope across a larger and larger number of neighboring frequency bands. The temporal quality was degraded by randomizing the phase of the carrier frequency for all frequencies above a progressively lower cutoff.

HVc neurons showed a very high sensitivity to song degradation along these dimensions. In particular, the responses to synthesized song with correct amplitude envelopes but random phase were greatly reduced and often not significantly different from background; preliminary results indicate that the phase of the carrier must be preserved for frequencies below 4 KHz. These results suggest that the exact natural spectral and temporal qualities of a song are essential for the selectivity seen in HVc neurons in adult birds. Investigating neural responses to systematic song alterations along other parametric dimensions, such as those that may be used to distinguish between similar natural songs, will help in further characterizing the tuning of these neurons and their potential role in song discrimination.

Supported by the Sloan Foundation and the Markey Charitable Trust.

66.5

METABOTROPIC GLUTAMATE RECEPTORS HYPERPOLARIZE NEURONS IN NUCLEUS HVc OF THE ADULT ZEBRA FINCH. D. J. Perkel*[1] and M. F. Schmidt[2]. [1]Dept. Neuroscience, University of Pennsylvania, Philadelphia, PA 19104 and [2]Division of Biology, Caltech, Pasadena, CA 91125.

In nucleus HVc of the songbird brain, glutamate excites neurons by activation of ionotropic glutamate receptors of the AMPA and NMDA types. We now report that activation of metabotropic glutamate receptors (mGluRs) has the unusual action of hyperpolarizing HVc neurons.

Intracellular recordings (pipettes filled with KAc) were obtained from HVc neurons in coronal or parasagittal brain slices (400 µm thick) prepared from adult zebra finches and maintained *in vitro*. Bath application of the mGluR agonist 1S,3R-aminocyclopentane dicarboxylic acid (ACPD; 100 µM) hyperpolarized most HVc neurons from resting potential by 9.1 ± 3.5 mV (n = 10/12). One cell showed no potential change and one cell was depolarized by 6 mV. The hyperpolarizing action persisted in the presence of a cocktail of tetrodotoxin (1µM) and glutamate and GABA receptor antagonists (CNQX, APV, bicuculline methiodide and picrotoxin), suggesting a direct action on the impaled cell and not an indirect effect of network activity. The mGluR antagonist MCPG (500 µM) blocked the ACPD-induced hyperpolarization. An increase in conductance accompanied the hyperpolarization, likely reflecting opening of potassium channels.

The ACPD-induced hyperpolarization resembles the response to the $GABA_B$ receptor agonist baclofen, suggesting that both are mediated by increased K conductance. In most other neurons examined, mGluR activation depolarizes neurons by reducing K conductance or increasing nonspecific cation conductance. However, ACPD-induced hyperpolarization similar to that observed here, due to increased K conductance, has been observed in the mammalian amygdala. The response in zebra finch HVc could reflect an unusual inhibitory role of glutamate.

Supported by NIH Grant DC-02477 and the Helen Hay Whitney Foundation.

66.6

Neural Responses to Auditory Feedback During Singing in Field L of Adult Zebra Finches. Marc F. Schmidt*, Division of Biology 216-76, California Institute of Technology, Pasadena, CA 91125.

Birdsong is a highly stereotyped behavior that is learned by adjustment of vocal output to a memorized song template by way of auditory feedback. Because song learning involves the interaction of vocal motor output and auditory feedback, a characterization of the relationship between these two events is critical for understanding the neural bases of song learning. In this study, we have recorded from awake freely moving adult male zebra finches and characterized the temporal relationship between song pre-motor activity in nucleus HVc and neural responses to that vocalization in Field L. In addition, because it has been reported that auditory responses in nucleus HVc of adult song birds become inhibited during song and following vocalization (McCasland and Konishi, 1981), we also asked whether this inhibition is restricted to HVc or whether it occurs in auditory stations afferent to HVc. Our results indicate that neurons in all three subdivisions of Field L (L1, L2 and L3) have the capacity of responding to auditory feedback of the bird's own vocalization and neural responses in Field L typically occur 10-15 msec after vocal output. Pre-motor neural activity in nucleus HVc generally precedes the onset of vocalization by 35-40 msec implying that the time delay between the onset of syllabic pre-motor activity and auditory feedback of that same syllable in Field L ranges from 45 to 60 msec. These results demonstrate that higher auditory centers such as Field L are able to monitor the bird's own vocal output and suggest that vocalization-induced inhibition of auditory feedback in adult birds likely first occurs at the level of either Field L or HVc and not in more afferent auditory stations. Supported by NIH Grant NRSA-DC00125

66.7

LOCAL INHIBITORY NETWORKS IN THE ZEBRA FINCH RA REVEALED WITH DUAL INTRACELLULAR RECORDINGS AND PHOTOSTIMULATION. J.E. Spiro*, M.B. Dalva and R. Mooney. Dept. Neurobiology, Box 3209, Duke Univ. Med. Ctr. Durham, NC 27710

Projection neurons (PNs) of the forebrain song control nucleus (SCN) RA are the vocal premotor neurons for learned song. They receive excitatory synaptic inputs from two other forebrain SCN, HVc and L-MAN, but less is known about the synaptic connections intrinsic to RA. To characterize how these intrinsic connections might influence RA's output during singing, we mapped them with intracellular recordings and scanning laser photostimulation techniques in brain slices made from male zebra finches.

Dual intracellular recordings made from both local and distant pairs of PNs (as confirmed by Neurobiotin (NB); n=18 pairs) showed that although they spontaneously fired action potentials (APs), these APs were not synchronized, even though they were similar in frequency (ca. 20 Hz). Furthermore, synaptic coupling between these pairs of cells was not observed electrophysiologically (n=10), nor did NB reveal dye-coupling. To map local inputs onto these cells, we used the focal uncaging of glutamate via a UV laser combined with whole cell recordings in voltage clamp conditions to detect evoked synaptic currents (PSCs). Strong inward PSCs (Vm=-60 mV) could be elicited from PNs by photostimulation of cells in a ring-like zone around the soma (n=6). These PSCs reversed between -50 and -30 mV; similar spontaneous PSCs in other PNs were blocked by picrotoxin (50 µM), suggesting that they arose from GABAergic interneurons. Current clamp recordings coupled with focal puffer-pipette applications of glutamate within RA showed that evoking this inhibition could silence PNs' spontaneous APs for tens to hundreds of milliseconds. Since stimulating HVc efferents often produced a similar effect, RA's local inhibitory network might provide one way that RA's output is coordinated by HVc during singing.

Supported by NIH DC02524 and a McKnight Scholars Award to R.M.

66.8

INTRINSIC AND SYNAPTIC PROPERTIES OF L-MAN NEURONS IN THE ADULT MALE ZEBRA FINCH F. Livingston* and R. Mooney. Department of Neurobiology, Box 3209, Duke University Medical Center, Durham, NC 27710.

The lateral part of the magnocellular nucleus of the anterior neostriatum (L-MAN) plays a pivotal role in the normal development of learned song in the zebra finch. As a first step towards a better understanding of its role in this process, we have used intracellular recording techniques both in acute brain slices and *in vivo* to study the electrophysiological and morphological properties of L-MAN neurons in the adult male zebra finch (>90 days posthatch).

L-MAN neurons have input resistances between 35-80 MΩ, with typical resting potentials between -70 and -85 mV, and fire trains of action potentials upon DC depolarization that show spike frequency adaptation over the course of several hundreds of milliseconds. Intracellular Neurobiotin staining revealed that many of these cells (27/98) have the morphological features of projection neurons, elaborating not only local axon collaterals but also projection axons that bifurcate, with one branch coursing ventral towards area X, and the other branch traveling caudal in the lamina hyperstriatica (LH), which is known to be the main route by which L-MAN axons travel to reach their synaptic targets in the robust nucleus of the archistriatum. L-MAN neurons are spontaneously active both in vitro and in vivo, displaying periodic bursts of synaptic activity. These synaptic bursts are present in the majority of cells that we have recorded from, and possess both excitatory and inhibitory components. Pharmacological studies suggest that these synaptic bursts are composed of inhibitory and excitatory components. In addition, electrical stimulation in areas ventral to L-MAN can drive both excitatory and inhibitory responses, as can antidromic activation via electrical stimulation of L-MAN projection axons in the LH.

Supported by NIH DC02524 and McKnight Scholars Award to RM.

66.9

INTRACELLULAR CHARACTERIZATION OF ELECTROPHYSIOLOGICAL PROPERTIES AND SYNAPTIC RESPONSES IN THE ZEBRA FINCH SONG NUCLEUS LMAN. C.A. Boettiger* and A.J. Doupe. Neuroscience Graduate Program, Keck Center, Depts. of Psychiatry and Physiology, UCSF, San Francisco, CA 94143-0444.

In the zebra finch (*Taeniopygia guttata)*, LMAN is thought to play a vital role in song learning. Lesions of this nucleus in juveniles severely disturb song learning. Furthermore, during learning, auditory LMAN neurons progress from being non-selective to a state of strong preference for the bird's own song. It is therefore of interest to investigate the nature of cellular changes occurring in LMAN during that period. As an initial step towards identifying these changes, we have used *in vitro* slice techniques to characterize the neurons in LMAN. Acute brain slices were prepared from zebra finches in the early stages of sensory learning (18-25d), when LMAN neurons are broadly responsive to auditory stimuli. Electrophysiological properties of LMAN neurons were assessed with intracellular recording techniques; the location of some neurons was verified by intracellular injection of biocytin. The cells were characterized by membrane resting potentials of 62 ± 5 mV, input resistances of 116 ± 43 MΩ, and membrane time constants of 31 ± 15 msec (n=25). Current-voltage and frequency-intensity relationships were examined by applying 500 msec square current pulses of variable amplitude. Cells displayed an approximately linear current-voltage relationship over the subthreshold range examined. During suprathreshold current pulses the frequency of firing adapted markedly; mean firing rates increased monotonically with suprathreshold current intensity. These input-output relationships resemble those of neocortical regular-spiking neurons.

Synaptic responses of LMAN neurons were also examined by activating intrinsic inputs and inputs from the thalamic nucleus DLM. Responses displayed both excitatory and inhibitory components. The excitatory components of both local and thalamic input activation were almost entirely abolished by bath application of the NMDA-type and non-NMDA-type glutamate receptor antagonists D,L-APV (100μM) and CNQX (10μM). Further studies will compare cellular and synaptic properties of LMAN neurons at various time points during song acquisition and development of song-selectivity in LMAN.

Supported by the Searle Scholars Program and a UCSF Opportunity Fellowship.

66.10

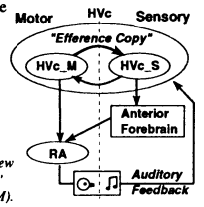
AVIAN INSPIRATORY PREMOTOR NEURONS: CONNECTIONS WITH RESPIRATORY AND VOCAL NUCLEI. H. Reinke and J.M. Wild*, Dept. of Anatomy, Univ. of Auckland, Auckland, New Zealand.

To locate inspiratory motor and premotor neurons that are possibly involved in vocal control, Mm. scalenus, levatores costarum and intercostales externi were injected with either cholera toxin B-chain (CTB) or WGA-HRP in pigeons, finches and budgerigars. The motoneurons were located in medial lamina IX of lower brachial segments and at the tip of the ventral horn of upper thoracic segments. Their dendrites ramified in the lateral funiculus and near the central canal. Following injections of either CTB or WGA-HRP in the lateral and ventral funiculi in pigeons, labeled neurons in the medulla formed predominantly contralateral dorsomedial and ventrolateral clusters that extended from nucleus retroambigualis to about 0.8 mm rostral to the obex. Extracellular single unit recordings primarily from the ventrolateral tegmentum at levels straddling the obex in all species revealed inspiratory related activity, with almost all units being of the decrementing type. Following iontophoretic injections of biotinylated dextran amine at the recording sites, labeled axons were observed primarily in the contralateral lateral funiculus, and at lower brachial and upper thoracic levels they terminated in close proximity to the CTB-labeled dendrites of inspiratory motoneurons. There were also projections to the column of Terni. The same injections also revealed projections to XIIts, and to and from other nuclei involved in respiratory-vocal control, *viz.* retroambigualis, the rostroventrolateral nucleus of the medulla, the ventrolateral parabrachial nucleus, and the dorsomedial nucleus of the intercollicular complex. No afferents were noted from pulmonary-recipient regions of nTS. Supported by grants from Whitehall Foundation, Inc. to JMW and the DFG to HR.

66.11

AN ASSOCIATIONAL MODEL FOR SENSORIMOTOR LEARNING OF BIRDSONG. T. W. Troyer*, A. J. Doupe and K. D. Miller, Keck Center for Integrative Neuroscience, Univ. of California, San Francisco CA 94143

Two hypotheses in the field of songbird learning locate sequence generation in HVc and the sensory template in the Anterior Forebrain Pathway (AFP). However, there is no direct anatomical connection between the AFP and HVc, and auditory feedback delay is likely to cause reinforcement signals to reach RA in the motor pathway during the subsequent burst of motor activity. An hypothesis of the sensorimotor phase of learning is outlined in which simple associational and reinforcement learning accomplish the task given known birdsong anatomy. Feedback delay is addressed by Hebbian association of song premotor activity in RA-projecting HVc neurons (HVc_M) with the resulting sensory activity in the AFP- projecting (HVc_S) neurons, hypothesized to receive most of the auditory feedback to HVc. This "efference copy map" or "forward model" within HVc is learned using direct auditory feedback without reference to the template. The AFP can then use this sensory prediction to reinforce the proper connections within RA without waiting for the auditory feedback signal. This first stage in the hypothesis is supported by a network model that learns template syllable representations, encoded as random activity patterns in RA. We further hypothesize that sequence generation results from reciprocal sensory->motor->sensory activity within HVc. This framework constitutes a testable hypothesis concerning the interplay of motor and sensory information in the song system and makes specific anatomical and behavioral predictions. *McDonnell-Pew Prog. in Cognitive Neuroscience (TWT); Searles Scholars' Prog. and Lucille P. Markey Charitable Trust (AJD&KDM).*



66.12

SOME OF THE TEMPORAL COMPLEXITY OF BIRD SONG MAY ARISE FROM NONLINEAR DYNAMICS OF THE SYRINX. M.S. Fee*, B. Pearson, P.P. Mitra and B.I. Shraiman, Bell Laboratories, Lucent Technologies, Murray Hill, NJ 07974.

Within a single zebra finch song note, there is complex spectral and temporal structure. We have observed that during frequency swept notes, the fundamental frequency may exhibit sudden transitions that occur in less than one period of the fundamental (<1 ms). In cases we have studied, the fundamental frequencies before and after such transitions are related by small integer ratios, characteristic of a phenomenon in nonlinear dynamics known as mode locking. Furthermore, we have observed notes exhibiting an abrupt offset of alternate spectral lines, indicative of another phenomenon known as period doubling.

Recent advances in chronic neural recording in singing birds have made it possible to study the neural organization of song production. It is therefore essential to understand which aspects of bird song are intrinsic to the vocal apparatus, and which are controlled by the brain. We find it unlikely that the rapid transitions observed could be controlled overtly by neural activity. In support of this view, we have developed a simple mathematical model in which the tympaniform membrane undergoes nonlinear relaxational oscillations driven by the Bernoulli effect. The model demonstrates how slow changes in a parameter controlling the resonant frequencies of the model membrane produce sudden transitions in the observed oscillation spectrum.

To further develop and test models of the syrinx, we are investigating excised zebra finch syringes, which are readily induced to oscillate in vitro and exhibit spectral characteristics similar to those found in zebra finch song.

Internal Funding

66.13

MECHANISMS UNDERLYING BINAURAL PHENOMENA IN BUDGERIGARS (*Melopsittacus undulatus*). M. L. Dent, O. N. Larsen* & R. J. Dooling. Dept. of Psychology, University of Maryland, College Park, MD 20742.

The existence of an interaural pathway in birds has motivated a considerable amount of research on the mechanisms of avian hearing, particularly those related to sound localization. Poor high frequency hearing in birds and small interaural distances have implicated the interaural canal as a mechanism by which sound may propagate from one ear to the other. Inherently, this makes the ears directional pressure difference receivers. Changes in middle ear pressure have consequences for frequency responses of the eardrums and the directionality of the ears. The pressure changes may also affect the efficacy of masking noise and the susceptibility to damage from acoustic overexposure. In a series of experiments on 5 species of birds, only budgerigars show evidence of pressure changes in response to external sounds-evidence of a middle ear muscle reflex. The shape of this function is remarkably similar to the shape of the audibility function measured behaviorally in this species.

In spite of their small heads budgerigars show a significant binaural masking release, especially at low frequencies. These results suggest that budgerigars may use their interaural pathways to increase signal-to-noise ratios when signal and noise are spatially separated. Intercranial air pressure may play a more significant role in avian hearing than heretofore realized.

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66.14

STUDIES OF VOCAL PRODUCTION IN BUDGERIGARS USING FOOD REWARD. K.Manabe and R. J. Dooling*. Dept. of Psychology, University of Maryland, College Park, MD 20742.

Song learning in birds has led to a number of insights into the neurobiology of learning. Generally much more is known about vocal production than about auditory perception, yet the interaction between hearing and vocalization is the critical issue in understanding vocal development and selective vocal learning in birds. We performed a number of experiments to examine which features of calls can be modified and whether these birds monitor their vocal production by hearing as humans do.

Budgerigars (parakeets) are small Australian parrots capable of vocal learning throughout adulthood. In the present experiments, birds were trained to produce or modify their species-specific call using food reward. Budgerigars were trained to modify the specto-temporal pattern of their calls as well as the intensity. In producing call variants of their species-specific calls in order to match a stored template, birds made changes in the overall frequency range as well as the duration of their species-specific calls. Birds also performed better when the template to be matched was played to the bird immediately prior to call production. In other experiments, call intensity was shown to vary as a function of level of background noise in the spectral region of species-specific calls. These results suggest that all features of species-specific contact calls in budgerigars are under voluntary control.

More dramatic effects on hearing from exposure to intense noise or from administration of ototoxic drugs also affected vocal production. Both noise exposures and injections of kanamycin decreased call production accuracy usually for a period of days to weeks. In most cases, call accuracy was restored before the auditory papillae were repaired through hair cell regeneration and before auditory sensitivity returned to normal. These results suggest that budgerigars can maintain and recover their vocal production accuracy by relying on other cues such as tactile and proprioceptive feedback.

(Supported by NIH Grants DC-00198 and MH-00982 to RJD)

67.1

SEX ON STEROIDS: TESTOSTERONE CYPIONATE, STANOZOLOL, AND OXYMETHOLONE EFFECTS ON THE SEXUAL BEHAVIOR OF MALE RATS.
A.S. Clark* and E.V. Harrold. Dept. of Psychology, Dartmouth College, Hanover, NH 03755.

We have previously described the effects of the anabolic-androgenic steroid (AAS) compounds 17α-methyltestosterone, methandrostenolone, and nandrolone decanoate on the sexual behavior of male rats. In this report, we extend our analysis to include testosterone cypionate, stanozolol, and oxymetholone. Three doses of each AAS were administered daily to a) intact male rats for 12 weeks and b) castrated male rats for 6 weeks. Sexual behavior was quantified weekly.

Testosterone cypionate, as expected, induced no behavioral deficits in intact males and effectively maintained sexual behavior in castrated males. In contrast, stanozolol and oxymetholone, at the highest doses administered, interfered with the expression of sexual behavior in intact males. Serum testosterone levels were also suppressed in males which received the high doses of stanozolol and oxymetholone. In castrated males, neither stanozolol nor oxymetholone effectively sustained sexual behavior throughout the 6 week testing period. These results illustrate that the bioactivity of individual AAS compounds falls along a continuum. Furthermore, human AAS abusers may expose themselves to side effects and health consequences which vary as a function of the dose and type of AAS they consume.
Supported by NIDA-08574 to ASC.

67.2

FOS IMMUNOREACTIVITY IN MALE RAT BRAIN FOLLOWING EXPOSURE TO CONDITIONED AND UNCONDITIONED SEX ODORS.
T.E. Kippin*, C. Manitt, S. Talianakis, & J.G. Pfaus. CSBN, Department of Psychology, Concordia University, Montréal, QC, Canada H3G 1M8.

Previous research has shown that pairing an artificial olfactory stimulus (almond odor) with copulation produces a mate-choice preference for females bearing that odor over females without it. However, the neural processes underlying this conditioned effect are unknown. Accordingly, the present study examined the induction of Fos immunoreactivity (IR) in response to conditioned and unconditioned sexual odors. Training procedures were identical to those used in previous experiments: 9 trials at 4-day intervals in which sexually-naive male Long-Evans rats were exposed either to estrous females with almond extract applied to their backs and anogenital regions (A+E) or to estrous females with distilled water applied to their backs and anogenital regions (E-alone). Fos IR was assessed in the brains of males from both groups 2 hr after presentation of almond, estrous, or no odors in bedding. The almond odor increased Fos IR in the nucleus accumbens and piriform cortex of A+E-trained, but not E-alone-trained males. A more complete analysis of the Fos induction in other brain regions will be presented. These data indicate that an odor paired with copulation is capable of inducing Fos within different brain regions. *Supported by NSERC of Canada (OGP-0138878).*

67.3

NEURAL AND BEHAVIORAL EFFECTS OF TESTOSTERONE ON THE SEXUAL BEHAVIOR OF CASTRATED MALE RATS.
S. Centeno*, A. Jacques, & J.G. Pfaus. CSBN, Department of Psychology, Concordia University, Montréal, QC, Canada H3G 1M8.

The present study was conducted to examine Fos activation in different brain regions during the return of appetitive and consummatory sexual behaviors in castrated male rats given testosterone (T) replacement. Intact Long-Evans male rats (N=80) acquired baseline rates of sexual behavior with receptive females in bilevel chambers prior to surgical castration. Following recovery, males were tested for sexual behavior in bilevel chambers once every 4 days until all sexual activity ceased. Males were then assigned to one of two T dose conditions, 30 or 50 μg/day, and tested for the reemergence of sexual behaviors with receptive females at 4-day intervals in bilevel chambers. A progressive increase in the proportion of males showing conditioned level changing, pursuits, mounts, intromissions, and finally ejaculations, was observed over a 24-day period (7 test sessions), with the higher dose group showing a faster return. On each test day, 4 males from each group were selected randomly, perfused, and their brains prepared for Fos immunocytochemistry. Males in both groups showed progressive and significant increases in the number of Fos-positive cells in the mPOA that were correlated with the reemergence of level changes, mounts, and intromissions with ejaculation, respectively. Fos expression in other brain areas (n. accumbens, striatum, hypothalamus, medial amygdala, and ventral tegmentum) is currently being examined and will be reported. These data show that the mPOA is activated by both appetitive and consummatory aspects of male sexual behavior. *Supported by NSERC of Canada (OGP-0138878).*

67.4

FLUOXETINE INHIBITION OF MALE RAT SEXUAL BEHAVIOR: REVERSAL BY OXYTOCIN.
J.M. Cantor[1,2]*, Y. Binik[1], & J.G. Pfaus[2]. [1]Dept. Psychol., McGill Univ. & [2]CSBN, Dept. Psychol., Concordia Univ., Montréal, Québec H3G 1M8 Canada.

In the first phase of the experiment, 40 sexually experienced, male Long-Evans rats were assigned to four groups and tested for sexual behavior in bilevel chambers once every 4 days. Rats received daily injections of fluoxetine (0, 1, 5, or 10 mg/kg, ip) for 11 experimental sessions. Each test of sexual behavior lasted 35 min and was scored for frequency and latency of level changes, mounts, intromissions, and ejaculations. Consistent with the effects reported for clincal use of fluoxetine in human males, fluoxetine reduced sexual excitement and decreased ejaculations in male rats. In the second phase of the experiment, the same rats were maintained on daily fluoxetine treatment at the same doses and tested in the same manner. However, 60 min before tests of sexual behavior, the neuropeptide oxytocin (200 ng, ip) or vehicle was administered in an A-B-B-A design. Oxytocin treatments reversed the inhibitory effects of chronic fluoxetine on sexual behavior. These data are consistent with the hypothesis that serotonin reuptake inhibitors (SRIs) interfere with sexual behavior by inhibiting the release of oxytocin during sexual behavior. Co-administration of SRIs with oxytocin or oxytocin agonists may therefore provide antidepressant action without antisexual side-effects.
Supported by NSERC of Canada to J.G.P. (OGP-0138878)

67.5

MATING-INDUCED FOS EXPRESSION IN MALE AND FEMALE MUSK SHREWS
C.J. Gill*, W.J. Cholbi and E.F. Rissman. Dept. of Biology, University of Virginia, Charlottesville, VA 22903

Neuronal activity, as indicated by Fos protein immunoreactivity (ir), is increased in specific brain areas after mating in several mammalian species. We examined the induction of neural Fos in the musk shrew, a species in which both female sexual behavior and ovulation are induced by exposure to a receptive male. Sexually inexperienced, 54 to 58 day old males (n=6) and females (n=7) were paired with receptive opposite sex individuals until one ejaculation occurred. Matched control animals (n=7, each sex) were placed alone in a clean cage with no exposure to opposite sex cues for an equivalent time. Experimental animals and their matched controls were perfused one hour after the mating and their brains processed for Fos-ir (Cambridge Research Biochemicals). Mated animals had significantly greater numbers of Fos-ir cells in the bed nucleus of the stria terminalis (BNST), the medial preoptic nucleus (mPOA) and the premammillary nucleus, with the number in the mPOA in mated males significantly greater than in mated females. No significant differences have been observed in Fos-ir in the ventral medial nucleus (VMN) or the medial amygdala. These data show that, like rats, but unlike the female ferret, another induced ovulator, stimulation received during mating increases c-fos activity in the mPOA and BNST of both males and females. Increased Fos-ir is observed in the VMN of females but not males an hour after of mating showing a sex difference in c-fos activation in this region. Additionally, in four brains in each group processed for GnRH-ir and Fos-ir, we only rarely found cells double labeled for Fos and GnRH. Since female musk shrews mate when estrogen levels are low, this supports the hypothesis that steroid activation is necessary for Fos induction in GnRH cells.
This work was supported by NIH NRSA 1F31MH10570-01A1 and NSF grant IBN 94-12605.

67.6

SEX DIFFERENCE IN STRIATED PENILE MUSCLES AND INNERVATING MOTONEURONS IN THE MUSK SHREW
L.M. Freeman* and E.F. Rissman. Dept. of Biology, University of Virginia, Charlottesville, VA 22903

The motoneurons (MNs) innervating the sexually dimorphic striated penile muscles are sexually dimorphic in either size or number in a variety of mammalian species, including rodents, carnivores and primates. We examined this neuromuscular system in an insectivore, the musk shrew *Suncus murinus*.

The bulbocavernosus (BC) and ishiocavernosus (IC) muscles are present in male but not female musk shrews. Horseradish peroxidase (HRP) injections into either the BC or the IC labeled MNs in the L5 and S1 regions of the spinal cord. At these levels, four distinct MN pools are present. Based on their position and apparent homology to the rat lumbar spinal cord, we refer to these motor nuclei as retrodorsolateral nucleus (RDLN), dorsolateral nucleus (DLN), ventrolateral nucleus (VLN) and ventromedial nucleus (VMN). HRP injections of the BC and IC muscles in males labeled a few MNs in the VMN, but the majority of filled cells were localized to the DLN. In both males and females, injections of a foot muscle, the flexor digitorum brevis, filled motoneurons in the RDLN.

To determine if there was a sex difference in these motor pools, MNs were identified and counted in alternate Nissl-stained sections (50 micron) of spinal cords from 11 males and 9 females. Raw counts are given ± SEM.

	VMN	DLN	RDLN
MALE	139.3±12.5	369.9±12.5	253.7±12.5
FEMALE	133.9±13.9	229.1±13.9*	245.3±13.9

Two-way ANOVA showed main effects of both nucleus and sex and a significant interaction. Planned comparisons found a significant sex difference in MN number only in the DLN ($p<.05$*). We will examine these MNs to determine if they are sexually dimorphic in size and if early androgen treatments increase MN number in females.
Supported by NIH T32-DKO7646 and NSF IBN 94-12605.

67.7

CO-DISTRIBUTION OF ESTROGEN RECEPTOR AND AROMATASE IMMUNOREACTIVE NEURONS IN THE MALE MUSK SHREW. S.L. Veney* and E.F. Rissman. Biology Department, University of Virginia, Charlottesville, VA 22903.

Aromatase enzyme catalyzes the conversion of testosterone (T) into estradiol (E_2). There are several lines of evidence to suggest that this process is important for the activation of male and female sexual behavior. In this study we describe the distribution of neurons immunoreactive for estrogen receptors (ER-ir) and cells immunoreactive for aromatase enzyme (AROM-ir) using standard immunocytochemistry techniques. Adult male musk shrews were castrated and given a T implant (s.c., 10 mm). In addition, they received an aromatase inhibitor (vorozole: generously provided by Janssen Research Foundation). Previous studies have shown that injections of vorozole enhance the staining of AROM-ir neurons (Rissman et al., J. Neuroendo., in press). After processing and sectioning the brain tissue, ER-ir was localized with H222 monoclonal antibody (generously provided by Abbott Labs) and AROM-ir cells were visualized with the polyclonal antibody R-8-1 (kindly supplied by Dr. Y. Osawa). We found an overlapping distribution of ER-ir and AROM-ir neurons in the VMN. In the POA however, two distinct populations of AROM-ir neurons are seen. In the mPOA a nucleus of AROM-ir neurons that does not contain estrogen receptors is present. In the lateral POA, there is a cell-zone that contains neurons with both ER-ir and AROM-ir. These data suggest that within the mPOA T is converted to E_2, however, the E_2 may bind to cells in the lateral POA. This work is supported by NSF IBN 94-12605 (EFR).

67.8

LESIONS OF THE POSTERIOR MEDIAL NUCLEUS OF THE AMYGDALA DELAY SEXUAL SATIETY. DB Parfitt*[a], LM Coolen[c], SW Newman[ab], and RI Wood[c]. Neuroscience Program[a] and Department of Anatomy and Cell Biology[b], University of Michigan, Ann Arbor, MI 48109. Department of Obstetrics and Gynecology[c], Yale University, New Haven, CT 06520.

In the male Syrian hamster, the anterior and posterior subdivisions of the medial amygdaloid nucleus (Me) have differential effects on mating behavior. Lesions of anterior Me (MeA) eliminate the behavior presumably by interrupting chemosensory signals, while posterior lesions of Me (MeP) alter the temporal sequencing of the behavior by interfering with somatosensory cues from the brainstem. Recent studies indicate that neurons in caudal MeP only express Fos protein in males mated to sexual satiety. This study tested the hypothesis that MeP facilitates the onset of sexual satiety, and thus elimination of MeP should enhance copulation. Mating behavior was observed in sexually-experienced adult male Syrian hamsters before and after lesions of MeP. All animals (n=27) were mated to sexual satiety for 3 consecutive days to establish a prelesion baseline. Following prelesion testing, 21 males received bilateral electrolytic lesions of MeP using Teflon coated stainless steel microelectrodes (0.5mA, 10 sec). The 6 remaining males served as sham operated controls. After a 1 week recovery, males were mated to sexual satiety for 3 consecutive days on 2 occasions one week apart. Males were then perfused with 4% paraformaldehyde and 40µm brain sections were processed to assess the location and extent of the lesion. Analysis revealed 10 animals with bilateral Me lesions, 5 in caudal MeP and 5 in mid-Me (MeA through rostral MeP); these 2 groups differed significantly in their postoperative behavior. The caudal MeP lesion group had a progressive increase in the number of ejaculations before satiety from 0.6±0.6 on prelesion day 3 to 2.6±0.2 and 3.8±0.6 on postlesion day 3 rounds 1 and 2 ($p \leq 0.05$). Similar increases were not observed in either the sham operated controls or the mid-Me lesioned males. These results suggest that caudal MeP is important in terminating copulation at sexual satiety. (Supported by NIH HD-32669 and T-32-DC-00011)

67.9

DEFICITS IN NONCONTACT ERECTION AND COPULATION IN RATS AFTER LESIONS OF BED NUCLEUS OF STRIA TERMINALIS OR NUCLEUS ACCUMBENS. Y.-C. Liu*, J.D. Salamone, and B.D. Sachs. Department of Psychology, University of Connecticut, Storrs, CT 06269-1020, U.S.A.

These studies continue our survey of brain areas mediating the rat's erectile response to inaccessible estrous female ("noncontact erection", NCE; Sachs et al., Physiol. Behav., 1994). BST: We made radiofrequency (RF) lesions in the bed nucleus of the stria terminalis (BST), which has rich connections with the medial amygdala (MeA) and the medial preoptic area (MPOA). MeA lesions eliminate NCE (Kondo et al., SNS Abstr.,1995). MPOA lesions cause a smaller deficit in NCE (Liu et al., SNS Abstr., 1995), but eliminate copulation. NCE was displayed by 3/14 BSTx males vs. 9/10 Sham males ($p<.01$) 10-12 days after surgery, and by 6/14 BSTx males vs. 10/10 Sham males ($p<.01$) 16-18 days after surgery. In mating tests 1-2 days after the first NCE test, 8/14 BSTx males and 10/10 Sham males copulated to ejaculation ($p<.03$); BSTx males had longer interintromission intervals ($p<.01$) and ejaculation latencies ($p<.01$); intromission ratios were unchanged. The results implicate projections from the MeA through the BST in mediating NCE. However, comparing the magnitude of lesion effects on NCE, MeAx > BSTx > MPOAx; hence, some MeA efferents may mediate NCE by a pathway not involving the BST and MPOA. NAcc: We also made RF or 6-OHDA lesions in the nucleus accumbens (NAcc). After 6-OHDA lesions, which caused a mean 78% reduction in NAcc dopamine, NCE was displayed by 5/11 NAccx males vs. 9/10 Sham males ($p=.06$) one week after surgery, and by 2/11 NAccx males vs. 8/10 Sham males ($p<.01$) two weeks after surgery. After RF lesions, there was not a reliable difference between groups in the incidence of NCE in Test 1 (3/10 NAccx, 5/10 Sham) or Test 2 (4/10 NAccx, 9/10 Sham, $p=.06$); however, all Sham males responded in at least one post-lesion test vs. 5/10 NAccx males ($p=.03$), and NAccx males had longer NCE latencies in Test 2 ($p<.02$). All males copulated to ejaculation with no significant change in parameters. The modest effect of both types of lesion on NCE supports a hypothetical role for NAcc dopamine in sexual arousal, at least in response to remote cues from estrous females, if not during copulation. [UConn Research Foundation]

67.10

CONVERSION OF TESTOSTERONE TO ESTROGEN IS NOT NECESSARY FOR THE EXPRESSION OF SEXUAL BEHAVIOR IN MALE SYRIAN HAMSTERS. T.T. Cooper*[1], A.N. Clancy[2], & H.E. Albers[1], [1]Lab. of Neuroendocrinology & Behavior, Depts. of Biology and Psychology, Georgia State Univ., Atlanta, GA 30303, [2]Dept. Of Psychiatry and Behavioral Sciences, Emory Univ. Sch. Of Med., GMHI, 1256 Briarcliff Rd, Atlanta, GA 30306, and Biology Dept., Georgia State Univ., Atlanta, GA 30303.

Male sexual behavior in some species is primarily controlled by androgens. In contrast, the sexual behavior of male rats is dependent on estradiol formed by the aromatization of testosterone to estradiol within the CNS. The present study examined the role of testosterone aromatization in controlling sexual behavior in male hamsters by determining whether systemic administration of the non-steroidal aromatase inhibitor, Fadrozole,decreases sexual behavior in hamsters. Hamsters were assigned randomly to one of two groups. Group 1 received 2.0mg/kg/day of Fadrozole administered s.c. by osmotic minipump for 8 weeks. Pilot data indicated that this dose of Fadrozole was effective at inhibiting aromatase activity in hamsters as revealed by anti-estrogen receptor immunocytochemical labeling in brain. Group 2 was implanted with empty minipumps. All animals were paired with a receptive female weekly for 8 weeks. Measures recorded included latency to mount, mounts, intromissions, and ano-genital investigation. No significant decline in sexual behavior was observed in the Fadrozole treated hamsters. Furthermore, weekly comparisons revealed no significant differences between groups on any measure. These data suggest that aromatization of testosterone to estradiol is not necessary for the expression of male hamster sexual behavior.

(Supported by NSF IBN-9222099)

67.11

DOPAMINE AGONISTS INCREASE PARAVENTRICULAR NITRIC OXIDE PRODUCTION IN MALE RATS: CORRELATION WITH PENILE ERECTION AND YAWNING. A. Argiolas*, S. Succu & M.R. Melis. Bernard. B. Brodie Dept. Neurosci., Cagliari Univ., 09124 Cagliari, Italy.

The effect of apomorphine (APO), a mixed D1/D2 agonist that induces penile erection (PE) and yawning (Y), on the concentration of NO_2^- and NO_3^- in the paraventricular nucleus of the hypothalamus (PVN), was studied in male rats by in vivo microdialysis. APO (80 µg/kg s.c.) increased the concentration of NO_2^- from 1.12 ± 0.45 µM to 3.8 ± 0.75 µM and of NO_3^- from 5.53 ± 0.82 to 11.25 ± 2.30 µM in the PVN dialysate. NO_2^- was increased also by LY 171555 (50 µg/kg s.c.), a D2 agonist that induces PE and Y, but not by SKF 38393 (5 mg/kg s.c.),a D1 agonist ineffective on PE and Y. Conversely, APO effect on NO_2^- was prevented by haloperidol (0.5 mg/kg i.p.), a mixed D1/D2 antagonist, and by L-sulpiride (25 mg/kg i.p.), a D2 antagonist, but not by SCH 23390 (50 µg/kg s.c.), a D1 antagonist, although all three compounds prevented PE and Y. The APO effect on NO_2^-, PE and Y, was also prevented by the nitric oxide (NO) synthase inhibitor N^G-nitro-L-arginine methylester (200 µg i.c.v.). The NO scavenger hemoglobin also (200 µg i.c.v.) prevented the NO_2^- increase, but was ineffective on PE and Y. In contrast, the oxytocin antagonist d$(CH_2)_5$Tyr(Me)-Orn^8-vasotocin (1µg i.c.v.) and the guanylate cyclase inhibitor methylene blue (300 µg i.c.v.) were ineffective on the NO_2^- increase, but prevented PE and Y. We infer that dopamine agonists induce PE and Y by acting on D2 receptors that increase NO synthase activity in the cell bodies of PVN oxytocinergic neurons projecting to extra-hypothalamic brain areas and mediating these behavioral responses.

67.12

OXYTOCIN INCREASES PARAVENTRICULAR NITRIC OXIDE PRODUCTION IN MALE RATS: CORRELATION WITH PENILE ERECTION AND YAWNING. M.R. Melis*, S. Succu & A. Argiolas. Bernard. B. Brodie Dept. Neurosci., Cagliari Univ., 09124 Cagliari, Italy.

The effect of a dose of oxytocin, that induces penile erection (PE) and yawning (Y), on the production of NO_2^- and NO_3^- in the paraventricular nucleus of the hypothalamus (PVN), was studied in male rats by in vivo microdialysis. Oxytocin (50 ng i.c.v.) increased the concentration of NO_2^- from 0.98 ± 0.29 µM to 4.2 ± 0.79 µM and of NO_3^- from 5.6 ± 0.33 µM to 8.03 ± 0.99 µM in the PVN dialysate. NO_2^- and NO_3^- concentration increase was parallel with the increase in the number of PE and Y episodes. NO_2^-, PE and Y were increased also by the oxytocin-related peptides [Thr^4,Gly^7]-oxytocin (100 ng i.c.v.) and oxytocin(1-8)(1 µg i.c.v.), which increased PE and Y, but not by oxytocin(1-6)(1 µg i.c.v.) or oxytocin(7-9)(1 µg i.c.v.), which were unable to induce PE and Y. The oxytocin effect on NO_2^-, PE and Y were prevented either by the oxytocin receptor antagonist d$(CH_2)_5$Tyr(Me)-Orn^8-vasotocin (1 µg i.c.v.), or by the nitric oxide (NO) synthase inhibitor N^G-nitro-L-arginine methyl ester (200 µg i.c.v.). In contrast, the mixed dopamine D1/D2 receptor antagonist haloperidol (0.5 mg/kg i.p.) was unable to prevent either NO_2^- increase or PE or Y. The NO scavenger hemoglobin (200 µg i.c.v.) prevented oxytocin-induced NO_2^- increase, but was unable to prevent PE and Y. Methylene blue (300 µg i.c.v.), an inhibitor of guanylate cyclase, was ineffective on NO_2^- increase, but prevented PE and Y. The results suggest that oxytocin induces PE and Y by acting on PVN oxytocin receptors that increase NO synthase activity in the cell bodies of paraventricular oxytocinergic neurons projecting to extra-hypothalamic brain areas and mediating these behavioral responses.

67.13

REGULATION OF REPRODUCTIVE BEHAVIORS AND BRAIN ANDROGEN RECEPTOR (AR) BY TESTOSTERONE IN PREPUBERTAL AND ADULT MALE GOLDEN HAMSTERS. R. D. Romeo, L. R. Meek*, C. M. Novak, and C. L. Sisk*, Dept. Psych., Neuroscience Program, Michigan State Univ., E. Lansing, MI 48824 and *Dept. Psych., Univ. of Minnesota, Morris, MN 56267

Prepubertal males are less responsive than adults to the activational effects of testosterone (T) on reproductive behavior. This study examined whether the ability of T to increase AR immunoreactivity (AR-IR) in brain regions involved in reproductive behavior is also less in prepubertal males. Sexually naive male hamsters were castrated under anesthesia at 21 or 42 days of age, and implanted subcutaneously with either a 0, 2.5, or 5 mg T pellet. One week later, males were given a 20 min mating session with a receptive female. Animals were euthanized one hour after the behavioral test, and blood samples and brain tissue were collected. Plasma T levels were equivalent in prepubertal and adult males that had been administered the same dose of T. Despite similar T levels, adult males exhibited more mounts and intromissions compared with prepubertal males, demonstrating that prepubertal males are less responsive than adults to the behavioral actions of T. In both age groups, increased circulating levels of T were associated with increased density of AR-IR cells in several brain regions involved in steroid-dependent activation of male sexual behavior, including the medial preoptic area, medial amygdala, posteromedial bed nucleus of the stria terminalis and magnocellular preoptic nucleus. Surprisingly, T increased AR-IR cell density in the latter two regions to a greater extent in prepubertal males than in adults. Thus, prepubertal males are more responsive to the effects of T on AR-IR cell density in these regions. These results indicate that a testosterone-dependent pubertal increase in ARs may be necessary, but is not sufficient, for the pubertal increase in behavioral responsiveness to T. (Supported by HD 26483)

67.14

ANDROGEN-RECEPTOR AND ESTROGEN-RECEPTOR CO-LOCALIZING NEURONS IN THE MEDIAL PREOPTIC AREA AND EXPRESSION OF MATING-INDUCED FOS IN MALE RATS. B. Greco*[1], D. A. Edwards[1] and A. N. Clancy[1,2,3]. [1]Psychology Dept., Emory Univ., Atlanta, GA 30322, [2]Biology Dept., GA St. Univ., Atlanta, GA 30303 and [3]Dept. of Psychiatry and Behavioral Sciences, Emory Univ. Sch. of Med., GMHI, 1256 Briarcliff Rd., Atlanta, GA 30306.

Conversion of testosterone into estradiol is important for male rat mating behavior and both steroids probably contribute to mating. An overlap in the distributions of neurons containing androgen receptors (AR) and estrogen receptors (ER) has been documented, and many AR-immunoreactive (AR-ir) neurons express mating-induced Fos-immunoreactivity (Fos-ir). Because mating-induced Fos-ir in the male rat occurs mainly in AR-ir neurons and because both steroids are important for mating, we hypothesized that AR-ir and ER-ir are co-localized. We examined, in adjacent sections from the medial preoptic area, the co-localization of ER-ir in: i) AR-ir containing neurons, and ii) Fos-ir expressive neurons. PG21 anti-AR (G. Prins), anti-c-fos (Genosys), and H222 anti-ER (Abbott Lab) primary antibodies were visualized, respectively, with cyanine-conjugated, fluorescein or cyanine-conjugated, and fluorescein-conjugated secondary antibodies (Jackson Lab). Five days after subcutaneous implant of a minipump delivering 0.28 mg/kg/day of the aromatase inhibitor, Fadrozole (Ciba-Geigy Corp), male rats were killed 1 hour after ejaculating with a receptive female. Preliminary analysis of sections incubated with anti-ER and anti-AR primary antibodies showed that ER-ir labeling occurred predominantly in AR-ir expressive neurons but that not all AR-ir neurons were ER-ir positive. In sections incubated with anti-ER and anti-c-fos primary antibodies, ER-ir labeling occurred in cells expressing mating-induced Fos-ir but cells which were single-labeled with either ER-ir or Fos-ir were also present. Because in the male rat almost all mating-induced Fos-ir is found in AR-ir positive neurons, these new findings permit the tentative conclusion that two populations of neurons control mating in male rats; the first containing AR-ir only and the second co-localizing AR-ir and ER-ir. (Funded by NSF grant IBN-9421208 to DAE).

67.15

EFFECTS OF VARIATIONS IN REPRODUCTIVE ACTIVITY ON HYPOTHALAMIC, PITUITARY, ADRENAL, AND TESTICULAR HORMONES IN MALE RATS. M. T. Williams*, A. E. McCrea, M. B. Hennessy and H. N. Davis. Dept. of Psychology, Wright State Univ., Dayton, OH 45435.

Male rats exhibit a stereo-typical pattern of sexual behavior in which a variable number of mounts (no vaginal penetration) and intromissions (mounts with vaginal penetration) precede each ejaculation, and as many as 8 to 12 ejaculations precede the onset of sexual satiety. At present, few data are available as to the cause of sexual satiety, although several studies suggest that various hormones, particularly those of the HPA axis, may be involved. The specific purpose of the present study was to systematically investigate the effects of variations in copulatory behavior on the production and release of hormones from hypothalamic, pituitary, adrenal or testicular origins. Male rats were either allowed to achieve 1 intromission (1-INTRO), 1 ejaculation (1-EJAC), or 5 ejaculations (5-EJAC), or were merely removed from their home cage (BASAL) and trunk blood, hypothalamii and testes were collected. Hypothalamic CRF levels tended to decline during sexual behavior, while plasma titers of ACTH were increased, as were the adrenal hormones corticosterone (CORT) and aldosterone (ALDO). Furthermore, 5-EJAC males had heightened serum testosterone titers. Testicular testosterone and CRF were also assayed. Testicular T increased during sexual activity, but was significantly elevated over BASAL males only after 5 ejaculations. Testicular CRF, however, was not affected by sexual activity. The decrement in hypothalamic CRF levels following sexual activity suggests a greater release as sexual activity continues and this is supported by the rise in ACTH titers. The present results clearly indicate that a variation in the amont of copulatory activity can significanly affect the production and release of a variety of hormones. Whether, and in what manner, these changes relate to sexual satiety remains to be determined.

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67.16

CELL-BODY LESIONS OF THE POSTERODORSAL PREOPTIC NUCLEUS (PdPN) AND THE POSTERODORSAL MEDIAL AMYGDALA (MeApd) DISRUPT SEX BEHAVIOR IN MALE GERBILS. M. M. Heeb* and P. Yahr. Department of Psychobiology, University of California, Irvine, CA 92697.

The sexually dimorphic area (SDA) of the gerbil hypothalamus is essential for male sex behavior. Using c-Fos immunocytochemistry we previously determined that the SDA, and many areas connected to or near the SDA, are activated when males are exposed to a variety of sex-related stimuli. Two of these areas, the PdPN and the lateral part of the MeApd, are activated only when males copulate to ejaculation.

To test the hypothesis that these two areas affect sex behavior in sexually experienced male gerbils, we aimed cell-body lesions at either or both cell groups. Three groups of males were given *N*-methyl-D-aspartate (NMDA), a neuronal cytotoxin, via iontophoresis, bilaterally in either the MeApd, the PdPN or both cell groups. A fourth group was given *N*-methyl-L-aspartate, NMDA's inactive isomer, or water. Males were retested weekly for four weeks for male sex behavior with receptive females.

All eight control males intromitted in every test, and only one ever failed to ejaculate. Twelve of 13 males with lesions aimed at the PdPN also intromitted, but eight of them failed to ejaculate on at least one test. The last male never mounted. In contrast, only three of nine males with lesions aimed at the MeApd mounted in more than one test, and all three failed to ejaculate on at least one test that involved intromission.

These results suggest that the MeApd may be involved in the control of both mounting and ejaculation, but that the PdPN may be specifically involved in the control of ejaculation.

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67.17

PLASMA TESTOSTERONE AND TISSUE 17α-HYDROXYLASE ACTIVITY IN CASTRATED AND FADROZOLE TREATED MALE ZEBRA FINCHES N. Lane, W. Grisham, S. Brown*, L. Thompson, A. Arnold and B. Schlinger, Dept.of Physiological Science and Lab of Neuroendocrinology of the BRI, UCLA, Los Angeles, CA 90095.

In songbirds, song and the neural circuits that control song, are highly sensitive to activational and organizational effects of estrogens (E). Nevertheless, manipulations of E physiology (gonadectomy, treatments with aromatase inhibitors or E-receptor antagonists) fail to block presumed E-dependent neural events and may fail to eliminate circulating E. Since E is derived from androgen (A), these data suggest that songbirds possess an important extragonadal site of A synthesis. To test this possibility, male zebra finches were castrated or left intact and were then injected daily with saline or with Fadrozole (Fad), a specific and effective aromatase inhibitor. 5 days later, birds were bled for measures of circulating testosterone (T) by RIA and sacrificed for measures of tissue P45017α-hydroxylase/C17-C20 lyase (P450-17α) activity (measured by the conversion of ^{3}H-progestin to ^{3}H-A). Castration significantly reduced circulating T levels whereas Fad increased T significantly in both castrates and intact birds. Though we have detected P450-17α activity previously in adrenals, it was not detected in homogenates of adrenals or of brains in this experiment. P450-17α was detected in testes. ^{3}H-A was formed in greater amounts by testes of males treated with Fad than with saline, perhaps contributing to the higher T levels found in these birds. Since we found no extragonadal P450-17α activity, we do not know the site of synthesis of the T detected in castrated + Fad treated males. Supported by IBN-9120776 and DC0217.

67.18

THE SYNERGISTIC ACTION OF TESTOSTERONE AND ESTROGEN IN THE RESTORATION OF MALE SEXUAL BEHAVIOR. M.E. Vagell*, and M.Y. McGinnis. Department of Cell Biology & Anatomy, Mount Sinai School of Medicine, City University of New York, New York, NY 10029

This study assessed the role of aromatization in male reproductive behavior by testing the effects of the aromatase inhibitor, fadrozole, on the restoration of male sexual behavior and partner preference in testosterone-treated castrate rats. We measured nuclear estrogen receptor occupation to determine whether fadrozole blocked brain aromatase. In addition, nuclear androgen receptor assays were used to verify that fadrozole does not block androgen receptors. Mini-osmotic pumps fitted to brain infusion cannulas were used to deliver fadrozole (20 μg/kg/day) into the right lateral ventricle. The majority of animals receiving fadrozole treatment with two, 10 mm testosterone filled Silastic capsules (T/F group) did not show any sexual behavior during the tests 7 and 14 days following implant surgery. However, animals receiving fadrozole treatment implanted with two, 10 mm testosterone capsules and one, 5 mm 1% estradiol capsule copulated normally, indicating that fadrozole's inhibition of male sex behavior was specifically due to blocking aromatase activity. Moreover, most of the animals which received only one, 5 mm 1% estradiol capsule (E group) also did not show any sexual behavior over this time course. Partner preference was measured in a three chambered apparatus as an index of sexual motivation. Repeated measures contrasts on the group x test interaction indicated that the T/F group was not significantly different from the T group. However, the E group did not show a preference for the receptive females and was significantly different from the T group. Fadrozole treatment resulted in a 59% decrease in brain nuclear estrogen receptor occupation relative to the T group. Fadrozole had no significant effect on brain or pituitary nuclear androgen receptor occupation. Our work suggests that both testosterone and estrogen are necessary for the restoration of male sexual behavior in rats. However, estrogen is neither necessary nor sufficient for the restoration of partner preference. This research was supported by NIH grant HD 27727.

67.19

SUICIDE TRANSPORT LESIONS OF STEROID-RESPONSIVE NEURONS IN THE MALE HAMSTER MATING BEHAVIOR CIRCUITRY. R.I. Wood* and L.M. Coolen, Dept OB/GYN, Yale Univ Med School, Box 208063, New Haven, CT 06520.

Copulatory behavior in the male Syrian hamster is dependent on chemosensory and hormonal stimuli, and is prevented if either signal is interrupted. Lesions of brain regions that relay chemosensory signals abolish copulation. However, lesions of steroid-responsive regions fail to block behavior. We hypothesize that the steroid-responsive nuclei in the mating behavior circuit are redundant, for intracerebral implants of testosterone in medial amygdala or bed nucleus of the stria terminalis and medial preoptic area will facilitate behavior. If so, multiple lesions of these regions will be neccesary to block mating behavior. This was tested in the present study using the suicide transport lectin, volkensin.

Volkensin is a toxic lectin from *Adenia volkensii* which lesions cells at the site of injection, and is taken up by terminals to lesion afferents to the injection site. Volkensin was injected (50 nl) unilaterally in the posteromedial subdivision of the bed nucleus of the stria terminalis (BNSTpm) via a glass micropipette. BNSTpm receives input from both posterior medial amygdala (MeP) and medial preoptic nucleus (MPN) and lesions centered in the BNSTpm fail to prevent copulation. One week after surgery, males were tested for mating behavior to determine the effects of the lesion. One hour after the mating test, males were perfused and brains were stained for Fos and androgen receptor immunoreactivity (AR-IR), and cresyl violet. AR-IR and Fos-IR were reduced unilaterally at the site of the injection and throughout MPN and MeP, indicating that volkensin lesioned BNSTpm and its afferents. With small unilateral lesions restricted to steroid-responsive brain regions, males continued to mate. However, when volkensin injection in BNSTpm was combined with contralateral bulbectomy mating was abolished.

These results demonstrate that volkensin is a useful tool to create multifocal lesions of steroid-responsive neurons and support the concept of functional redundancy within the hormone-sensitive portions of the circuitry for male sexual behavior. (Supported by NIH HD-32669).

MONOAMINES AND BEHAVIOR I

68.1

VTA DOPAMINE BURST ACTIVITY IN RELATION TO SENSORY EVENTS, J.C. Horvitz*, T. Stewart, B.L. Jacobs, Program in Neuroscience, Princeton University, Princeton, NJ 08544.

While DA neurons have previously been shown to exhibit single-spike activity with occasional bouts of burst firing (a train of 2-4 spikes with ISI's < 80 msec), the behavioral significance of these firing modes is unknown. In this study, single-unit activity of dopamine (DA) neurons in the ventral tegmental area (VTA) was recorded in freely-moving cats during the presentation of auditory and visual stimuli of 1 msec duration. The presentation of auditory and visual stimuli typically produced a 20-fold increase in the probability of burst firing, with the maximum probability occurring approximately 80 msec after stimulus presentation. The burst activity was followed by a 250 msec period during which burst activity had a near-zero probability of occurrence.
NIMH (MH-10296)

68.2

PREPULSE INHIBITION OF THE ACOUSTIC STARTLE REFLEX IS RELATIVELY INSENSITIVE TO DISRUPTION BY INDIRECT DOPAMINE AGONISTS. J.P. Druhan* and R.J. Valentino, Dept. of Psychiatry, Med Coll of PA and Hahnemann Univ, Phila., PA 19102.

Dopamine (DA) receptor agonists can interfere with the ability of moderate intensity prepulse stimuli to inhibit startle reflexes in rats. This influence of DA agonists on prepulse inhibition (PPI) suggests that mesotelencephalic DA neurons may modulate sensorimotor gating processes involved in regulating responses to salient environmental stimuli. However, studies examining the effects of amphetamine on sensorimotor gating have reported only moderate disruption of PPI by doses that are known to increase DA release. The present study examined this discrepancy further by comparing the effects of several direct and indirect agonists on PPI of the acoustic startle reflex in rats. Experiments with direct DA receptor agonists confirmed that PPI could be completely disrupted by low doses of the mixed D1/D2 DA receptor agonist, apomorphine (0.2 to 0.8 mg/kg) and by the selective D2 agonists, propylnorapomorphine (0.2 to 0.8 mg/kg) and quinpirole (0.2 to 0.8 mg/kg). In contrast, tests with indirect DA agonists failed to reveal significant effects of the monoamine reuptake inhibitor, cocaine (10.0 to 40.0 mg/kg), or the selective DA reuptake blocker, GBR 12909 (5.0 to 20.0 mg/kg). PPI was significantly disrupted by d-amphetamine, but only at the highest dose tested (4.0 mg/kg). These findings indicate that PPI is relatively insensitive to manipulations that increase extracellular DA, and they raise questions as to whether sensorimotor gating deficits in humans can be attributed to increased activity of mesolimbic DA neurons. (Supported by the Scottish Rite Benevolent Foundation Schizophrenia Research Program).

68.3

THE DAD2-LIKE AGONIST QUINPIROLE PRODUCES MONOTONIC REDUCTIONS IN MOTOR ACTIVITY OF C57 MICE. L.D. Middaugh*, J.P. Halberda, and B.E. Gard, Dept. of Psychiatry, CDAP, Med. Univ. of SC, Charleston, SC 29425.

As reported for rats, the DAD1-like agonists SKF 38393 and SKF 82958 elevated or reduced the locomotion of C57 mice, the particular effect depending upon dose and testing conditions. Although DAD2-like agonists are also reported to have a biphasic action on locomotion of rats, quinpirole only reduced locomotion of C57 mice in this study. Reduced activity was observed whether the mice were male or female, were young or mid-aged adults, were naive or well habituated to the test environment, or were injected with very low or very high doses administered either IP or SC. Since the monotonic reduction in motor activity occurred over a wide range of testing conditions, it is unlikely that methodological variables can account for the apparent species differences in the effects of quinpirole. Thus, we conclude that the receptor systems mediating the effects of DAD1-like agonists on motor activity of C57 mice, and probably other strains, are similar to those of rats; however, that the systems mediating the effects of the DAD2-like agonist quinpirole are dissimilar for the two species. Possible reasons for the differences include species differences in DA sub-receptor numbers or affinity states, in their anatomical distribution including pre- and post-synaptic location, or in the interaction of D1, D2, and D3 subreceptor systems. (Funding source DA08034.)

68.4

D1 AND D2 DOPAMINE RECEPTOR INVOLVEMENT IN THE BEHAVIORAL EFFECTS OF MK-801 IN 6-OHDA LESIONED RATS. K.E. Asin* and L. Bednarz Neuroscience Research, Pharmaceutical Products Division, Abbott Laboratories, D-47U, Bldg AP9A, Abbott Park, IL 60064

In rats bearing unilateral 6-OHDA forebrain lesions, the NMDA receptor antagonist, MK-801, induces ipsilateral rotation, but interacts differentially with D1 and D2 dopamine (DA) receptors. Although both D1 and D2 receptor agonists produce contralateral rotation in these animals, co-treatment with MK-801 potentiates D1 -agonist induced rotation but antagonizes the rotation produced by the D2/D3 receptor agonist, quinpirole. In a recent study, it appeared that repeated l-DOPA treatment subsequently interfered with MK-801's interactions with selective DA agonists (Engber et al 1994). Since repeated l-DOPA treatment differentially alters D1/D2 receptor sensitivity (Asin et al 1995), we investigated D1/D2 contributions to the effects of MK-801 in DA agonist treated, 6-OHDA lesioned rats.

In the first study, lesioned rats were treated with the D2 antagonist haloperidol (0-0.38 mg/kg, sc) 20 min prior to MK-801 (0 or 0.10 mg/kg, ip). Twenty min later rats were injected with the D1 DA receptor agonist, A-85653 (0.06 mg/kg, sc), and rotation was measured for 6h. Haloperidol dose-dependently blocked the potentiation of A-85653-induced rotation produced by MK-801. In a second study, rats were injected with MK-801 (0 or 0.10 mg/kg) 10 min prior to treatment with the D1 antagonist SCH 23390 (0- 1 mg/kg, sc). Ten min later, rats were treated with quinpirole (0.15 mg/kg, sc) and rotation was measured for 3h. The reduction in quinpirole-induced contralateral rotation produced by MK-801 was dose-dependently blocked by SCH-23390. In a third study, rats were pretreated with either haloperidol or SCH 23390 prior to treatment with MK-801 alone (0.25mg/kg,sc) and rotation was recorded for 3 h. Both DA antagonists dose-dependently blocked the ipsilateral rotation produced by MK-801. The results of these studies indicate that the interactive effects of MK 801 with D1 and D2 agonists may rely on increases in DA tone and receptor synergism, perhaps in the intact striatum, resulting from NMDA receptor blockade. Asin et al., JPET 273, 1995; Engber et al. NeuroReport 5, 1994.

This research was supported by Abbott Laboratories.

68.5

DOPAMINE D_1 RECEPTOR AGONIST-INDUCED GROOMING IN RATS: AN EXAMINATION OF THE LINK TO ADENYLATE CYCLASE. P.R. Rau, R.B. Mailman , D.E. Nichols[1]*, and C.P. Lawler. Neuroscience Center, Univ. of North Carolina, Chapel Hill, NC 27599, and [1]Purdue Univ., W. Lafayette, IN 47907.

Dihydrexidine (DHX) and the phosphodiesterase (PDE) inhibitor rolipram were used to assess the roles of adenylate cyclase and the second messenger adenosine cyclic 3',5'-monophosphate (cAMP) in mediating the grooming induced by activation of D_1 dopamine (DA) receptors. Both drugs were evaluated for their ability to stimulate grooming in rats when administered alone, as well as when administered together. DHX (0.1, 1.0, and 3.0 mg/kg s.c.) produced the expected overall increases in total grooming time, whereas rolipram (7.5, 15 and 30 µg/kg s.c.) produced inconsistent effects on this measure. Most importantly, there was no interaction attributable to the combined effects of rolipram and DHX on total grooming time. An examination of grooming bout structure revealed striking differences between the actions of the two compounds. DHX produced moderate increases in both grooming frequency and bout duration. Rolipram produced larger increases in grooming bout duration, but these were offset by decreases in bout frequency. The ability of rolipram to depress grooming frequency was evident when it was coadministered with the two lowest doses of DHX (0.1 and 1.0 mg/kg), but not at the highest dose (3.0 mg/kg). In no case did rolipram produce a synergistic enhancement in any aspect of grooming behavior induced by DHX. This lack of synergism contrasts with a previous study in this laboratory in which a similar experimental strategy in a drug discrimination paradigm showed synergism. This inability of rolipram to enhance DHX-induced grooming adds support to the hypothesis that grooming induced through D_1 receptor activation is not mediated by the AC/cAMP system. (Supported by MH42705, MH40537, HD03110, MH33127, and GM07040.)

68.6

THE EFFECT OF DOPAMINERGIC AGENTS ON REACTION TIME IN RHESUS MONKEYS M.R. Weed* and L.H. Gold. Department of Neuropharmacology, The Scripps Research Institute, La Jolla, CA 92037.

Measures of reaction time have been shown to be sensitive to CNS manipulations such as treatment with dopaminergic drugs or infection with viruses like HIV. Rhesus monkeys (4) performed a 5-choice reaction time procedure adapted from a human neuropsychological battery (CANTAB; CAmbridge Neuropsychological Test Automated Battery; Paul Fray, Ltd., Cambridge, UK). Monkeys depressed a lever for a variable duration (0.75-2.5 s) until a stimulus flashed in 1 of 5 circles on a touch-sensitive computer monitor. Releasing the lever and touching the appropriate circle within 2 s from stimulus onset resulted in the delivery of two 190 mg food pellets. The behavior was shaped by hand until responding was reliable with stimuli of 0.6-0.1 s (16-26 sessions). One 30-trial training session was conducted Mon., Wed. and Thurs., and cumulative-dosing tests were conducted on Tues. and Fri. Stimuli were randomized by duration (1, 0.1 or 0.01 s) and circle postition across each of four 30-trial test sessions. Baseline reaction times were established with 20 m saline pretreatments (i.m.) prior to each session. Saline injections had no effect upon either response latency or latency to release the lever, and there were no effects of the multiple tests (N=2). Amphetamine (1.0, 1.7 and 3.0 mg/kg, i.m., N=2) had little effect upon either measure until responding was suppressed (5.6 mg/kg, N=2). Selective dopamine receptor antagonists (D1) SCH 39166 (1.7-30 µg/kg, i.m., N=2) and (D2) raclopride (10-56 µg/kg, i.m., N=2) produced dose-dependent increases in both latency measures. The increases in latency with dopamine receptor antagonists verify that the current paradigm is sensitive to neuropharmacological manipulation. Verification of the sensitivity of this task to CNS manipulations is critical prior to its use in the investigation of the CNS effects of immunodeficiency disease and/or drug-induced neurotoxicity in rhesus monkeys. Supported by U.S.P.H.S. grants: MH 47680 and DA 09111.

68.7

DOPAMINE D-2 RECEPTORS MODULATE THE BEHAVIORAL RESPONSE TO ACTIVATION OF SEROTONIN 5-HT_{2A} RECEPTORS IN THE RAT MEDIAL PREFRONTAL CORTEX. **David L. Willins* and Herbert Y. Meltzer, Laboratory of Biological Psychiatry, Case Western Reserve University, Cleveland, Ohio 44106**

The direct injection of serotonin$_{2A}$ (5-HT_{2A}) agonists, (e.g. DOI, mCPP) into the medial prefrontal cortex (mPFCx) of rats has been shown to elicit a head twitch response (HTR). This response is blocked by pretreatment with selective 5-HT_{2A} receptor antagonists (e.g. ketanserin, MDL 100,907). In the present study the role of dopamine (DA) in the mPFCx in the HTR to DOI was evaluated. The direct administration of the D-1 agonist, SKF38393 (3 µg/0.5 µl/side) bilaterally into the mPFCx elicited a HTR. Co-injection of SKF38393 with DOI (3 µg/0.5 µl/side) produced a HTR which was not greater than additive with the effects of DOI. Systemic administration of a low dose (10 µg/kg) of SCH23390, a D-1 antagonist, inhibited the HTR to SKF38393 but did not block DOI-stimulated HTR. The direct injection of the D-2/D-3 agonist, quinpirole (3 µg/0.5 µl/side) into the mPFCx produced a slight, but significant, increase in the HTR. However, co-administration of quinpirole with DOI inhibited the HTR produced by DOI. Co-injection of a low dose of the selective D-2 antagonist, sulpiride (1µg) with DOI potentiated the effects of DOI. We have previously demonstrated that the systemic administration of DA receptor antagonists inhibits the HTR to intra-cortical DOI (Willins and Meltzer, Soc. Nsci. Abst., 809.9, 1995). This suggests that the behavioral effects of activating 5-HT_{2A} receptors in the mPFCx are suppressed by DA in the cortex and facilitated by DA in other brain regions. The present findings further indicate that DA elicits its effects on sertonin-mediated behavior in the mPFCx through activation of postsynaptic D-2 receptors. **(Supported by MHCRC47808 and a NARSAD Young Investigator Award)**

68.8

DIFFERENTIAL EFFECTS OF D1 AND D2 DOPAMINE RECEPTOR AGONISTS AND ANTAGONISTS ON APPETITIVE AND CONSUMMATORY ASPECTS OF MALE SEXUAL BEHAVIOR IN JAPANESE QUAIL. J. Balthazart*, C. Castagna, and G. F. Ball. Lab. Biochemistry, Univ.of Liège, B-4020, Belgium and Dept. of Psychology, Johns Hopkins Univ. Baltimore, MD 21218, USA.

Previous pharmacological studies in quail demonstrated that general dopaminergic agonists affect appetitive as well as consummatory aspects of male sexual behavior. Inhibition of both components of behavior were observed with the general agonist, apomorphine while the indirect dopamine agonists, nomifensin or amfonelic acid, enhanced certain aspects of these behaviors. These inhibitory effects of apomorphine in quail may reflect its predominant action at the level of D2-like dopaminergic receptors while the two other compounds primarily exert their effects through an interaction with D1 receptors. This was tested by studying the behavioral effects of specific D1 and D2 dopaminergic receptor agonists and antagonists in castrated male Japanese quail that were chronically treated with exogenous testosterone (subcutaneous Silastic implants). Appetitive male sexual behavior was measured by the time spend in front of, and looking through, a small window that provides a view of a female who is subsequently released into the cage to evaluate copulatory behavior per se. The effects of five compounds were tested: one D1 (SKF38393) and two D2 (PPHT or N-0434 and quinpirole or LY171555) agonists, and one D1 (SCH23390) and one D2 (Spiperone) antagonist. All compounds were tested at a low and a high dose (100 µg and 1 mg/kg respectively for all drugs except spiperone where the doses were 2 and 10 mg/kg). A consistent effect of all drugs on consummatory sexual behavior was observed: it was stimulated by the D1 agonist and the D2 antagonist but inhibited by the D1 antagonist and the D2 agonists. Far fewer effects of the treatments were detected on the two measures of appetitive behavior. The time spent in front of, and looking through the window was decreased by the two D2 agonists but not affected by the other treatments. These data therefore demonstrate that, as in rodents, male copulatory behavior in quail is stimulated by the action of dopamine at the level of its D1 receptors but inhibited by activation of the other receptor subtype (D2). The partial dissociation observed between the effects of the same treatments on appetitive and consummatory aspects of sexual behavior also suggests that these two behavioral systems may be controlled by the action of dopamine on different neuronal networks.
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68.9

DEVELOPMENTAL AND BEHAVIORAL DEFICITS OF DOPAMINE-DEFICIENT MICE. Q.-Y. Zhou, M. Szczpka, J. E. Erickson, M. Fujanaga, H. Van Tol* and R. D. Palmiter. Clarke Institute of Psychiatry, University of Toronto, Toronto, ON M5T, 1R8, Canada and Howard Hughes Medical Institute, University of Washington. Seattle, WA 98195, USA.

A strain of DA-deficient mice was developed by combined gene knock-out and knock-in techniques. Dysregulated expression of neuronal genes in the striatum was observed in DA-deficient mice. However, general brain development appear normal in the absence of DA signaling including neurogenesis of both DA neurons and their major targets. DA-deficient mice exhibited a number of abnormal individual and social behaviors. All DA-deficient pups were severely hypoactive, failed to feed themselves and died within a few weeks of birth. Importantly, DA-deficient pups were rescued to adults by repeated administration of L-DOPA. Rescued adult DA-deficient mice also exhibited severe hypoactivity, adipsia and aphagia and performed poorly in rota-rod test in the absence of DA (8-12 hr after L-DOPA administration). The feeding and motor deficits were reversed by administration of L-DOPA. Preliminary work of intrastriatal delivery of L-DOPA indicated that the nigrostriatal pathway is responsible for both motor and feeding functions. Male DA-deficient mice displayed abnormal aggressive behavior towards intruders after L-DOPA administration whereas male TH heterozygous mice, which have reduced DA level, were less aggressive. Male DA-deficient mice also exhibited excessive and inappropriate sexual behavior towards females after L-DOPA administration. These results indicate that intact dopamine systems are essential for normal expression of a number behaviors of mice and the availability of reversible DA knock-out mice should help to study modulatory role of DA on target neurons including finding the target molecules of DA signaling. Supported by Howard Hughes Medical Institute and Clarke Foundation.

68.10

ASSESSMENT OF BEHAVIORAL ORGANIZATION DIFFERENTIALLY CHARACTERIZES D2 AND D4 KNOCK OUT MICE. M.P. Paulus*, D. Grandy, M. Low, M. Kelly, S. C. Dulawa, and M.A. Geyer. UCSD Dept Psychiatry, La Jolla, CA 92093.

Targeting genes using homologous recombination that generates mouse strains with distinct mutations in their genome enables the investigation of gene-related behavioral organization. In this investigation, D2 and D4 knockout (KO) mice were tested in an unconditioned motor paradigm to assess overall levels of activity and the geometrical characterization of movements based on a scaling approach. Briefly, unconditioned motor activity was recorded with high temporal and spatial resolution from D2 or D4 KO male and female mice and wild type controls using a video-based system consisting of 41 × 41 × 38 cm white Plexiglas boxes. The amount of locomotor activity was assessed by measuring the number of micro-events, *counts*, within two 15-minute windows. In addition, the geometric characteristics of movement sequences was measured using the spatial scaling exponent, d, and the fluctuation spectrum of local spatial scaling exponents, $f(d)$. A mixed model ANOVA (*gender* × *genotype*) × (*within–session habituation*) was calculated for the D2 KO experiment. Neither the genotype ($F(2, 23) = 0.36$, n.s.) nor gender ($F(1, 23) = 0.66$, n.s.) factors had significant main effects on the amount of activity. There was a significant effect of time ($F(1, 23) = 14.40$, $p < 0.001$) but no interaction with genotype ($F(2, 23) = 1.20$, n.s.) or gender ($F(1, 23) = 0.76$, n.s.) on the *counts* measure. In contrast, there was a significant main effect of genotype on the geometrical patterns of movements ($F(2, 23) = 13.89$, $p < 0.0001$). While gender did not exert a main effect on geometrical patterns ($F(1, 23) = 0.65$, n.s.), there was a significant interaction between genotype and gender ($F(2, 23) = 3.64$, $p < 0.05$). Thus, D2 KO mice in general and female KO mice in particular are characterized by a significantly increased d suggesting an increased engagement in predominantly circumscribed movement sequences. Support provided by DA02925, MH01223, and NARSAD.

68.11

APOMORPHINE SUSCEPTIBILITY IN RATS IS DETERMINED BY BOTH GENETIC AND ENVIRONMENTAL FACTORS. B.A. Ellenbroek* and A.R. Cools Dept. Psychoneuropharmacology P.O. Box 9101, 6500 HB Nijmegen, the Netherlands.

It is well known that animals show a large individual variability to the behavioural effects of dopaminergic drugs like apomorphine. It has been speculated that this susceptibility for apomorphine may be linked to the development of drug dependence and schizophrenia. The present study was designed to investigate the role of genetic and early postnatal environmental factors in determining apomorphine susceptibility. In order to investigate the role of genetic factors, the stereotyped gnawing response upon a challenging dose of apomorphine (1.5 mg/kg s.c) was studied in a number of inbred strains of rats. The results show that the apomorphine response was highest in Sprague Dawley rats, and lowest in the ACI and Fischer rats. In order to investigate the role of early postnatal factors we separated the mother from her puppies at days 3, 6 or 9 after birth (day of birth is day 0). The puppies showed an enhanced stereotyped response to 1.5 mg/kg s.c. apomorphine at adult age. In a second series we studied the interaction between genetic and environmental factors by studying the effects of cross-fostering and maternal separation in rats bred selectively for apomorphine susceptibility (APO-SUS and APO-UNSUS). The results show that maternal separation enhanced apomorphine susceptibility in APO-UNSUS but not in APO-SUS, whereas cross-fostering reduced apomorphine susceptibility in APO-SUS but not APO-UNSUS. These data strongly suggest that the development of apomorphine susceptibility is determined by a complex interaction between genetic and environmental factors. Given the above mentioned relationship between apomorphine susceptibility and drug dependence and schizophrenia, these data may have important implications for the etiological theories regarding these mental illnesses.

68.12

THE EFFECTS OF TEMPORARY LESIONS OF THE INSULAR AND MEDIAL PREFRONTAL CORTEX ON FIXED-INTERVAL SCHEDULE-CONTROLLED BEHAVIOR IN THE RAT. S.B. Evans*, and D.A. Cory-Slechta. Department of Neurobiology and Anatomy, University of Rochester School of Medicine and Dentistry, Rochester N.Y. 14642.

Sixteen food-deprived rats were trained to press a lever for food reinforcement on a one-minute-fixed-interval (FI) schedule. Previous work in this laboratory has shown that the rate of responding on this fixed-interval schedule of reinforcement is sensitive to dopamine (DA) neurotransmission in the nucleus accumbens (N.Acc.), such that temporarily inactivating D1 and D2 receptors with EEDQ, an irreversible DA antagonist, markedly decreases overall rate of responding. This decrease in rate can be prevented by protecting the D1 or D2 receptors with an antagonist during the EEDQ treatment. Therefore, if behavior on the FI schedule is sensitive to changes in the N.Acc. DA system, manipulations of other regions that regulate N.Acc. DA should have predictable effects on FI performance. It has been demonstrated by others that the rat prefrontal cortex (PFC) regulates DA neurotransmission in the N.Acc. Our FI-trained rats were therefore implanted with cannulae aimed at the medial PFC (n=8) or the Agranular Insular cortex (n=8). Lidocaine at varying concentrations was injected into these areas to observe the effect on subsequent FI behavior. Preliminary results from the temporary-lesion studies suggest that over an FI session, lidocaine into the PFC causes an initial *decrease* in run rate at the beginning of the session, followed by an *increase* in run rate at the end of the session. These changes were sometimes accompanied by decreased post reinforcement pause times. It is our hypothesis that the *increase* in run rate reflects an increase in DA release in the N.Acc. This work is supported by ES05017, ES05903, ES01247, and 5T32 MH19963-01.

68.13

PHASIC INCREASES IN EXTRACELLULAR DOPAMINE DURING A FREE-CHOICE NOVELTY TASK AS MEASURED BY FAST-SCAN VOLTAMMETRY. G.V. Rebec*[1], J.R.C. Christensen[1], C. Guerra[1], and M.T. Bardo[2]. [1]Program in Neural Science and Dept. Psychology, Indiana University, Bloomington, IN 47405; [2]Dept. Psychology, University of Kentucky, Lexington, KY 40506.

Response to novelty in rats is used as an analog of human sensation-seeking activity, which is associated with an increased risk for drug abuse. Previous research with slow-scan voltammetry has shown an increase in the catechol signal in the nucleus accumbens of rats allowed free-choice entry into a novel compartment (Rebec et al., Soc. Neurosci. Abstr. 20:826, 1994). To identify the catechol and to obtain a more precise time-course of the novelty-related increase, we repeated the novelty task using fast-scan cyclic voltammetry. Recordings were obtained at 100-ms intervals with carbon-fiber microelectrodes placed in either the shell or core region of the nucleus accumbens. In accumbal shell, we observed a pronounced increase in oxidation current (> 100% above baseline) that began immediately upon entry into novelty and terminated in < 10 s. Voltammetric analysis revealed that the increase in signal was due to dopamine. No novelty-related voltammetric changes were observed in accumbal core. Random motor activity either before or after entry into novelty had no effect in either accumbal region. Our results provide real-time evidence for mesolimbic dopamine release in novelty-seeking behavior, suggesting a common action with drugs of abuse. It also appears that phasic, rather than tonic, dopaminergic activation plays a critical role in free-choice novelty.

Supported by NIDA.

68.14

ON THE ROLE OF DOPAMINE IN THE EFFECT OF NICOTINE ON THE EEG IN RATS B.Ferger and K.Kuschinsky* *Institute of Pharmacology and Toxicology, Faculty of Pharmacy, University of Marburg, D-35032 Marburg, Germany*

In a previous study (Ferger and Kuschinsky, Naunyn-Schmiedeberg's Arch. Pharmacol 350: 346 (1994)), it was shown that a moderate dose of nicotine (0.2 mg/kg s.c.) produced desynchronization in the EEG of rats and a decrease of power which was not antagonized by blockade of D1-like dopamine receptors, although this EEG pattern seemed to be characteristic for activation of D1-like rather than D2-like receptors. This seemed surprising since nicotine is known to enhance dopaminergic neurotransmission in the basal ganglia. Due to a strong reciprocal connection between cortex and striatum, dopaminergic effects on the striatum should lead to alteration in the cortical EEG.

Therefore, the release of dopamine was studied in the striatum by microdialysis in awake rats and, in parallel studies, the cortical EEG was measured after administration of 0.4 mg/kg of nicotine s.c.. This dose produced a desynchronization in the EEG and a decrease of power in all of the frequency bands. There was very little increase of striatal extracellular dopamine (maximal increase: 28 % of baseline level).

We conclude that activation of striatal dopaminergic neurotransmission does not seem to be relevant for the EEG effects observed. *Supported by VERUM Foundation*

68.15

REGULATION OF DOPAMINE BY PROGESTERONE IN THE MEDIAL PREOPTIC AREA OF FEMALE RATS, L. Matuszewich*, D. S. Lorrain, & E. M. Hull. State Univeristy of New York at Buffalo, Buffalo, NY 14260.

Progesterone is frequently used to enhance the receptive and proceptive behaviors in estrogen-primed, ovariectomized female rats. One mechanism by which progesterone influences behavior may be through up or down regulation of the release of neurotransmitters in specific brain sites. The medial preoptic area (MPOA) contains progesterone receptors and plays a role in female rat sexual behavior. The present experiment explored the regulation of dopamine release by progesterone in the medial preoptic area. On the test day, microdialysis probes were inserted into the MPOA in 9 ovariectomized, estrogen primed female rats. Following a 2 hour stabilization period, samples were collected every 6 minutes for 36 minutes. A subcutaneous injection of either 500 ug progesterone or oil was then given to each subject. Three hours following the injection, 6 minute samples were again collected. All females that received progesterone showed a significant decrease in dopamine release compared to samples prior to injection. The oil treated females, however, did not show a decrease in dopamine activity. Dopamine's metabolites DOPAC & HVA also were significantly depressed in progesterone treated females compared to oil treated controls. Therefore, progesterone may influence female rat sexual behavior by decreasing dopamine activity in the MPOA.

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68.16

SEX DIFFERENCES IN POSTURAL COMPENSATION TO AN INCLINED PLANE DURING HALOPERIDOL CATALEPSY: THE ROLE OF NEONATAL ANDROGEN EXPOSURE S. M. Pellis*, E. F. Field and I. Q. Whishaw. Dept. of Psychology, University of Lethbridge, Lethbridge, AB Canada T1K 3M4.

Rats exhibiting haloperidol-induced cataleptic immobility can be induced to jump by placing them on a tilting inclined plane. Males and females injected with 5.0 mg/kg of haloperidol differ in their response to this. The angle of the incline plane was measured at the point where the rats jump. Females jump at a lower angle than do males. Males also shifted their body backwards and made backward adjusting steps. In contrast, females did not shift their body backwards and are more likely to step forward. This difference is modified by neonatal androgen manipulation. Females injected with testosterone propionate at birth were more male-like and males castrated at birth were more female-like. This suggests that sex differences in postural support mechanisms are partly determined by neonatal androgen exposure. This research was supported by NSERC.

68.17

SEX DIFFERENCES IN THE ORGANIZATION OF LATERAL MOVEMENTS ARE PRESERVED DURING APOMORPHINE INDUCED STEREOTYPY E. F. Field*, S. M. Pellis and I. Q. Whishaw. Dept. of Psychology, University of Lethbridge, Lethbridge AB Canada T1K 3M4.

Apomorphine is known to produce circling in rats. This circling involves modifications of the hindlimb stepping patterns commonly seen during turning in rats injected with saline. During turning behaviors by rats, sex differences in the lateral movement of the pelvis and associated hindlimb stepping patterns have also been reported. In this study the effects of apomorphine (1.1 mg/kg), on differences in turning patterns of male and female rats are compared. Males display more lateral movement of the pelvis, relative to the movement of the snout, during apomorphine-induced turning than do females. This difference reveals that while stepping patterns of male and female rats are modified by apomorhine treatment, the differences in lateral movement of the pelvis are not. This suggests that sex differences in lateral pelvic movement are independent of hindlimb stepping patterns. This research is supported by NSERC.

68.18

CHRONIC AMPHETAMINE TREATMENT INDUCES SENSITISATION OF PREPULSE INHIBITION IN THE RAT. J. Zhang, J. A. Engel, B. Söderpalm and L. Svensson*. Department of Pharmacology, Göteborg University, Göteborg, Sweden.

Male Sprague-Dawley rats were repeatedly treated with amphetamine (AMP, 1 mg/kg, s.c.) at an interval of 3 days and tested for prepulse inhibition of acoustic startle after each treatment. This treatment regimen induced sensitisation in the animals as evidenced by a progressive increase in the disrupting effect of AMP on prepulse inhibition. Persistent changes in brain function was indicated since an increase in sensitivity was observed in sensitised animals also after a 22 days long drug- and test-free period. The development of sensitisation was blocked by pretreatment with haloperidol (0.1 mg/kg, s.c.), which suggests that sensitisation of the disrupting effects of AMP was dependent on dopamine (DA) D_2 receptor activation. Furthermore, the development of sensitisation was blocked by adrenalectomy, which suggests that sensitisation was dependent also on circulating adrenal hormones. Increased DA-ergic activity has been implicated in the pathophysiology of schizophrenia and AMP-induced sensitisation of the neuronal functions that modulate prepulse inhibition may be an experimental model to investigate this hypothesis.

This investigation was supported by grants from the Swedish Medical Research Council (4247).

68.19

AMPHETAMINE AND THE ELICITATION AND INHIBITION OF THE STARTLE REFLEX IN ADULT AND AGED MICE. S. B. Schwarzkopf*, V. Thai, P. Agrawal, E. Gutierrez, & J. R. Ison, Brain & Cognitive Sciences, University of Rochester, Rochester NY 14627.

Aged mice, like aged humans, show a diminished acoustic startle reflex (ASR), an effect too severe to be due to changes in auditory function. Here we tested the hypothesis that this decrement results from reduced arousal, which suggests that it may be partially remediated by a small dose of amphetamine (AMPH). We also examined prepulse inhibition for gaps in noise (1 to 15 ms long, 60 ms before the ASR) to see if gap thresholds were sensitive to AMPH. F1 offspring of CBA/Ca and C57Bl/6J mice were tested at 8 and 27 m (n = 18), first at AMPH levels of 0, .05, .1 and .5 mg/kg, and later, at 4 mg/kg vs 0. The old mice had lower ASRs, which were unaffected by low AMPH. There were no effects of age or low AMPH on relative PPI. With high AMPH ASRs were reduced to near activity values in old but not young mice. Thresholds were not affected even in old mice, as long as they had a response that could be measured. Rather than being engaged in interfering stereotyped acts, old mice seemed "behavioral inert" in the testing cage, but remained capable of processing small gaps in noise. These data suggest the testable hypothesis that normal dopamine release in mesolimbic regions was reduced in the aged mouse. Perhaps a primary effect of AMPH in old mice was to increase extracellular serotonin, which would be expected to diminish the ASR. Supported by NIH Grants AG09524 and EY01319, and by RICHSR

68.20

R-(+)-HA-966, A GLYCINE-NMDA ANTAGONIST, ACTS IN THE VENTRAL TEGMENTAL AREA TO BLOCK CONDITIONED FEAR AND ASSOCIATED METABOLIC ACTIVATION OF MESOCORTICAL DOPAMINE NEURONS. J.D. Elsworth*, B.A. Morrow, E.J.K. Lee and R.H. Roth. Depts. of Pharmacology and Psychiatry, Yale University School of Medicine, New Haven, CT 06520.

Systemic administration of the glycine-NMDA antagonist, R-(+)-HA-966, in the rat blocks the expression of conditioned fear behavior and the associated increase in mesocortical dopamine (DA) metabolism. In the present study, R-(+)-HA-966 was injected in the ventral tegmental area (VTA) to see whether the drug acted in the VTA to achieve its effects in the conditioned fear paradigm, an animal model of anxiety.

Rats underwent 3 sessions each lasting 30 min on 3 consecutive days: habituation (no tones, no footshocks), acquisition (10 tones paired with 0.4 mA x 0.5 sec footshocks), and extinction (10 tones, no footshocks). R-(+)-HA-966 (15 µg/0.5 µl) was administered into the VTA immediately before the session on either the acquisition day or the extinction day. Sacrifice was directly after the last session.

R-(+)-HA-966 given either on acquisition or extinction days prevented the conditioned fear-induced increase in DA metabolism in the medial prefrontal cortex, but not in the nucleus accumbens. On the acquisition day, exposure to R(+)-HA-966 blunted the acquisition of fear, compared to saline controls, as measured by shock-induced immobility. On the extinction day, rats given R(+)-HA-966 on the acquisition day displayed less immobility in response to the tone, compared to controls, both initially and throughout the test period. Rats treated with R(+)-HA-966 on the extinction day had a greater rate of extinction than control rats. In another study, R(+)-HA-966 (15 mg/kg i.p.) prevented the shock-induced increase in the number of tyrosine hydroxylase positive cells with Fos-LI in the VTA.

Thus antagonism at VTA glycine-NMDA sites blocks conditioned fear behavior and associated metabolic activation of mesocortical, but not mesoaccumbens DA neurons. *Supported by US PHS MH-14092.*

MONOAMINES AND BEHAVIOR II

69.1

LOCAL APPLICATION OF GABA AGONISTS AND ANTAGONISTS FAILS TO MODULATE THE PREFRONTAL CORTICAL DOPAMINE STRESS RESPONSE. M. D. Doherty* and Alain Gratton. McGill Univ., Douglas Hosp. Res. Ctr., Montreal, Canada, H4H 1R3.

Stress stimulates DA release in prefrontal cortex (PFC) and in nucleus accumbens (NAcc); increasing evidence suggests that meso-PFC DA projection modulates the meso-NAcc DA response to stress. We have recently shown that D1-, but not D2-, receptor blockade in PFC enhances (Doherty and Gratton, 1996), and that PFC GABA-B but not GABA-A receptor activation inhibits, stress-induced DA release in NAcc (Soc. for Neurosci., 1995). While it is clear from these studies that PFC DA and GABA both contribute to the regulation of NAcc DA transmission, the interaction between DA terminals and GABA neurons in PFC is not yet completely understood. While DA is known to influence GABA release, it is not clear if the PFC DA response is modulated locally by GABA. To investigate this, we used voltammetry to monitor the DA stress response in PFC following local injections (0.01, 0.10, and 1.00 nmole in 0.5 µl) of GABA-A and GABA-B agonists (baclofen and muscimol) and GABA-A and GABA-B antagonists (bicuculline and phaclofen). Neither the GABA-A or GABA-B agonists or antagonists altered the PFC DA stress response. We also report that intra-PFC injections of the D2/D3 DA agonist quinpirole inhibited, whereas local DA uptake blockade with GBR 12909 potentiated, the PFC DA stress response. These data indicate that the PFC DA response to stress is not modulated locally by GABA. It would appear therefore that the inhibitory effect of intra-PFC baclofen on the NAcc DA stress response is mediated by GABA-B receptors located on PFC interneurons or output neurons. *Supported by the Medical Research Council of Canada.*

69.2

MESOCORTICAL DOPAMINE RESPONSES TO PHYSICAL AND PSYCHOLOGICAL STRESS: RELATIONSHIP WITH LATERALITY AND PLASMA CORTICOSTERONE. R.M.Sullivan* and A.Gratton. Douglas Hosp. Research Ctr., McGill University, Montréal, CANADA H4H 1R3.

Increasing evidence suggests that activation of the DA projection to the medial prefrontal cortex (mPFC) is asymmetric and functionally related to the ability to cope with stress. In the present study voltammetry was used to monitor changes in DA levels in the left and right mPFC of rats exposed to mild physical and psychological stress. These were 2 min. of tailpinch (TP), and 15 min. exposure to cat feces (CF), respectively. Fourteen male Long-Evans rats with bilateral mPFC carbon fibre recording electrodes were tested on four consecutive days, during which the electrochemical response to each stress was recorded in each mPFC in counterbalanced order. A week later, animals underwent a 20 min. restraint stress, with plasma taken at 0, 20 and 80 min. to determine corticosterone (CORT) levels. It was found that the DA response to TP was significantly longer-lasting in the left than in the right mPFC, while a trend in the opposite direction was noted for the DA response to CF. The peak increases in DA elicited by the two stressors were significantly correlated only in the right mPFC. The magnitude of stress-induced increases in plasma CORT was positively correlated to DA mPFC responses to both stressors, but significant only for TP. Finally, CORT responses were more pronounced and longer-lasting, when asymmetries in the DA response to CF favoured the right mPFC. The data suggest that asymmetric mesocortical DA activation depends on the type of stress, and that regulation of DA responses to both types of stress is most tightly coupled in the right mPFC. Also, while neuroendocrine and DAergic stress responses are positively linked, this relationship is only asymmetrical for the psychological stressor, which may reflect a specialized role for right mPFC mechanisms in the integration of emotional and physiological responses to stressful situations. (Supported by FRSQ and MRC).

69.3

FLUCTUATIONS IN PREFRONTAL CORTICAL DOPAMINE DURING OPERANT RESPONDING FOR FOOD: AN IN VIVO ELECTROCHEMICAL STUDY IN RAT. N.R. Richardson* and A. Gratton, McGill Univ., Douglas Hosp. Res. Ctr., Montreal, Canada, H4H 1R3.

Lesions to the prefrontal cortex (PFC) result in aphagia; feeding and food-related stimuli activate the meso-PFC dopamine (DA) system as does operant behavior rewarded by food. In the present study, we used voltammetry in combination with monoamine-selective probes to monitor DA levels in PFC of rats lever-pressing for a reward of condensed milk. Under standard conditions, each response was rewarded by 0.2 ml of milk delivered over 30 sec paired with a 30 sec light cue. Typically, lever-presses were immediately followed by small decreases in DA signal and these began increasing again once consumption of the milk reward was completed. The magnitude of signal decreases during milk consumption did not appear to change as a function of satiation or experience. Delaying milk delivery by 20 or 30 sec after each lever-press caused a corresponding decrease in DA signal accompanying milk consumption; these were preceded by increases time-locked to the duration of the delay. Decreases in signal preceded responses in the delay condition. Lever-presses rewarded with three times the usual volume of milk (0.6ml/30sec) were followed by more pronounced decreases while halving the expected reward (0.1ml/30sec) reduced the magnitude of signal decreases; withholding the milk reward altogether (0 ml) caused increases in DA signal. Orderly changes in DA signal were observed in response to increasing the response requirements to earn each reward (FR 3, FR 5, or FR 10). A decline in signal coincided with additional operant responding. The signal began to increase immediately after the rewarded lever-press before decreasing 12-20 sec into the period of milk delivery. The present data suggest that there are reward-related changes in PFC DA transmission. The magnitude of these changes is comparatively smaller and, in all but a few exceptions, opposite to those observed in nucleus accumbens (NAcc) under the same conditions. These findings are also in agreement with the idea that meso-PFC DA indirectly modulates certain reward-related changes in NAcc DA transmission. *Supported by the MRC of Canada and McGill Graduate Scholarship program.*

69.4

PERINATAL ANOXIA FACILITATES DEVELOPMENT OF SENSITIZATION TO AMPHETAMINE IN ADULT RAT. W. Brake*, P. Boksa, and A. Gratton, Dept. of Psychiatry, McGill Univ., Douglas Hosp Res Ctr, Montréal, Canada.

There is increasing evidence linking schizophrenia to obstetric complications (such as transient anoxia) at birth. We have found evidence in rat suggesting that anoxia at birth can later facilitate the development of sensitized dopamine (DA) transmission in nucleus accumbens (NAcc). Animals as well as humans will sensitize to the behavioral stimulant effect of amphetamine. We examined the possibility that the locomotor stimulant action of amphetamine would sensitize to a greater extent in rats that had been subjected to an episode of perinatal anoxia. Dams were decapitated on the last day of gestation, the uterus was removed by Caesarian (C)-section and immersed in a 37°C saline bath for 15 min (C+15). Control groups comprised pups either delivered immediately after C-section (C+0) or born vaginally (VAG). Three months later, the spontaneous locomotor activity was monitored. Animals were then assigned to receive one of three pretreatment conditions: either 5 consecutive once daily injections of D-amphetamine (2.0 mg/kg ip) or of equal volume of saline or no injection. One week later, all animals received a challenge injection of D-amphetamine (0.5 mg/kg ip). No differences were evident between the three birth groups that had received amphetamine pretreatment. However, of those animals that had been pretreated with saline, the C+15 group showed a significantly greater locomotor response to the amphetamine challenge than did the C+0 and VAG groups. There were no differences between birth groups in the no-pretreatment condition. It appears that the stress associated with repeated intraperitoneal injection of saline was sufficient to sensitize C+15 animals to the challenge of amphetamine. Stress will cross-sensitize with the behavioral effects of amphetamine. Stress also stimulates DA release in NAcc and we have reported evidence that sensitization of this response is enhanced by perinatal anoxia. Together, these findings provide neurobiological evidence in support of the idea that obstetric complications involving anoxia may contribute to the pathophysiology of schizophrenia.

69.5

PREFRONTAL CORTEX DOPAMINE AND SEROTONIN: MICRODIALYSIS DURING AGGRESSION AND ALCOHOL SELF-ADMINISTRATION IN RATS A.M.M. van Erp* and K.A. Miczek, Department of Psychology, Tufts University, Medford, MA 02155

Prefrontal cortex (PFC) dopamine (DA) has been shown to increase during exposure to stressors, including threat of attack, but little is known about PFC DA or serotonin (5-HT) in aggressive animals. Resident rats confronting an intruder were characterized neurochemically, using *in vivo* microdialysis of DA and 5-HT in PFC and n. accumbens (NAC). Male Long-Evans rats, housed with a female, attacked a smaller male intruder in their home cage during 5 min tests. After 2 encounters the residents were implanted with a guide cannula aimed at NAC or PFC. A microdialysis probe was inserted 18 hours before an intruder confrontation. Ten min samples were taken during 60 min baseline, 10 min intruder test and 120 min recovery. During the intruder test aggressive residents displayed up to 10 bites and threats, for a total duration of 110 seconds. In PFC, DA was increased to 160% after the intruder confrontation in aggressive animals, whereas 5-HT was decreased to 50% of baseline. These changes did not preceed but followed the fight. In NAC, no such changes in DA or 5-HT were observed. In a second group of residents, intruder tests were combined with ethanol self-administration. During microdialysis an ethanol drinking solution was presented for 10 min, followed by a 10 min intruder confrontation, leading to increased DA levels in NAC; no such increase in DA was observed during intruder confrontations without ethanol. Effects of ethanol on PFC DA and 5-HT are under current investigation. The present results point to phasic changes in DA and 5-HT that are triggered by the display of aggressive behavior. (USPHS grant R37AA05122)

69.6

(+)HA966 AND CLONIDINE, BUT NOT PROPRANOLOL, PREVENT FG7142- INDUCED INCREASES IN PREFRONTAL CORTICAL DOPAMINE TURNOVER AND AMELIORATE IMPAIRMENT OF SPATIAL WORKING MEMORY IN RATS AND MONKEYS. BL Murphy*, AFT Arnsten, JD Jentsch, and RH Roth, Department of Pharmacology and Section of Neurobiology, Yale University School of Medicine, New Haven, CT 06510.

The benzodiazepine inverse-agonist, FG7142, produces a selective increase in dopamine (DA) turnover in the rodent prefrontal cortex (PFC). Administration of FG7142 to rats and monkeys produces impairment of PFC-dependent, but not non-PFC-dependent, cognitive tasks. The level of FG7142-increased DA turnover in the rodent PFC correlates with the degree of the cognitive deficits, and the impairment can be blocked in rats and monkeys by DA-receptor antagonists (Murphy et al., 1994; 1996). The current study examines the effects of drugs which do not act at DA receptors, but rather alter the stress-induced increase in DA turnover, in order to determine their influence on the FG7142-induced impairment of spatial working memory. Systemic administration of clonidine, a noradrenergic agonist, and (+)HA966, a glycine/NMDA antagonist, prevented the FG7142-induced increase in DA turnover in the rodent PFC. In addition, a direct infusion of (+)HA966 into the ventral tegmental area also blocked the increase in rodent PFC DA turnover. (+)HA966 and clonidine treatment prevented the FG7142-associated PFC-dependent cognitive deficits in both rats and monkeys. (-)HA966, a GHB-like agent, also blocked the FG7142-induced increase in PFC DA turnover and attenuated the FG7142-associated cognitive deficits in the rat. Propranolol was ineffective at blocking FG7142-associated cognitive impairment in rats. These studies support our previous conclusion that excessive PFC DA turnover leads to impaired PFC-dependent cognition. Support for these studies came from NIH grants #MH14092, #AG0636 and from NSF #GER9253954 (BLM).

69.7

MESOLIMBIC DOPAMINERGIC NEURONS AND AFFECTIVE PERCEPTION : SPECIFICITY OF AMYGDALA- NUCLEUS ACCUMBENS INTERACTIONS. A. Louilot* and C. Besson, CNRS UMR 5541 - Université de Bordeaux II - 146 rue Léo Saignat - 33076 Bordeaux Cedex - France.

We demonstrated previously that the responses of mesolimbic dopaminergic (DAergic) neurons to an affective stimulus are regionalized and lateralized in the nucleus accumbens (ACC). Furthermore, we showed that DA responses in the left *core* are directly dependent on the basolateral nucleus of the amygdala (BLA) if the stimulus is attractive and that other structures interact with the BLA if the stimulus presents an aversive value. In the present study, we investigated the involvement of the BLA-ACC interactions on the DA responses at the level of the left *shell*.

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

The following results were obtained : during the 2nd presentation of the CS an increase of about 50±20% was observed in the control group+PBS whereas DA signal reached about 120±20% above the baseline in the experimental group+PBS; in the control group+TTX a maximal increase in DA release of about 160±20% was observed whereas in the experimental group+TTX the olfactory stimulus induced an increase of about 80±35% above the basal level. The present study strongly suggests that BLA is only involved in the perception of attractive stimulus in the *shell* part of ACC and that BLA interacts in this context with other forebrain structures.

69.8

MICROINJECTION OF THE D1 ANTAGONIST SCH23390 INTO THE LEFT OR RIGHT MEDIAL PREFRONTAL CORTEX DIFFERENTIALLY ALTERS THE BEHAVIORAL AND NEUROCHEMICAL RESPONSES TO FOOTSHOCK STRESS. D.M. Nielsen*, R.W. Keller, Jr. and S.D. Glick, J.N. Carlson, Dept. of Pharmacology and Neuroscience, Albany Medical College, Albany, NY 12208

Interactions between lateralized cortical, limbic, and striatal dopamine (DA) systems have been related to the response to stressors. During the stress response asymmetrical changes take place in these DA systems. The behavioral response to stressors may be modulated by lateralized DA systems in the medial prefrontal cortex (mPFC). One manifestation of endogenous lateralization of DA systems is rotation (or circling) behavior in normal unlesioned rats. In the present studies mPFC DA lateralization was augmented pharmacologically in male Long Evens rats of differing rotational biases. Animals were then placed in a shuttlebox and exposed to a footshock stressor. Escape behavior and resultant neurochemical responses were assessed. Intracerebral microinjections of the selective D1 antagonist SCH23390 into the left mPFC increased shuttlebox footshock escape latency, while right injections had no significant effect. Neurochemical differences were influenced by side of injection as well as by turning preference. DA, serotonin (5HT) and their metabolites were measured in the mPFC, nucleus accumbens (NAS) and striatum of both the left and right sides. Left drug injections had greater effects than right injections on DA and 5HT neurochemistry in the mPFC and striatum. Thus injections of the D1 antagonist SCH23390 into the left, but not right, mPFC altered both the behavioral and neurochemical responses to footshock stress.

(Supported by MH45539)

69.9

EFFECTS OF D-AMPHETAMINE INJECTED INTO THE NUCLEUS ACCUMBENS OR VENTRAL PALLIDUM ON LOCOMOTION AND RESPONDING FOR A CONDITIONED REWARD. P.J. Fletcher*[1], M.S. Sabijan and N.J. De Sousa. Clarke Institute of Psychiatry[1], Toronto, M5T 1R8 & Dept. of Psychology and Psychiatry[1], University of Toronto, Toronto, Canada M5S 1A1.

Both the nucleus accumbens (NAcc) and ventral pallidum (VP) receive dopamine (DA) projections from the mesencephalon. Although DA inputs to the NAcc are implicated in both locomotion and reward processes, very little is known of the behavioural significance of DA in the VP. Therefore, these studies examined the effects of microinjections of d-amphetamine (AMPH; 0,1,3 and 10 μg) into the NAcc or VP on locomotor activity and responding for a conditioned reward (CR). Male rats were prepared with cannulae aimed at either the NAcc or VP. In Experiment 1, AMPH injected into the NAcc dose dependently (1,3 and 10 μg) increased locomotor activity measured as photobeam interruptions. Intra-VP microinjections of AMPH also increased activity (10 μg). In Experiment 2, food deprived rats were first trained to associate a light/tone stimulus (subsequently the CR) with food delivery. In the test phase, they were allowed access to a lever delivering the CR, and an inactive (NCR) lever. Responding on the CR lever was greater than responding on the NCR lever indicating that the light/tone stimulus functioned as a CR. Responding for the CR was selectively and dose-dependently potentiated by NAcc AMPH (3 & 10 μg). AMPH at 10 μg also enhanced CR responding when injected into the VP. However, the magnitude of this responding appeared to be lower than responding elicited from NAcc injections of AMPH. The results suggest that release of endogenous DA in the VP enhances locomotion and responding for a CR. However, these effects appear to be less intense than those resulting from DA release in the NAcc. (Funded by NSERC).

69.10

A HISTOLOGICAL METHOD FOR VERIFYING THE SOURCE OF ANALYTES RECOVERED IN MICRODIALYSIS STUDIES. R.L. Meisel*, R.K. Rowe and J.G. Kohlert. Department of Psychological Sciences, Purdue University, West Lafayette, IN 47907.

Verification of probe placements in microdialysis studies typically rely on determination of the location of the probe in Nissl stained brain sections. Because the goal of microdialysis studies is to sample from a defined functional neurochemical system and because the distribution of terminal fields from such systems often defy conventional cytoarchitecture, we have begun developing a method to identify the cellular origins of the terminal field from which microdialysis samples are recovered. For our studies, we have been interested in the modulation of nucleus accumbens dopamine. Following a dialysis experiment, we flush the probes with fluorogold and then process the brains histochemically for tyrosine hydroxylase. Quantitative cell counts of midbrain neurons containing both fluorogold and tyrosine hydroxylase indicated that about 92% of the dopaminergic projection to the nucleus accumbens arose from the A10 ventral tegmental region. There were no quantitative differences between rostral and caudal accumbens probe placements, nor was the midbrain labeling of rostral bed nucleus of the stria terminal placements distinguishable from the accumbens placements. The number of neurons labeled was positively related to the volume of the injection site, and basal dopamine levels were also positively correlated with the number of fluorogold-labeled cells. These results validate assumptions that dopamine recovered from the nucleus accumbens in microdialysis studies is primarily of A10 origin, and that basal dopamine levels reflect the size of the terminal field from which samples are recovered.

Supported by NSF grant IBN-9412543 (RLM).

69.11

VAGINO-CERVICAL STIMULATION DURING FEMALE SEXUAL BEHAVIOR INCREASES DOPAMINE RELEASE IN THE NUCLEUS ACCUMBENS. J.G. Kohlert*, R.K. Rowe and R.L. Meisel. Dept. of Psychological Sciences, Purdue Univ., West Lafayette, IN 47907.

In vivo recovery of extracellular dopamine concentrations in the nucleus accumbens of female hamsters and rats have shown increases during mating. We tested the possibility that vagino-cervical stimulation is involved in the increase in accumbens dopamine during copulation in female Syrian hamsters. Using microdialysis we sampled accumbens dopamine during mating in females with vaginal masks which prevented penile insertion and in females receiving intromissions. Dopamine concentrations significantly increased during the first 30 min in copulation tests for the hormonally-primed females without vaginal masks, while no increases were seen in hormonally-primed females with vaginal masks or in oil-treated females not engaging in sexual activity. However, the total time spent in lordosis was not different between the two groups of hormonally-primed females. These results indicate that vagino-cervical stimulation is necessary to induce increases in extracellular dopamine in female hamsters during copulation and that neither the mere presence of the male nor the simulation received during mounting of the male is sufficient to produce these changes.

Supported by NSF grant IBN-9412543 (RLM).

69.12

ENDOGENOUS GLUTAMATE IN SUBSTANTIA NIGRA MEDIATES THE STRESS-INDUCED INCREASE IN STRIATAL EXTRACELLULAR DOPAMINE. S. L. Castro* and M. J. Zigmond. Department of Neuroscience and Psychiatry, Univ. of Pittsburgh, Pittsburgh, PA 15260.

We have shown that antagonists of excitatory amino acids (EAAs) infused into neostriatum attenuate the stress-induced increase in striatal DA synthesis, suggesting a role of endogenous glutamate in this process (Castro et al., 1996). However, we have failed to obtain any direct evidence for a role for endogenous EAAs acting within the striatum in regulating DA *release* (Keefe et al., 1992, 1993). EAA-containing terminals from cortex project to the cell bodies, as well as terminals, of the nigrostriatal DA neurons, and thus, may influence striatal DA release indirectly via these cell bodies. Several recent studies have suggested that such an interaction is possible, but that endogenous EAAs in the substantia nigra (SN) or ventral tegmental area do not alter basal DA release from striatum or nucleus accumbens. In this study we examined the influence of EAAs in SN on striatal DA release during stress. Microdialysis probes were directed to both the SN (1-mm probe; AP -5.2, ML -5.5, DV -9.6 angled 25o in the mediolateral plane) and the neostriatum (4-mm probe; AP +0.5, ML -2.5, DV -7.0) of adult male rats. Rats receiving vehicle infusion into SN showed a 46 ± 9% (n=3) increase in extracellular DA levels when exposed to 30 min of intermittent footshock. Intranigral infusion of the NMDA receptor antagonist APV (0.1 mM) into SN via reverse microdialysis, which did not significantly alter basal extracellular DA levels in ipsilateral neostriatum, significantly attenuated the footshock-induced increase (13 ± 4% above baseline, n=5). These data suggest that the stress-induced release of striatal DA may be may be mediated by an action of glutamate in SN. (Supported in part by USPHS grants MH29670 and MH45156.)

69.13

MECHANISMS UNDERLYING THE EFFECTS OF DOPAMINE LOSS IN THE MEDIAL PREFRONTAL CORTEX ON THE ACTIVITY OF MESOLIMBIC DOPAMINE NEURONS D. King*, D.G. Harden, A.A. Grace, M.J. Zigmond & J.M. Finlay. Depts of Neuroscience & Psychiatry, Univ. of Pittsburgh, Pittsburgh, PA 15260.

We previously reported that dopamine (DA) loss in the medial prefrontal cortex (mPFC) potentiated the stress-induced increase in DA efflux in the nucleus accumbens (NAS) shell but not the NAS core. To determine whether this effect results from lesion-induced changes in the electrophysiological activity of DA neurons, we used extracellular recordings to examine the activity of DA neurons in the ventral tegmental area of control and lesioned rats (n=10/group). Depletion of DA in the mPFC decreased the firing rate (-24%) and the incidence of burst-firing (-23%) of these neurons [control rate = 4.7±0.2 Hz and bursts = 39±3%; n=113-148/group). Because the magnitude of evoked responses of DA cells is dependent upon basal activity, we propose that this decrease would result in larger stress-evoked responses. We also examined whether the differential effects of lesions on DA efflux in the NAS shell and core are paralleled by differences in the severity of DA loss between the mPFC subareas that preferentially innervate the NAS shell (ventral mPFC) and the core (dorsal mPFC). The distribution of DA terminals remaining in the ventral and dorsal mPFC after lesions was determined by examining tissue DA content and tyrosine hydroxylase immunoreactivity in control and lesioned rats (n=15/group). The absolute amount of DA remaining in the mPFC after lesions was greater in the ventral than in the dorsal area (0.04±0.00 vs 0.02±0.00 ng/mg tissue), although the percent loss was similar in the two areas (-74% vs -68%). In addition, more tyrosine hydroxylase immunoreactivity remained in the ventral mPFC. These data rule out the possibility that the selective effect of lesions on DA efflux in the NAS shell is due to greater DA loss in the ventral mPFC. Rather, we support the view that the selective effect of DA depletion in the mPFC on DA efflux in the NAS shell is due to the selective responsiveness of DA terminals in the ventral mPFC to stress [*Supported by NARSAD, USPHS grants MH45156 & MH10773*].

69.14

INTRA-ACCUMBENS INFUSION OF PREFERENTIAL D_3 RECEPTOR AGONISTS REDUCE SPONTANEOUS AND DOPAMINE INDUCED-LOCOMOTION IN THE RAT A. Ouagazzal* and I. Creese. Center for Molecular & Behavioral Neuroscience, Rutgers University, Newark, NJ 07102.

The objective of the present study was to test the hypothesis that D_3 dopamine (DA) receptors located within the nucleus accumbens exert an inhibitory influence on psychomotor functions. We first examined the effects of intra-accumbens infusion of the D_3-preferring agonists, PD 128,907 and 7-OH-DPAT, on spontaneous locomotion. For comparison we also studied the preferential DA autoreceptor agonist, B-HT 920. Bilateral intra-accumbens infusion of PD 128,907 (1.5 and 3 μg/0.5 μl), induced a dose-dependent hypolocomotion, whereas its enantiomer, PD 128, 908 was inactive. Local infusion of 7-OH-DPAT and B-HT 920 also decreased spontaneous locmotion. In addition, both drugs induced yawning with the B-HT 920 producing the greatest effect. In a second experiment, the ability of these agonists to reduce the hyperactivity induced by bilateral intra-accumbens infusion of DA (10 μg/0.5 μl) was studied. Prior infusion of PD, 128,907 or 7-OH-DPAT (3 μg) into the nucleus accumbens markedly reduced the locomotor hyperactivity induced by DA. However, local infusion of B-HT 920 (3 μg) failed to antagonize the locomotor effects of DA. These findings support the hypothesis that D_3 receptors exert an inhibitory influence on psychomotor functions and further suggest that these D_3 receptors may be located postsynaptically in the nucleus accumbens. Our data also indicate the involvement of dopamine autoreceptors, likely D_2 receptors, within the nucleus accumbens in the modulation of the yawning behavior in the rat.

Supported by NIMH.

69.15

THE INVOLVEMENT OF NUCLEUS ACCUMBENS DOPAMINE IN LEVER PRESSING: A BEHAVIORAL AND NEUROCHEMICAL STUDY OF CORE AND SHELL REGIONS. J.D. Sokolowski* and J.D. Salamone, Dept. of Psychology, University of Connecticut, Storrs, CT, 06269-1020.

A series of three experiments was undertaken to investigate the involvement of nucleus accumbens core and shell dopamine (DA) in lever pressing tasks. In the first experiment, rats were trained to lever press on a fixed-ratio 5 (FR5) operant schedule with lab chow concurrently available in the chamber. In the second experiment, rats were trained to lever press on the FR5 alone. In both experiments, rats were initially trained and then were injected with the neurotoxic agent 6-hydroxydopamine (6-OHDA) in either the nucleus accumbens core or shell. Injections of 6-OHDA into the core significantly decreased lever pressing and increased chow consumption in the first experiment. If lever pressing tests were conducted with no lab chow concurrently available (experiment 2), core injections of 6-OHDA produced a modest but significant decrease in lever pressing. Injections of 6-OHDA into the medial/dorsal region of shell produced much smaller behavioral effects, with only a slight reduction in lever pressing in both experiments. Although both core and shell injections produced similar DA depletions in the target region (about 90%), core injections produced larger depletions of total accumbens DA. In experiment 3, microdialysis probes were implanted in either the core or the shell following training on the FR5 schedule. There were increases in extracellular DA in both the core and shell during the lever pressing session, with a tendency towards a higher percentage increase in the shell. Thus, although DA release is increased in core and shell during lever pressing, the results of the DA depletion experiments indicate that rats with accumbens DA depletions in either core or shell remain directed towards the acquisition and consumption of food.

(Research supported by a grant from NSF)

DRUGS OF ABUSE: NICOTINE

70.1

NICOTINE SELF-ADMINISTRATION IN RATS HAVING UNLIMITED ACCESS TO THE DRUG. J.D. Valentine*, J.S. Hokanson, S.G. Matta, and B.M. Sharp, Minneapolis Medical Research Foundation and Departments of Medicine, Hennepin County Medical Center and University of Minnesota, Minneapolis, MN 55404.

Identifying the neural substrates involved in chronic drug use will require the increased use of models in which animals choose to engage in patterns of drug-taking behavior similar to those of humans. We are currently using such a model to study the substrates affected by chronic nicotine self-administration. In this model, jugular-canulated rats living in operant chambers are allowed 24-hour access to 2 levers; presses of one lever are programmed to deliver 0.03 mg/kg nicotine base in 50 µl saline over 1.2 sec, with each injection being followed by a 1-min timeout; presses of the other lever have no programmed consequences. Initially, under these conditions, 24-hour self-administration rates can be quite erratic, but stablize after approximately 1-3 weeks. Importantly, the vast majority of injections are self-administered during the active portion of the light/dark cycle. Changing the schedule of reinforcement from a FR1-->FR3-->FR5 leads to systematic increases in the number of presses on the active lever, without affecting on the number of presses on the inactive lever or the total number of injections administered per 24-hour session. Moreover, discontinuing the nicotine injections causes pressing of the active lever to extinguish. Thus, rats allowed unlimited access to nicotine self-administer the drug in a pattern comparable to humans, and the behavior is maintained by the reinforcing properties of the drug. We are currently using this model to investigate various types of neuronal plasticity that might underlie the chronic self-administration of nicotine. (Supported by DA03977 and T32-DA07234)

70.2

SELF-ADMINISTERED NICOTINE MAY PRODUCE DIFFERENT EFFECTS THAN YOKED NICOTINE. E. C. Donny, A. R. Caggiula,* C. Rose, S. Knopf, C. G. McAllister, A. F. Sved, K. A. Perkins, *University of Pittsburgh, Pittsburgh, PA. 15260*

Injected nicotine results in activation of the hypothalamic-pituitary-adrenocortical (HPA) axis and sympathetic nervous system (SNS) and suppression of immune function. These findings form the basis of several hypotheses regarding the effects of nicotine on human smokers. However humans self-administer nicotine and other evidence indicates that self-administered drug produces different effects than experimenter-administered drug. This led us to ask whether the effects of experimenter-administered nicotine also result when rats self-administer the drug. Rats were trained to lever press for food and were implanted with IV catheters. One group self-administered nicotine while the other two groups received yoked infusions of either nicotine or saline. On the first day, self-administered and yoked nicotine significantly elevated plasma corticosterone (CORT) levels above a preinfusion baseline within 15 min after the first infusion (0.03 mg/kg) and showed partial tolerance by the end of the 1 hr session. In contrast yoked, but not self-administered nicotine elevated plasma epinephrine at 15 min and both epinephrine and norepinephrine at 1 hr. This experiment points to the importance of using self-administration as a model which more closely approximates human nicotine use. Supported by NIDA grants DA-07546 and DA-08505.

70.3

INTRAVENOUS NICOTINE SELF-ADMINISTRATION IN LABORATORY ANIMALS: CHASING THE ENIGMA. M.A. Bozarth* & C.M. Pudiak, *Addiction Research Unit, Department of Psychology, University at Buffalo, Buffalo, NY 14260.*

Two standard intravenous self-administration procedures effective in demonstrating cocaine and heroin reinforcement were used to assess the reinforcing effect of nicotine -- direct acquisition and cross-substitution procedures. The first experiment examined the ability of nicotine to **establish** self-administration behavior. Experimentally naive rats were tested using 10-hr sessions for a total of 20 days. Manipulations which enhance responding were not used, e.g., lever training, food deprivation. The second experiment examined the ability of nicotine to **maintain** responding in animals previously trained to intravenously self-administer cocaine (1 mg/kg/inf). Various nicotine doses were substituted for the reinforcing cocaine injections during 3-hr self-administration tests; each nicotine dose was tested for 5 consecutive days. Both studies used nicotine bitartrate (10, 30, or 100 µg/kg/inf; dose expressed as free-base weight; solution pH adjusted to 7 ± 0.2) infused in a 0.25 ml/500 g volume over 30 sec/500 g.

In the direct acquisition study, several rats showed periodic response levels suggesting nicotine self-administration during some days of testing. However, nicotine intake was erratic both across days and within sessions even for these subjects. When tested for cocaine self-administration after completion of the nicotine tests, all subjects showed reliable cocaine self-administration. In the cross-substitution study, response rates for 10 and 30 µg/kg/inf nicotine unit-doses exceeded saline response rates. However, both nicotine doses produced nearly identical intake patterns decreasing across the 5-day tests. The 100 µg/kg/inf nicotine dose failed to maintain responding above saline levels.

These data suggest that nicotine does not serve as an effective reinforcer when tested using standard procedures that readily demonstrate intravenous cocaine and heroin reinforcement. Unlike the self-administration of highly addictive drugs, nicotine self-administration appears enigmatic, requiring special testing conditions. This finding is consistent with other tests suggesting the reinforcing efficacy of nicotine is very low.

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70.4

NICOTINE ABSTINENCE PRECIPITATED BY CENTRAL BUT NOT PERIPHERAL HEXAMETHONIUM. D.H. Malin*, C.K. Schopen, J.W. Kirk, E.E. Sailer, B.A. Lawless, T.P. Upchurch, M. Shenoi, N. Rajan & J.R. Lake. Univ. of Houston-Clear Lake, Houston, TX 77058.

Nicotine dependence has been induced in the rat by 7 days s.c. infusion of 9 mg/kg/day nicotine tartrate via Alzet osmotic minipump. Abstinence syndrome has been precipitated by pump removal or by s.c. injection of the noncompetitive nicotinic antagonist mecamylamine, which freely crosses the blood-brain barrier (BBB). In contrast, the noncompetitive nicotinic antagonist hexamethonium crosses the BBB very poorly. Groups of 7 nicotine dependent rats were injected s.c. with 0.5 or 1 mg/kg hexamethonium dichloride (HEX) or saline alone and observed for 20 min. Few abstinence signs were observed in any group; one-way ANOVA revealed no significant drug effect. In a 2nd experiment, 18 rats were cannulated in the 3rd ventricle and rendered nicotine dependent. I.C.V. injection of 18 ng HEX precipitated an average of 42.8 signs in 20 min, 12 ng HEX precipitated 32.3 signs, and saline alone precipitated 9.2 signs. Both dose groups differed significantly from the saline group and there was a significant linear trend of signs as a function of dose. The higher dose had no significant effect in nondependent rats. It is concluded that HEX is at least 22 million times as potent by the central route, and there is a major CNS component in nicotine dependence. (NIDA DA08260)

70.5

NICOTINE ALTERS MET-ENKEPHALIN CONTENT AND PREPROENKEPHALIN mRNA IN THE STRIATUM OF MICE. M. Hadjiconstantinou*, R. Isola, T.A. Wemlinger, G.A. Tejwani and N.H. Neff. Departments of Psychiatry and Pharmacology, The Ohio State University College of Medicine, Columbus, Ohio, 43210.

In mice, acute administration of nicotine increased the content of met-enkephalin (MET-ENK) in the accumbens, dorsal striatum and hypothalamus, but not in the hippocampus, frontal cortex and olfactory tubercle. The increase of the opioid in the striatum was prolonged and was accompanied by a rise of the mRNA for its precursor peptide preproenkephalin (PPE). Discontinuation of nicotine after chronic administration caused a withdrawal syndrome, that lasted longer than 72 hr. Early during the withdrawal, the content of MET-ENK in the accumbens and dorsal striatum fell, and remained low for more than 48 hr. In contrast, the abundance of PPE mRNA increased by 12 hr into withdrawal, and remained elevated for longer than 96 hr. Our results indicate that endogenous opioids might play a role in nicotine dependance/withdrawal.

70.6

EFFECT OF CHRONIC NICOTINE (NIC) ON RESPONSIVENESS OF TERMINAL REGIONS OF DOPAMINERGIC PATHWAYS TO A LOCAL NIC CHALLANGE. D.L. Marshall*, P.H. Redfern and [1]S. Wonnacott. Sch. Pharmacy & Pharmacol. and [1]Sch. Biol. & Biochem., Univ. Bath, Bath BA2 7AY, U.K.

Given that nerve terminal nAChRs are thought to play an important role in the central actions of NIC (McGehee *et al.*, 1995), we have compared the effect of two chronic NIC regimes on the responsiveness of nAChRs in the terminal regions of the 3 ascending dopaminergic pathways to a local application of NIC using *in vivo* microdialysis. Male Sprague-Dawley rats (250-350g) received NIC (0.4mg/kg/day s.c.) either by daily injection or osmotic pump for 7 days, after which dialysis probes were implanted into the striatum, accumbens or cortex. The following day the effect of a local application of NIC on dopamine (DA) levels was determined as previously described (Marshall *et al*, 1996). Differences between chronic NIC and saline control were compared using two way ANOVA for repeated measures.

	Saline Inj	Nicotine Inj	Saline Pump	Nicotine Pump
Striatum	372±122%	1370±317%*	397±173%	264±73%
Accumbens	368±128%	663±199%	349±137%	351±151%
Cortex	173±32%	283±102%	211±47%	199±72%

Data shown are peak DA levels (expressed as % of basal, mean ± s.e.m.) after local nicotine challange, n=3-8. *=$p<0.05$.

These data suggests that intermittent exposure to peak NIC levels sensitises nAChRs in the striatum whereas steady state, trough levels, produced by chronic infusion, do not.

McGehee, D.S., *et al.* (1995). *Science* 269: 1692-1696.
Marshall, D.L., *et al.*(1996) *Br. J. Pharmacol.* Proceedings Suppl. (in press).

70.7

LOCALLY APPLIED NICOTINE ENHANCES THE CLEARANCE OF EXOGENOUSLY ADMINISTERED DOPAMINE IN THE RAT NUCLEUS ACCUMBENS. N.A. Kisro* and C. Ksir. Department of Psychology and Neuroscience Program, University of Wyoming, Laramie, WY 82071.

Many researchers believe that the mesolimbic system is one of the common biological pathways through which addictive drugs, including nicotine, exert their actions. Currently there is debate as to where in the mesolimbic system nicotine is exerting its actions: at the cell bodies located in the ventral tegmental area (VTA) or at the cell terminals located in the nucleus accumbens (NAc).

In vivo voltammetry was used to estimate changes in the clearance of exogenously applied dopamine after acute, local application of nicotine (0.003µM, 0.01µM nicotine, or 0.03µM nicotine) into the NAc of rats. The peak dopamine concentration was significantly lower after 0.03µM nicotine application when compared to those in the saline group, implying an increase in the reuptake of dopamine. This nicotine effect was partially blocked when mecamylamine (1 mg/kg) was administered systemically 20 minutes prior to nicotine application. In addition, peak dopamine concentrations were significantly higher after 0.10µM cocaine application. These results suggest that nicotine acts at dopamine terminals, perhaps causing an increase in the release of dopamine into the synapse. This could then lead to a facilitation of the dopamine reuptake process.

70.8

CHANGES IN EXTRACELLULAR DOPAMINE IN THE NUCLEUS ACCUMBENS AND IN THE PREFRONTAL CORTEX OF NICOTINE DEPENDENT RATS ARE DISSOCIATED FROM PHYSICAL ABSTINENCE SYNDROME. E. Carboni*, C. Giua, and G. Di Chiara.
Dept. of Toxicology, Univ. of Cagliari, Viale Diaz 182, 09126 Cagliari, Italy

The nicotinic antagonist mecamylamine and the opiod antagonist naloxone are able to precipitate a physical abstinence syndrome in rats cronically exposed to nicotine. The aim of the present study was to investigate the realationship between the physiscal abstinence syndrome and changes of dopamine release in the n. accumbens and in the medial prefrontal cortex induced by mecamylamine and naloxone, in rats exposed chronically to nicotine delivered by osmotic minipumps. Male Sprague Dawley rats (260 g) were implanted with Alzet osmotic minipumps that deliver 9 mg/Kg/day of nicotine tartrate. Nine days thereafter, rats were implanted with a dialysis probe in the nucleus accumbens or in the medial prefrontal cortex. 24 hour later dopamine was measured in the perfusate. Steady state dopamine output from the nucleus accumbens was increased by 44 % in rats implanted with nicotine minipumps. Mecamylamine at the dose of 1 mg/Kg s.c. induced a physical abstinence syndrome and decreased by about 40 % dopamine release in rats implanted with nicotine minipumps but was ineffective in controls. Mecamylamine (1 mg/Kg s.c.) increased dopamine output in the medial prefrontal cortex by about 40 % in nicotine dependent but not in control rats. Naloxone (2 mg/Kg), failed to modify extracellular dopamine the n. accumbens and in the prefrontal cortex of control and of nicotine implanted rats. Blockade of nicotine receptors by mecamylamine causes a modification of dopamine output in the n. accumbens and in the prefrontal cortex that is associated with the abstinence syndrome. Naloxone, although able to precipitate the abstinence syndrome in nicotine dependent rats, did not modify dopamine output in both the accumbens and the prefrontal cortex, thus suggesting that the changes in dopamine output in the mesolimbic system of nicotine dependent rats are dissociated from the physical signs of abstinence.

70.9

STIMULATION OF ENERGY METABOLISM AND DOPAMINE RELEASE IN THE ACCUMBAL SHELL BY NICOTINE. F.E. Pontieri*, G. Tanda#, E. Carmenini, F. Orzi^ and G. Di Chiara#. Dept. Neuroscience, University 'La Sapienza', Rome; #Dept. Toxicology, University of Cagliari; ^INM 'Neuromed', Pozzilli (IS), Italy.

Intravenous administration of psychostimulants and opiates, at dosages corresponding to those that sustain self-administration, preferentially or selectively increases extracellular dopamine and energy metabolism in the shell of the rat nucleus accumbens. Then, enhanced energy metabolism and increased dopamine transmission in the shell represent distinctive neurobiological markers of the addictive potentials of drugs, independently from their specific mechanisms of action. This study was designed to investigate whether nicotine produces in the shell neurochemical effects which resemble those of typically addictive substances. Local rates of glucose utilization and extracellular dopamine were therefore measured in the shell and core of the nucleus accumbens following intravenous administration of nicotine (0.025-0.05 mg/kg, free base) in the rat, using the 2-deoxyglucose method and brain microdialysis, respectively. Nicotine produced dose-dependent increases of both glucose metabolism and dopamine output in the shell, without affecting values in the accumbal core. These results demonstrate that acute intravenous administration of nicotine in the rat selectively modifies dopamine transmission and functional activity in the accumbal shell. They, then, provide functional and neurochemical evidence for the existence of specific neurobiological commonalities between nicotine and typically addictive drugs.

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70.10

NIMODIPINE BLOCKS NICOTINE-INDUCED LOCOMOTION BUT NOT LOCOMOTION INDUCED BY GBR-12909 C. Hart*, N.A. Kisro, S.L. Robinson, and C. Ksir. Department of Psychology and Neuroscience Program, University of Wyoming, Laramie, WY 82071

Several studies report an attenuation of psychostimulant-induced locomotion in rats when calcium channel blockers are administered beforehand. Nicotine and GBR-12909 are both potent inducers of locomotor activity in rats. However, the effects of pretreatment with calcium channel blockers on nicotine- and GBR-12909 induced locomotion have not been assessed.

The present study examined the effects of nimodipine, a dihydropyridine L-type calcium channel antagonist, on nicotine- and GBR-12909-induced locomotor activity in drug-naive rats. Nicotine (.4 mg/kg IP) and GBR-12909 (10 mg/kg IP) both produced significant increases in locomotion following acute administration. However, when rats were given injections of nimodipine (10 mg/kg IP) one hour before test drugs, nicotine-induced locomotor activity was blocked. In contrast, pretreatment with nimodipine delayed the onset of, but did not block, GBR-12909-induced locomotor activity. These results suggest that nicotine and GBR-12909 induce increases in locomotor activity through different mechanisms: nicotine effects being calcium-dependent and GBR-12909 effects being calcium-independent.

70.11

EFFECTS OF LOW DOSE MECAMYLAMINE ON REGIONAL CEREBRAL GLUCOSE METABOLISM IN RATS RECEIVING CHRONICAL NICOTINE. M. Takagishi, A.S. Kimes* and E.D. London, Brain Imaging Section, Intramural Research Program, NIDA, P.O. Box 5180, Baltimore, MD 21224

As a therapeutic strategy for smoking cessation, Rose *et al.* (1992) have proposed concurrent administration of nicotine (Nic), delivered transdermally along with orally administered mecamylamine (Mec), a noncompetitive antagonist of nicotinic cholinergic receptors. In the rationale for this treatment, Nic would provide adequate occupancy of receptors to prevent a withdrawal syndrome and Mec would attenuate the reinforcing effects of rapid dosing with nicotine self-administered by smoking. In human studies, low doses of Mec reduce the consumption of cigarettes without producing self-reports of withdrawal in smokers (Tennant *et al.*, 1984, Rose *et al.*, 1994). Although Nic/Mec treatment seems to have utility in clinical smoking cessation programs, and a theoretical basis for therapeutic success has been proposed, no data on the interaction of low dose Mec with chronic Nic (cNic) in the brain are available. To elucidate this interaction *in vivo*, we assayed regional cerebral metabolic rates for glucose (rCMRglc), using the 2-deoxy-D-[1-^{14}C]glucose (2DG) technique in rats receiving cNic and given a challenge dose of Mec. Rats received an infusion of saline or 9 mg/kg Nic (as the base) per day in saline via osmotic pumps. After 7 days, rats were given Mec (0.3 mg/kg, s.c.) or saline 15 min before the 2DG injection. The dose of Mec was about one-third of what was used previously to precipitate Nic withdrawal (Malin *et al.*, 1994). In rats given cNic, rCMRglc was higher in some brain regions, including anteroventral and interanteromedial thalamic nuclei, and the interpeduncular nucleus, compared with control values. These 3 brain regions previously showed increased rCMRglc after acute Nic challenge (London *et al.*, 1988). Generally, Mec appeared to reverse rCMRglc increases in rats given cNic. The findings suggest that a low dose of Mec partially nullifies the effect of cNic on rCMRglc without producing a generalized withdrawal syndrome. These findings support the rationale of concurrent agonist-antagonist therapy in smoking cessation.

70.12

IBOGAINE BLOCKS THE DOPAMINE RESPONSE TO NICOTINE. *I.M. Maisonneuve*, G.L. Mann and S.D. Glick, Department of Pharmacology and Neuroscience, Albany Medical College, Albany, NY 12208.*

Ibogaine, an alkaloid found in Tabernanthe iboga, has been claimed to be effective in interrupting the nicotine dependence syndrome (Lotsof, 1991). Since there is increasing evidence that the rewarding effect of nicotine is mediated by the dopamine mesolimbic system, we investigated whether ibogaine could alter the acute dopamine response to two 5 minute i.v. infusions of nicotine (0.32 mg/kg/infusion), administered one hour apart. Experiments were conducted using *in vivo* microdialysis in awake and freely moving male Sprague-Dawley rats. Pretreatment with ibogaine (40 mg/kg, i.p.) 19 hours prior to the first nicotine infusion significantly attenuated the increase in extracellular dopamine levels induced by the nicotine infusions (treatment x time interaction, $F_{(3,36)}$= 5.39, p<0.004). Ibogaine also attenuated nicotine-induced increases in dopamine metabolites (DOPAC and HVA). These results suggest that ibogaine may decrease the rewarding effect of nicotine. Further studies are warranted to explore possible mechanisms of this ibogaine-nicotine interaction as well as to determine the effects of ibogaine after chronic administration of nicotine. (Supported by DA03817).

70.13

IBOGAINE AND KAPPA OPIOID AGONISTS HAVE A SELECTIVE INHIBITORY ACTION ON NICOTINIC RECEPTOR-MEDIATED CATECHOLAMINE RELEASE, A.S. Schneider*, S.J. Mah, P.E. Liauw, Y.M. Tang and J.E. Nagel. Dept. Pharmacology & Neuroscience, Albany Medical College, Albany, NY 12208

We have tested the effects of ibogaine on catecholamine release from a model neuronal system, cultured bovine chromaffin cells, in an effort to determine sites and mechanisms of ibogaine action. Various modes of stimulating catecholamine release were used including nicotinic ACh receptor activation, membrane depolarization with elevated K^+ (70 mM) and Na^+ channel activation with veratridine (100 μM). In addition, because ibogaine has been reported to interact with kappa opioid receptors, we tested whether kappa receptor ligands either mimic or inhibit ibogaine's effects on catecholamine release. Ibogaine (< 10 μM) and the kappa agonists, cyclazocine (30μM) and U50,488 (10μM), were found to selectively inhibit nicotinic receptor mediated catecholamine release. The ibogaine and cyclazocine inhibitory actions were not prevented by the opioid antagonists, norBNI or naltrexone. At higher doses (100 μM) ibogaine also inhibited veratridine- and K^+-evoked release. Ibogaine (100μM) effects were not rapidly reversible and persisted for at least 19 hours following ibogaine washout. The results would be consistent with an action of ibogaine and cyclazocine at a site within the nicotinic receptor cation channel and may be relevant to claims of ibogaine interruption of nicotine addiction. Supported in part by PHS grant 5R01DA06787, Subcontract A10165b.

70.14

EFFECTS OF SMOKING IN SCHIZOPHRENIC PATIENTS, MEASURED BY EVENT-RELATED POTENTIALS. E. Kodama, K. Morita*, I. Nakamura, S. Kinoshita, N. Kawamura, A. Miyahira and H. Maeda, Department of Psychiatry, Kurume University School of Medicine, 67 Asahi-Machi, Kurume -City, 830, Japan

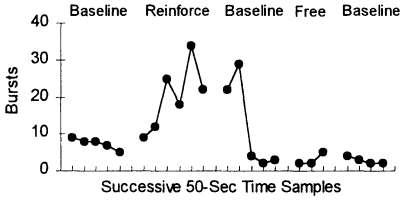
The present study examined the effects of acute nicotine administration following 18 hours of abstinence from cigarette smoking on event-related potentials (ERPs), using an auditory oddball paradigm in 11 male shizophrenic patients. Informed consents were obtained from all subjects at each test. The P300 component as reflected by cognition was analysed. The P300 peak amplitudes were negatively correlated with daily nicotine intake under daily-life condition (r=-0.65). The peak amplitude and the area and latency of the P300 were not changed significantly during withdrawal following smoking abstinence. Significant decreases in both the peak amplitude and the area were observed after the resumption of smoking. No decrease in the peak P300 amplitude caused by the resumption of smoking was found with nicotine gum pretreatment. No reliable correlations were observed between these P300 values (the peak amplitude, the area and the latency) and psychotic scores (BPRS scores) or dosages of neuroleptics (CPZ equivalent dosages). The present data suggest that information processing as reflected by the P300 may be inhibited by both the chronic effects of smoking and the acute effects of the resumption of smoking following abstinence. The mechanisms involved in CNS sensitivity to schizophrenic patients to smoking may be different from that of healthy subjects.

DRUGS OF ABUSE: OTHER I

71.1

ANANDAMIDE: IN VITRO REINFORCEMENT OF HIPPOCAMPAL BURSTING BY THE NATURAL LIGAND OF THE CANNABINOID RECEPTOR. L. Stein*, B.G. Xue and J.D. Belluzzi, Department of Pharmacology, College of Medicine, University of California, Irvine, CA 92717.

Involvement of cannabinoid receptors in reward and addiction is suggested by widespread human use of marihuana, although animal tests of cannabinoid reinforcement have produced mixed results. Cannabinoid receptors are found in high density in rat hippocampus and other brain areas. Using the hippocampal-slice preparation, we attempted to demonstrate in vitro reinforcement of CA1 bursting with local micropressure applications of the endogenous cannabinoid ligand, anandamide. Approximately 60% of the CA1 neurons tested showed progressive increases in burst activity after a series of brief, burst-contingent applications of anandamide at pipette concentrations of 20-40 µM (Figure). Identical "free" microinjections of anandamide administered independently of cellular activity did not increase (and usually suppressed) hippocampal bursting, thus ruling out drug-induced stimulation of CA1 firing. We conclude that anandamide, like dopamine and dynorphin A, may serve as a natural reinforcement transmitter. *(Supported by NIDA grant DA05107)*



71.2

CORTICAL NEURONS PRODUCE *SN-2* MONOARACHIDONOYLGLYCEROL, A PUTATIVE ENDOGENOUS CANNABINOID LIGAND. N. Stella* and D. Piomelli, The Neurosciences Institute, 10640 John J. Hopkins Drive, San Diego CA 92121.

Endogenous cannabinoids constitute a family of lipid molecules that specifically activate cannabinoid (CB) receptors. The first member of this family to be characterized was anandamide, an ethanolamine derivative of arachidonic acid. Recently, another derivative of arachidonic acid, *sn-2* monoarachidonoylglycerol (MAG) was shown to bind to and activate both CB1 and CB2 receptors with EC50s in the µM range.

We have addressed two questions relevant to the possible role of MAG as an endogenous cannabinoid. Is MAG produced by neurons? And if so, under what conditions? By using bidimensional thin layer chromatography and reversed-phase HPLC, we determined that rat cortical neurons in primary cultures contain MAG. We also found that the amount of MAG in these neurons is significantly enhanced by the Ca^{2+} ionophore ionomycin in a concentration-dependent manner (0.3 µM - 3 µM). This effect of ionomycin is completely prevented when extracellular Ca^{2+} was chelated with EGTA (1 mM). EGTA alone reduced the MAG levels by about 50% of basal. Depolarizing concentrations of KCl (40 mM) stimulates MAG formation. The effect of KCl is also dependent on extracellular Ca^{2+} and is inhibited by Cd^{2+} ions (30 µM), indicating the involvement of voltage-activated Ca^{2+} channels. In several cell types, formation of 2-monoacylglycerols results from activation of diacylglycerol (DAG) lipase. We found that both the KCl- and ionomycin-induced formation of MAG were inhibited by the specific DAG lipase blocker, RHC 80267 (100 µM). Our results indicate that MAG is present in cultured neurons, and that its levels are controlled by Ca^{2+} ions, probably acting through stimulation of DAG lipase activity. It remains to be determined whether mechanisms exist for the release and inactivation of MAG, as expected of an extracellular signaling molecule.
Supported by Neurosciences Research Foundation

71.3

FORMATION OF AN ENDOGENOUS CANNABINOID PRECURSOR IN CULTURED NEURONS AND ITS CONTROL BY CALCIUM AND cAMP D. Piomelli*, H. Cadas, S. Gaillet#, M. Beltramo, L. Venance◊, The Neurosciences Institute, 10640 John J. Hopkins Drive, San Diego, CA92121; #Université Montpellier II, France; ◊Collège de France, 75321 Paris, France.

Anandamide, an endogenous lipid that activates cannabinoid CB1 receptors, and N-palmitoylethanolamine (which activates a CB2-like receptor in mast cells) are produced in an activity-dependent manner by primary cultures of rat cortical neurons. Formation of these compounds may occur through cleavage of a phospholipid precursor, N-acyl phosphatidylethanolamine (NAPE), catalyzed by a calcium-activated phosphodiesterase activity. We report here that NAPE in cultured cortical neurons is synthesized via a calcium-dependent reaction that is stimulated by neuronal activity (membrane depolarization) and potentiated by cAMP-dependent protein phosphorylation. We also show that extracts of cortical neurons contain a calcium-dependent enzyme activity that produces NAPE, by catalysing the transfer of an acyl group from phosphatidylcholine to the amino group of phosphatidylethanolamine. We solubilized and characterized this N-acyl transferase (NAT) activity in a rat brain particulate fraction. We found that, in the presence of Ca^{2+}, brain NAT activity catalyzes the formation of NAPE (20:4), as well as NAPE (16:0), NAPE (18:0) and NAPE (18:1), from endogenous substrate. In the rat, NAT activity is most abundant in brain, testes and skeletal muscular tissues. Within the brain, NAT activity is greatest in thalamus and striatum, and lowest in hypothalamus and olfactory bulb.

Our results indicate that NAPE biosynthesis in neurons is controlled by Ca^{2+} and cAMP. Such regulatory effect may participate in maintaining a supply of cannabimimetic N-acylethanolamines during synaptic activity, and prime target neurons for release of these bioactive lipids. Our results also show that NAPE, particularly the anandamide precursor NAPE (20:4), is produced in discrete regions of the brain by a Ca^{2+}-dependent NAT activity.

Supported by Neurosciences Research Foundation

71.4

CLONING AND CHARACTERIZATION OF THE MOUSE CB1 CANNABINOID RECEPTOR GENE Kara Ditto, Qing Tao and Mary E. Abood* Dept of Pharmacology/ Toxicology, Medical College of Virginia, Virginia Commonwealth University, Richmond VA 23298.

The mechanisms underlying the development of tolerance to cannabinoids are not completely understood, but do not appear to result from pharmacokinetic changes; therefore biochemical and/or cellular changes are responsible for this adaptation. Studies in our laboratory have demonstrated reduced cannabinoid receptor (CB1) binding with a concomitant increase in receptor mRNA in cerebellar tissue isolated from mice chronically exposure to CP55,940. This suggests that tolerance to cannabinoids may be mediated, in part, by alteration of neuronal (CB1) cannabinoid receptor gene expression. In order to elucidate the role of transcriptional vs. post-transcriptional control during the development of tolerance, we have cloned the gene for the mouse CB1 receptor. The gene was isolated from a P1 genomic library. Sequence analysis of a 6 kb BamHI fragment of the mouse CB1 genomic clone indicates 95% nucleic acid identity between mouse and rat (100% amino acid identity) and 90% nucleic acid identity (97% amino acid identity) between mouse and human. The human and rat sequences diverge about 60 bp upstream of the translation initiation codon, suggesting the possibility of splice variants of the CB1 receptor as well as divergence of other regulatory sequences between these genes. Indeed, examination of the 5' untranslated sequence of the mouse CB1 genomic clone indicates a splice junction site approximately 60 bp upstream from the translation start site. In parallel studies, we are examining the effect of chronic exposure to Δ^9-THC on CB1 gene expression in mouse N18TG2 neuroblastoma cells. We demonstrate specific CB1 binding sites on these cells using [3H]SR141716A as a radioligand. Preliminary studies indicate the CB1 mRNA is altered in N18TG2 cells following 24 hours of Δ^9-THC treatment. These results indicate that the N18TG2 neuroblastoma cell line may represent a model system with which we can examine transcriptional and post-transcriptional regulation of CB1 receptor gene expression. DA-05274.

71.5

SUPPRESSIVE EFFECTS OF VARIOUS CANNABINOID AGONISTS ON INSTRUMENTAL LEVER PRESSING: A MICROANALYSIS OF THE BEHAVIORAL EFFECTS. J. Aberman*, D. Carriero, S-Y. Lin, A. Hill, A. Makriyannis, and J.D. Salamone. Depts. of Pharmaceutical Sciences and Psychology, U. of Connecticut, Storrs, CT 06269-1020.

In the present study, operant conditioning methods were used to characterize some of the motor effects of the cannabinoid agonists CP 55,940, AM 356 and delta 8 tetrahydrocannabinol (delta 8 THC). Behavioral output during lever pressing on a fixed ratio 5 schedule was recorded by a computerized system that measured response initiation time (time from offset of one response to onset of the next) and duration for each lever press. All three cannabinoid drugs substantially depressed lever pressing response rate. Cannabinoid-induced deficits in responding were largely due to substantial increases in the average response initiation time. Analysis of the distribution of response initiation times indicated that cannabinoid-treated rats made relatively few responses with fast initiation times (e.g. 0-125 msec). The cannabinoid drugs also increased the number of pauses in responding (i.e. response initiation times > 2.5 sec). In addition, there was an overall increase in average response duration among animals that received the higher doses of cannabinoids. The effects of cannabinoids on response initiation and duration were somewhat similar to those effects produced by other response-suppressing drugs, such as neuroleptics and cholinomimetics. The rank order of potency was CP 55,940 > > delta 8 THC $\geq$ AM 356, with CP 55,940 being approximately 50 times more potent than the other drugs. Animals were ataxic and cataleptic at the higher doses of cannabinoids that produced an almost complete cessation of responding. The relative potencies of these behavioral effects are consistent with the affinities of these drugs for cannabinoid receptors, which suggests that suppression of lever pressing can be used as a behavioral test for the assessment of in vivo cannabinoid activity.

71.6

CANNABINOID WITHDRAWAL AND CENTRAL AMYGDALA: A CORTICOTROPIN RELEASING FACTOR (CRF) IN VIVO MICRODIALYSIS AND A C-FOS IMMUNOHISTOCHEMISTRY STUDY IN RATS. F.Rodríguez de Fonseca*[1], R.A.Carrera[2], M. Navarro[1], M.Wilson[2],G.F.Koob[2] and F.Weiss[2]. [1] Dpt. Psicobiología, Facultad de Psicología, Universidad Complutense, 28223-Madrid (SPAIN). [2] Dpt. Neuropharmacology, The Scripps Research Institute, La Jolla, CA (USA).

Previous research has suggested a role for CRF in the anxiogenic response to cannabinoids. The present study was designed to evaluate the effects of acute, chronic and withdrawal from cannabinoid treatment on CRF release from central amygdala in rats, monitored by *in vivo* microdialysis. A parallel study was conducted to asses the anatomical distribution of the early gene *c-fos* protein product using an immunohistochemistry approach. Acute exposure to the highly potent cannabinoid HU-210 (100 μg/kg) resulted in both a reduction of extracellular CRF levels, starting 60 min. after the injection, and in a blockade of the 4-aminopyridine-induced release of CRF, when this K^+-channel blocker was added to the perfusion medium. Acute treatment with HU-210 resulted in a marked increase in c-fos expression in stress-related nuclei (including central amygdala, shell of nucleus accumbens, bed nucleus of the stria terminalis and paraventricular nucleus of the hypothalamus). Acute exposure to the novel cannabinoid receptor antagonist SR141716A (3mg/kg) did not alter CRF levels in naive animals but induced a sharp release of CRF from central amygdala in animals treated for 14 days with a daily dose of HU-210 (100 μ/kg). The time course of SR141716A-induced CRF release paralleled the onset of behavioral indices of cannabinoid withdrawal. C-fos expression was found to be intense in withdrawal group but not in control animals treated with SR141716A. The present results support the hypothesis of a central amygdala involvement in the neuropharmacological effects of cannabinoids.

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71.7

THE STIMULATORY EFFECTS OF Δ^9-TETRAHYDROCANNABINOL ON ADRENOCORTICOTROPIN HORMONE AND CORTICOSTERONE SECRETION DURING DIFFERENT STAGES OF THE ESTROUS CYCLE IN FEMALE RATS. R. Cadena, L.L. Murphy, and R.W. Steger*. Dept. of Physiology, SIU School of Medicine, Carbondale, IL 62901.

Our previous studies have shown that Δ^9-tetrahydrocannabinol (THC), the major psychoactive component of marijuana, stimulates the hypothalamic-pituitary-adrenal axis in ovariectomized rats. The purpose of this study was to investigate the effects of THC on adrenocorticotropin hormone (ACTH) and corticosterone (CORT) release in intact, cycling female rats. Adult female Sprague-Dawley rats were subjected to daily vaginal lavage in order to determine the stages of the estrous cycle. Rats were implanted with indwelling jugular cannulae for THC administration and blood sampling. The rats were administered either THC (0.5 or 1.0 mg/kg b.w.) or its vehicle and blood was withdrawn every 30 minutes for 2 hours with the first sample obtained immediately prior to THC or vehicle administration. Plasma was collected for ACTH and CORT determinations. Results showed that there were significant dose-related increases in ACTH and CORT from 0 to 60 minutes following THC administration when compared to basal hormone levels ($p<.05$). Even though basal levels of ACTH and CORT are elevated during estrus, it appears that the greatest percent increase in both ACTH and CORT following THC treatment occurs during proestrus. Therefore, THC stimulates both ACTH and CORT secretion in intact cycling rats. Furthermore, the finding that the greatest percent increase of ACTH and CORT occurred during proestrus suggests that the hormone milieu may affect the response of the hypothalamic-pituitary-adrenal axis to cannabinoids.

71.8

THE ROLE OF CANNABINOID RECEPTORS IN THE LUTEINIZING HORMONE (LH) AND ADRENOCORTICOTROPIN (ACTH) RESPONSE TO CANNABINOIDS. L.L.Murphy* and B.C. Statham, Dept. of Physiol., So. IL Univ. School of Med., Carbondale, IL 62901.

The hypothesis that cannabinoid receptors mediate THC-induced inhibition of LH release and stimulation of ACTH secretion was investigated by using selective cannabinoid receptor agonists (CP 55,940 and WIN 55,212) and an antagonist selective for the brain cannabinoid receptor (SR141716A). Adult rats were ovariectomized and 4 weeks later implanted with polyethylene cannulae inserted into the jugular vein for drug administration and blood withdrawal. CP 55,940 (CP; 0.01, 0.05 or 0.1 mg/kg b.w.; Pfizer, Inc.), WIN 55,212-2 (WIN; 0.05, 0.1, or 0.5 mg/kg; RBI), THC (0.5, or 1.0 mg/kg) or their respective vehicles were administered at time 0 and sequential blood samples were taken for 2 hr for plasma LH and/or ACTH determinations. Plasma LH levels were significantly suppressed by CP, WIN and THC ($p<0.05$); THC>CP>WIN with respect to magnitude and duration of suppression. ACTH levels were significantly stimulated from 20 to 100 min in a similar dose-related manner after either THC or WIN administration when compared to vehicles ($p<0.05$). SR141716A (SR; 0.5 mg/kg b.w., ip; Sanofi Recherche) pretreatment blocked the inhibitory effects of WIN and THC on LH release but did not appear to block cannabinoid-induced ACTH release. In fact, SR itself produced a significant increase in basal ACTH levels 30 to 70 min after its administration relative to vehicle control ($p<0.05$). Whereas the results with the receptor agonists suggest that cannabinoid receptors mediate THC-induced alterations in LH and ACTH, the results using SR suggest that cannabinoids affect LH and ACTH release via different cannabinoid receptor-mediated pathways.

71.9

EFFECTS OF DELTA-9-THC PERINATAL TREATMENT OF MOTHERS ON MORPHINE AND FOOD OPERANT REINFORCED BEHAVIORS IN THE ADULT OFFSPRING.

S.MARTIN, J.A.CRESPO, R.FERRADO, C.GARCIA-LECUMBERRI, L.GIL(#), J.A.RAMOS(#), J.J.FERNANDEZ-RUIZ(#), N.DIEZ, J.MANZANARES*(@) AND E.AMBROSIO. Dept. Psicobiología.UNED.28040 Madrid.(#)Dept.Bioquímica y Biología Molecular (Fac.Medicina) and(@)Dept.Farmacología(Fac.Farmacia).UCM.28040 Madrid.Spain

Perinatal exposure to cannabinoids has been shown to alter the development and differentiation of central nervous system(CNS) and produce CNS impairment in rodents and other species. Different neurotransmitter and neuropeptidergic systems have been reported to be affected by perinatal administration of cannabinoids, including the dopaminergic and opioid systems. These changes can elicit long term neuroendocrine alterations and modifications in different behavioral patterns. The purpose of these experiments was to study the effect of perinatal treatment of delta-9-tetrahydrocannabinol(THC) of the mothers on operant morphine self-administration and food reinforced behavior in both sexes of adult offspring. Male and female age-matched adult offspring born from mothers Wistar rats perinatally exposed to THC(5mg/Kg) or vehicle(VEH) from day 5 of gestation until day 24 after birth were tested on acquisition (6 days) of intravenous morphine self-administration or on food reinforced behavior under a Progressive Ratio schedule (3,5,7,9...775). Subjects were surgically prepared with an intravenous catheter in jugular vein and placed in operant chambers for morphine self-administration studies. Operant drug-reinforced behavior was examined in a 23h access paradigm in which each lever press resulted in 1 mg/Kg morphine injection with a 30s timeout period (FR1: TO 30"). After morphine self-administration studies, operant reinforced behavior was examined in the same chambers daily during 3hr sessions in a separate group. Offspring of THC treated mothers showed a statistically significant increase of acquisition of morphine-reinforced behavior compared to the offspring of vehicle treated mothers. This increase was significantly greater in female than in male on the last days of acquisition period. On the contrary, there was not statistically significant differences between both mother pretreatment(THC or VEH) and sex in the offspring. These results suggest that the offspring of mothers treated with THC could be more vulnerable to morphine reinforcing effects. Moreover, this THC-induced susceptibility to morphine reinforcing effects may be differentially affected by sex. Supported by DGICYT PB93-0290.

71.10

DEVELOPMENTAL PCP EXPOSURE PRODUCES DECREASED LOCOMOTOR RESPONSES TO PCP AND MK-801. F.M. Scalzo.* Dept of Peds., Univ. Arkansas for Med. Sci., Little Rock, AR 72202.

Previous studies from our laboratory have shown that subchronic exposure to phencyclidine (PCP) on postnatal days (PNDs) 24-37 (but not PNDs 4-17) results in long-lasting (20 days) alterations in the behavioral response to a pharmacological challenge with PCP or the NMDA antagonist, MK-801. The present studies were designed to further define the critical period for obtaining these effects. Rats were dosed with saline or 7.5 mg/kg PCP on PNDs 1-9, 11-19, 21-29 or 31-39. Ten and 20 days post-dosing, locomotor activity and ataxia were measured for one hr following a challenge with either saline, 2.5 or 5.0 mg/kg PCP, 0.1 mg/kg MK-801 or 1.0 mg/kg d-amphetamine. Activity was measured via an automated system and ataxia was measured by observation. Subchronic PCP treatment after PND 11 resulted in decreased locomotor responses to both PCP and MK-801 challenges. These decreases were observed in the absence significant increases in ataxia in most instances. The locomotor response to a saline or d-amphetamine challenge was not affected by pretreatment with PCP. These results suggest that subchronic (9 days) exposure to PCP results in long-lasting decreases in the locomotor response to both PCP and MK-801. The mechanism(s) mediating this change in responsiveness are unclear but might involve alterations in NMDA systems or PCP treatment-induced alterations in PCP metabolism. Supported by NIDA.

71.11

AN IN VITRO MODEL OF PHENCYCLIDINE NEUROTOXICITY. M. Phillips, S. Alagarsamy* T.C. Pappas and K.M. Johnson. Dept. of Pharmacology, University of Texas Medical Branch, Galveston, TX 77555-1031.

Phencyclidine (PCP) and other NMDA antagonists have neurotoxic effects after acute administration in rats. This toxicity is largely confined to the cortex and related limbic areas. It is blunted by D1, muscarinic and kainate antagonists as well as by pentobarbitol. To better understand this mechanism, we are attempting to model this in primary culture. Cortical cultures derived from rat fetuses (E16) were used for experiments on day 9-21 in vitro. Cultures were exposed to drugs in HEPES buffered saline lacking Mg^{2+} for 30 minutes followed by a 24h exposure to serum containing medium. Both the salt solution and the medium were assayed for lactate dehyrodenase (LDH) activity. The cultures were also visually examined using a LIVE/DEAD kit from Biomol. 100 μM DA produced a profound increase in LDH release and ethidium homodimer staining indicating massive cell death. 10 μM DA produced a smaller, but significant increase in LDH release. Addition of SOD/catalase slightly attenuated the death caused by 100 μM DA and almost completely prevented that caused by 10 μM DA. These data suggest that formation of $OH^{\cdot}$ and $O_2^{\cdot}$ may be responible for DA-induced damage. However, there may also be an NMDA receptor mediated component since MK-801 reduced cell death by ~50 %. Bicuculline (BIC) at 1mM produced substantial increases in LDH release, but 100 μM produced a marginal increase in cell death. Carbachol at 1 mM also produced a marginal increase in LDH release, but in combination with BIC, this effect was potentiated. Finally, the combination of DA (10 μM), carbachol and BIC (100 μM) produced an additive effect on LDH release and apparent cell death. These data suggest that this approach may be useful in understanding the mechanisms underlying PCP-induced neurotoxicity. Supported by DA 02073 and T32 DA 07287.

71.12

RISPERIDONE THERAPY IN PHENCYCLIDINE (PCP) INTOXICATION. A. James Giannini*, Gale L. Colapietro, Deirdre K. Cook. Ohio State University, Boardman, Ohio 44512.

PCP is a drug of abuse with agonist activity at both DA-2 (dopaminergic) and 5-HT-2 (serotonergic) activity. Heretofore, haloperidol, a mixed dopamine antagonist with predominant DA-2 activity, has been the standard treatment for PCP psychosis.

Risperidone is a novel neuroleptic which has both 5-HT-2 and DA-2 antagonist activity. Its efficacy was compared against that of haloperidol in sixteen consecutive male patients presenting with PCP psychosis. Eight patients were given haloperidol 5 mg. at three 30-minute intervals and the other 8, 3 doses of risperidone 3 mg. at similar intervals. Responses were measured using the BPRS by two blinded raters.

Results indicated that haloperidol was superior to risperidone at 30- ($p < .02$), 60-minute ($p < .02$) intervals. There was no difference at the 90 minute interval. Afterwards, risperidone gave superior responses at 120- ($p < .02$), 150- ($p < .01$) and 180-minutes ($p < .01$). It is hypothesized that haloperidol's initial superior response was due to increased rate of absorption, whereas the increased efficacy of risperidone was due to activity at both 5-HT-2 and DA-2 receptors.

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71.13

STRUCTURE-ACTIVITY AND STRUCTURE-LIPOPHILICITY RELATIONSHIPS FOR 7-ARYLIDENE AND 7-HETEROARYLIDENE MORPHINAN-6-ONES. Hemendra N. Bhargava*, Guo-Min Zhao, Jing-Tan Bian, Y. Nan, S.P. Upadhyaya, W. Xu, W.J. Dunn, III, and L. Bauer, Dept. Pharmaceutics/Pharmacodynamics and Dept. Med. Chem./Pharmacognosy. Univ. Ill., Chicago, IL 60612.

Five compounds related to 7-benzylidenenaltrexone (BNTX) were synthesized as potential selective and potent δ-opioid receptor antagonists. The compounds were evaluated for their activity in vitro at the μ-, δ- and κ-opioid receptors using ligand binding assays, functional activity in mouse vas deferens (MVD) and guinea pig ileum (GPI) and in vivo (analgesic) activity against μ-, δ- and κ-opioid receptor agonists. In addition, the potential role of lipid solubility in the actions of these compounds was determined by measuring the partition coefficients between octanol/water. Most compounds showed greater selectivity towards δ opioid receptors in comparison to μ or κ-opioid receptors. All compounds showed opioid antagonistic activity in GPI and MVD preparations. BNTX and 4-pyridyl derivatives were equally effective against δ_1 and δ_2 agonist induced analgesia but BNTX was more potent. 3-Pyridyl and methylimidazolyl derivatives were more selective for δ_2 in comparison to δ_1 -opioid receptor. The 4-chlorophenyl and 4-fluorophenyl were twice as potent against δ_2 - than for δ_1 - opioid receptor. The apparent lipid solubility (CLOGP) values in decreasing order were: 4-chlorophenyl > 4-fluorophenyl > BNTX > 3-pyridyl = 4-pyridyl > methylimidazolyl. In general, BNTX and 4-pyridyl which had medium lipid solubility were equally effective against δ_1 and δ_2 receptors; 3-pyridyl and methylimidazolyl derivatives were more selective for δ_2 than δ_1 and had lower lipid solubility; 4-chlorophenyl and 4-fluorophenyl derivatives were the most lipid soluble and had intermediate activity (Supported by grants DA-08867 and a Research Scientist Development Award K-02-DA-00130 from the National Institute on Drug Abuse).

71.14

PLASMA AND BRAIN PHARMACOKINETICS OF ENADOLINE AT NEUROPROTECTIVE DOSES IN HEALTHY NON-ANESTHETIZED RATS. J. P. Hinton* and G. Hudson, Department of Pharmacokinetics and Drug Metabolism, Parke-Davis Pharmaceutical Research, Division of the Warner-Lambert Company, 2800 Plymouth Road, Ann Arbor, MI 48105.

A rat brain microdialysis study of enadoline (CI-977), a κ-opioid agonist, was conducted in non-anesthetized healthy rats to determine brain extracellular fluid (ecf) concentrations of enadoline associated with neuroprotective subcutaneous (SC) doses. Three groups of 3 to 4 non-anesthetized yet restrained Sprague-Dawley rats with jugular cannulas and implanted brain (striatum) microdialysis probes received single SC doses of 0.3, 1.0, or 3.0 mg/kg enadoline. Blood and microdialysate samples were collected over a 12 hour period. Enadoline equivalent concentrations were determined with a validated radioimmunoassay. Plasma and ecf pharmacokinetic parameters were calculated by standard non-compartmental methods. At each dose, brain ecf concentration-time profiles were nearly coincident with enadoline plasma free fraction concentration-time profiles. Furthermore at each dose the ratio of AUC_{ecf} /$AUC_{fplasma}$, which represents the distribution of drug between plasma and brain, was determined to be unity within experimental error. These results suggest that unbound plasma concentrations may predict brain ecf concentrations of enadoline.

Research was supported by Parke-Davis Pharmaceutical Research.

71.15

ASPIRIN'S ABILITY TO ATTENUATE THE DEVELOPMENTAL DELAYS OF PRENATAL EXPOSURE TO 1,1,1-TRICHLOROETHANE. H.E. Jones, S.E. Bowen and R.L. Balster.* Dept. Pharmacology and Toxicology, Medical College of Virginia-Virginia Commonwealth University, Richmond, VA 23298.

Many abused solvents share behavioral and pharmacological properties with established CNS depressant drugs like alcohol. Some infants exposed *in utero* to solvents have craniofacial anomalies similar to those associated with perinatal alcohol exposure (Pearson et al., Pediatrics, 93:211, 1994). Since fetal alcohol and solvent exposure share many phenotypic endpoints, perhaps similar cellular mechanisms govern their etiology. Although the exact mechanisms of the Fetal Alcohol Syndrome have yet to be elucidated, one possibility may be the overproduction of prostaglandins. In animals, aspirin, a cyclooxyngenase inhibitor, attenuated the teratogenic effects of alcohol on fetal mortality and dysmorphology (Randall and Anton, Alcohol Clin Exp Res 8:513, 1984). Based on our previous observations showing that exposure to 1,1,1-trichloroethane (TCE) (8000 ppm; 60 min; 3 times/day) during gestation days 12-17 produced developmental and behavioral delays in mice, we attempted to attenuate these deficits by administering a s.c. injection of aspirin (300 mg/kg) 60 min prior to each exposure. Dams were assigned to one of four groups: 1) TCE 8000 ppm/vehicle injection, 2) sham exposure/saline injection 3) TCE 8000 ppm /aspirin injection or 4) sham exposure/aspirin injection. Upon parturition, dams exposed to TCE and aspirin had, on average, statistically smaller litter sizes (number of pups) relative to their sham counterparts. No other differences were observed in malformations, litter weight, sex ratio or length of gestation. Although we replicated our earlier findings of TCE-associated delays in weight gain, landmark development, righting reflex, rooting reflex, grip strength, negative geotaxis and the inverted screen task, aspirin did little to attenuate these effects. In fact, in some tasks, such as the rooting reflex, prenatal exposure to aspirin exacerbated the TCE-induced behavioral delays. Research Supported by NIDA grant DA03112 and predoctoral fellowship DA05665.

71.16

DISCRIMINATIVE STIMULUS EFFECTS OF ALKYLBENZENE SOLVENTS IN ETHANOL-TRAINED MICE. S. E. Bowen*, H.E. Jones and R. L. Balster. Department of Pharmacology & Toxicology, Medical College of Virginia, Richmond, VA 23298-0613.

Drug discrimination procedures were used to compare the discriminative stimulus effects of ethanol (ETOH) and several volatile alkylbenzenes. Male albino mice were trained to discriminate between i.p. injections of ETOH (1.25 g/kg) and saline in a two-lever operant task in which responding was under the control of a fixed-ratio 20 (FR20) schedule of food presentation. Stimulus generalization was examined after 20-min inhalation exposures to toluene (1000-6000 ppm), ethylbenzene (500-4000 ppm) and propylbenzene (500-4000 ppm). Testing is in progress with benzene (500-4000 ppm), xylene (500-4000) and cumene (500-4000 ppm). Concentration-related increases in ETOH-lever responding were observed for all compounds tested to date. For toluene, maximal-levels of > 85% ETOH-lever responding were obtained at 6000 ppm. For ethylbenzene and propylbenzene, the maximal mean percentages of ETOH-lever responding were lower, but 5 out of 10 mice showed full substitution at one or more concentrations. The shared discriminative properties of these compounds with ETOH suggest that these compounds may share some of ETOH's pharmacological properties. These data, in combination with previous research results, are beginning to suggest that alkylbenzenes produce a qualitatively similar profile of acute effects, differing only somewhat in potency. The increasing evidence for commonalities in the behavioral effects of solvents and ETOH may contribute to the understanding of the mechanisms underlying solvent abuse. In addition, this research may also provide a basis for selecting procedures for studying other homologous series. Supported by NIDA grant DA-03112 and NIDA fellowship DA-05670.

DRUGS OF ABUSE: OPIOIDS I

72.1

CHRONIC TREATMENT WITH MORPHINE INCREASES PROTEIN KINASE C βI IMMUNOREACTIVITY IN RAT CEREBRAL CORTEX. M. M Garcia* and R. E. Harlan. Dept. Otolaryngol. and Anat., Tulane Med. Sch., New Orleans, LA 70112

The long-term consequences of chronic abuse of opiates are largely unknown. Although opiates are known to inhibit adenylate cyclase activity, several recent studies also have implicated protein kinase C (PKC) in the acute and long-term actions of opiates. To determine if chronic opiate exposure alters expression of specific isoforms of PKC, we implanted male rats with pellets containing 75 mg morphine sulfate or vehicle, daily for 5 days. On the sixth day, the rats were euthanized and perfused for immunocytochemical localization of the βI and βII isoforms of PKC. Produced by differential splicing of the same primary transcript, these two isoforms are expressed in markedly different populations of neurons throughout the rat brain. In the cerebral cortex, the βI isoform is found in scattered stellate neurons of layers 2 and 3, and in the neuropil and in stellate neurons of layer 4. Labeled neurons are nearly absent in layer 5, but numerous in layer 6, especially at the interface between layers 5 and 6. Immunoreactivity is found in somas, dendrites, axons and terminals. The βII isoform is found associated with the Golgi apparatus in numerous neurons of layers 2/3 and 6. Chronic treatment with morphine markedly increased the number of neurons containing PKC βI, especially in layer 4, in the 5/6 interface and in deep layer 6. The intensity of neuropil staining in layer 4 was also greater following morphine. The effect was most obvious in the somatosensory cortex. No marked changes were noted in the number of labeled cells in the caudate-putamen, nor in the number of cortical neurons containing the βII isoform of PKC. These results suggest a region-specific up-regulation of PKC βI in neocortex following chronic morphine treatment. Supported by LEQSF RDA-029 to MMG.

72.2

MORPHINE-ALTERED CHANGES TRANSCRIPTION GENES IN RAT BRAIN. H. Ujike*@, X.B. Wang@, J.T. You@, G.R. Uhl#@. @Mol. Neurobiol. Br., IRP, NIDA, NIH; #Dept. Neurol. & Neurosci., JHUSM, Baltimore, MD 21224

Abuse of opiates induces long-lasting behavioral changes, including tolerance and dependence, that are likely to reflect adaptations in CNS function. To elucidate potential molecular mechanisms underlying these phenomena, we used subtracted differential display PCR to identify changes in the expression of 23 genes upregulated in brains of rats sacrificed 4h after 20mg/kg morphine. Sequence analyses showed that one was dihydropteridine reductase (DHPR), which promotes the NADPH-mediated reduction of dihydrobiopterin to tetrahydrobiopterin, a co-enzyme for tyrosine hydroxylase. Northern analyses and quantitative RT-PCR confirmed that DHPR expression was increased in striatum and hippocampus after acute morphine treatments. Three different PCR products encoded a member of the K+ channel gene family whose expression was increased in striatum and decreased in frontal cortex and thalamus in animals sacrificed following a morphine injection. The 21 other sequences, largely 3' untranslated regions, showed no Genbank sequence matches. Elucidation of the patterns of region-specific changes in transcription after acute morphine and identification of the effects of chronic morphine treatments will help to select genes that are candidates for involvement in mechanisms of opiate dependence.

72.3

DIFFERENTIAL FOS IMMUNOREACTIVITY AFTER HEROIN SELF-ADMINISTRATION IN DEPENDENT AND NON DEPENDENT RATS: M.R.A. Carrera*, A. E. Ryabinin, M. C. Wilson and G.F. Koob. Dept. Neuropharmacology, The Scripps Research Insitute. La Jolla, CA 92037.

Chronic drug-use produces adaptations in molecular or cellular processes which may oppose the pharamacological effects of the drug resulting in dug dependence. The current study sought to investigate the neural sites for the reinforcing actions of opiates and the motivational effects associated with opiate abstinance, and how these substrates change during the induction of dependence. Male Wistar rats were trained on heroin self-administration (HSA), implanted with morphine or placebo pellets and treated with naloxone (nlx) (0.00, 0.003 or 0.03 mg/kg). In this behavioral model, dependent (D) but not non-dependent (ND) rats present a shift to the left of the dose-effect function for nlx-induced increases in HSA. An analysis of Fos immunoreactivity (FI) in both groups (ND and D) following HSA show an overall activation of substrates associated with opiate actions inlcuding the pontine nuclei, central gray, ventral tegmental area (VTA) various hypothalamic and thalamic nuclei, basolateral amygdala (BLA) and nucleus accumbens (Acb). In the ND condition, nlx treatment produced virtually no change with the exception of small increases in the dorsomedial hypothalamous, ventral bed nucleus of the stria terminalis and central amygdala at the higher dose. In contrast, D subjects exhibited a hightened overall activation in addition to significant FI increases in hippocampus, lateral and ventromedial hypothalamus, centromedial thalamus, caudate putamen, globus pallidus, various sepatal subregions and accumbens core (AcbC). Whereas at the low nlx dose there was pronounced activation in VTA, ventromedial hypothalamus, rhomboid thalamic nucleus, and Acb, the high dose induced expression in the nucleus of the solitary tract, area postrema, locus coeruleus; paraventricular, supraoptic and lateral hippothalmus, central but not basolateral amygdala and core but not shell aspect of the Acb. These findings suggest that while the onset of opiate dependence yields higher neural activation of reward pathways, different brain regions are also recruited by this event. Supported by: DA 04398, NIH-NIDA.

72.4

ANDROGENIC-ANABOLIC STEROIDS MODULATE C-FOS INDUCTION BY MORPHINE OR COCAINE IN MALE RAT CAUDATE-PUTAMEN. H. E. Brown, M. M. Garcia, P. S. Guth*, and R. E. Harlan. Depts. of Anatomy, Otolaryngology, and Pharmacology, Tulane U. Sch. Med., New Orleans, LA 70112.

Androgenic-anabolic steroids (AASs) have been reported to produce symptoms of abuse and dependence in human athletes. Induction of c-Fos antigen in the caudate-putamen (CPu), which may index the reinforcing properties of abused drugs, was used in the current studies to examine the view that AASs impact neural reward systems. Male rats were injected over 15 days with increasing dosages of testosterone cypionate (TC) plus nandrolone decanoate (ND), or oil. Initial and final TC + ND dosages were 0.5 + 0.25 mg/kg and 1.0 + 0.5 mg/kg body weight (BW). On Day 16, groups of rats received 1 or 10 mg/kg BW morphine sulfate, 1 or 10 mg/kg BW cocaine hydrochloride, or saline. Rats were euthanized 2 h later. c-Fos immunocytochemistry was done on coronally-sectioned CPu anteriorly from the decussation of the anterior commisure (d-ac). c-Fos immunoreactivity (IR) in 3 sections/brain was quantified by digital image analysis of dorsomedial CPu, a region in which we observed morphine- and cocaine-induced c-Fos IR overlap. AAS treatment alone did not affect c-Fos IR. The higher morphine dosage, and both cocaine dosages, significantly increased the number of cells exhibiting c-Fos IR. In each case, AAS pretreatment significantly decreased the number of IR cells. In morphine-treated rats, c-Fos IR was confined to the dorsomedial CPu, whereas c-Fos IR was widespread in cocaine-treated rats. In AAS- or oil-pretreated rats given 10 mg/kg cocaine, we therefore counted cells from the dorsal half of the CPu in brain sections at 240 mm intervals anteriorly from the d-ac. In the anterior and posterior portions of this region, AAS pretreatment respectively decreased and increased c-Fos IR cell counts in rats challenged with 10 mg/kg BW cocaine. Although the precise loci of the AAS effects we report here are unclear, the data indicate that AAS actions in the brain are reflected by neostriatal neurons which might participate in the rewarding or reinforcing qualities of morphine and cocaine.
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72.5

DEPOLARIZATION OF MYENTERIC NEURONS BY CHRONIC MORPHINE TREATMENT IS DUE TO A REDUCTION IN THE SODIUM PUMP. J.-Q. Kong, J. Meng, D.A. Taylor and W.W. Fleming*. Dept. of Pharmacol. and Toxicol., R.C. Byrd Hlth. Sci. Ctr., West Virginia University, Morgantown, WV 26506-9223.

Chronic exposure to morphine leads to the development of heterologous subsensitivity of guinea pig myenteric plexus/longitudinal muscle to inhibitory agonists that is associated with a depolarization of myenteric 'S' neurons (MN). The mechanism underlying this depolarization was investigated by determining the effect of ouabain on MN from control and morphine treated (pellet implanted) guinea pigs. Intracellular electrophysiological recording from MN was accomplished using standard techniques. Morphine superfusion (1 μM) led to a hyperpolarization of 'S' neurons which was not significantly different in magnitude between placebo (6 ± 1 mV) and tolerant (5.5 ± 1.3 mV) preparations. Ouabain superfusion (5 μM) was performed in the absence or presence of TEA (0.5 mM) to eliminate the potential complicating interaction of ouabain with K channels in these neurons. Ouabain produced a depolarization of MN from placebo treated animals of 17 ± 3 mVin the absence and 14 ± 2 mV in the presence of TEA. However, in MN from animals implanted with morphine pellets, the same concentration of ouabain produced depolarizations of 8 ± 3 mV in the absence and 6 ± 2 mV in the presence of TEA. The magnitude of the depolarization produced by ouabain was significantly reduced in MN from morphine treated animals compared to controls either in the presence or absence of TEA. In addition, the resting membrane potential of MN from morphine treated animals (-45 ± 2 mV) was significantly reduced from that obtained in cells from placebo animals (-55 ± 2 mV) by 10 mV as previously observed in MN. These data suggest that the depolarization observed in MN following chronic treatment with morphine may be due to a reduction in electrogenic sodium pumping capacity of these neurons. Such an alteration in sodium pumping capacity could be responsible for the 10 mV reduction in membrane potential as well as the heterologous changes in sensitivity observed following chronic morphine treatment. A similar reduction in sodium pumping capacity has been implicated as a mechanism for adaptive sensitivity changes in a variety of other excitable tissues. Supported in part by NIH grant DA 03773.

72.6

THE EFFECTS OF CHRONIC EXPOSURE TO MORPHINE ON MU OPIOID RECEPTOR CHARACTERISTICS IN C57 AND DBA MICE. R.L. Petruzzi, T.N. Ferraro* G.T. Golden, and W.H. Berrettini, Department of Psychiatry, Thomas Jefferson University, Philadelphia, Pennsylvania

Given a choice, C57 mice will self administer substantial quantities of morphine compared to DBA mice and much of this difference has been attributed to a genetic locus on chromosome 10 in the vicinity of the mu opioid receptor. To compare binding characteristics of mu opioid receptor populations between the two strains, mice were given single daily injections of morphine sulfate (0.1 mg/kg, sc) or saline for a period of seven days and sacrificed six hours after the last injection. Brains were removed and dissected into specific regions. Receptor binding studies were preformed on frontal cortex, striatum, substantia nigra, and nucleus accumbens. Data were analyzed using Scatchard analysis, and K_d and B_{max} comparisons between strains and treatments were done by the Mann-Whitney U test. Results for nucleus accumbens and substantia nigra were inconclusive while frontal cortex results were not significant. Results in striatum show that before morphine injection, DBA mice have fewer opioid receptors than C57 mice ($p=0.0080$); after injection, C57 mice have fewer receptors than DBA ($p=0.0037$). In addition, C57 mice chronically exposed to morphine exhibit a decreased receptor density compared to C57 controls ($p=0.0013$), a phenomenon not observed in DBA mice. No differences or changes in receptor affinity were indicated by K_d values. These results are consistent with the fact that C57 mice exhibit a significantly greater preference for oral and intravenous opioids compared to DBA mice. This work was supported by NIDA grant RO1-DA08041.

72.7

ESTIMATION OF THE EXPRESSION OF MUSCARINIC m1 mRNA BY QUANTITATIVE PCR DURING AND AFTER SPONTANEOUS MORPHINE WITHDRAWAL . L. Zhang* and J.J. Buccafusco. Department of Pharmacology and Toxicology, Medical College of Georgia, and Department of Veterans Affairs Medical Center, Augusta, GA, 30912.

Previous studies in this and other labs have demonstrated that central cholinergic neurons play an important role in the expression of morphine withdrawal symptoms. Also, selective muscarinic receptor antagonists exert marked antiwithdrawal activity. Rats were made dependent upon morphine using a chronic i.v. infusion schedule of increasing concentrations of morphine over 5 days. Spontaneous withdrawal was initiated by termination of the morphine infusion. Withdrawal-induced cardiovascular and behavioral symptoms were recorded and correlated with changes in the transcription of mRNA encoding the m1 muscarinic receptor in cerebral cortex and hippocampus. For the RT-PCR reaction, an internal standard was generated by using an Apo B gene fragment linked to the primers for the m1 muscarinic receptor. The forward composite primer included a linker sequence that allowed extension of the T7 RNA polymerase promoter sequence in a subsequent PCR reaction, and, using the T7 primer and the reverse primer of the target message. After the T7 promoter sequence was extended, cRNA was obtained using an *in vitro* transcription system. The molecular size of the m1 target and the internal standard PCR products were, respectively, 274 and 373 bp. PCR products were separated on electrophoretic gels and the bands stained and quantified by densitometry. Morphine infusion was shown to produce about a 50% increase in the level of mRNA encoding the m1 subtype of muscarinic receptors in the cortex. During withdrawal levels were reduced by 50%. At the completion of withdrawal m1 mRNA levels returned to the high levels observed prior to withdrawal. In contrast, m1 receptor mRNA expression was normal after morphine infusion in hippocampus, but levels were reduced by about 60% at the peak of withdrawal, and were reduced by 80% at the end of the withdrawal phase. Thus, in the hippocampus, these changes which occur after withdrawal may underlie the conditioned component of addiction. Supported by the VA Medical Research Service.

72.8

CHANGES IN QUANTITY AND PHOSPHORYLATION STATE OF L-TYPE CALCIUM CHANNEL α_1 SUBUNIT WITH ACUTE AND CHRONIC MORPHINE. M.A. Bernstein, D.R. Compton* and S.P. Welch, Medical College of Virginia, Dept. of Pharmacology and Toxicology, Richmond, VA 23298.

The involvement of dihydropyridine-sensitive, or L-type, calcium channels in tolerance to morphine-induced antinociception has been implicated by in vivo studies showing cross-tolerance between parenterally administered morphine and intrathecal Bay K 8644. Such changes may be due to an increase in the number of L-type channel α_1 subunits, the pore-forming subunit of the channel, or may result from changes in cAMP-dependent protein kinase phosphorylation of the subunit, which has been shown to affect calcium gating. To quantitate α_1 channel subunit protein, Western immunoblot analysis was performed using membrane preparations from mice treated with morphine acutely or chronically. Changes were noted in α_1 subunit quantity in the spinal cord, suggesting channel sequestration following acute morphine treatment, and channel upregulation following chronic exposure. No changes were found in the brainstem. The phosphorylation state of the subunit is currently being assessed using a back-phosphorylation method followed by immunoprecipitation and SDS-PAGE separation.

Supported by NIDA grants DA07027, DA00186, DA06031.

72.9

CHANGES IN THE DOPAMINERGIC SYSTEM OF THE ADULT RAT BRAIN AFTER ACUTE AND CHRONIC HEROIN EXPOSURE
A.Stadlin*, H.L.Choi, B.N.Ladenheim[1], J.L.Cadet[1] and S.F.Ali[2] Department of Anatomy, Chinese University of Hong Kong, Shatin, N.T., Hong Kong; [1]Molecular Neuropsychiatry Section, Addiction Research Center, NIDA/NIH, Baltimore, MD 21224 and [2]Neurochemistry Lab, Division of Neurotoxicology, National Center for Toxicological Research, FDA, Jefferson, AR 72079, USA.

The dopaminergic system of the brain plays an important role in the acute and chronic effects of drugs of abuse. The present study investigates the effects of acute and chronic heroin exposure in this system. Male adult Sprague-Dawley rats received an acute dose of 20 mg/kg (s.c.) high-grade street heroin. The chronic group received 10 mg/kg/day for the first week (s.c.) followed by 20 mg/kg/day for the second week. Control animals received saline injections. Changes in the dopamine transporter (DAT), dopamine (DA), D1 and D2 receptors were studied in the striatum using receptor ligand binding assays and quantitative autoradiography. The DA and DOPAC levels were examined using HPLC/EC methods. Results showed that, after an acute dose of heroin, there is a 20% decrease in DAT, D1 and D2 binding with a concomitant increase in DOPAC levels in the striatum. Contrary to the acute heroin-exposed animals, chronic heroin-exposed animals showed a 36%, 22% and 48% increase in DAT, D1 and D2 receptor binding respectively. A corresponding increase in DA levels was also observed in these animals. The present results showed that acute and chronic exposure to heroin resulted in differential changes in the dopaminergic system. These results suggest that chronic administration of heroin may result in an overall upregulation of both presynaptic and postsynaptic elements of the dopaminergic system.

72.10

EFFECTS OF CHRONIC ADMINISTRATION OF MORPHINE OR NALTREXONE ALONE OR IN COMBINATION ON THE ACTIVITY OF CENTRAL NITRIC OXIDE SYNTHASE (NOS) IN MICE. Shailendra Kumar, Robert V. House* and Hemendra N. Bhargava, Dept. Pharmaceutics/Pharmacodynamics, Univ. Ill., Chicago, IL 60612 and IIT Res. Inst., Chicago, IL 60616.

The time course of the effects of subcutaneous implantation of morphine (25 mg) or naltrexone (NTX) (10 mg) pellets alone or in combination on the NOS activity was investigated in male Swiss-Webster mice. Mice implanted with appropriate placebo pellets served as controls. NOS activity was determined in brain regions (cerebellum, cortex, midbrain, and remainder of the brain, and spinal cord) by the formation of [^{3}H]citrulline from [^{3}H]arginine. NOS activity decreased in all brain regions, but not in spinal cord, 24h after morphine pellet implantation. It was accompanied by high level of motor activity. NOS activity increased 48 and 72 h after pellet implantation in the cerebellum and cortex. Implantation of NTX pellets along with the morphine pellets completely antagonized the changes in central NOS activity induced by morphine. It is concluded that initial decrease in NOS activity may be related to the increased motor activity induced by morphine in the mouse. On the other hand, on subsequent days the induction of NOS in certain brain regions may be related to morphine tolerance -dependence process. Furthermore, the alteration in NOS activity by morphine involves opioid receptors since it is blocked by NTX (Supported by a Research Scientist Development Award, K02-DA-00130 from the National Institute on Drug Abuse).

72.11

EFFECTS OF MULTIPLE INTRACEREBROVENTRICULAR INJECTIONS OF δ_1- or δ_2 - OPIOID RECEPTOR AGONISTS ON BRAIN δ OPIOID RECEPTORS IN MICE. Guo-Min Zhao, Nicholas P. Plotnikoff* and Hemendra N. Bhargava, Dept. Pharmaceutics/Pharmacodynamics, Univ. Ill., Chicago, IL 60612.

The effects of D-Pen^2, D-Pen^5 enkephalin (DPDPE), and D-Ala^2, Glu^4 deltorphin II (deltorphin II), the selective agonists for δ_1- and δ_2-opioid receptors, injected i.c.v. for different duration on the brain δ-opioid receptors were determined in male Swiss Webster mice. Twice daily injections of DPDPE (20 μg/mouse) or deltorphin II (20 μg/mouse) for 4 days resulted in the development of tolerance to their analgesic action. Under this condition, the binding constants (B_{max} and K_d) for [^{3}H]DPDPE in brain could not be determined, possibly due to destruction of binding sites. Twice daily injections of DPDPE (20 μg/mouse) or deltorphin II (20 μg/mouse) for two days also resulted in 45% and 56% decrease in their analgesic action compared to vehicle injected controls. Under this condition, DPDPE treatment decreased the B_{max} but had no effect on the K_d of binding of [^{3}H]DPDPE. On the other hand, deltorphin II treatment decreased the B_{max} but increased the K_d of [^{3}H]DPDPE in brain membranes. It is concluded that both DPDPE and deltorphin II in short term or long term treatment produce tolerance to their analgesic effects. The short term treatment of both drugs down regulates brain δ-opioid receptors whereas long term treatment apparently results in neurotoxicity as seen by total loss of binding sites (Supported by grants DA-08867 and a Research Scientist Development Award, K02-DA-00130 from the National Institute on Drug Abuse).

72.12

EXPOSURE TO A DELTA OPIOID AGONIST DURING THE PREWEANING PERIOD ALTERS DOPAMINE RECEPTORS IN THE 28-DAY-OLD RAT BRAIN. D.E. Walters*, G.-J. Shieh and C.C. Edge. Div. Pharmacology, School of Pharmacy, Auburn Univ. AL 36849.

To determine if exposure to a delta opioid receptor agonist during suckling could alter the development of brain dopamine (DA) receptors, on postnatal day 7 (PD7), 14-day osmotic pumps containing SNC 80, 37.5 mg/ml, or pH adjusted 0.9% saline vehicle were implanted into chloral hydrate anesthetized lactating rats. On PD21, the pups were weaned and on PD28, the striata of male offspring were dissected and assayed for DA D1 and D2 receptors.

There was a significant 24% increase in the density (Bmax) and a 44% decrease in the affinity (Kd) of DA D1 receptors in the striatum of 28-day-old offspring exposed to SNC 80. However, SNC 80 had no significant effect on the Bmax or Kd of DA D2 receptors in the striatum of 28-day-old offspring. Exposure to SNC 80 did not significantly alter the body weights at 28 days of age or the food consumption of offspring between weaning and sacrifice; nor was there a significant effect of SNC 80 on maternal food consumption during the nursing period. The results suggest that delta opioid mechanisms might be involved in the neurological changes observed in offspring of mothers addicted to opioids during nursing. The SNC 80 was provided by K. Rice. Supported by NIDA grant DA07968.

72.13

A NEONATAL RAT MODEL OF OPIOID TOLERANCE AND DEPENDENCE. S. R. Thornton, F. L. Smith and L. S. Harris*. Department of Pharmacology/Toxicology, MCV/VCU, Richmond, VA.

Human neonates and infants continuously infused with fentanyl or morphine become tolerant and dependent. Morphine has been shown to produce tolerance and dependence in neonatal animals, but there is no published data for fentanyl tolerance and dependence. We developed an animal model to investigate whether continuous fentanyl administration would render neonatal rats tolerant and physically dependent to fentanyl. Postnatal day 6 rat pups were anesthetized and then remained either naive or were surgically implanted with Alzet 1003D osmotic mini-pumps containing either saline (1 μl/hr) or fentanyl (100 μg/kg/hr) and challenged with fentanyl 72 hrs after implantation. The ED_{50} values for the naive and saline treated animals were not significantly different (20 μg/kg for both groups). However, fentanyl treated animals demonstrated good tolerance when compared to the saline treated animals (90 μg/kg vs. 20 μg/kg, respectively). Fentanyl exposed animals injected with naloxone (0.1 mg/kg, s.c.) exhibited withdrawal signs, such as increased spontaneous activity, micturation, defecation and vocalization. Utilizing the osmotic mini-pump technology, fentanyl tolerance and dependence have been compared to the classical opioid, morphine. Future studies will assess the long-term cellular/molecular changes in rats neonatally exposed to fentanyl which may indicate a predisposition for juvenile and adult rats to self-administer more opioids than neonatal opioid naive rats. Research was supported by NIDA grant DA05274.

72.14

EFFECTS OF POSTNATAL NALOXONE EXPOSURE ON NEUROPEPTIDE mRNA EXPRESSION IN HYPOTHALAMIC NUCLEI OF NEONATAL RATS PRENATALLY EXPOSED TO METHADONE. J.W. Nemitz*. Division of Structural Biology, WV School of Osteopathic Medicine (WVSOM), Lewisburg, WV 24901.

Pregnant Sprague-Dawley rats were made physically dependent to methadone (9mg/kg/day) using subcutaneous (sc) osmotic minipumps (Alza). The pregnancies were brought to term and the methadone exposed rat pups were injected with either naloxone (5mg/kg sc) or saline 24 hours postnatally. Both male and female rat pups were used in the study. Naloxone precipitated withdrawal signs, including, hyperactivity, increased oral behaviors and vocalizations, were observed for prenatally methadone exposed rat pups. Following an observation period of 1 hour the pups were sacrificed by decapitation. Frozen serial coronal sections (20 um), made at the level of the hypothalamus, were processed for in situ hybridization, using ^{35}S-oligonucleotide probes (DuPont), and emulsion autoradiography. Arginine-vasopressin (AVP) and oxytocin (OXY) mRNA levels were decreased in the supraoptic nucleus (SON) and paraventricular nucleus (PVN) of methadone exposed rat pups as compared to controls following a postnatal injection of saline. However, when methadone exposed rat pups were postnatally injected with naloxone, AVP and OXY mRNA levels in the SON and PVN were increased as compared to the neuropeptide mRNA levels observed in these nuclei of methadone exposed rat pups that were postnatally injected with saline. These data indicate that the decrease in AVP and OXY mRNA expression in the SON and PVN of neonatal rat pups prenatally exposed to methadone can be reversed by postnatal naloxone injection, thus providing evidence for an opioid mechanism of action. Supported by intramural WVSOM funds.

72.15

GESTATIONAL MORPHINE ALTERS THE DENSITY OF CATECHOLAMINE FIBERS IN THE HYPOTHALAMUS OF ADULT FEMALE RATS. I.Vathy*, H-J. He, M. Iodice and A. Rimanóczy[1]. Dept. Psych. and Neurosci., Albert Einstein Coll. Med., Bronx, NY. 10461. [1]Dept. Psych., A. Sz-Györgyi Med. Sch., Szeged, Hungary

The present study investigated the distribution of norepinephrine (NE) and dopamine (DA) fibers in different brain regions of adult female rats exposed to morphine in utero (10 mg/kg/twice daily on days 11-18 of gestation). Females were ovariectomized (OVX) at least one week prior to sacrifice, and some were injected with 3 μg of estradiol benzoate 48 hours before sacrifice. Animals were perfused with 4% paraformaldehyde, brains were removed, post-fixed, saturated with sucrose, frozen and stored in -80° C. Coronal sections of 10 μm were cut with a cryostat, and the distribution of tyrosine hydroxylase-immunoreactive (TH-IR) and DA β-hydroxylase (DBH)-IR fibers were identified in alternate sections. Prenatal morphine reduced the density of both TH- and DBH-IR fibers in several brain areas including the lateral hypothalamus of females. Estrogen treatment did not affect the density of TH or DBH-IR fibers in OVX females from either prenatal treatment group. The reduction in hypothalamic catecholamine fiber density may be related to the reduction in NE content and turnover rate observed in the hypothalamus of prenatally morphine-exposed females in previous studies (Vathy and Kátay, Dev. Brain Res., 68:125-134, 1992; Vathy et al., Brain Res., 662:209-215, 1994). The number of DA and NE cellbodies will also be investigated and analyzed later. Supported by DA 05833 awarded to IV.

73.1

DRD3 AND DRD4 GENE MARKER FREQUENCIES IN POLYSUBSTANCE ABUSERS AND CONTROLS[1]. L.A. Rodriguez[2*], D.J. Vandenbergh[2], E. Bendahhou[2], and G.R. Uhl[2,3]. [2]Molecular Neurobiology Branch, National Institute on Drug Abuse, Intramural Research Program, Baltimore, MD 21224. [3]Departments of Neurology and Neuroscience, Johns Hopkins University School of Medicine, Baltimore, MD 21205.

Dopamine D_3 (Drd3) and D_4 (Drd4) receptor genes are implicated in substance abuse vulnerability due to their primary expression in limbic brain regions and recently reported links between measures of novelty seeking behaviors and specific Drd4 genotypes (Benjamin *et al.*, Nat Genet 12:81-84, 1996). Allelic association of Drd3 and Drd4 polymorphisms was sought in a sample of caucasian polysubstance abusers and controls largely ascertained through the Addiction Research Center in Baltimore, MD. Drd3 polymorphisms were identified in 111 drug abusers and 74 controls via an exonic A to G substitution producing a serine (A1 allele) to glycine (A2 allele) substitution at position 9 in the extracellular *N*-terminus. Drd4 polymorphisms were identified through exon III variants in 60 drug abusers and 66 controls via the presence of long (6 or more) or short (less than 6) repeats of a 48 bp sequence. No significant allelic associations were detected for either dopamine receptor gene (*Drd3*: $\chi^2=0.04$; df=1, $p=0.84$; *Drd4*: $\chi^2=0.55$; df=1, $p=0.31$, one-tailed), suggesting that variants of the Drd3 and Drd4 genes in linkage disequilibrium with these markers may not play large roles in the genetic mediation of drug abuse risk.

[1]Supported by the NIDA/NIH Intramural Research Program.

73.2

BIOCHEMICAL DIFFERENCES BETWEEN DARK AGOUTI AND FISCHER 344 RATS IN THE MESOLIMBIC DOPAMINE SYSTEM. E.S. Brodkin*, R.Z. Terwilliger, C.N. Haile, and E.J. Nestler. Laboratory of Molecular Psychiatry, Departments of Psychiatry and Pharmacology, Yale Univ Sch of Med, New Haven, CT.

Previous studies have found that Lewis (LEW) and Fischer 344 (F344) rats differ in levels of specific proteins in the mesolimbic dopamine system, in their behavioral and endocrine responses to stress and drugs of abuse, and in their susceptibility to autoimmune disease (LEW more autoimmune-prone). To determine whether these traits covary in other inbred strains, we studied another autoimmune-prone inbred strain, the Dark Agouti, relative to F344 rats under basal conditions. In the ventral tegmental area (VTA), we found by immunoblotting that Dark Agouti rats had significantly higher levels of GluR1 and glial fibrillary acidic protein and lower levels of neurofilament proteins than F344 rats. There also was a trend for Dark Agouti rats to have higher levels of tyrosine hydroxylase than F344 rats in the VTA. In contrast, in the nucleus accumbens (NAc), there was no difference in levels of protein kinase A, $Gi_{1/2\alpha}$, or tyrosine hydroxylase, proteins that do differ in LEW rats. Thus, the VTA, but not the NAc, of the Dark Agouti is "LEW-like" as compared to F344 rats. Further studies will compare behavioral responses to drugs of abuse in Dark Agouti and F344 rats. With genetic maps now available, these phenotypic differences may be amenable to quantitative trait locus analysis. (Supported by DA08227.)

73.3

INCREASED SENSITIVITY TO THE STIMULANT ACTIONS OF MORPHINE AFTER INFECTION OF VTA WITH AN HSV VECTOR EXPRESSING GLUR1. W. A. Carlezon Jr.*, V. A. Boundy, R. G. Kalb^, R. Neve^^, and E. J. Nestler. Laboratory of Molecular Psychiatry and ^Dept. Neurology, Yale University School of Medicine, New Haven CT, and ^^Molecular Genetics Laboratory, Harvard Medical School, Belmont MA.

Repeated administration of morphine upregulates expression of the AMPA receptor subunit GluR1 in the ventral tegmental area (VTA), the cell body region of the mesolimbic dopamine system. Increased GluR1 expression would be expected to alter the excitability of VTA dopamine cells, and could contribute to the neurobiological adaptations that accompany repeated morphine treatment. We now report that direct and specific upregulation of GluR1 in VTA—through the use of a recombinant defective Herpes Simplex Virus (HSV) vector expressing GluR1—increases sensitivity to the locomotor-stimulating actions of morphine. Sprague-Dawley rats were first screened for locomotor activity after morphine injection (1.0 mg/kg, SC). Rats (12 per group) then received either unilateral microinjections (2.0 µl at 2 X 10^8 pfu/ml) of pHSVGluR1 or of pHSVLacZ, or sham surgery (injection needle not lowered into brain). Rats were retested with morphine (1.0 mg/kg, SC) on day 3 and day 4 after surgery. The locomotor activity of rats given VTA microinjections of pHSVGluR1 increased 70%, whereas 25% increases were seen after microinjections of pHSVLacZ or after sham surgery. Immunohistochemical analysis 96 hr after infection revealed moderate numbers of highly GluR1-immunoreactive cells in VTA in the injected hemisphere of rats given pHSVGluR1, whereas GluR1 immunoreactivity was at normal low basal levels in VTA on the non-injected side of the brains or on the injected side of the brains of rats given pHSVLacZ. These studies suggest that altered expression of AMPA receptor subunits in VTA may underlie some behavioral adaptations to opiates. (Supported by DA08227)

73.4

ORAL INTAKE OF DRUGS OF ABUSE BY RATS BRED SELECTIVELY FOR OPIOID ACCEPTANCE. K. R. Carlson* and L. Perez. Dept. of Pharmacology & Molecular Toxicology, U. Massachusetts Medical Center, Worcester, MA 01655-0126.

Lines which accept or reject solutions of the potent opioid etonitazene, and a randomly bred control line, have been established by selective breeding. In order to test the specificity of selective breeding, drug-naive subjects from generation 8 were offered a continuous choice in their home cages between water and a 10% ethanol solution for 20 days. There was no difference between the accepting and rejecting lines in preference for one fluid or in dose of ethanol received. The same rats were then given a choice between water and increasing concentrations (0.08-0.64 mg/ml) of a cocaine solution, seven days at each concentration. There were no differences among the lines in preference for the drug, but the rejecting line ingested a higher dose than the accepting line. Finally, these animals were subjected to the same regimen as used in choosing rats for selective breeding: 2 days of 2.5 µg/ml etonitazene alone, followed by 4 days of a water-etonitazene choice. In all measures the order of the lines was as expected: accepting > control > rejecting. Thus, selection has been rather specific and not for a generalized tendency to become intoxicated. Supported by NIH DA06539

73.5

BLOCKADE OF TYPE II GLUCOCORTICOID RECEPTORS REDUCES BEHAVIORAL AND DOPAMINERGIC RESPONSES TO MORPHINE.

M. Marinelli, B. Aouizerate, M. Barrot, M. Auriacombe, M. Le Moal and P.V. Piazza. SPON: European Brain and Behavior Society
INSERM U. 259, Université de Bordeaux II. 33077 Bordeaux Cedex, France.

Previous data show that glucocorticoid hormones facilitate the behavioral and dopaminergic effects of morphine. In this study we examined the role of glucocorticoid receptors on the behavioral and dopaminergic responses to morphine by selectively blocking glucocorticoid receptors via i.c.v. administration of receptor antagonists 2hr before the administration of morphine. In the first experiment we studied the effect of type I or type II receptor antagonists on the locomotor effects of morphine. Blockade of type II receptors by either RU 38486 (100 ng/6µL), or by the more selective RU 39305 (200 ng/6µL), which is void of antiprogesterone action, largely reduced the response to morphine whereas the type I antagonist spironolactone (100 ng/6µL) had no significant effect. In the second experiment we determined if the influence of type II glucocorticoid receptors on this drug-induced behavior was dopamine-dependent by injecting morphine directly in the VTA. Indeed, type II receptor blockade reduced the locomotor response to intra-VTA morphine. In a final experiment we examined how blockade of type II receptors could exert its effects on the dopaminergic system. To this end, we monitored extracellular concentrations of dopamine (DA) in the nucleus accumbens shell by means of microdialysis in freely moving rats. Blockade of type II receptors reduced both baseline values and morphine-induced DA release. In conclusion, these results show that type II glucocorticoid receptors modulate dopamine-dependent behavioral effects of morphine and that changes in the basal and drug-induced activity of DA neurons may, at least in part, account for these effects. Furthermore, these results also indicate that opioid effects may be decreased by acute pharmacological manipulations of glucocorticoid hormones.

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73.6

GLUCOCORTICOIDS SELECTIVELY MODULATE DOPAMINERGIC ACTIVITY IN THE NUCLEUS ACCUMBENS SHELL.

M. Barrot, M. Marinelli, F. Rougé-Pont, N. Abrous*, M. Le Moal, P.V. Piazza. Psychobiologie des Comportements Adaptatifs, INSERM U259, Univ. de Bordeaux II, 33077 Bordeaux Cedex, France.

Previous investigations have shown that glucocorticoids can modify basal and drug-induced dopamine (DA) release. We studied the possible anatomical selectivity of these effects by means of microdialysis in freely-moving rats. DA levels were monitored at rest or after vehicle or morphine (2mg/kg, s.c.) injection in sham-operated (sham) and adrenalectomized (ADX) rats and in ADX rats with corticosterone replacement. Identical probes (CMA11/2mm) were used in the core and shell of the nucleus accumbens and in the dorso-lateral striatum. In sham animals, there were basal and morphine-induced differences in DA levels between the three regions, the shell being the most reactive. The shell was also the only region in which a s.c. vehicle injection increased DA levels. In the core and in the striatum, no significant difference was observed between sham and ADX rats. In contrast, ADX decreased basal DA levels by 66% in the nucleus accumbens shell; ADX also suppressed the response to vehicle injection, and the morphine-induced DA release in these rats just reached the basal levels of sham animals. The effects of ADX were reversed by restoring corticosterone circadian secretion. These differences between core, shell and striatum in sham rats and in the anatomic specificity of glucocorticoid action, point out that the three regions are physiologically different. This fact must be taken into account in dialysis studies of the nucleus accumbens, especially for phenomena in which glucocorticoids could be implicated. In conclusion glucocorticoids, which act on behavioral and motivational processes, selectively modulate dopaminergic activity in the shell, which is considered the limbic portion of the nucleus accumbens.

Supported by INSERM, Université de Bordeaux II and Conseil Régional d'Aquitaine.

73.7

NATURE OF STRESS INDUCED POTENTIATION OF MORPHINE'S REWARDING PROPERTIES. **M. J. Will*, L. R. Watkins & S. F. Maier. Dept. of Psychology, University of Colorado, Boulder, CO 80309.**

We have previously shown that inescapable tailshock (IS) potentiates the rewarding properties of morphine when measured by conditioned place preference. The physical nature of IS and the multiple pharmacological properties of morphine make interpretation of these data difficult. The present experiments were designed to address some of these interpretive issues. One approach involved attempting to produce the effects of IS with the benzodiazepine inverse agonist DMCM, a drug shown to mimic other IS induced behavioral effects. This approach would exclude external physical stress and possible injury. Initial preferences were measured over a 20 min trial in which rats explored 2 distinct environments separated by a neutral area. On day 2, the inverse agonist DMCM (.3mg/kg i.p.) or vehicle was administered and subjects then restrained for a period equal to the shock session. Conditioning occurred 24 and 48 hours after DMCM/restraint with 3mg/kg-ml s.c. morphine in one context &, 4 hours later, 1ml/kg s.c. saline vehicle in the other context. Conditioning assignment was counter-balanced randomly. Testing of preference shift was conducted over a 20 min period approximately 24 hours following the last conditioning pairing. Difference in time spent in compartments before & after conditioning with drugs was determined. Significant potentiation of morphine conditioned place preference was observed in the DMCM treated groups compared to controls. Time course and stressor controllability effects were also investigated. Supported by Grant NIH MH50479.

73.8

CORTICOSTERONE IS NOT INVOLVED IN STRESS- AND HEROIN-INDUCED RELAPSE TO HEROIN-SEEKING. Y. Shaham*, D. Funk, S. Erb, T. Brown, C.-D. Walker, J. Stewart. Center for Studies in Behavioral Neurobiology, Concordia Univ., Montreal, and Addiction Research Foundation, Toronto, Canada.

We have found previously that brief exposure to footshock and to priming injections of heroin reinstate heroin-seeking behavior following prolonged extinction. Here we report on the role of the adrenocortical hormone, corticosterone, in stress- and heroin-induced relapse. Rats were trained to self-administer heroin (100 µg/kg infusion, IV) during four 3-h sessions per day, over a 2-week period. Extinction sessions with saline were given for 4-6 days. Reinstatement of heroin-seeking was then studied after exposure to priming injections of saline and heroin (0.25 mg/kg, SC), and 15 min of intermittent footshock (0.5 mA). Neither adrenalectomy (performed at the end of the training phase), nor chronic exposure to a synthesis inhibitor of corticosterone, metyrapone (100 mg/kg, twice daily from day 1 of extinction), nor an acute injection of metyrapone, given 3 h before testing, altered reinstatement of heroin-seeking induced by either footshock or a priming injection of heroin. Surprisingly, acute exposure to metyrapone, alone, potently reinstated extinguished heroin-seeking behavior. Thus the ability of footshock stress and priming injections to induce relapse to heroin-seeking would appear to be independent of the actions of corticosterone in the central nervous system. Supported by NIDA R01-09750

73.9

EFFECTS OF HALOPERIDOL ON THE RESPONSE-REINSTATING PROPERTIES OF DISCRIMINATIVE STIMULI IN AN OPERANT MODEL OF DRUG RELAPSE. K. McFarland* and A. Ettenberg. Behavioral Pharmacology Laboratory, University of California, Santa Barbara, CA 93106.

Olfactory stimuli (orange and almond food extract) served as discriminative cues about the availability (S+) or unavailability (S-) of heroin reinforcement (a single 0.1 mg/kg infusion) in the goal box of a straight arm runway. Following discrimination training, the running response was extinguished in the absence of the olfactory cues. On a single trial, the discriminative stimuli were tested for their ability to reinstate running behavior *prior* to presentation of any reinforcement. Subjects presented with the S+ on test day traversed the alley significantly more quickly than did those subjects presented with the S-. These results demonstrate, in an animal model of drug self-administration, that environmental discriminative cues possess the ability to reinstate drug seeking behavior following a period of abstinence. The response-reinstating properties of the S+ odor was unaltered by pretreatment with any of three doses of haloperidol (0.0, 0.15 or 0.3 mg/kg IP), suggesting that the motivating properties of heroin-predictive stimuli are independent of endogenous dopamine activity.

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73.10

CADMIUM EXPOSURE PREVENTS BEHAVIORAL SENSITIZATION TO MORPHINE. D. Miller, C.L. Livermore and J.R. Nation*. Texas A&M University, College Station, TX 77843.

Adult male rats were exposed to diets containing 100 ppm cadmium chloride (Group Cadmium) or no added cadmium (Group Control) for 45 days prior to testing for the effects of acute and repeated ip morphine administration (10 mg/kg/daily session) on locomotor activity. Relative to saline injected animals, an initial (acute) challenge of morphine on Day 1 of testing decreased activity (total distance traveled) among control animals, but diminished responding was not exhibited by cadmium-exposed animals given morphine. With continued daily administration of morphine or saline for 14 days, the augmented locomotor stimulating effects of morphine (behavioral sensitization) apparent in controls were not evident in cadmium-exposed animals. These findings suggests that cadmium, which exists in high levels in all tobacco products, produces a hyposensitivity to morphine, and this effect may alter drug selection and use.

United States Public Health Grant DA07932

73.11

DELTA-OPIOID RECEPTOR BLOCKADE PREVENTS SENSITIZATION TO THE CONDITIONED REINFORCING EFFECTS OF MORPHINE

A.C. Thompson* & T.S. Shippenberg, Neuroimaging/Drug Action Section, NIDA-ARC, NIH, Baltimore, MD 21224

Opioid antagonists can block the reinforcing effects of cocaine as measured by the conditioned place preference (CPP) paradigm. The present study used an unbiased place preference conditioning procedure to examine the influence of selective δ-opioid receptor antagonists on the development of, and sensitization to, the conditioned reinforcing effects of morphine. Male Sprague Dawley rats received once daily injections of morphine (5.0 mg/kg s.c.) or vehicle on days 1-5 either alone, or in combination with naltrindole or naltriben. Conditioning, consisting of 2 morphine and 2 saline sessions, was conducted on days 8-9 and rats were tested for place preference on day 10. Rats pretreated with morphine alone spent significantly more time on the drug-paired side than on the vehicle-paired side. Low doses of both naltrindole and naltriben (0.03-0.1mg/kg), when paired with morphine pretreatment, blocked the morphine-induced enhancement of CPP. In a second experiment without pretreatment, rats were conditioned for 3 days with morphine + naltrindole or naltrindole alone (3 drug and 3 saline sessions). Naltrindole was ineffective in blocking the development of CPP to morphine, and failed to produce conditioning when given alone. These data suggest that δ-opioid receptors have an important role in modulating sensitization, but are not involved in the development of place preference to morphine. Furthermore, the effectiveness of naltriben, a selective δ2 receptor antagonist, in blocking sensitization suggests the involvment of the δ2 subtype in this effect.

Supported by NIDA Intramural Research Program

73.12

CHRONIC EXPOSURE TO LEAD ALTERS THE INITIATION OF BEHAVIORAL SENSITIZATION TO MORPHINE. C.L. Livermore, J.R. Nation, D.K. Miller, and M. W. Meagher.* Texas A&M University, College Station, TX, 77843-4235.

To date, investigators have largely restricted their analyses of chemical co-treatment effects to naturally occurring or synthetic drugs that present know pharmacologic profiles. However, environmental pollutants may also influence drug sensitivity and therein disturb patterns of selection and use of drugs with substantial abuse potential. Continuing this line of research, the present investigation examined the effects of recurrent lead exposure on behavioral sensitization to morphine. Locomotor activity (total distance traveled) was monitored for control and lead-treated adult male rats (45 days exposure to 250 ppm lead acetate via *ad lib* food supply) across 14 daily one-hour test sessions. In each session, a daily i.p. injection of 10 mg/kg morphine or vehicle was administered for 14 successive days. Post-injection activity was then examined for 80 minutes. On Day 15, all of the animals received saline injections to test for effects due to conditioning components. A 10 mg/kg morphine challenge injection was given to all groups on Day 16 to compare acute and chronic administration of the drug. The findings show that lead exposure retards the initiation of sensitization, without any apparent contextual cueing. These data suggest that xenobiotic contamination may alter drug responsiveness and therein may influence patterns of drug selection and use.

R01DA07932-02

73.13

FLUOXETINE BLOCKS THE EXPRESSION BUT NOT THE DEVELOPMENT OF MORPHINE-INDUCED SENSITIZATION OF ORAL STEREOTYPY IN THE RAT. H.K. Wennemer and C. Kornetsky*. Departments of Pharmacology and Psychiatry, Boston University School of Medicine, Boston, MA 02118.

Repeated high doses of morphine sulfate (MS) administered to a rat causes an intense repetitive biting and gnawing behavior that can be reexpressed by a low dose of MS as long as 6 months after the initial morphine treatment (Pollock and Kornetsky, PB&B, 1996). The expression of this biting behavior has been found to be blocked by both D1 antagonists and the NMDA antagonist MK-801 (Livezey et al. Brain Res., 1995) with the latter also capable of blocking the development of the sensitization. The present experiment was designed to determine the role of serotonin in the acute expression and development of sensitization of morphine-induced stereotypy. Ten F-344 rats were administered four 10 mg/kg doses of MS (s.c.) in a 36 hour period. Half of these were administered 5.0 mg/kg (i.p.) of fluoxetine (FLX), a potent reuptake inhibitor of serotonin, while the rest received the vehicle injections 60 min prior to each of the four MS doses. Following each injection of MS, behavior was rated for 2 consecutive hours by an observer blind to the treatment history. By the final dose of MS the mean duration of stereotypic behavior was 40 min for the MS plus vehicle group and 4 min for the MS plus FLX group. At 1 and 3 weeks after the sensitizing treatment both groups were challenged with 4.0 mg/kg MS. Both groups of rats expressed approximately 75 min of biting with no significant difference between them. At 2 and 4 weeks both groups were challenged with 4.0 mg/kg of MS plus 10.0 mg/kg of FLX, little or no biting was seen in either group. The mean duration of biting in each group was approximately 3 min. These results indicate that although serotonin appears to play a role in the expression of MS-induced stereotypy, it is not involved in the development of sensitization. (Supported by NIDA grants DA02326 and K05-DA00099 to CK).

DRUGS OF ABUSE: OPIOIDS III

74.1

DIFFERENTIAL EFFECTS OF COMPETITIVE AND NON-COMPETITIVE ANTAGONISM ON INTRAVENOUS SELF-ADMINISTRATION IN DRUG-NAIVE MICE. Thøger Rasmussen* and Michael D.B. Swedberg. Novo Nordisk A/S, Behavioral Pharmacology, Novo Nordisk Park, DK-2760 Måløv, Denmark.

Intravenous self-administration in drug-naive mice has the potential for rapidly assessing drug abuse liability. Typically, bell-shaped dose-dependent response curves are obtained with compounds possessing behaviorally reinforcing properties. With the aim of further characterizing the acute nose-poke self-administration model, we investigated the effects of morphine (0.025, 0.125, 0.225, 0.325 and 0.425 mg/kg/infusion) after prior treatment with the opiate antagonist naltrexone (3.0 mg/kg), and nicotine (0.025, 0.075, 0.125, 0.175 and 0.225 mg/kg/infusion) after prior treatment with the nicotine antagonist mecamylamine (0.1, 0.3 and 1.0 mg/kg). Whereas naltrexone shifted the morphine dose-response curve to the right, mecamylamine shifted the lower end of the nicotine dose-response curve to the right but as the dose of nicotine increased, the dose-response curves flattened out. These data support the idea that the mechanisms underlying self-administration in drug-naive mice are receptor mediated. The patterns of antagonism seen here fit the distinction between naltrexone as a competitive antagonist and mecamylamine as a non-competitive antagonist. Funded by Novo Nordisk A/S.

74.2

KAPPA-OPIOID RECEPTORS ANTAGONIZE HEROIN SELF-ADMINISTRATION AND DOPAMINE RELEASE IN THE NUCLEUS ACCUMBENS: AN IN VIVO FAST-CYCLIC VOLTAMMETRY STUDY. Z-X. Xi, S.A. Fuller & E.A Stein*. Dept. of Psychiatry and Behav. Medicine, Medical College of Wisconsin, Milwaukee, WI 53226

Microdialysis and behavioral studies have demonstrated that μ and κ opioid agonists exert different and/or opposite effects on basal mesolimbic dopamine (DA) release and rewarding/aversive stimulus properties. However, changes in DA release during heroin self-administration (SA), possible interactions between μ and κ receptors in the mesolimbic DA system, and the relationship between mesolimbic DA transmission and heroin-induced reinforcing effects are still unclear. By monitoring extracellular DA changes in the nucleus accumbens (NAcc) with fast-cyclic voltammetry (Nafion-coated 8μm carbon fiber electrode) combined with heroin-SA (0.06, 0.1 & 0.2mg/kg/inj), we observed: (1) dose-dependent monophasic increases in the DA-dependent electrochemical signal in NAcc during both heroin-SA and passive administration; (2) pre- or co-administration with U50,488H (a selective κ opioid agonist, 0.025-0.05mg/kg/inj, iv) or pretreatment with dynorphin A (1-2 μM/μl, icv) dose-dependently depressed heroin-induced DA release in the NAcc during heroin-SA; (3) pretreatment with Nor-BNI (a selective κ receptor antagonist, 1-2 μM, icv) significantly potentiated heroin-evoked DA release; (4) 0.025 mg/kg/inj U50,488H or dynorphin A (2μM, icv) significantly increased heroin-SA, while 0.05 mg/kg/inj U50,488H co-administered reduced or completely blocked heroin-SA; (5) Nor-BNI (1-2μM, icv) also slightly increased heroin-SA. These findings that activation of central κ receptors can antagonize both heroin-induced DA release in the NAcc and heroin SA behavior suggest that κ receptors may play a role in modulating heroin reinforced behaviors. Supported by NIDA/INVEST Fellowship N01DA-3-0002.

74.3

EFFECTS OF NALTREXONE ON HEROIN- AND COCAINE-MAINTAINED BEHAVIOR IN RHESUS MONKEYS RESPONDING UNDER A PROGRESSIVE-RATIO SCHEDULE. J. K. Rowlett* and W. L. Woolverton. Dept. Psychiatry & Human Behav., U. Mississippi Med. Ctr., Jackson, MS 39216.

The opioid receptor antagonist naltrexone has been shown to block heroin-maintained behavior and either suppress or not alter cocaine-maintained behavior. This study assessed the effects of naltrexone on the reinforcing efficacy of heroin and cocaine using a progressive-ratio (PR) schedule. Four rhesus monkeys were prepared with intravenous catheters and trained to press a lever for cocaine injection. The PR schedule consisted of a 5-component daily session, each component with 4 trials at the same fixed ratio (FR). The FR sequence was 120, 240, 480, 960, 1920. Trials were separated by a 30 min time-out. A session ended when all injections were taken or 2 consecutive FRs were not completed (limited hold=15 min). Behavior was maintained on a single-alternation sequence in which drug (cocaine, 0.10 mg/kg/inj or heroin, 0.013 mg/kg/inj) and saline sessions alternated. When inj/session were stable (3 drug and 3 saline sessions with ≤ 10% variation and no upward or downward trends), test sessions were conducted according to the sequence: DSTDSDT (D=drug, S=saline, T=test). Naltrexone (0.001-0.8 mg/kg, i.m.) or saline (0.1 ml/kg, i.m.) was given 10 min before the test session, in which heroin (0.0010-0.025 mg/kg/inj) or cocaine (0.013-0.10 mg/kg/inj) was available. Naltrexone dose-dependently decreased heroin-maintained behavior (0.013 mg/kg/inj) to saline levels. Naltrexone (0.006 mg/kg) produced a rightward shift in the heroin dose-response function, with a mean apparent pK_B value of 8.0 (range=7.7-8.3), consistent with μ-opioid receptor antagonism. Naltrexone had no effect on cocaine-maintained behavior at doses over 100 times higher than those that antagonized heroin-maintained behavior. These results indicate that naltrexone reduced the reinforcing efficacy of heroin, but this reduction was surmounted by increasing the heroin dose (i.e., competitive antagonism). Naltrexone did not alter cocaine-maintained behavior, indicating no involvement of opioid receptors in the reinforcing effects of cocaine. Supported by NRSA DA-05625 from NIDA (JKR).

74.4

THE EFFECTS OF DIETARY MACRONUTRIENT MANIPULATIONS ON INTRAVENOUS MORPHINE SELF-ADMINISTRATION.
B.A. Gosnell*, D.D. Krahn, J.M. Yracheta and B. J. Harasha.
Department of Psychiatry, University of Wisconsin-Madison, Madison, WI 53719.

Manipulations of diet composition have been shown to influence opioid peptides, opioid receptors and the analgesic response to morphine. It is not known, however, whether changes in diet composition influence the self-administration of opiates. We therefore conducted an experiment to determine the effects of such manipulations on morphine self-administration. Jugular catheters were implanted in male rats (n=8). After recovery, morphine self-administration was measured in daily 18 hr sessions (FR 1 schedule, 0.1 mg/kg/infusion). During the session and during the 6 hr spent daily in the home cage, water and powdered lab chow were available. After self-administration stabilized, the lab chow was replaced with either a high-fat diet (FAT) (80% fat, 20% protein) or a high-carbohydrate diet (CARB) (80% carb., 20% protein). All rats were tested for 7 days on each diet, with a 7 day period of availability of only lab chow intervening between the test diet phases. Order of presentation of the diets was counter-balanced. Mean daily caloric intakes were 71 ± 3, 80 ± 4 and 106 ± 8 kcal in the chow, CARB and FAT conditions, respectively; these represent increases over chow intake of 12 % (CARB) and 49 % (FAT). Maintenance on the CARB diet caused a slight but non-significant decrease in the number of infusions obtained; maintenance on the FAT diet had no detectable effect on the number of infusions. Mean numbers of infusions were 86 ± 10, 72 ± 5 and 89 ± 10 for the chow, CARB and FAT conditions, respectively. These results indicate that imposed changes in the macronutrient composition of the maintenance diet have only minimal effects on concurrent morphine self-administration. This finding does not, however, rule out the possibility that baseline macronutrient preferences may be useful as predictors of morphine self-administration. Possible relationships between diet preferences and self-administration are currently under investigation. Supported by NIDA DA05471 and DA00210.

74.5

CAN THE REWARDING EFFECTS OF DIFFERENT DRUGS BE DETERMINED USING A PROGRESSIVE RATIO REINFORCEMENT SCHEDULE IN A DRUG SELF ADMINISTRATION MODEL? C.L. Duvauchelle*, T. Sapoznik and C. Kornetsky. Departments of Psychiatry and Pharmacology, Boston University School of Medicine, Boston, MA 02118.

In order to compare the relative reinforcement value of cocaine alone, heroin alone and their combination ("speedball"), a progressive ratio (PR) reinforcement schedule of the respective drugs was used to determine breakpoints. The initial training for all rats was on a combined dose of 18 µg/kg/inj of heroin (H) and 300 µg/kg/inj of cocaine (COC). Breakpoints for the training dose and individual component doses were determined for both half and double the training dose. Of the three doses of each treatment, only cocaine self-administration yielded the expected monotonic increase in breakpoint as a function of the self-administered dose. Also the breakpoint for COC was greater than for the combination of COC and H. Because these results suggested that H plus COC is less rewarding than is COC alone, we determined the doses for these three drug treatments that produced saline level responding. 1.2 H and 18.8 COC (µg/kg/inj) combined, and the same dose of COC alone produced breakpoints indistinguishable from saline. 4.5 µg/kg was the highest H dose resulting in a breakpoint no different than saline. However, a speedball combination of 2.3 µg/kg of H plus 37.5 µg/kg of COC, produced a breakpoint significantly higher than that for the COC component alone. These results suggest that a PR reinforcement schedule is not a reliable measure of relative rewarding value between drugs. In addition, because increased motor performance equals increased reward in this schedule, it is extraordinarily vulnerable to drug-induced influences on motor activity. Supported by NIDA grants DA02326 and K05-DA00099 to CK

74.6

RESPONSES OF NUCLEUS ACCUMBENS AND VTA NON-DOPAMINE NEURONS TO HEROIN IN FREELY-MOVING RATS. R.-S. Lee*, P. Griffin, S. C. Steffensen, S. Casalman, and S. J. Henriksen. The Scripps Research Institute, La Jolla, CA 92037.

The nucleus accumbens (NAcc) has been hypothesized to function as an integrator of motor and complex limbic information. Other evidence indicates that the NAcc and ventral tegmental area (VTA) may be critical sites for reward-seeking behaviors. To further investigate NAcc-related and VTA-related physiological correlates of drug-seeking behaviors, we have employed the following paradigm: Naive rats were allowed to explore freely in an operant chamber and to self-administer heroin intravenously (via an indwelling catheter) through nosepoking behavior (FR-1 schedule). Behaviorally related and drug dependent changes in neuronal activity of these regions were recorded using stainless steel microwires (62 µm) implanted into the NAcc and/or VTA under anesthesia. Our observations indicate that: 1) The acquisition of heroin self-administration (0.06 mg/kg/injection) is associated with specific changes in NAcc neuronal activity, beginning from as few as four 2 hour daily sessions of nosepoking behavior; 2) Presumptive VTA non-dopamine neurons (criteria: spike duration < 500 µs, and histological identification) also are inhibited during heroin self-administration behavior; 3) VTA non-dopamine neurons appear to be more sensitive than are NAcc to non-contingent heroin injection (0.2 mg/kg, i.v.); and 4) acute tolerance (desensitization) of NAcc neuron and presumptive VTA non-dopamine to heroin self-administration occurrs during repeated, chronic heroin self-administration sessions. These data suggest that neuronal discharges in NAcc and in VTA are altered in a complex fashion during the acquisition and maintenance of heroin self-administration behavior. (Supported by DA-08301 to SJH).

74.7

ENSEMBLE NEURAL ACTIVITY IN THE NUCLEUS ACCUMBENS (NAc) AND PREFRONTAL CORTEX (PFC) OF THE AWAKE RAT DURING FOOD AND WATER REINFORCEMENT, AND HEROIN SELF-ADMINISTRATION. *RV Subrahmanyam*, JM Paris, J-Y Chang, DJ Woodward.* Program in Neuroscience and Dept. of Physiology/Pharmacology, Bowman Gray School of Medicine, Winston-Salem, NC 27157.

The purpose of this study was to analyze and compare the firing patterns elicited by different reinforcers in regions of the mesolimbic reward circuit. Adult male rats were surgically implanted with chronic multiwire recording electrodes into the NAc and PFC. Neural activity was examined while awake rats performed an operant task under a multiple FR5/FR1 schedule maintained by food and water. Neural activity was examined at specific nodes in the task sequence, including two conditioned stimuli (a light indicating lever activation and a tone signaling reinforcer delivery). A typical rat recorded over 5 sessions yielded a total of 36 NAc and 20 PFC neuron-sessions. Four patterns of neural activity were observed in both the NAc and PFC during food and water reinforcement: 1) increases in firing rate during lever-pressing; 2) increases *prior to* light presentation (anticipatory excitatory activity); 3) decreases *prior to* tone presentation (anticipatory inhibitory activity); and 4) decreases *during* tone presentation. The responses were relatively less robust in the same neurons during drinking segments. Increased activity during pellet consumption was also observed in both regions. Finally, increases and decreases in firing rate before lever-pressing (anticipatory activity) were observed in the NAc in a separate group of animals during heroin self-administration. This procedure allows a direct comparison of food- and heroin-reinforced neural patterns in the same animal under a multiple FR5/FR5 schedule. From these data, it appears that neural signals exist in the mesolimbic reward circuit that are specific to individual reinforcers as well as those which appear general across reinforcers. *Supported by NIDA-DA2338 (DJW). Poster available at http://biogfx.neuro.wfu.edu/*

74.8

NMDA ANTAGONIST, MK-801, ANTAGONIZES THE DEVELOPMENT MORPHINE-INDUCED CONDITIONED PLACE PREFERENCE. M. C. Clavier, W.L. Nores, R.D. Olson & A. L. Vaccarino*, Department of Psychology, University of New Orleans, LA 70148.

Several lines of evidence show a critical role of the *N*-methyl-D-aspartate (NMDA) receptor in the development of morphine-induced tolerance and analgesia. In the present study we examined the involvement of the NMDA receptor in the development of morphine-induced conditioned place preference (CPP). The CPP apparatus consisted of a grey start-box and two treatment compartments (white/wood chip floor and black/wire grid floor). On the day before conditioning, rats were placed in the start-box and allowed 15 min free access to both compartments to establish a baseline preference. Rats received 2 conditioning trials per day (6 h apart) on days 1, 3, 5, 7 and 9. On one conditioning trial rats were injected ip with MK-801 (0.1 mg/kg) + morphine (5 mg/kg), MK-801 + saline, saline + morphine, or saline + saline and were confined to the non-preferred side for 50 min. On the other conditioning trial rats received saline + saline and were confined to the other compartment for 50 min. The order of conditioning trials was counterbalanced. To examine the development of CPP rats were placed in a drug-free state in the start-box and allowed free access to both compartments for 15 min on days 2, 4, 6, 8 and 10. Morphine produced a significant CPP after one conditioning session (i.e., day 2), which increased after the second conditioning session (i.e., day 4), and then steadily decreased on subsequent trials. MK-801 did not affect morphine CPP on day 2, but MK-801 attenuated morphine CPP on subsequent days. MK-801 alone did not produce any changes. These results suggest that MK-801 disrupts the learning of the association between morphine and the environment, but does not affect morphine reward.

74.9

AGONIST AND ANTAGONIST EFFECTS OF *MU* OPIOIDS IN RATS TRAINED TO DISCRIMINATE A HIGH DOSE OF FENTANYL FROM SALINE. L. Zhang, M. Evola and A. M. Young*. Department of Psychology, Wayne State University, Detroit, MI 48202.

We studied the discriminative stimulus effects of a high training dose of the high efficacy μ agonist fentanyl. Male Sprague-Dawley rats (N=16) were trained to discriminate 0.04 mg/kg of fentanyl from saline under a fixed-ratio 15 schedule of food delivery. The higher efficacy μ agonists etonitazene, fentanyl, methadone, and morphine evoked full fentanyl-lever responding and suppressed response rates in the same potency order (etonitazene > fentanyl > methadone >= morphine). The lower efficacy μ agonists nalbuphine and nalorphine evoked less than 50 percent fentanyl-lever responding. The κ agonist spiradoline and the nonopioids *d*-amphetamine and ketamine evoked saline-lever responding and suppressed rates. *In vivo* apparent pA2 analysis showed that nalbuphine (3.2-32 mg/kg) and nalorphine (1.0-10 mg/kg) antagonized the stimulus effects of fentanyl with an apparent pA2 of 5.4 for nalbuphine and 6.2 for nalorphine. Naltrexone (0.01-0.32 mg/kg) also antagonized stimulus and rate-altering effects of fentanyl with an apparent pA2 of 7.7. In concert with previous findings from our laboratory that nalbuphine and nalorphine evoked full stimulus effects of 0.01 mg/kg fentanyl (Sutherland *et al.*, 1995, *Soc. Neurosci. Abstr.* 21: 727), these results suggest that the training dose of fentanyl can modulate the likelihood that certain μ opioids (e.g., nalbuphine, nalorphine) will evoke or antagonize the stimulus effects of fentanyl. For nalbuphine and nalorphine, their observed antagonism of the stimulus effects of high-dose fentanyl is likely due to low efficacy μ agonist actions. [Supported by DA03796, KO2 DA00132, GM08167]

74.10

ASSESSMENT OF THE STIMULUS PROPERTIES OF MDA AND MDMA STEREOISOMERS IN A LSD-SALINE-AMPHETAMINE DISCRIMINATION Michele M. Taylor and Lisa E. Baker. Department of Psychology, Western Michigan University, Kalamazoo MI 49008

The stereoisomers of 3,4-methylenedioxyamphetamine (MDA) and 3,4-methylenedioxymethamphetamine (MDMA) have been demonstrated to differ in the extent to which they produce stimulus generalization in subjects trained to discriminate between either amphetamine or a hallucinogen and a vehicle. Inconsistencies among previous findings seem to indicate that the extent to which the discriminative stimulus effects of the optical isomers of both MDMA and MDA are amphetamine or hallucinogen-like depends on the training drug and the discrimination procedures utilized. The present study employed a three-choice discrimination procedure in order to further delineate the discriminative stimulus properties of the stereoisomers of MDA and MDMA. Eight male Sprague-Dawley rats were trained to discriminate amphetamine (1.0 mg/kg) and LSD (.08 mg/kg) from saline in a three-lever, food reinforced (sweetened condensed milk) drug discrimination procedure. A fixed-ratio (FR) 20 schedule with a reset condition for incorrect responses was employed. When criteria (80% over 10 consecutive sessions) were met, (+)-MDA, (-)-MDA, (+)-MDMA (.31-1.25 mg/kg) and (-)-MDMA (.88-3.5 mg/kg) were tested for substitution. All of these compounds elicited a greater percentage of responding on the LSD lever than on the amphetamine lever. These results suggest that the discriminative stimulus properties of both MDMA and MDA isomers resemble those of LSD more closely than those of amphetamine. It is suggested that the use of a three-lever discrimination procedure affords a greater degree of precision in the assessment of the compound stimulus properties of these designer drugs.

74.11

INDIVIDUAL DIFFERENCES IN THE EFFECTS OF COCAINE AND AMPHETAMINE IN SQUIRREL MONKEYS DISCRIMINATING MORPHINE. Doreen M. Grech* and Roger D. Spealman, Harvard Medical School, NERPRC, Southborough, MA 01772-9102.

The effects of cocaine and amphetamine were studied in squirrel monkeys trained to discriminate morphine from vehicle using a two-lever drug-discrimination procedure. In about half the subjects tested, both cocaine and amphetamine substituted for morphine in a dose-related manner and enhanced the discriminative stimulus effects of morphine when given as a pretreatment. In the remaining subjects, neither cocaine nor amphetamine substituted appreciably for morphine and typically attenuated the effects of morphine when given as a pretreatment. The former, but not the latter, subjects also showed full or partial substitution for morphine when tested with the dopamine transport inhibitor GBR 12909 and the delta opioid agonist BW 373U86. All subjects showed full substitution for morphine when tested with the mu opioid agonist fentanyl and none of the subjects showed consistent substitution for morphine when tested with the norepinephrine transport inhibitor talsupram, the serotonin transport inhibitor citalopram, or the kappa opioid agonist U50488H. The results suggest a role for both dopaminergic and delta opioid mechanisms in the morphine-like stimulus effects of cocaine and amphetamine in individual monkeys. Supported by DA 00499, DA 06303, MH 07658, MH 14275 and RR00168.

74.12

STIMULUS PROPERTIES OF A DEXTROMETHORPHAN-DIPHENHYDRAMINE COMBINATION IN RATS

S.A. Vanecek[1]*, D.V. Gauvin[1], C.A. Sannerud[2], K.D. Hutchinson[2], R.I. Briscoe[1], T.J. Baird[1], M. Vallett[1], K.L. Carl[1], & F.A. Holloway[1]; [1]Dept Psychiat & Behav Sci, O.U.H.S.C., Okla. City, OK 73190 & [2]Drug & Chem. Eval. Section, Drug Enforcement Admin.,Washington,D.C. 20537

The stimulus properties of a combination of dextromethorphan and diphenhydramine (DEXTROMETH + DIPHEN) were evaluated using a two-choice discrimination task and a self-administration task in rats. Twelve male Sprague-Dawley rats were trained to discriminate between 10 mg/kg DEXTROMETH + 10 mg/kg DIPHEN versus saline in a food-motivated lever-press task. Once trained, each rat demonstrated a dose-dependent increase in drug-lever responding with increasing increments in the test combinations. Partial cross-generalization was engendered by both cocaine and *d*-amphetamine. Some rats demonstrated complete cross-generalization to low cocaine doses (1.0 - 3.2 mg/kg), while others cross-generalized to the higher doses (10-32 mg/kg). The drug discrimination data suggested that a "stimulant" dimensionality was produced by the DEXTROMETH+DIPHEN combination in rats.

Three-day substitution tests were conducted with various combinations of DEXTROMETH+DIPHEN in 4 rats trained to self-administer 0.5 mg/kg/inj. i.v. cocaine in daily 4 hr sessions. This combination failed to maintain avid self-administration. These data suggested that, while the combination of DEXTROMETH+DIPHEN could produce subjective effects that cross-generalized to those of cocaine and amphetamine test stimuli, the abuse potential assessed by self-administration appeared to be minimal. Therefore, the predicted abuse liability of these OTC combinations are, at best, equivocal.

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74.13

CONDITIONED TASTE AVERSION AND DRUGS OF ABUSE: A REINTERPRETATION. P.S. Grigson*, H. Li, K. Smith, P. Smith, P. Lyuboslavsky, and R. Norgren. Department of Behavioral Science, College of Medicine, The Penn State University, Hershey, PA 17033.

Rats treat morphine and cocaine as rewards. They will increase running speed, bar pressing, and preference for a compartment when associated with either drug. Paradoxically, however, the same drugs appear to function as aversive unconditioned stimuli (US) in a conditioned taste aversion (CTA) paradigm. The following data support a new hypothesis that potentially synthesizes these discrepant findings. That is, following saccharin-morphine pairings, for example, rats decrease intake of a saccharin CS, not because of the aversive properties of the drug, but because the reward value of the saccharin CS is outweighed by that of the morphine. Two experiments showed that morphine and cocaine selectively suppressed intake of a palatable CS (saccharin), but not of a more neutral CS (NaCl). In the same paradigm, a LiCl US reduced intake of both gustatory CSs. Two other experiments demonstrated that the suppressive effects of morphine, but not LiCl, increased as the concentration, and presumably the palatability, of the saccharin CS was increased. Together, the results support a reward comparison, rather than a CTA, account. Specifically, they show that the suppressive effects of the drugs of abuse on CS intake depend upon the well-established rewarding, rather than upon the hypothetical, aversive, properties of the US. Supported by NIH grants DC 02016, DC 00240, and MH 00653.

74.14

SHORT TERM MEMORY ABILITIES APPEAR TO BE DISRUPTED IN HUMANS WITH EXPOSURE TO METHADONE. M.C. Fratzke*, T.L. Anderson, J.L. Perez, J.K. Shepherd, and H.L. Roitblat. University of Hawaii, Psychology Department, Honolulu, HI 96822

Present data suggest that methadone disrupts short term memory. Clearly, this may be detrimental to the effectiveness of substance abuse counseling, and possibly contributes to a poor prognosis. This study of short term memory demonstrates some of the possible consequences of methadone use on performance and cognitive functioning.

To provide a better understanding of the effects of chronic exposure to methadone, the study compares fifty-five volunteer subjects enrolled in a methadone maintenance program, all of whom have at least two weeks of methadone use as part of their treatment for opiate dependence, ninety-two subjects with no documented drug use and sixteen subjects enrolled in a "drug free" substance abuse treatment program. Subjects' short term memory was assessed with a modified form of the digit-span memory test (LETTSPAN). This instrument provides separate measures of the subjects' ability to recall items and to remember a given item's position in a sequence. Subjects who were administered methadone as part of their treatment scored significantly lower on the ability to recall the identity of items and to recall an item's position in a sequence, presented either successively or simultaneously. These results call for a reevaluation of the potentially disruptive effects of methadone on memory, effects which have not been fully delineated.

74.15

OPIOID EFFECTS ON EXCITATORY AFFERENT-DRIVEN NEURONAL ACTIVITY IN THE MEDIAL PREFRONTAL CORTEX. J.L. Giacchino* and S.J. Henriksen, Dept. of Neuropharmacology, The Scripps Research Institute, La Jolla, CA 92037.

The medial prefrontal cortex (mPFC) is a significant component of the reinforcement pathways which contribute to development and maintenance of opiate addiction. We previously reported that the spontaneous activity of mPFC neurons and their response to excitatory neurotransmitters are modified by both systemic and electrophoretic administration of mu-selective opioid agonists. We have now investigated the effects of these opioids on mPFC neuronal activity evoked by stimulation of major afferents including putative glutamatergic input from both mediodorsal thalamus (Gigg et al, 1992; Pirot et al, 1994) and CA1/subiculum (Jay et al, 1992; Gigg et al, 1994), as well as the basolateral amygdala (Pérez-Jaranay & Vives, 1991; Matsuda & Fujimara, 1995).

Extracellular recordings were made from neurons in the prelimbic subarea of the mPFC in halothane-anesthetized rats. Electrical stimulation of the mediodorsal thalamus, CA1/subiculum and the basolateral amygdala evoked short latency orthodromic single cell activity (3-8 msec, 6-14 msec, 8-15 msec, respectively). Systemic morphine (2.5mg/kg, sc) significantly decreased the response of mPFC neurons activated by stimulation of the thalamus and amygdala. This effect was reversed by naloxone (5mg/kg, sc). The effect of an electrophoretically-applied mu opioid agonist on driven mPFC neuronal firing was also investigated. Preliminary data indicates that local electrophoretic application of DAMGO (1 mM) does not affect the driven activity of mPFC neurons, although spontaneous activity may be altered. These data argue that the effect of systemic opiates on mPFC neuronal activity may be mediated through their effects on excitatory afferent inputs, although local effects at mPFC mu opioid receptors cannot be excluded.

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74.16

PHARMACOLOGICAL CHARACTERIZATION OF OPIATE INTERACTIONS WITH NAcc NEURONS IN VIVO. J.R. Criado*, G.I. Berg, R.-S. Lee, S. C. Dulawa and S.J. Henriksen, Department of Neuropharmacology, The Scripps Research Institute, La Jolla, CA 92037. Department of Neuroscience, U. California San Diego, La Jolla, CA 92093.

We previously reported electrophysiological data obtained in vivo suggesting that the effects of systemically administered opiates on NAcc neurons must occur through both the VTA dopaminergic projection to the NAcc, as well as directly within the NAcc (J. Neurosci.,9:3538). To characterize further the effects of heroin on limbic-NAcc inputs, we studied the effects of heroin and [D-Ala^2,NMe-Phe^4,Gly-ol]-Enkephalin (DAMGO), a selective μ agonist, on amygdala- and glutamate-activated NAcc neurons. Our results showed that while local application of DAMGO (1.0 mM) and systemic heroin (0.5 mg/kg, s.c.) suppressed only 25% of the recorded amygdala-activated NAcc neurons, these compounds markedly inhibited glutamate-activated NAcc neurons. Furthermore, MK-801 (0.5 mg/kg, i.p.), a selective non-competitive NMDA antagonist, or apomorphine (100 μg/kg, i.p.), a mixed dopamine receptor agonist, had no effect on amygdala-activated NAcc neurons that were insensitive to heroin. In a subgroup of NAcc neurons that were characterized by stable spontaneous activity and a reliable excitatory response to amygdala stimulation, heroin markedly suppressed spontaneous activity, but it had no effect on amygdala-activated NAcc activity. In these neurons, heroin-induced suppression of spontaneous activity was clearly reversed by naloxone (5 mg/kg, i.p.). However, amygdala-activated NAcc neurons that were inhibited by heroin were not reversed by naloxone. These findings suggest that heroin-induced suppression of spontaneous activity in the NAcc is mediated primarily by μ receptors, but opioid-induced effects on excitatory afferent inputs might be mediated indirectly via other as yet unidentified mechanisms (Supported in part by DA-08301 to SJH).

75.1

RISPERIDONE INCREASES SEROTONIN RELEASE IN THE RAT FRONTAL CORTEX VIA α_2-ADRENOCEPTOR ANTAGONISM IN THE NERVE TERMINAL REGION

P. Hertel*, G.G. Nomikos, B. Schilström, L. Arborelius and T.H. Svensson. Dept. of Physiology and Pharmacology, Div. of Pharmacology, Karolinska Institutet, S-171 77 Stockholm, Sweden.

We have previously shown that risperidone (RISP), an antipsychotic drug with high affinity for 5-hydroxytryptamine (5-HT)$_{2A}$ and dopamine (DA)$_2$ receptors as well as for α_1 and α_2 adrenoceptors, enhances 5-HT metabolism selectively in the rat frontal cortex (FC). To further study the influence of RISP on central 5-HT systems, we compared its effects on 5-HT release in the FC with those obtained with other antipsychotic drugs, i.e. clozapine (CLOZ), haloperidol (HAL) and amperozide (AMP), as well as, with the selective α_2 or 5-HT$_{2A}$ receptor antagonists idazoxan (IDAZ) or MDL 100907 (MDL), respectively. The underlying mechanism for the stimulatory action of RISP on 5-HT release in the FC was also analyzed. RISP (0.2, 0.6 and 2.0 mg/kg, s.c.) dose-dependently increased 5-HT levels in the FC measured by microdialysis. This facilitatory effect was mimicked by AMP (10.0 mg/kg, s.c.) and, to some extent, by IDAZ (0.25 mg/kg, s.c.). In contrast, CLOZ (10.0 mg/kg, s.c.), HAL (2.0 mg/kg, s.c.) and MDL (1.0 mg/kg, s.c.) exerted only minor effects on 5-HT release in brain. Local administration of RISP or IDAZ (1-1000 μM) in the FC dose-dependently increased 5-HT release in this area. Using single cell recording techniques, it was found that RISP (25-800 μg/kg, i.v.) dose-dependently decreased the firing rate of 5-HT cells in the dorsal raphe nucleus (DRN), an effect that was largely antagonized by pretreatment with the selective 5-HT$_{1A}$ receptor antagonist WAY 100635 (5μg/kg, i.v.). These results indicate that the increased 5-HT release in the FC by RISP is related to its α_2 adrenoceptor antagonistic action, a property shared with both AMP and IDAZ, and seems to be executed at the nerve terminal level. The inhibition of 5-HT cell firing by RISP is probably secondary to increased 5-HT release, e.g. in the DRN, since it was antagonized by a 5-HT$_{1A}$ autoreceptor antagonist. The enhanced 5-HT release in the FC by RISP may be of particular relevance for the treatment of schizophrenia when associated with depression and in schizoaffective disorder. Supported by the Swedish Medical Research Council (4747), Karolinska Institutet and Janssen Pharmaceutica Ltd, Beerse.

75.2

DIFFERENTIAL INDUCTION OF CHRONIC AP-1 BINDING ACTIVITY BY TYPICAL AND ATYPICAL ANTIPSYCHOTICS.

P. Rogue*, G. Vincendon, A. N. Malviya. UPR416-CNRS, Center for Neurochemistry, 5 rue Blaise Pascal, Strasbourg 67084 France.

Using gel-shift assays, we studied the effect of antipsychotics on AP-1 binding activity in the CNS. A single injection of haloperidol (2 mg/kg I.P.), which transiently induces c-*fos* and *jun* B expression in the striatum (Str) and nucleus accumbens (NAc) of the rat, produces a transient increase in AP-1 binding activity in these regions. Acute clozapine (20 mg/kg) raises c-*fos* mRNA and AP-1 activity in the NAc and in the prefrontal cortex (PFC). The therapeutic effects of antipsychotics are delayed, and the effects of chronic treatments were studied. After prolonged haloperidol administration (2 mg/kg for 15 days), though c-*fos* and *jun* B induction in the Str and NAc were dessensitized, AP-1 binding activity remained elevated. Similar results were found in the NAc and PFC after prolonged clozapine treatment. *Jun* D expression was slightly but significantly incresed in the Str by chronic haloperidol. Thus prolonged antipsychotic administration alters the composition of AP-1 transcription factor complex in a regionally specific maner.

75.3

INDUCTION OF FOS PROTEIN IMMUNOREACTIVITY IN THE RAT FOREBRAIN BY THE DOPAMINE D3-SELECTIVE LIGAND NNC 00-3094

T.S. Ludvigsen[1], P.J. Larsen[2], N. Korsgaard[1], R. Hohlweg[1], M.A. Scheideler[1] and A. Fink-Jensen[1]*. [1]Health Care Discovery, Novo Nordisk A/S, DK-2760 Måløv, Denmark and [2]Institute of Medical Anatomy, University of Copenhagen, Denmark.

The benzindole NNC 00-3094 has been identified as a dopamine D3 receptor selective ligand in radioreceptor binding experiments at the cloned dopamine receptor subtypes (Scheideler et al., Soc Neurosci Abstr, 252.13, 1995). In vivo, NNC 00-3094 exhibited a neuroleptic-like profile in several preclinical tests suggestive of antipsychotic activity without inducing the motoric side effects often associated with antipsychotic medication (Fink-Jensen et al., Soc Neurosci Abstr, 252.14, 1995). In the present study, we have used FOS protein immunohistochemistry as a functional index of the effect of NNC 00-3094. NNC 00-3094 (0.1, 1 and 10 mg/kg s.c.) induced a strong FOS protein immunoreactivity in the limbic medial prefrontal cortex and shell region of the nucleus accumbens and only a very weak effect in the non-limbic dorsolateral striatum at the highest dose (10 mg/kg). In contrast, the partial selective dopamine D3 agonist quinpirole (0.1 and 1 mg/kg s.c.) was without effect. The NNC 00-3094 induced specific pattern of FOS protein immunoreactivity in these forebrain areas resembles the effect of the "atypical" neuroleptic clozapine. The distribution is in concordance with the regional distribution of dopamine D3 receptors and with the pharmacological effects of NNC 00-3094.
This research was supported by Novo Nordisk A/S.

75.4

THE ADENOSINE A_{2A} RECEPTOR AGONIST CGS 21680 AS A POTENTIAL "ATYPICAL" ANTIPSYCHOTIC IN ANIMAL MODELS.

R. Rimondini, S. Ferré*, S. O. Ögren and K. Fuxe. Dept. of Neuroscience, Karolinska Institute, S-171 77 Stockholm, Sweden.

We have previously found experimental evidence for a predominant antagonistic interaction between adenosine A_{2A} and dopamine D_2 receptors in the ventral compared to the dorsal striatum. It was therefore postulated that adenosine A_{2A} agonists could have antipsychotic properties with an "atypical" profile (low extrapyramidal side effects liability), since the antipsychotic and extrapyramidal side effects of neuroleptics seem to be mediated, at least partially, by the blockade of dopamine D_2 receptors in the ventral and dorsal striatum, respectively. The systemic (i.p.) administration of CGS 21680 was found to dose-dependently antagonize spontaneous and amphetamine-induced (1 mg/kg i.p.) motor activity with a similar ED50 value (about 0.2 mg/kg). High doses of CGS 21680 were needed to induce mild catalepsy with the bar test and it never reached a significant effect with the grid test. The highest dose of CGS 21680 used, 10 mg/kg, produced much less catalepsy than haloperidol 1 mg/kg i.p. With that high dose the animals were clearly hypotonic and they had signs of pronounced peripheral side effects (tachycardia and diarrhea). A high ratio between the ED50 value for induction of catalepsy and the ED50 value for antagonizing amphetamine-induced motor activity was therefore obtained (>20). It has been suggested that PCP-induced behavioural effects in animals represent an animal model of schizophrenia, since it reproduces both positive and negative symptoms of schizophrenia, while amphetamine only mimics positive symptoms. CGS 21680 induced a stronger depressant effect on the motor activity induced by PCP (2 mg/kg s.c.) than on the motor activity induced by an equipotent dose of amphetamine (1 mg/kg i.p.) (ED50 values of 0.03 versus 0.2 mg/kg, respectively). In conclusion, the present results show a clear "atypical" antipsychotic profile of the adenosine A_{2A} agonist CGS 21680 in animal models.
(Work supported by MFR and Wenner Gren and Marianne and Marcus Wallenberg Foundations)

75.5

ELUCIDATION OF ANTIPSYCHOTIC MECHANISMS BASED ON ASSUMPTIONS OF BRAIN STATE: A MULTIVARIATE APPROACH

N Waters*, E S Holm, P Martin, ML Carlsson, A Carlsson and L O Hansson
Dept. of Pharmacology, Göteborg University, Medicinaregatan 7, 413 90 Göteborg, Sweden

Due to the interconnectivity of brain structure, antipsychotic drugs must have effects on all neurons in the brain. They create a new, transient, equilibrium of brain state which is expressed behaviourally. Depending on the initial brain state antipsychotic drugs may have different global effects. To characterise drug effects, and accepting these assumptions one has to measure many variables at different locations in the brain, but also monitor drug effects at different initial brain states and use data evaluation methods that are capable of recognising systematic patters in large data sets.

Our approach to the elucidation of drug action is based on the glutamate hypothesis of schizophrenia, some recent biochemical findings in post-mortem brain tissue of schizophrenics and effects of NMDA-receptor antagonists in the rat. Data are investigated by means of multivariate evaluation techniques. This approach offers a link between pathological biochemical aberrations in man and biochemical changes in rodents under experimental conditions along with a description of global effects of drugs in the normal and hypoglutamatergic brain state.

We find that NMDA receptor antagonists can induce a brain state resembling schizophrenia. Antipsychotics can partially normalise the hypoglutamatergic brain state via different mechanisms. Neuroleptics introduce other perturbations that counteract normalisation. Atypical antipsychotics interfere with a mechanism that is operative in the hypoglutamatergic, but not in the normal brain state. Neuroleptics block the utilisation of this mechanism.

The Stanley foundation is gratefully acknowledged for financial support.

75.6

MULTIVARIATE MODELLING OF RELATIONS BETWEEN MONOAMINERGIC BRAIN BIOCHEMISTRY, THALAMIC GAD mRNA AND BEHAVIOUR: EFFECTS OF ANTIPSYCHOTICS.

ES Holm*, N Waters, A Carlsson, P. Martin, LO Hansson and ML Carlsson.
Dept of Pharmacology, Göteborg University, Medicinareg 7, S-413 90 Göteborg, Sweden.

The thalamus appears to have a crucial position in the cortico-subcortical feedback circuitry, regulating the incoming flow of information from the sense organs to protect the cortex from over exposure and to focus attention. The central thalamic nuclei are controlled by GABAergic projections from the thalamic reticular nucleus. The activity of these GABA neurons may be reflected in GAD mRNA levels, which have been found to change quickly in response to several psychoactive drugs. Thus GAD mRNA might be used as an indicator of influence on thalamic function.

To further explore the significance of thalamic GAD mRNA, the functional relationship between monoaminergic brain biochemistry, thalamic GAD mRNA and locomotor activity was evaluated using multivariate techniques. Studies were performed on rats receiving various antipsychotic drugs as well as on non-treated rats.

A robust relationship between locomotor activity and the biochemical variables measured could be established regardless of treatment. Common to all conditions is that GAD mRNA covaries strongly with the level of behavioural activity. In contrast, under the influence of various drugs, the interrelations between monoaminergic indices, GAD mRNA and behaviour are different, yet predictable.

These different relationships between biochemistry and behaviour can be thought of as the expression of different brain states, which all produce a particular behaviour. In all these brain states, thalamic GAD mRNA displays the same relation to motor activity. This may suggest that thalamic functioning is closely and constantly related to behavioural output. Such a constant relationship cannot be established between other brain regions and behaviour.

HoechtMarionRousell is gratefully acknowledged for financial support.

75.7

CHRONICAL HALOPERIDOL OR CLOZAPINE TREATMENT IN RATS: DIFFERENTIAL RNA DISPLAY ANALYSIS, BEHAVIORAL STUDIES AND PLASMA LEVEL DETERMINATION V. Fischer, U. Schmitt, B. v. Keller, S. Reuss[$], C. Hiemke* and N. Dahmen. *Departments of Psychiatry and Anatomy[$], University of Mainz, 55131 Mainz, Germany.*

Differential display, a method developed for the visual comparison of mRNA expression, is useful in the identification and cloning of specifically regulated genes. To study whether long-term administration of the typical neuroleptic haloperidol or the atypical neuroleptic clozapine changes neuronal gene expression, rats were treated orally for 21 days with 1.6 mg/kg haloperidol or 36 mg/kg clozapine per day. During the neuroleptic treatment the animal behavior was recorded in an open field. Compared with untreated controls, the total distance moved was reduced on day 5 by 22% under haloperidol and by 41% under clozapine. Blood samples were taken at the end of the experiment. Plasma analysis revealed mean concentrations of 22 ng/ml for haloperidol and 14 ng/ml for clozapine. RNA was isolated from each brain, without cerebellum, and subjected to differential RNA display. cDNAs were generated from mRNA using oligo-dT primers anchored to the beginning of the poly(A) tail and were then PCR amplified using the same 3'primer and a second 10 mere arbitrary 5'primer. Amplified products were analysed on a sequencing gel. Up to date we have analysed 22 out of 312 suggested primer combinations. Gene fragment patterns were highly reproducible between control and treated groups without revealing any consistent regulatory effect so far. However, preliminary data support the notion of gene fragments being regulated by chronical neuroleptic treatment.

Supported by the Deutsche Forschungsgeminschaft (grant Da 370/1-1).

75.8

D-AMPHETAMINE REVERSAL AS A MODEL FOR ANTIPSYCHOTIC EFFICACY IN CEBUS APELLA MONKEY. C. L. Christoffersen*, D. Johnston, F.W. Ninteman, L.T. Meltzer and T.G. Heffner. Parke-Davis Pharmaceutical Research, Division of Warner-Lambert Company, Ann Arbor, MI 48105.

We report the modification of a d-amphetamine (AMP) antagonism test in cebus monkeys (Peacock and Gerlach, Eur. J. Pharmacol. 237:329, 1993) that was used to determine doses of antipsychotics that produce CNS effects presumably related to efficacy. This test allows a within species comparison of therapeutic doses with those that induce extrapyramidal side-effects (EPS) in haloperidol-sensitized cebus monkeys, a standard test for EPS liability. These data are compared with the therapeutic dose/EPS dose ratios derived from testing the inhibition of Sidman avoidance (SA) and EPS liability in squirrel monkeys. Five cebus apella monkeys were tested with various doses of AMP to determine the minimum dose required to reliably produce mild stereotypy and motor unrest. Monkeys were rated by an observer, blinded to the treatment condition, for the intensity of stereotypy and motor unrest present. With the dose of AMP(0.75 mg/kg IM) established, compounds were tested for their ability to block the effects of AMP. Compounds were given PO two hours before AMP and the monkeys were rated every 15 min. after AMP for 90 min. There was no separation between the minimal effective EPS dose and the anti-amphetamine(A-AMP) dose for haloperidol or SDZ 208-912. Clozapine had some A-AMP effects but did not produce EPS at the doses tested. The separation between the minimal EPS and the A-AMP dose in cebus monkeys is smaller than the separation between the SA ED50 and minimal EPS dose in squirrel monkey. However, the compounds tested remain in the same rank order for ratio of efficacy to side-effect in both species. (Supported by Warner-Lambert.)

75.9

LEVELS OF EXTRACELLULAR DOPAMINE IN PREFRONTAL CORTEX AND NUCLEUS ACCUMBENS RESPOND DIFFERENTLY TO LOCAL ANTIPSYCHOTIC DRUG INFUSION. K.D. Youngren*, E. A. Andrusiak, and R.H. Roth. Neuroscience Program, Depts. of Psychiatry and Pharmacology, Yale University School of Medicine, New Haven, CT 06510.

Previous studies in this laboratory have indicated that the atypical antipsychotic drug clozapine preferentially affects dopamine (DA) systems in the prefrontal cortex (PFC) of both the rat and the monkey. In order to examine this preferential effect of clozapine on the mesocortical DA system further, microdialysis was used to measure the extracellular levels of DA in both the PFC and the nucleus accumbens (NAS) of the rat. Clozapine (100 µM) or haloperidol (100 µM) was infused through the dialysis probe directly into the PFC and NAS. Infusion of clozapine into the PFC or the NAS produced significant increases in extracellular DA, although the increase in the PFC was of longer duration. Clozapine infusion into the PFC produced increases in extracellular DA levels that were significantly greater than in the NAS. Infusion of haloperidol into either the PFC or the NAS also produced significant increases, typically of short duration, in extracellular DA within the respective brain region. Infusion of lower concentrations of the two drugs is also being examined. The results indicate that differences within the terminal field regions of these two dopaminergic projections account for at least part of the differing responses of the two brain regions to antipsychotic drug treatment. Although the PFC and the NAS share a source of dopaminergic afferents in the A10 cell group, the two regions respond differently to local antipsychotic drug administration. The present results thus provide further evidence that the atypical antipsychotic drug clozapine preferentially affects dopaminergic transmission within the PFC. Supported in part by USPHS Award MH-14092.

75.10

PERSISTENT DOWNREGULATION OF TYROSINE HYDROXYLASE (TH) IN SUBSTANTIA NIGRA (SN) BUT NOT VENTRAL TEGMENTAL AREA (VTA) FOLLOWING WITHDRAWAL FROM HALOPERIDOL.

A. J. Levinson, S. Garside, P. I. Rosebush and M. F. Mazurek*. Departments of Medicine (Neurology) and Psychiatry, McMaster University Medical Centre, Hamilton, ON L8N 3Z5.

The dopamine antagonist haloperidol (HAL) can cause tardive side effects that may persist after the drug is withdrawn. We studied the time course of changes in dopaminergic neurons of SN and VTA following withdrawal (WD) of HAL. Rats received daily intraperitoneal injections of saline (n=9) or HAL 2 mg/kg/day (n=21) for 8 weeks and were killed at 2, 4 or 12 weeks *after* the final injection. Sections of SN and VTA were processed for tyrosine hydroxylase (TH) and calbindin (CB) immunohistochemistry. In HAL-treated rats, TH-positive cell counts were normal in VTA but were decreased in SN by 34% at 2 weeks WD and by 52% at 4 weeks, with preferential loss in medial portions of SNc; TH cell counts were almost fully recovered by 12 weeks WD. CB positive neurons were not spared, but rather showed the same pattern as TH positive cells. Using TH staining, the cross-sectional area of SN was reduced by 34% at 2 weeks WD and by 17% at 4 weeks, with full recovery by 12 weeks WD. Mean TH+ve cell size, by contrast, was essentially unchanged at 2 and 4 weeks WD, but significantly decreased in SN at 12 weeks WD. These results indicate that HAL can produce selective changes in midbrain dopamine neurons that persist long after discontinuation of the drug.

75.11

CLOZAPINE-INDUCED ACh RELEASE IN THE PREFRONTAL CORTEX (PFC): NON-TOLERANCE AFTER CHRONIC TREATMENT. M.A.Parada*, M.Puig de Parada, and L.Hernández. Department of Physiology, School of Medicine, Universidad de los Andes, Mérida 5101-A, Venezuela.

We have shown that acute ip clozapine increases ACh preferentially in the PFC by reference to the nucleus accumbens (NAC) and striatum (STR) (1). We postulated that a similar ACh release in the frontal cortex could be therapeutic for schizophrenic patients, but that proposition requires the demonstration of some pharmacological properties. This study focused on non-tolerance. Five male Wistar rats received a daily ip clozapine injection (20 mg/Kg) for 21 days. Simultaneous microdialysis was used to monitor in 25-min samples the effects of the first and last injections on the ACh outflow in the NAC, STR and PFC. Only values (pmol/20µl) for the last area are reported here. Removable probes were inserted at the beginning and at the end of the treatment period. Basal levels (0.83 ± 0.16) increased to 2.16 ± 0.23 (310 ± 77.2 %) fifty min after the first injection [F(1/8): 22.9; $p<0.002$)]. Basal levels during the last day (0.63 ± 0.14) were slightly but not significantly lower than levels during the first day [F: 0.86; ns). They increased to 1.9 ± 0.3 (329.2 ± 54.8 %) in the 2nd sample after the 21st injection (F: 13.7; $p<0.01$). There were no differences between the above-basal ACh release after the first (1.34 ± 0.25) and the last (1.27 ± 0.27) clozapine injection (F: 0.03; ns). These results show that the PFC clozapine-induced ACh release does not display tolerance, which fulfills one requirement for the suggestion that it could be an index of one of the therapeutic effects in human patients.

1.- Parada et al. JPET 1996 (In Press).

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75.12

PCP-INDUCED DEFICITS IN SQUIRREL MONKEY SOCIAL BEHAVIOR ARE DIFFERENTIALLY AFFECTED BY CLOZAPINE AND HALOPERIDOL.

Rod J. Fishkin*, Lily Zhou and James T. Winslow, Ph.D. Hoechst Marion Roussel, Inc., Neuroscience Therapeutic Domain, Bridgewater, New Jersey, 08807

Phencyclidine (PCP) and other glutamate channel blockers can induce a model psychosis in humans and precipitate psychotic symptoms in schizophrenic patients. This syndrome mimics both the positive and the more treatment resistant negative symptoms. The purpose of this study was to examine the pharmacological profiles of PCP and MK-801 on primate social interactions in small groups (2-3 males per group). Differential efficacies of clozapine and haloperidol in antagonizing these effects were also examined. PCP and MK-801 both produced significant and reliable decreases in the frequency and amount of time allocated to social initiatives expressed by treated squirrel monkeys (*Saimiri sciureus*). Changes in social behavior produced by MK-801 but not PCP were associated with marked decreases in all indicies of motor activity at each of the doses tested. Clozapine reversed PCP-induced deficits at moderate doses and enhanced these decreases at higher doses where motor activity was also decreased. Haloperidol did not significantly affect PCP-induced deficits in social behavior at any dose tested. We are currently examining activities of both PCP and clozapine in an intruder challenge test associated with increased social interaction in the same animals. Initial findings suggest that PCP-induced deficits in the social behavior of squirrel monkeys may provide a discriminating test for antipsychotics against the treatment resistant social deficits associated with schizophrenia.

75.13

THE NMDA-RECEPTOR ANTAGONIST MEMANTINE IS NOT PSYCHOTOMIMETIC IN YOUNG HEALTHY VOLUNTEERS J. Kornhuber*[1], W. Retz[2], L. Sitzmann[2], A. Schmidtke[2], M.K. Herbert[3]. [1]Dept. of Psychiatry, University of Göttingen, 37075 Göttingen; Depts. of [2]Psychiatry and [3]Anesthesiology, University of Würzburg, 97080 Würzburg, Germany.

High-affinity uncompetitive antagonists at the N-methyl-D-aspartate (NMDA) type glutamate receptor, e.g. MK-801 or phencyclidine (PCP), share a high probability of inducing psychotomimetic side effects. Low-affinity NMDA receptor antagonists, like memantine and amantadine, are frequently used for the treatment of Parkinson's disease, dementia and spasticity. Their potential to cause psychotomimetic side effects, however, is still a matter of debate. In order to assess the psychotomimetic profile of memantine, we have conducted a monocentric, double-blind, two-arm, randomized study comparing one group of young male healthy students (n=20) receiving memantine 10 mg once daily for one week and 20 mg for a second week with another group (n=20) receiving placebo. Medication was given under supervision to ensure compliance. We have shown previously that this memantine dosage yields steady-state plasma and CSF concentrations significantly interacting with the NMDA receptor. Each volunteer was tested *before* and *during* (day 15) memantine/placebo treatment with (1) a self-rating scale for current subjective feeling ("Befindlichkeitsskala"), (2) a self-rating scale designed to detect dissociative symptoms, (3) the Brief Psychiatric Rating Scale, and (4) a concentration task ("d2-Test"). Test values *during* treatment were analyzed with the ANCOVA using test values *before* treatment as covariates. When looking at total test values, in none of the four test instruments significant effects of treatment were found ($P > 0.05$). This study confirms our clinical observations that low-affinity NMDA receptor antagonists rarely induce significant psychotomimetic side effects during therapeutic use. Low affinity and associated fast voltage-dependent channel unblocking kinetics are likely to be responsible for the better tolerance of memantine and also of amantadine.

75.14

NEUROANATOMICAL LOCALIZATION OF DIZOCILPINE-INDUCED DEFICITS IN PREPULSE INHIBITION OF THE STARTLE RESPONSE. V.P. Bakshi* and M.A. Geyer. Dept. of Neuroscience, UCSD, La Jolla, CA 92093.

Intense auditory or tactile stimuli elicit an involuntary startle response that is attenuated when a weak stimulus (a prepulse) is presented immediately prior to the startling stimulus. This phenomenon of "prepulse inhibition" (PPI) is thought to be an operational measure of sensorimotor gating. Because deficits in sensorimotor gating are characteristic of several disorders including schizophrenia, it is of interest to determine the neural substrates underlying deficient PPI. Systemic or intracerebroventricular administration of dizocilpine (DZ) and other non-competitive NMDA antagonists such as the psychotomimetic phencyclidine markedly disrupt PPI in rats. The purpose of the present investigation was to identify the specific brain regions that mediate these effects. Six regions were examined in separate groups of rats (male Sprague-Dawley): nucleus accumbens (NA), dorsomedial thalamus (DMT), ventral hippocampus (VH), amygdala (AM), medial prefrontal cortex (MPFC), and dentate gyrus (DG). In each study, rats received bilateral infusions of DZ (0, 0.25, 1.25 and 6.25 μg/0.5 μl) in a counterbalanced order over 4 test days. Five min after drug administration, animals were tested in startle chambers. The test session consisted of presentations (in a counterbalanced order) of a 120 dB "pulse" (the primary startling stimulus) either alone or immediately preceded by non-startling "prepulses" that were 3, 6, or 12 dB above the background noise (65 dB). DZ markedly reduced PPI following administration into VH, AM, MPFC and DG, but not NA and DMT. These results indicate that the PPI-disruptive effects of DZ are mediated by multiple discrete brain regions.

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75.15

PHARMACOKINETICS OF PHENCYCLIDINE AND COCAINE IN NEONATAL PIGLETS S. Primozic[3], A. Mrhar[3], J. Valentine[1], R. Karba[4], I. Grabnar[3], L.J. Burge[1], T.M. Badger [1*], M.H. Creer [2], C. Nehus [2], and F.M. Scalzo[1]. [1]Dept. of Peds., Univ. of Ark. for Med. Sci., and [2]Dept. of Tox., Veteran's Admin., Little Rock, AR and Faculty of [3]Pharmacy and [4]Elect. & Comp. Engin., Univ. of Ljubljana, Ljubljana, Slovenia.

The acute and residual motor and cardiovascular effects of developmental exposure to phencyclidine (PCP) and cocaine are poorly understood. Few animal models of human neonatal exposure to these compounds have been established. In order to determine the relationship between blood levels of cocaine and PCP and their acute behavioral and cardiovascular effects in neonates, a pharmacokinetic study was conducted to characterize the disposition of a single iv bolus of 6.0 mg/kg cocaine and 1.5 mg/kg PCP in piglets. For cocaine, mean maximal blood levels (34 mg/L) were observed 30 sec post-dosing. The mean volume of distribution was 1.54 ± 0.47 L/kg. Systemic clearance was 0.041 ± 0.003 L/min/kg with average distribution and elimination half-lives of 0.3 ± 0.1 and 58.0 ± 18.0 min, respectively. The volume of distribution (V_c) was 0.059 L/kg. For PCP, a very rapid decline in concentrations was observed in the first 5 minutes post injection. Plasma concentrations of 0.8 ± 0.3 mg/L and 0.06 ± 0.03 mg/L were observed 5 min and 8 h post-dosing. Extensive distribution of PCP to tissues is reflected by a V_c of 3.06 ± 0.77 L/kg. Systemic clearance was 0.012 ± 0.002 L/min/kg. Average distribution and elimination half-lives were 2.7 ± 0.6 min and 2.5 ± 0.8 h, respectively; V_c was 0.86 L/kg. A comparison of the pharmacokinetic parameters for cocaine and PCP indicates that like in adults, the distribution of PCP is more extensive than cocaine and the elimination of PCP is prolonged compared to cocaine. The results also suggest that the blood pressure and heart rate increases following iv cocaine and PCP are related to high blood levels during the distribution phase of both compounds. Supported by NIDA, Nat. Res. Council, Min. of Sci. & Tech. of Slovenia and the JW Fulbright Schol. Bd.

75.16

ALTERATIONS IN ZEBRA FINCH SONG PRODUCED BY BLOCKADE OF DOPAMINERGIC INPUTS. **S. A. Helekar*, N. Viswanath, D. B. Rosenfield and J. R. Nimerick. Stuttering Center Speech Motor Control Laboratory, Department of Neurology, Baylor College of Medicine, Houston, Texas 77030.**

Analysis of the avian central nervous system has revealed dense catecholaminergic innervation of neural structures such as the higher vocal center and the robust nucleus of the archistriatum that control song production. As part of a program to study bird song as a model of human speech motor control, we have initiated a neuropharmacological study to investigate the functional contribution of the dopaminergic input in the production of song in zebra finches. We hypothesized that dopaminergic modulation of synaptic interactions within song-related structures is critical for the patterning of avian song output. We tested this hypothesis by blocking two predominant types of dopaminergic receptors, D1 and D2 with intramuscularly injected antagonist, haloperidol (0.2 - 10 mg/Kg body weight) in 8 adult zebra finches with well developed song. The female directed song in untreated birds although stereotypic showed 3 striking variations. These variations were as follows: 1) The numbers of introductory notes and motifs per song were highly variable. 2) The last syllable in a motif was frequently omitted (5/8 birds). 3) Repetitive sets of syllables were added to the end of a basic motif in >20% of motifs (3/8 birds). Haloperidol at lower doses (0.2 - 1 mg/Kg) significantly reduced the frequency of songs and the number of motifs per song. There was also an induction of the occurrence of abortive songs consisting only of a series of introductory notes in haloperidol treated birds (7/8). Strikingly, this blocker did not produce any significant alterations in the spectrographic characteristics of individual syllables within a song even at a dose (1mg/Kg) that produced a detectable impairment of the flight capability of birds. At high doses (2 - 10 mg/Kg), haloperidol produced a complete cessation of songs although occasional monosyllabic calls were still produced. These findings indicate that dopaminergic modulation within song structures influences only the duration of song output and exerts no intrinsic control over the neural microcircuitry that generates the complex acoustic patterns and timings of individual syllables within songs. This work was supported by Lowin Medical Research Foundation and M. R. Bauer Foundation.

75.17

REGULATION OF PKC ISOZYMES BY CHRONIC LITHIUM IN HUMANS. G. Chen, A. Jiang, M. E. Schmidt, R Risinger, O Kofman*, W. Z. Potter AND H. K. Manji. Dept. of Psy. & Beh. Neuro., WSU School of Medicine, Detroit, MI 48201; NIMH, Bethasda, MD 28091 and Ben Gurion Univ., Israel

Lithium is a widely used, clinically effective antimanic and mood-stabilizing agent, but its therapeutic mechanisms of action remain to be elucidated. A growing body of evidence has indicated that upon chronic administration, lithium reduces the immunolabeling of Protein kinase C (PKC) isozymes in rat brain. The possibility that these biochemical effects may have therapeutic relevance is suggested by the fact that another clinically effective antimanic and mood-stabilizing agent, Valproate produces similar changes in PKC isozymes, and that manic patients have been reported to have elevated levels of platelet membrane associated PKC activity. In view of PKC's effects on neuronal excitability, neurotransmitter release, and regulation of gene expression, attenuation of PKC activity by lithium may play a role in its therapeutic effects, but have not yet been clearly established in humans. In the present study, healthy volunteers were administered therapeutic doses of lithium carbonate, and PKC isozymes were investigated after both 5 days and 5 weeks of treatment using both Western blotting and ELISA methodology; we used healthy volunteers in order to avoid the potentially confounding effects of alterations in mood-state dependent variables. We find that similar to the effects observed in rat brain, chronic but not acute lithium administration decreases the levels of PKC α. Studies are underway to elucidate the role of PKC in the clinical efficacy of lithium.

76.1

MONOAMINERGIC DYNAMICS DURING CHRONIC FLUOXETINE ADMINISTRATION: *IN VIVO* MICRODIALYSIS IN AWAKE MONKEYS. T.D. Smith*, R. Kuczenski, and S.L. Foote. Psychiatry Dept, UCSD, La Jolla, CA 92093

Fluoxetine (FLU) (Prozac), the latest drug to be widely prescribed for treatment of depression, is classified as a selective serotonin (5-HT) reuptake inhibitor. However, results from *in vivo* microdialysis studies in rodent brain suggest that dopamine (DA) and norepinephrine levels also may be affected by FLU. Although FLU blocks the reuptake of 5-HT in brain tissue within minutes, its antidepressant effect may not appear for weeks. Results from experiments in rodents examining mechanisms underlying this delay in therapeutic efficacy are equivocal. Therefore, we used newly developed *in vivo* microdialysis techniques in awake monkeys to compare extracellular 5-HT and DA in caudate (CD), hippocampus (HC) and frontal cortex (FCtx) prior to and during chronic (21 days) administration of FLU (10 mg/kg, p.o.). 5-HT and DA were assessed under basal conditions and during local infusion of either FLU to block reuptake or 120 mM K^+ to induce neuronal depolarization. Basal 5-HT levels were dramatically enhanced (3- 6-fold) by 3-7 days of systemic FLU, but declined to double their original levels within 21 days in all brain regions. In contrast, basal CD and FCtx DA levels were reduced by half in 3-7 days of treatment and remained at this new lower level. Chronic systemic FLU progressively decreased, and finally abolished the ability of locally infused FLU to enhance extracellular 5-HT or cortical DA levels. In contrast, during the first week of systemic FLU, the striatal DA response was even more robust than in untreated monkeys. However, by day 14, DA levels in CD were no longer responsive. Chronic FLU administration also severely blunted the effects of locally infused 120 mM K^+ on 5-HT, and especially on striatal DA levels. These data indicate that, in non-human primates, chronic systemic FLU treatment differentially alters basal DA and 5-HT levels and reduces the enhancement of extracellular DA and 5-HT in response to locally-induced depolarization in terminal fields. Thus, diminished basal DA, as well as enhanced basal 5-HT levels may contribute to FLU's therapeutic efficacy. In addition, altered 5-HT and DA responses to acute challenges following chronic FLU suggest that changes in monoaminergic dynamics may also contribute to FLU's antidepressant effects. (Generously supported by DA08346)

76.2

ANTAGONISM OF AMPHETAMINE-INDUCED BEHAVIORAL CHANGES IN SELECTED MEMBERS OF A PRIMATE SOCIAL COLONY BY THE SELECTIVE 5-HT_{2A} ANTAGONIST MDL 100,907. L.E. Shapiro, R.F. Schlemmer*, J.E. Young, C.K. Fleming and J.M. Davis. University of Illinois at Chicago, Chicago, IL 60612.

Recent clinical evidence has suggested a potential role of serotonin (5-HT) in psychosis which has generated considerable interest in evaluating 5-HT antagonists as potential antipsychotics. The present study assessed the effect of the selective 5-HT_{2A} antagonist MDL100,907 (*R*-(+)-α-(2,3-dimethoxy-phenyl)-1-[2-(4-fluorophenylethyl)]-4-piperidine-methanol) (MDL) in an amphetamine (AMP)-induced screening model for antipsychotics in primates. During the study, 4 doses of MDL were tested alone and in combination with AMP. Four females from a stable social colony of 5 adult stumptail macaques (*Macaca arctoides*) were assigned to 2 treatment groups. The male was not treated. After determining baseline behavior, each group received drug treatment for two days/week in a cross-over design. First, AMP was given alone, then one of 4 escalating doses of MDL, 0.1-5.0 mg/kg, was given each week. MDL was given nasogastrically in the AM and late afternoon on Day 1 and in the AM of Day 2. AMP, 1 mg/kg, was given i.m. 15 min before the second observation. Two 60 min behavioral observations were conducted daily by a "blind" observer, the first 35 min after administration of MDL and the next started 90 min later. MDL produced a dose-dependent antagonism of AMP-induced increased submissive gestures of treated monkeys. In addition, AMP-induced increased visual scanning (checking) and social withdrawal (increased distancing from cagemates) was antagonized at selected MDL doses. MDL failed to antagonize AMP stereotypy and did not induce movement disturbances. The results demonstrate that selective 5-HT_{2A} antagonism reverses some behavioral changes induced by AMP in monkeys. Thus, selective 5-HT_{2A} antagonists may have a potential role as antipsychotics.

76.3

A DOUBLE-BLIND STUDY OF SERTRALINE VERSUS PLACEBO IN THE TREATMENT OF POSTPARTUM DEPRESSION: PRELIMINARY FINDINGS C.N. Epperson*, D. Ward-O'Brien, L.H. Price, C.J. McDougle. Clinical Neuroscience Research Unit, Dept. of Psychiatry, Yale Univ. School of Med. 34 Park St., New Haven, CT 06519.

It remains a point of debate whether postpartum depression (PPD), which occurs in 10% of childbearing women, is the same as depression occurring at other times. To date, there has been no published report of a double-blind, placebo-controlled study of antidepressant treatment in PPD. The purpose of this ongoing study is to further our knowledge of the phenomenology, neurobiology and pharmacological treatment of PPD. **Methods:** Subjects were outpatients of the Yale Postpartum Mood Disorders Clinic and met DSM-IV criteria for major depression within 6 months of childbirth. After a 1-week placebo lead-in, women were randomized to sertraline (Zoloft) or placebo for 6 weeks. The main outcome measures for antidepressant response were the Hamilton Rating Scale for Depression and the Clinical Global Impression Scale. **Results:** To date, 6 of the 13 women enrolled have completed the study, while two are still in the double-blind phase. Four of the six completers were considered "responders"; 3 randomized to sertraline, one to placebo. Two women who were randomized to placebo were "nonresponders". After the placebo lead-in 3 were not randomized, one due to improvement, two due to decompensation. Finally, two women dropped out of the study. **Conclusions:** Preliminary findings from this ongoing study indicate that sertraline may be efficacious in the treatment of PPD. More women will need to be enrolled before making definitive conclusions regarding the role of biological and psychosocial stressors in PPD. **Funding Source: NARSAD**

76.4

THE PHARMACOKINETIC EFFECTS OF VENLAFAXINE ON IMIPRAMINE METABOLISM. Albers LJ, Reist C*, Vu R, Tang SW. VA Medical Center, Long Beach, CA, 90822, Department of Psychiatry, University of California - Irvine

Selective reuptake inhibitors have become important agents in the treatment of psychiatric illness. These drugs have a more benign side effect profile than tricyclic antidepressants and are less toxic in overdose. Important effects on cytochrome P450 enzymes, however, have emerged as relevant considerations in their clinical use. Venlafaxine (VF) is a new antidepressant which affects both serotonin and norepinephrine uptake. In vitro inhibition studies with human microsomes indicate minimal effects of VF on CYP2D6, CYP1A2, CYP3A4 and, CYP2C9 isoenzyme function. This suggests that VF should have little interaction with drugs metabolized by these isoenzymes. To evaluate these findings in vivo, the metabolism of a single 100 mg dose of imipramine (IMI) was studied in 7 healthy volunteers before and after three days of treatment with 150 mg per day of VF. Serial blood samples were collected and assayed by HPLC for imipramine (IMI), desipramine (DMI), 2OH-IMI and 2OH-DMI levels to determine any pharmacokinetic changes in metabolism. Preliminary data indicate modest but significant elevations of C_{max} and AUC for desipramine after venlafaxine treatment. No significant difference was seen in imipramine C_{max} or AUC with venlafaxine.

76.5

IN VITRO BINDING PROFILE OF MODERN ANTIDEPRESSANTS AND THEIR METABOLITES: CORRELATION BETWEEN AFFINITIES AT VARIOUS RECEPTORS. D. L. Knight*, W. N. Morgan, M. J. Owens and C. B. Nemeroff. Lab. of Neuropsychopharmacology, Dept. Psychiatry & Behavioral Sciences, Emory University School of Medicine, Atlanta GA 30322.

We have recently examined the affinity of a number of modern antidepressants at the rat and human serotonin (SERT) and norepinephrine (NET) transporters, rat 5-HT_{2A}, 5-HT_{1A}, muscarinic, guinea pig histamine, receptor, and human α_1 and α_2 receptors. The antidepressants studied included: nefazodone, hydroxynefazodone, *m*CPP, the desethyltriazoledione metabolite of nefazodone, trazodone, amitriptyline, desipramine, paroxetine, sertraline, citalopram, fluoxetine, fluvoxamine, venlafaxine, and *O*-desmethylvenlafaxine. Binding affinity (pK_i) was highly correlated between the rat and human versions of the SERT or NET, but not between same species versions of the two transporters themselves. The affinity at the NET was positively correlated with muscarinic receptor binding. A less robust positive correlation was observed between SERT and muscarinic binding. Moreover, binding amongst the 5-HT_{2A}, 5-HT_{1A}, α_1, α_2, and H_1 receptors were significantly positively correlated with each other in most instances. 5-HT_{2A} binding correlated with 5-HT_{1A}, H_1, α_1, and α_2 binding. 5-HT_{1A} binding correlated with 5-HT_{2A}, α_1, and α_2 binding. H_1 binding correlated with 5-HT_{2A} and α_1 binding. α_1 binding correlated with 5-HT_{2A}, 5-HT_{1A}, α_2, and H_1 binding. α_2 binding correlated with 5-HT_{2A}, 5-HT_{1A}, and α_1 binding. These findings suggest that unique structural features of the indivual compounds confer a certain degree (i.e., high, intermediate, or low) of potency/affinity at more than one type of receptor. Supported by a grant from Bristol Myers-Squibb and RISP MH51761.

76.6

EFFECT OF CHRONIC ANTIDEPRESSANT TREATMENT ON CRE- AND SP1-BINDING ACTIVITY IN RAT HIPPOCAMPUS AND FRONTAL CORTEX. D. Frechilla, A. Otano and J. Del Río*. Department of Pharmacology, School of Medicine, University of Navarra, Pamplona, Spain.

There is a lag time for the onset of clinical efficacy of antidepressant treatment and it has been suggested that the long-term adaptative changes induced by these drugs may also involve changes in gene expression. We have addressed this issue by studying the effect of chronic antidepressant administration on CRE- and SP1-binding activity in rat hippocampus and frontal cortex. Fluoxetine and desipramine (3 and 10 mg/kg/day respectively) were given daily to rats for 3 weeks and the animals were sacrificed 3 hours after the last injection. Nuclear extracts were prepared and the DNA-protein binding reaction was performed with consensus CRE and SP1 oligonucleotide probes. Gel-shift assays showed that CRE-binding activity was increased in both frontal cortex and hippocampus after chronic fluoxetine treatment. However, desipramine only enhanced CRE-binding activity in the frontal cortex. Opposite effects on SP1-binding activity were obtained after chronic antidepressants. This activity was reduced by fluoxetine in both brain regions and only in the frontal cortex by desipramine. The results show a differential and region-specific effect of the two antidepressants suggesting that these drugs may affect gene transcription by promoting changes in transcription factors. (Supported by CICYT, SAF94-1381).

76.7

POTENT BEHAVIORAL INTERACTIONS BETWEEN DESIPRAMINE AND THE NMDA RECEPTOR ANTAGONIST MK-801 IN RATS. K.A. Trujillo*, J.M. Van Treijen, C. Lanning and A.B. Chinn, Psychology Program, California State University, San Marcos, CA 92096-0001.

There is increasing interest in the potential role of N-methyl-D-aspartate (NMDA) receptors in the effects of antidepressant drugs. While some investigators suggest that antidepressants may produce their therapeutic actions by direct effects on NMDA receptors, others suggest that NMDA receptors may have a more indirect role, mediating the adaptive changes thought to be critical to the therapeutic actions of antidepressants. The present study was undertaken to examine potential behavioral interactions between the tricyclic antidepressant drug desipramine (DES) and the non-competitive NMDA receptor antagonist MK-801.

Adult, male Sprague-Dawley rats were placed in a 16"x16" plexiglass open field chamber. After 30 minutes of habituation, animals received an injection of saline, DES (5.0 mg/kg), MK-801 (0.1 or 0.3 mg/kg) or a combination of DES plus MK-801. Activity was automatically recorded for 3 hours by computer-interfaced photocell arrays and MK-801-induced motor abnormalities were scored by experimenters.

As has been previously described, MK-801 produced a dose-dependent increase in activity -- while 0.1 mg/kg produced mild, non-significant increase in photocell counts, 0.3 mg/kg doubled activity relative to saline controls. Although DES produced no changes in activity alone, this drug potently increased the response to the high dose of MK-801. These results suggest a potent interaction between DES and NMDA receptors, and are consistent the idea that NMDA receptors may be involved in the therapeutic actions of antidepressants. Studies are currently underway examining chronic interactions between these drugs.

Supported by a Young Investigator Award from the National Alliance for Research on Schizophrenia and Depression (NARSAD) and by a CSU San Marcos Faculty Development Award.

76.8

BLOCKADE OF 5-HT AND NE REUPTAKE BY VENLAFAXINE: *IN VIVO* ELECTROPHYSIOLOGICAL STUDIES IN THE RAT. J.C. Béïque*, C. de Montigny, P. Blier and G. Debonnel. Neurobiological Psychiatry Unit, McGill University, Montréal, Québec, Canada, H3A 1A1.

Venlafaxine (VLX) is an antidepressant agent which blocks *in vitro* the reuptake of both 5-HT and NE. The present *in vivo* electrophysiological studies were undertaken to assess the effect of VLX on the 5-HT and NE systems. Administered acutely, VLX prolonged dose-dependently the time required for a 50% recovery (RT_{50}) of the firing activity of dorsal hippocampus CA_3 pyramidal neurons from the suppression induced by microiontophoretic application of 5-HT and NE. The RT_{50} values for 5-HT were increased by 1 mg/kg i.v. of VLX, whereas 10 mg/kg i.v. were required for increasing the RT_{50} values for NE. A two-day treatment with VLX (delivered s.c. by osmotic minipumps) also dose-dependently increased the RT_{50} values for both 5-HT and NE applications: the RT_{50} values for 5-HT were increased at a dose of 10 mg/kg/day, whereas those for NE were at 20 mg/kg/day. Acute i.v. administration of VLX suppressed the spontaneous firing activity of dorsal raphe 5-HT and locus coeruleus NE neurons with ED_{50}'s of 233 and 754 µg/kg, respectively. The selective 5-HT_{1A} receptor antagonist WAY 100635 (100 µg/kg, i.v.) reversed this effect of VLX on 5-HT neurons and the α_2-adrenoceptor antagonist piperoxane (1 mg/kg, i.v.) reversed that on NE neurons. Taken together, these results indicate that, *in vivo*, VLX blocks both reuptake processes, its potency being greater for the 5-HT than for the NE reuptake process. This differential potency of VLX might constitute the biological substratum of the positive dose-response relationship of the efficacy of VLX in major depression.

76.9

ANTIDEPRESSANTS PREVENT THE GLUCOCORTICOID STIMULATION OF TRH EXPRESSION IN CULTURED HYPOTHALAMIC NEURONS IVOR. MD JACKSON* AND LU-GUANG LUO Division of Endocrinology, Rhode Island Hospital, Brown University School of Medicine, Providence, RI 02903

This study used a well established fetal rat hypothalamic neuronal culture system to directly examine the effect of antidepressants on TRH expression. Glucocorticoids which are known to enhance TRH production *in vitro* were used in conjunction with each antidepressant. Cultured 17 day fetal hypothalamic neurons were treated with different concentrations of antidepressants for 7 days following which TRH content was measured by radioimmunoassay(RIA). The results showed that imipramine(IMIP), a tricyclic antidepressant, decreased the TRH content in a dose-dependent manner (from 80.7 ± 4.9, at 10^{-9}M, to 14.1 ± 0.6, at 10^{-5}M, fmol/well; $p<0.05$). Desipramine(DESI), another tricycle antidepressant, also decreased the TRH content (from 63.6 ± 2.5, at 10^{-9}M, to 12.6 ± 0.4, at 10^{-5}M, fmol/well; $p<0.05$) though the response was not dose-dependent. Sertraline(SERT) and fluoxetine(FLUO), serotonin selective reuptake inhibitors (SSRI), also decreased TRH content in a dose dependent manner(from 83.9 ± 7.9, at 10^{-10}M, to 7.6 ± 0.4, at 10^{-5}M, and from 41.66 ± 2.5, at 10^{-8}M, to 17.54 ± 0.92, at 10^{-6}M, fmol/well respectively; both $p<0.05$). However, tranylcypromine(TRAN), a monoamine oxidase inhibitor (MAOI), showed no effect on TRH expression. We then tested the effect of these antidepressants on the Dex stimulation of TRH content. IMIP, DESIP and FLUO at 10^{-6}M reduced the TRH response to glucocorticoid stimulation (36.4 ± 4.0, 56.6 ± 2.4, 23.75 ± 4.0 respectively *vs* 107 ± 7.5 fmol/well; $p<0.05$). These findings suggest that the enhanced thyroid function in depression, which we postulate may be secondary to glucocorticoid stimulation of TRH gene expression (Endocrinology 136:2793-4,1995), can be reversed by antidepressants through a direct effect on the TRH neuron.

76.10

EFFECT OF CHRONIC COADMINISTRATION OF SERTRALINE AND PINDOLOL ON THE OPEN FIELD ACTIVITY OF OLFACTORY BULBECTOMIZED RATS. R.J. Thielen* & A. Frazer. Dept. of Pharmacol., UTHSCSA and Audie L. Murphy Memorial Veterans Hospital, San Antonio, TX 78284.

There is current interest in whether adjunctive treatments, such as the 5-HT_{1A} receptor/β adrenoceptor antagonist pindolol, increase the rate of development of antidepressant (AD) efficacy. To study this phenomenon mechanistically, a test system in animals in which ADs alone exert results only after chronic treatment would be useful. Olfactory bulbectomized (OBX) rats show a number of behavioral deficits, some of which are reversed only by chronic AD treatment. The purpose of the present study was to examine whether coadministration of sertraline (5 mg/kg, ip) with pindolol (2 mg/kg, sc) would reduce the time necessary to see an AD-like effect in OBX rats. Rats were anaesthetized, two holes were drilled into the skull 2 mm anterior to bregma, and the olfactory bulbs were removed by aspiration. Sham animals were treated identically, except the olfactory bulbs were not removed. After allowing two weeks for recovery, sham rats were treated with vehicle and OBX rats were treated with vehicle, pindolol-vehicle, vehicle-sertraline or pindolol-sertraline, twice daily for seven days. Rats were tested for open field activity for 5 min, 15 hr after the last drug administration. Sham and OBX rats did not show any differences at any time in total activity in the open field; however, OBX rats demonstrated a 2.5 fold increase in the number of crosses through the center of the open field during the last 3 min of the test compared to the sham rats. Pindolol alone had no effect on this increased center crosses; however, OBX rats treated either with sertraline alone or in combination with pindolol did not show an increase in center crosses compared with that of sham rats. These results demonstrate that seven day treatment with sertraline alone reverses some aspects of the open field behavioral alteration seen with OBX rats. Modification of this procedure should lead to a test able to detect changes in the onset of antidepressant activity.

(Supported by Research Funds from the VA and USPHS Grant MH29094).

76.11

ANTAGONISM BY WAY 100635, A 5-HT_{1A} ANTAGONIST, OF THE EFFECTS OF FLUOXETINE AND BEFLOXATONE IN THE FORCED SWIM TEST. P.C.Moser* and D.J.Sanger. CNS Pharmacology Group, Synthélabo Recherche, 10 rue des Carrières, 92500 Rueil-Malmaison, FRANCE.

Recent clinical data suggests that co-administration of pindolol with an antidepressant (AD), particularly the 5-HT reuptake inhibitor fluoxetine (FLX), can shorten the time to onset of clinical activity and increase the proportion of responders. It is hypothesised that pindolol blocks somatodendritic 5-HT_{1A} receptors in the raphé nuclei, thereby mimicking the desensitisation of these receptors that occurs after chronic AD treatment and preventing the reduction in raphé cell activity that occurs after acute AD treatment. Although most animal tests use acute treatment to demonstrate AD activity, it remains possible that blockade of these 5-HT_{1A} receptors may potentiate these effects. We examined this possibility using the selective 5-HT_{1A} antagonist WAY 100635 (WAY) in combination with either FLX or the MAO-A inhibitor befloxatone using the forced swim test (FST) in rats. FLX (10-80 mg/kg po) and befloxatone (0.03-1 mg/kg po) dose-dependently reduced immobility time. WAY (0.01-0.1 mg/kg sc) was without effect itself. A combination of the ADs with WAY (0.1 mg/kg) demonstrated that rather than potentiate their effects, WAY significantly reduced their activity in this test. These results demonstrate that blockade of 5-HT_{1A} receptors does not potentiate the effects of ADs in the FST. In contrast, they suggest that 5-HT_{1A} receptors mediate, at least in part, the AD-like effects of FLX and befloxatone in the FST.

76.12

ANTI-IMMOBILITY EFFECTS OF FLUOXETINE AND DESIPRAMINE IN RATS SELECTIVELY BRED FOR HIGH SENSITIVITY TO 8-OH-DPAT. D.S. Janowsky* and D.H. Overstreet. Skipper Bowles Center for Alcohol Studies, University of North Carolina, Chapel Hill, NC 27599-7178.

Rats which were selectively bred for increased hypothermic responses (HDS) to the 5-HT-1A receptor agonist, 8-OH-DPAT, were found to have innately higher immobility in the forced swim test compared to their parallel bred low sensitive group (LDS) or a randomly bred control (RDS). The present study compared the anti-immobility effects of chronic fluoxetine (10 mg/kg) treatment in the three lines and included a HDS group treated chronically with desipramine (5 mg/kg). Vehicle (isotonic saline), fluoxetine, or desipramine were injected intraperitoneally once daily between 08:00 and 10:00 for 14 days. Approximately 22 hr after the 10th injection, baseline temperatures were recorded and the rats were challenged with 8-OH-DPAT (0.5 mg/kg) and temperatures were recorded again 45 min later. Approximately 22-26 hr after the 14th injection, the rats were placed individually in the swim tank for a single 5-min recording. As found previously, the HDS rats were more hypothermic after 8-OH-DPAT and more immobile in the swim test. Fluoxetine both blunted the hypothermic response and reduced immobility in all groups. In the randomly bred RDS group, the correlation between the hypothermic response and immobility was 0.8 ($n=8$, $p < 0.05$). The responses of HDS group to desipramine appeared to countradict this association, as there was a highly significant decrease in immobility, but no significant group change in the hypothermic response to 8-OH-DPAT. However, 5 of the 8 rats did in fact exhibit blunted responses and when a correlation was carried out between the hypothermic response and immobility in these rats, it was 0.90 ($n=8$, $p<0.01$). These findings support previous work implicating a relationship between 5-HT-1A sensitivity and immobility and are consistent with the literature suggesting an involvement of 5-HT-1A receptor function in affective disorders.

76.13

PINDOLOL BOOSTS OR BLOCKS FLUOXETINE IN THE RAT FORCED SWIMMING TEST (FST) DEPENDING ON THE FLUOXETINE DOSE.
M. J. Detke*, J. Ph. Reneric and I. Lucki. Departments of Psychiatry, Pharmacology and Psychology, University of Pennsylvania, Philadelphia, PA 19104.

The combination of 5-HT_{1A} receptor antagonists with selective serotonin reuptake inhibitors (SSRIs) has been shown to enhance the release of serotonin, presumably by blocking presynaptic autoreceptors in the midbrain raphe nuclei. Pindolol has also been shown to enhance the effects of SSRIs in the treatment of clinical depression in humans (Artigas et al., 1994; Arch Gen Psychiatry, 51:248-251). In the FST, an animal model of depression, SRIs reduce immobility and increase swimming (Detke et al., 1995; Psychopharmacology, 121:66-72). This model was used to examine the effects of the combined treatment of pindolol with fluoxetine, an SSRI. Room-temperature water 30 cm deep was used to prevent the rats from supporting themselves with tails or hind limbs. After a 15-min pretest, drugs were administered 24, 5, and 1 hr prior to the 5-min swim test.

Fluoxetine at a dose of 20 mg/kg SC reduced immobility and increased swimming. Pindolol at doses of 0.06 - 2 mg/kg SC blocked the effects of fluoxetine in a dose-dependent manner. Fluoxetine, at a subthreshold dose of 3.2 mg/kg SC, did not produce statistically reliable behavior changes. In this case, pretreatment with pindolol at doses of 0.06 - 2 mg/kg SC enhanced the effects of fluoxetine, reducing immobility and increasing swimming behaviors. Pindolol administered alone at these doses did not produce significant changes in behavior.

These results confirm earlier findings showing that SSRIs produce antidepressant-like effects in the FST, and that these effects are behaviorally specific. The fact that low doses of pindolol enhance (sub-maximal) effects of fluoxetine may be mediated by pindolol's ability to block presynaptic 5-HT_{1A} autoreceptors. Blockade of maximal fluoxetine effects by pindolol is presumed to be mediated by blockade of post-synaptic 5-HT_{1A} receptors.

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76.14

SEROTONERGIC AND NORADRENERGIC MECHANISMS INVOLVED IN THE EFFECTS OF ANTIDEPRESSANTS IN THE RAT FORCED SWIMMING TEST (FST).
J. Ph. Reneric and I. Lucki*. Departments of Psychiatry and Pharmacology, University of Pennsylvania, Philadelphia, PA 19104.

Recent studies of the Porsolt behavioral despair test or FST using a time-sampling scoring technique have shown that serotonin reuptake inhibitors (SSRIs) decrease immobility while increasing swimming behavior, whereas norepinephrine (NE) reuptake inhibitors (NRIs) decrease immobility while increasing climbing. (Detke, et al., 1995; Psychopharmacology,121:66-72). These different behaviors were used to examine the effects of combined treatment of an SSRI (fluoxetine) with a NRI (desipramine). In addition, the paradigm was used to assess the effects of three antidepressant compounds with mixed activity as serotonin-NE reuptake inhibitors (S-NRIs), venlafaxine, milnacipran and duloxetine. Room-temperature water was 30 cm deep to prevent the rats from supporting themselves with tails or limbs. After a 15-min pretest, drugs were administered 24, 5, and 1 hr prior to the 5-min swim test.

Desipramine and fluoxetine both reduced immobility, and these effects were additive. Desipramine increased only climbing behavior, whereas fluoxetine increased only swimming behavior. Desipramine administered with fluoxetine interacted to decrease high levels of swimming. The reduction in swimming was presumably secondary to spending more time climbing.

Compounds with mixed activity at inhibiting serotonin and NE uptake produced different behavioral patterns in the FST. Venlafaxine reduced immobility and increased swimming. These effects began at 10 mg/kg and were dose-dependent. Venlafaxine also increased climbing at the highest dose tested (80 mg/kg). These behaviors are consistent with venlafaxine being slightly more effective at inhibiting serotonin than NE reuptake. Milnacipran reduced immobility and increased climbing. These effects were dose-dependent and were statistically reliable at doses of 20 - 40 mg/kg. There was no effect on swimming. This is consistent with milnacipran's slight selectivity for NE reuptake blockade. Duloxetine dose-dependently reduced immobility at doses of 10 - 40 mg/kg. This appeared to be due to increased climbing rather than swimming behavior. *Supported by MH 36262.*

76.15

THE EFFECT OF SELECTIVE SEROTONIN RELEASING AGENTS IN THE CHRONIC MILD STRESS MODEL OF DEPRESSION IN RATS.
D. Marona-Lewicka* and D. E. Nichols. Department of Medicinal Chemistry and Molecular Pharmacology, Purdue University, West Lafayette, Indiana 47907

Chronic exposure of rats to a sequence of mild unpredictable stressors causes an antidepressant-reversible decrease in responsiveness to reward (anhedonia) which can be monitored by a substantial decrease in the consumption of palatable weak (1%) sucrose solution. Stress-induced behavioral deficits may be maintained for a long time, however the chronic administration of clinically effective antidepressants can restore normal behavior. Usually this effect of antidepressants, in animals undergoing chronic mild stress, is observed following 3-4 weeks of drug treatment, whereas no effect is observed in non-stressed control rats. MMAI (5-methoxy-6-methyl-2-aminoindan) and MTA (p-methylthioamphetamine) are non-neurotoxic, highly selective neuronal serotonin (5-HT) releasing agents. In drug discrimination substitution tests, MMAI fully mimicked citalopram and partially substituted in sertraline-trained rats, whereas partial substitution occurred with citalopram and sertraline in MMAI-trained rats. Citalopram and sertraline are selective 5-HT reuptake inhibitors (SSRI) and very clinically effective antidepressants. Chronic treatment with MMAI (5 mg/kg 2 x day i.p.; 4 weeks) reverses the deficit in rewarded behavior measured as a decrease in the consumption of 1% sucrose solution in a chronic mild stress model of depression in rats. The magnitude of this effect was comparable to that observed following administration of citalopram (10 mg/kg i.p.; 4 weeks), used as a standard antidepressant drug. These results indicate that MMAI has antidepressant activity in this animal model of depression. Studies in progress are also examining the effect of MTA in this chronic mild stress model of depression.

76.16

CHRONIC ELECTROCONVULSIVE SEIZURE (ECS) INDUCES MOSSY FIBER SPROUTING.
V. A. Vaidya* and R. S. Duman. Neuroscience Program, Laboratory of Molecular Psychiatry, Depts. of Psychiatry and Pharmacology, Yale University School of Medicine, New Haven, CT 06508.

Recent work from our laboratory has shown that chronic ECS, as well as other antidepressant treatments, increases the expression of BDNF in the hippocampus. BDNF plays a role in neuronal survival, differentiation and growth raising the possibility that chronic ECS influences the morphology of hippocampal neurons. This is supported by recent work from our lab demonstrating that chronic ECS alters the phosphorylation state and amount of neurofilament subunits. To further study the influence of chronic ECS on hippocampal morphology we used the Timm method, which stains the mossy fiber pathway because of it's high Zn^{+2} content. Rats received 10 daily ECS treatments (50 mA, 0.3 sec) and were sacrificed 12 days after the last treatment. Chronic ECS treated animals showed a dramatically different pattern of staining with Timm granules present throughout the supragranular layer of the dentate gyrus, which is indicative of mossy fiber collateral sprouting. This prominent sprouting appears to arise in the absence of gross cell damage which has been associated with lesion induced sprouting. This synaptic reorganization is reversible since 35 days after the last ECS treatment there appears to be no difference between the groups. Further experiments are being conducted to study the time course of this sprouting at the light and ultrastructural level. Experiments are also being conducted to study the effect of BDNF infusions and other antidepressant treatments on the pattern of Timm staining.
(Supported by MH45481)

76.17

REVERSAL OF FLUOXETINE-INDUCED SUPPRESSION OF SEROTONERGIC NEURONAL ACTIVITY BY WAY-100635: A DOSE-RESPONSE STUDY IN BEHAVING CATS.
C. W. Metzler *, C. A. Fornal, F. J. Martin and B. L. Jacobs. Program in Neuroscience, Princeton University, Princeton, NJ 08544.

Recent evidence suggests that 5-HT_{1A} autoreceptor antagonists may enhance the antidepressant efficacy of selective serotonin reuptake inhibitors (SSRIs) by blocking the negative neuronal feedback action of increased synaptic serotonin. We have previously shown that 5-HT_{1A} autoreceptor antagonists such as WAY-100635 rapidly restore the firing of serotonergic neurons in the dorsal raphe nucleus (DRN) after acute treatment with the SSRI fluoxetine (Fornal et al., Soc. Neurosci. Abstr. 1995). In the present study, we examine the effects of increasing doses of WAY-100635 on the suppression of serotonergic DRN neuronal activity produced by fluoxetine in behaving cats. Serotonergic neurons were recorded and identified as described previously (Fornal et al., JPET 270: 1345, 1994). Drugs were injected remotely via a jugular catheter. WAY-100635 was administered in increasing doses at 6-min intervals starting 15 min after fluoxetine administration (5 mg/kg). Fluoxetine decreased the activity of serotonergic neurons to approximately 10% of baseline levels. Subsequent administration of WAY-100635 reversed the inhibitory action of fluoxetine in a dose-dependent manner. Whereas 0.005 mg/kg of WAY-100635 had no effect, a cumulative dose of 0.01 mg/kg restored neuronal activity to about 50% of pre-fluoxetine baseline values. Cumulative doses $\geq$ 0.025 mg/kg significantly increased (~ 60%) neuronal activity above baseline levels. The effect of WAY-100635 appeared to be maximal at 0.05 mg/kg, since cumulative doses up to 1 mg/kg produced little or no further increase in neuronal activity. These results indicate that WAY-100635 is a highly potent and effective 5-HT_{1A} autoreceptor antagonist. Supported by grants from the AFOSR (F 49620-94-1-0128) and the NIMH (MH 23433).

76.18

THE ABILITY OF WAY-100635 TO INCREASE SEROTONERGIC NEURONAL ACTIVITY AFTER ACUTE FLUOXETINE TREATMENT IS DEPENDENT ON BEHAVIORAL AROUSAL.
B.L. Jacobs *, C.A. Fornal, F.J. Martín and C.W. Metzler. Program in Neuroscience, Princeton University, Princeton, NJ 08544.

The therapeutic efficacy of selective serotonin reuptake inhibitors (SSRIs) might be potentiated by agents which block the 5-HT_{1A} autoreceptor and thereby prevent the negative feedback action of serotonin on serotonergic neuronal activity. In the present study, we examined the influence of behavioral state on the ability of WAY-100635 (0.1 mg/kg, i.v.) to increase the activity of serotonergic dorsal raphe neurons in behaving cats after acute treatment with the SSRI fluoxetine (5 mg/kg, i.v.). Serotonergic single-unit activity was recorded using methods described previously (Fornal et al., JPET 270: 1345-1358, 1994). The ability of WAY-100635 to reverse the effect of fluoxetine was highly influenced by the behavioral state of the animal. WAY-100635 maximally elevated neuronal activity when cats were behaviorally active, and was less effective or completely ineffective in increasing neuronal activity after fluoxetine treatment during periods of behavioral quiescence (e.g., drowsiness or sleep), when serotonergic neurons normally display little or no spontaneous activity. Since GABAergic mechanisms appear to mediate the state-dependent decrease in serotonergic neuronal activity, pharmacological blockade of 5-HT_{1A} autoreceptors would not be expected to increase neuronal activity during sleep. These results suggest the potential utility of behavioral strategies (e.g., increasing general activity and/or sleep deprivation) in further augmenting the effects of combining a SSRI and a 5-HT_{1A} antagonist. Supported by grants from the AFOSR (F 49620-94-1-0128) and the NIMH (MH 23433).

76.19

CHRONIC ADMINISTRATION OF LITHIUM INCREASES THE EXPRESSION OF CREB AND BDNF IN RAT HIPPOCAMPUS. M. Nibuya*, A. Jung, E.J. Nestler, and R.S. Duman Lab. of Molecular Psychiatry, Dept. of Psychiatry and Pharmcology, Yale Univ. Sch. Med., New Haven, CT, 06508

Chronic administration of lithium appears to upregulate the cAMP pathway in brain. For example, we have shown that expression of adenylyl cyclase in specific brain regions is increased by chronic lithium treatment. We have also found that chronic antidepressant treatments upregulate the cAMP pathway, including increased levels of PKA (cAMP-dependent protein kinase) and expression of CREB (cAMP response element binding protein). In the present study we demonstrate that chronic lithium administration also upregulates these intracellular components of the cAMP pathway. Chronic lithium treatment (28 d) increased soluble, but not particulate, levels of PKA enzyme activity and increased the expression of CREB mRNA in the hippocampus. Chronic lithium administration also increased levels of CRE binding, determined by gel shift analysis, indicating that the induction of CREB mRNA is accompanied by an increase in CREB function. Upregulation of CREB indicates that lithium may influence target genes of this transcription factor: we have found that increased expression of CREB in response to antidepressant treatments is accompanied by an increase in the expression of BDNF (brain derived neurotrophic factor). Here we find that chronic lithium treatment also increases levels of BDNF mRNA in hippocampus. Regulation of CREB and BDNF could enhance the function and survival of neurons in the hippocampus and thereby contribute to the therapeutic actions of lithium. (Supported by MH 51399).

AGING BEHAVIOR

77.1

OPEN FIELD ACTIVITY AND HUMAN INTERACTION VARY AS A FUNCTION OF AGE AND BREED IN DOGS. J. Th. Rick[1]*, E. Head[1], B.J. Cummings[2], C.W. Cotman[3], W.W. Ruehl[4], B.A. Muggenberg[5] and N.W. Milgram[1]
[1]University of Toronto, Scarborough College, Scarborough, Ontario, Canada, M1C 1A4, [2]Harvard Med. School, Boston, MA, [3]Institute for Brain Aging and Dementia, University of California, Irvine, CA, [4]Deprenyl Animal Health Inc., Overland Park, KS, [5]Inhalation Toxicology Research Institute, Albuquerque, NM.

Open field activity was studied in kennel reared purebred beagles from two separate colonies and in pound source mixed breed dogs. Animals ranged in age from 9 months to 13 years. Dogs were placed in an observation room for a 10 minute period and 8 behaviors were recorded: distance traveled, time spent inactive, urination, directed sniffing, grooming, rearing, vocalizing and jumping frequencies (Head & Milgram, 1992). A human interaction test was also conducted using identical procedures except for the presence of a familiar or unfamiliar person in the observation room.

Open field measures of exploratory and locomotor behavior decreased as a function of age in pound source dogs, but not in the human interaction test. Although young pound source dogs did not differ from young beagles with respect to the parameters measured, beagles from both colonies did not show evidence of age-dependent decreases in open field activity. Cognitive test scores obtained from the same group of dogs were not correlated with open field activity measures, indicating that cognitive dysfunction in aged dogs is unrelated to sensorimotor deficits. These findings raise the possibility of genetic factors affecting age-dependent changes in open field activity. However, rearing conditions and differential neuropathology may also contribute to the behavioral differences observed among breeds. Supported by NIA Grant (AG 12694) to CWC, BJC, BAM and NWM.

77.2

REPRODUCTIVE STATUS AND AGE EFFECTS ON LEVELS OF ACTIVITY AND TESTOSTERONE IN MALE DEER MICE. K.-P. Ossenkopp*, T. S. Perrot-Sinal and M. Kavaliers. Neuroscience Program and Department of Psychology, University of Western Ontario, London, Ontario, Canada, N6A 5C2.

Deer mice are a long-lived species whose reproductive state is dependent on photoperiod. Locomotor activity was examined in a multivariate manner with an automated Digiscan monitoring system. Both level of activity and spatial distribution of activity (thigmotaxis) was quantified. Subsequently, plasma testosterone levels, testes and adrenal weights, and brain measures were obtained. Mice were grouped by age (old, 57 mo; middle aged, 38 mo; adult, 16 mo; young B, 3 mo) and kept under a long day photoperiod. An additional group of young mice (NB, 3.6 mo) was housed under a short day photoperiod. Old mice exhibited lower levels of activity for several measures as well as lower testosterone levels when compared to young B, but not young NB mice. The young B mice also had higher activity levels, but not testosterone values, than the adult and middle aged mice. The young NB mice had both lower activity and testosterone levels relative to young B mice. Only the young NB mice had lower corrected testes weights than the other groups. These findings indicate the need to consider reproductive status when examining age-related changes in behavior. (Supported by NSERC)

77.3

REACTIVITY TO NOVELTY IN AGED, COGNITIVELY IMPAIRED RATS. J. Rochford*, E. Spreekmeester, M. Meaney, R. Quirion and W. Rowe. Douglas Hospital Research Center, Dept. Psychiatry, McGill University, Montreal, Quebec.

Two populations of aged, Long Evans, rats can be identified on the basis of performance in the Morris Water Maze Task. Aged (24 month) unimpaired (AU) rats perform similarly to young (Y, 6 month) animals. Aged impaired rats (AI) display latencies to find the submerged platform greater than two standard deviations from the mean of the Y animals. Whereas these data indicate that some aged animals are deficient in terms of their spatial learning and memory capacities, the mechanism underlying this deficit has yet to be identified. A hallmark of efficient cognitive processing is the ability to cope with environmental change. Consequently, the present studies were conducted to assess reactivity to repeated exposure to novel stimuli. Reactivity was assessed by examining the degree and duration of (1) neophobia exhibited towards a novel gustatory/olfactory stimulus (sweetened milk) (2) hypoalgesia induced by exposure to a novel hot-plate (48.5° C) apparatus and (3) exploratory behavior in an elevated plus maze. AI rats exhibited a greater initial degree of neophobia (lower consumption on day 1) and protracted reactivity (lower consumption over days 2-8) in comparison to AU and Y animals. AI rats were more reactive to novelty on the hot plate test (as indicated by longer paw lick latencies); this novelty-induced hypoalgesia did not habituate in AI rats following repeated plate exposures. The degree of exploratory behavior (total arm entries)exhibited during three exposures to the elevated plus maze was reduced in AI rats. This effect was not a consequence of deficient affective mechanisms as measures of anxiety (open/total arm entries, time in open arms) were not different among AI, AU and Y animals. These data suggest that AI rats are hyper-reactive to novelty, and that this sensitivity persists following repeated stimulusexposure. (Funded by MRC and NSERC).

77.4

RETENTION OF OPERANT BEHAVIOR BY YOUNG AND MATURE RATS. K.S. Seybold*, R.W. Brown, G. Fisher and R.L. Port. Dept of Psychology, Slippery Rock University, Slippery Rock PA 16057.

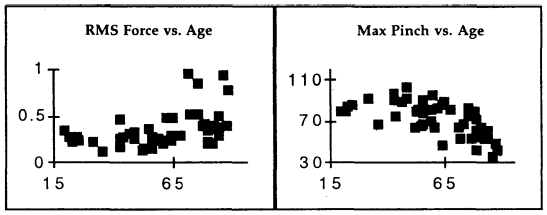
Aged subjects relearn classical (Coffin & Woodruff-Pak, 1993) and instrumental (Port, Murphy & Magee, 1996) responses as quickly as younger animals, but were found to show a marked deficit when retention of a classically conditioned response was examined without reinforcement (Solomon, et al, 1995.) The present study examined retention of operant responses in the absence of reinforcement.

Eight young (3 mos) and 8 mature (15 mos) male rats were autoshaped to barpress for food reinforcement. After reaching a criterion of >100 responses on two consecutive days, they received no training for a two week period. Retention was tested in a twenty min nonreinforced session. Mature subjects took significantly longer to reach criterion and retention performance (number of responses) was deficient in comparison to the younger subjects. Thus, age-related retention deficits seem to be generally exacerbated by testing in the absence of reinforcement. Supported by SRU.

77.5

DECLINE IN LATERAL PINCH STRENGTH AND STEADINESS WITH AGE. A.A. Overton, K.H. Olson and J.J. Pellegrini*. Departments of Biology and Occupational Therapy, College of St. Catherine, St. Paul, MN 55105.

This study quantitatively describes changes in hand motor function across the adult lifespan. We examined healthy female volunteers between 20 and 100 years of age with tests of strength, speed and steadiness. We assessed lateral pinch strength by having subjects apply maximum force to a pinch gauge. We measured speed of finely targeted movements by having subjects perform the Purdue pegboard test. We evaluated pinch steadiness by asking subjects to apply constant force to an isometric pinch gauge while they were provided with visual feedback of their force output on a computer screen; the target force was 2 N. Subjects used their dominant hand for all tasks. After finishing these tasks, subjects completed a self assessment questionnaire of manual dexterity.



77.6

AGE EFFECTS ON SIX TEMPORAL FACTORS IN VISUAL PERCEPTION. R.S. Kennedy*, Essex Corporation, Orlando, FL 32803; J.M. Ordy, Neuroscience Research, Pittsford, NY 14534; and W.P. Dunlap, Tulane University, New Orleans, LA 70118.

There is a loss of both temporal and spatial discrimination in vision with normal aging. In human temporal visual tests, there is an apparent loss of temporal resolution of closely succeeding visual events that are recognized as separate by young adults. Although optical mechananisms are also involved in temporal and spatial resolution, interest has focused on possible differential, age-related changes in magnocellular and parvocellular pathways extending from the ganglion cells of the retina to LGB, and V1 of the striate cortex. These parallel pathways mediate information for motion, form, depth, and color. In this study, the effects of age were evaluated in a temporal visual factors (TVF) battery, administered on a PC, and comprising (1) Flicker, (2) Phi-Apparent Movement, (3) Masking, (4) Simultaneity, (5) Group/Element Movement, and (6) Saccadic Accuracy. Forty-five subjects, ranging from 35-75 years were tested, and correlations were performed between age and TVF scores. The correlations between age and Group/Element Movement showed the strongest relationship and was $P < 0.001$, followed by Saccadic Accuracy ($P < .01$), Backward Masking ($P < 0.01$), then Simultaneity ($P < 0.05$). Phi-Apparent Movement and Flicker showed directionally consistent age effects, but they were not statistically significant. These findings indicated significant age effects on specific human temporal visual factors that appear to be critically involved in such dynamic psychomotor tasks as driving and flying.

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77.7

ALTERED TIME PERCEPTION IN ELDERLY HUMANS RESULTS FROM THE SLOWING OF AN INTERNAL CLOCK. P.A. Mangan*, P.K. Bolinskey, A.L. Rutherford, and C.D. Wolfe, Clinch Valley College of the University of Virginia, Wise, VA 24293.

Evidence suggests that the transformation of real time into psychological or subjective time involves the activity of an internal clock. Recent studies have shown that drugs that increase dopamine levels at the synapse, such as methamphetamine, accelerate this clock, and drugs that decrease dopamine levels, such as haloperidol, slow the clock. This evidence supports the position that dopamine plays an important role in the regulation of an internal clock.

While a general pattern of anatomical and physiological changes occur throughout the brain during normal aging, selective atrophy of the substantia nigra with a concurrent reduction in the production of dopamine and effectiveness of the dopamine system is a conspicuous characteristic of the aging brain. These findings lead to the hypothesis that the reduction in dopamine activity in the aging brain should alter the functioning of the internal clock, and that older adults should experience a slowing of their internal clock resulting in the underestimation of a duration of time.

A group of healthy active adults, 60-80 years of age, and a group of young adults, 18-22 years of age, performed two tasks; a simple counting task and a more complex visual comparison task. Each group was told to work on the task until they determined that 3 minutes had passed. The actual time spent on each task was recorded.

Comparison of the task performance showed that the older group underestimated the amount of time spent on both tasks, with the amount of time spent on the visual comparison task showing the most underestimation. The younger group underestimated the amount of time spent only on the visual comparison task, and their underestimation was significantly less than the older group. These findings are consistent with a selective decrease in the effectiveness of the dopamine system that results in the slowing of an internal clock during normal aging.

77.8

VOCAL MANIFESTATIONS OF DEMENTIA IN NURSING HOME RESIDENTS. K. Hammerschmidt[1], P. Werner[2], J. D. Newman[1]*, and J. Cohen-Mansfield[2]. [1]Lab. of Comparative Ethology, NICHD, NIH, Poolesville, MD 20837 and [2]Research Institute, Hebrew Home of Greater Washington, Rockville, MD 20852.

This study examined the vocal utterances of 25 vocally disruptive nursing home residents (20 female, 5 male, mean age 86.1 years), with the goal of identifying acoustic parameters associated with type and severity of dementia. Recordings were made during periods of spontaneous vocalization. Vocalizations were digitized at 12,500 samples/sec, using the RTS/SIGNAL sound analysis system (Engineering Design). A total of 419 utterances were saved as digital files. Dementia was rated as 'Alzheimer's,' 'vascular,' or 'unknown' by the attending physician. Statistical analyses were performed with SPSS. Eighty-five acoustic parameters were measured from each call, using LMA 5.0 (Hammerschmidt). These acoustic parameters were then used in a linear discriminant function analysis, with type of dementia as the grouping variable. Classification accuracy was 91.79%, well above chance, indicating the presence of acoustic differences in the vocalizations of the 3 groups. These findings indicate that acoustic analysis of vocalizations can be a useful tool in assessing cognitive impairment and degenerative disorders in aged humans.

Supported by NICHD (Hammerschmidt, Newman) and NIA (Werner, Cohen-Mansfield).

77.9

REGIONAL CEREBRAL BLOOD FLOW STUDIES OF MEMORY IN THE BALTIMORE LONGITUDINAL STUDY OF AGING S. Golski*, A. B. Zonderman, M. Kraut, R. N. Bryan, and S. M. Resnick. Lab. of Personality and Cognition, NIA, 4940 Eastern Ave., Baltimore, MD 21224.

Annual magnetic resonance imaging (MRI), positron emission tomography (PET), and neuropsychological assessments are being performed over 9 years in 180 BLSA participants, aged 60 and older, to assess the contribution of changes in brain structure and function to cognitive aging. PET-cerebral blood flow (CBF) scans are obtained using ^{15}Oxygen labelled water during a resting baseline and two activated conditions: performance of verbal and figural continuous recognition tasks. Accuracy in each task averaged 74%. Initial PET results for 107 right-handed individuals are reported. Activation patterns were examined separately for each of the 4 age X sex subgroups (35 men and 27 women aged 60-69; 27 men and 18 women aged 70-85) and for younger versus older subjects using statistical parametric mapping. Both recognition memory tasks elicited relative increases in CBF in the right frontal lobe, bilateral occipital lobes and posterior temporal and cerebellar region, and relative decreases in bilateral posterior cingulate gyrus. Comparisons between the verbal and figural tasks indicated relative left frontal-temporal activation for the verbal task and relative activation of right visual association areas for the figural task. Older women had less task-specific regional activations than younger women, but age groups were not significantly different for men. The relationship between individual performance and CBF activation is discussed. *NIH*

77.10

THE EFFECTS OF AGE ON THE NEURAL SUBSTRATE FOR VERBAL WORKING MEMORY. P.A. Reuter-Lorenz*[1] J. Jonides[1] E.E. Smith[1] A.A. Hartley[3] A. Cianciolo[1] E. Awh[1] C. Marshuetz[1] and R.A. Koeppe[2] Dept. of Psych.[1], Dept. of Nuclear Medicine[2], Univ. of Michigan, Ann Arbor, MI, 48109; Dept. of Psych., Scripps College, Claremont, CA[3].

Age-related impairments in cognition have been attributed in part to the decline of working memory with age. We used PET to investigate the neural substrates of verbal working memory in a group of young (mean age = 23 yrs.) and elderly adults (mean age = 70 yrs.). Performance on two different tasks was compared: An item-recognition task, in which on each trial subjects stored 4 letters in memory for 3 secs and then indicated whether an (opposite case) probe item was part of the memory set; A continuous performance task in which subjects viewed a sequence of letters and compared the current letter to the one 2-back in the sequence. Both tasks require the active maintenance/rehearsal of stored items; the 2-back task requires in addition maintenance, temporal coding and more continual updating of the stored contents. On the item recognition task older subjects were slower but as accurate as the younger group. The younger group showed activation in regions that previous research has identified with verbal storage and rehearsal. The older subjects showed a similar pattern of left hemisphere activations but there was additional activation of homologous right hemisphere sites, suggesting recruitment. On the 2-back task, analyses revealed bilateral activation in frontal and parietal sites and cerebellar activation in both groups. A comparison of high- and low-accuracy older subjects revealed that the low-accuracy subgroup had greater frontal and parietal activation whereas the high-accuracy subgroup showed higher cerebellar activation. The contribution of gender and differences in global activation levels to these differences are considered. These results suggest a change in working memory mechanisms with age.

Supported by NIA (AG 13027)

78.1

PRELIMINARY RESULTS OF MULTI MODALITY PRESURGICAL EVALUATION FOR INTRACTABLE EPILEPSY D.J.Vincent*[1], A.E.Bryant[2], D.R.Roberts[1], J.J.Halford[1], W.C. Worthington[2], C.L. Vera[1], M.S. George[1].
[1] Radiology, Med. Univ. of South Carolina, Charleston, SC 29425 and
[2] Epilepsy Unit, Roper Hospital, Charleston, SC 29401.

Numerous techniques for mapping behavior onto brain regions are now available. It is unclear which of the available methods (fMRI, WADA testing, surface EEGs, ERPs, depth and subcortical electrodes or cortical grids) are best for presurgical determination of behaviors. These techniques have varying effectiveness, invasiveness, safety and cost. As a first step in the process of ascertaining the optimal techniques and to guide future research as to when and how noninvasive methods may replace the invasive, we have begun by using all methods and cross comparing results.

To date we have studied 7 patients with intractable epilepsy, using varying combinations of structural MRI, functional MRI, WADA testing, angiography, EEGs, ERPs, depth and subdural electrodes, cortical grids and surgery. All fMRI studies used the FAS verbal fluency language task. All WADAs tested language and memory.

In 3 out of 4 the fMRI and WADA were in agreement as to the hemisphere of language dominance. While, in one, the WADA showed bilateral language and the fMRI was lateralized.

This correlation between the WADA and fMRI is promising. Future work is necessary to determine the proper methods for presurgical determination of eloquent cortex.

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78.2

DELINEATION OF SEIZURE ONSET, SEIZURE SPREAD, AND NORMAL BRAIN ACTIVITY BY FUNCTIONAL MAGNETIC RESONANCE IMAGING (fMRI). J. Hirsch[1,2], K. Kim*[1,2], J.D. Victor[2], N. Relkin[2], K. M. Lee[1,2], D.R. Moreno[1]. Dept. of Neurology[1], MSKCC; Dept. of Neurology and Neuroscience, Cornell U Med Coll[2], NY, NY 10021.

A 16 year old right handed female with a congenital malformation in the right posterior frontal lobe and a seizure disorder participated in a functional imaging study to identify sensorimotor areas. One of the patients' typical sensory seizures occurred during one of the runs. This enabled us to localize fMRI signals associated with the onset of a spontaneous seizure, its progression, and the relationship to normally activated motor cortex. Images were acquired on a 1.5-tesla scanner (GE) equipped for echo planar imaging with an in plane resolution of 1.56 X 1.56 mm. 16 contiguous slices were acquired each 4.7 mm thick. The fMRI signals associated with the seizure were first observed in an area adjacent to the normal motor activity. There was minimal movement artifact which was further reduced by alignment of the images prior to a voxel-by-voxel statistical analysis. MR signal amplitudes exceeded the normal functional activity by as much as a factor of 5. A sequential time analysis (6 sec intervals) revealed both local spreading of the onset focus and the emergence of subsequent foci first in the ipsilateral prefrontal areas, and the mesial surface areas. These were followed by activity in the homologous regions of the opposite hemisphere suggesting that the generalization followed a specific pattern of functional connectivity. These findings are the first to co-localize eloquent motor activity and seizure activity, and to quantify the onset, progression pattern, time course, and relative MR amplitudes of the seizure event.

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78.3

ABSENCE OF CORRELATION BETWEEN AMOBARBITAL DISTRIBUTION, ASSESSED WITH SPECT BRAIN PERFUSION IMAGING, AND BEHAVIORAL MANIFESTATIONS DURING THE INTRACAROTID AMOBARBITAL PROCEDURE (IAP). J.P. Soucy*, I. Rouleau, J. Robidoux and K. Laflamme, Hôpital Notre-Dame, Université de Montréal and UQAM, Montréal, Qc, Canada, H2L 4M1.

Direct intracarotid injection of Technecium-99m hexamethyl propylene amine oxime (^{99m}Tc-HMPAO) combined with SPECT has been used as a mean to assess the cerebral territories perfused by coinjected amobarbital during IAP in presurgical epileptic patients. However, the few available studies reporting memory testing results have been limited to the potentially functionnaly abnormal side ipsilateral to the determined epileptogenic focus. We have obtained right and left perfusion data in 9 patients, and unilateral injection (contralateral to the focus) in 1 patient, with well defined unilateral temporal lobe foci. Vigilance and memory were scored using a standardized procedure. Moderate to severe vigilance impairement was observed following 5 of the injections, without any correlation with either the distribution or number of territories perfused or the presence of contralateral crossover. As expected, significant memory deficits were observed after injection contralateral to the focus; however, performance varied from patient to patient and was in no way related to the perfusion pattern. Indeed, the patient with the best score was the only one with any (injected side) hippocampal visualization; the rarity of hippocampal perfusion during the IAP has previously been reported.

In conclusion, behavioural manifestations are determined not only by the directly perfused territories but also by the resulting deafferentation of structures not exposed to amobarbital.

LC Simard Res. Ctr. - PAFAC UQUAM

78.4

AN APPLICATION OF CORRELATION DIMENSION FOR PRE-PROCESS OF EPILEPTIC EEG SIGNALS. Xiaoyu Liu, Qiang Shen, Guoqing Chang*, Dazong Jiang, Biomedical Engineering Research Institute, Xi'an Jiaotong University, Xi'an 710049, P.R. China; *Department of Neurobiology, Duke University Medical Center, Durham, NC 27710.

Epilepsy is a common disease in clinics. When the epileptogenic focus appears in the brain, it discharges periodically and results in epileptiforms in EEG recorded from patients. Therefore, epilepsy can be diagnosed by detecting characteristic epileptiforms such as spikes and sharp waveforms in recorded EEG signals. However, it is usually difficult and tedious for the technician to find these characteristic epileptiforms in clinical practice because there are many normal waveforms and various artifacts in such epileptic EEG signals. Thus, it is necessary to pre-process the epileptic EEG signals to select the segments which may have characteristic epileptiforms. On the other hand, normal brain is a chaotic system. When epilepsy occurs, brain chaos status and the value of correlation dimension, one of chaos's parameters, will be reduced. Accordingly, in the present experiment, we chose the value of correlation dimension as an indicator to pre-process epileptic EEG signals and select the segments which may have characteristic epileptiforms. A moving window (width: 48000 points) was used to calculate the correlation dimensions of EEG data recorded from 6 patients, including the pre-evoked, evoked and after-evoked recordings. The threshold for correlation dimensions was set at 3.0. Any segment with lower correlation dimension was automatically selected. Results show that our method can be used to obtain epileptiforms from epileptic EEG data in a very short period. Therefore, this method might be very useful to pre-process epileptic EEG data and for EEG Holter monitoring in clinics.

78.5

TIME-FREQUENCY ANALYSIS OF MESIAL TEMPORAL LOBE SEIZURES USING THE MATCHING PURSUIT ALGORITHM

Piotr J. Franaszczuk,[1,3,4] Gregory K. Bergey,*[1,2,3] P.J. Durka[4]
Departments of [1]Neurology and [2]Physiology, [3]Maryland Epilepsy Center University of Maryland School of Medicine, Baltimore, Maryland and [4]Laboratory of Medical Physics, Warsaw University, Warsaw, Poland

The onset and propagation of partial or focal epileptic seizures is frequently an evolving and nonstationary process. Yet the ability to analyse seizure activity is often limited by methods that require stationary or quasistationary epochs of data. Time-frequency transform analysis offers the ability to analyze relatively long continuous segments of seizure activity even when the dynamics are rapidly changing. We report our early experience with the application of the time-frequency transform using the matching pursuit (MP) algorithm. Up to 80 seconds of continuous seizure activity was analyzed from selected channels obtained from recordings in patients made with simultaneous subdural grid (lateral temporal neocortical) and depth electrode arrays (mesial temporal) as part of their evaluation prior to seizure surgery. The MP approach permitted decomposition of the seizure epochs which were then displayed using Wigner plots. This permits identification of the predominant contributing frequencies for any given time period. Two patterns of seizure activity have been observed. In one pattern the predominant frequency of seizure activity has a gradual but relatively continuous change over time. In the other, many more contributing frequencies are seen with no clear pattern of change. These patterns of changes of seizure frequency may reflect different characteristics of the neuronal generators of rhythmic seizure activity.

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78.6

LANDAU-KLEFFNER SYNDROME: MODALITY INDEPENDENCE OF LINGUISTIC COMPETENCE J. Kegl[1]*, K. Baynes[2], D. Brentari[3], H. Poizner[1] Cntr. for Molec. and Behav. Neuroscience, Rutgers Univ., Newark, NJ 07102[1], Cntr. for Neuroscience[2] and Linguistics Dept.[3], Univ. of California, Davis, CA 95616

Landau-Kleffner syndrome (LKS) has been described as "acquired aphasia of childhood," and occurs secondary to epileptic activity. Symptoms include abnormal EEG, auditory agnosia, and bilateral, temporal lobe seizures, resolving at or before puberty. Our research strives to delineate the linguistic extent of the deficits associated with LKS. We present a case study of a 26 year old, right-handed female with LKS, who was a full-term birth, with mildly delayed speech milestones until age 4, when her speech deteriorated to babble. An EEG done in 1974 was severely abnormal bilaterally. Current EEG, MRI, and neurological exam are normal, but a PET scan demonstrates bilateral temporal lobe hypometabolism. This subject tests within the normal range on all tests of intelligence not requiring verbal mediation (WAIS-R (PIQ=110); RCPM; WMS-R; Wisconsin Card Sort; Trail Making Test A, B, etc.) In contrast, standard tests of language and vocabulary skills (WAIS-R) revealed severe impairment. Auditory agnosia severely compromises her spoken language production (Assessment of Phonological Properties-Revised=severely impaired)) and reception (discrimination of 172 phoneme pairs (at chance for "different" pairs)), but performance on parallel tasks using Signed English was unimpaired. Auditory processing deficits seriously impacted vocabulary development (Boston Naming Test: Spoken English (28/60=impaired), Signed English (30/60=impaired); WAIS-R (vocabulary (2)), but syntactic competence, as measured by grammaticality judgment tasks (Linebarger, et al., 1993) and argument structure distribution profile (Kegl, 1995) in Signed English, as well as written and spoken English is surprisingly spared. We conclude that LKS is characterized by a severe deficit in processing speech and speech-like auditory input, but does not have consequences for higher level linguistic processes or production and comprehension of language in non-auditory modalities. Funding: NIH/NIDCD #DC01656 & DC01664 (to Rutgers U.).

78.7

LOCALIZATION OF AN ICTAL ONSET ZONE USING A REALISTIC 4-SHELL HEAD MODEL OF SCALP, SKULL, LIQUOR AND BRAIN.

H. Nishijo*[1], H. Ikeda[2], K. Miyamoto[3], S. Endo[2], T. Ono[1]. Depts. [3]Physiol. and [2]Neurosurg., Toyama Med. & Pharmaceu. Univ., Toyama 930-01; [3]Chuo Electronics Co., Ltd, Tokyo 192, Japan.

We developed stereotaxic dipole localization (dipole tracing, DT) by a boundary element method using a realistic 4-shell head model of scalp, skull, liquor and brain (SSLB) with conductivity ratios of 1:1/80:3:1. The SSLB/DT can correct attenuation of potentials due to high resistance of a skull, and current-shunt effects of cerebrospinal fluid. To localize ictal onset zone, the SSLB/DT was applied to ictal electroencephalograms (EEGs) of a 9-month-old baby with hemimegalencephaly who had an enlarged subdural liquor space due to prior right occipital lobectomy and right parietal cortical resection. Generators of ictal EEGs were also estimated by DT using a realistic 3-shell head model of scalp, skull and brain (SSB). Estimated dipoles were superimposed on the three-dimensional magnetic resonance imaging (3-D MRI) of the patient's brain. The DT with SSLB model localized dipoles in the surface of the right parietal lobe, while the SSB/DT localized dipoles in the enlarged subdural liquor space. The electrocorticograms (ECoGs) during the second operation confirmed that ictal ECoGs (δ wave) propagated from the location of the dipoles estimated with SSLB model. The results suggest that the DT with SSLB model could accurately localize dipoles even when a subject has an enlarged subdural liquor space due to neurosurgical operation or brain atrophy.

78.8

CORTICAL INCREMENTAL AND DESYNCHRONIZING RESPONSES TO HUMAN THALAMIC CENTROMEDIAN (CM) STIMULATION. M. Velasco, * F. Velasco, A.L. Velasco, F. Brito and I. Marquez. Functional Neurosurgery, General Hospital, Neurophysiology, National Medical Center IMSS, México City.

Low frequency (6/s) threshold (320-640uA) stimulation of the basal, central and antero-basal portions of **CM** produced incremental recruiting, augmenting and primary responses, respectively. Recruiting and augmenting like responses showed a bilateral regional scalp distribution with emphasis at the ipsilateral frontal (recruiting) and central (augmenting) regions; while primary like responses showed a focal destribution at the ipsilateral parietal region. High frequency (60/s) suprathreshold (640-800uA) stimulation of the basal and central, but not the antero basal **CM** regions, produced EEG desynchoronization and a slow negative shift of the EEG baseline with same scalp distribution to that showed by recruiting and augmenting like responses. These data obtained from patients with intractable seizures and **CM** electrodes for treatment indicate that synchronizing /desynchronizing **CM** influences share the same regional path ways posible related to thalamo-cortical modulation of sen sory information.

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78.9

CORTICAL SPIKE-WAVE COMPLEX RESPONSES TO HUMAN THALAMIC CENTROMEDIAN (CM) STIMULATION. F. Velasco, * M. Velasco, A.L. Velasco, F. Jimenez and B. Rojas. Functional Neurosurgery, General Hospital and Neurophysiology National Medical Center IMSS. México City.

Low frequency (3/s), high intensity (30=2400uA) combined stimulation of left CM and right Non-specific Mesencephalic Pathways elicited a reliable response similar to the 3/sec spike-wave EEG discharges and symptoms and "irresponsiveness" of the typical Absence Attacks, spontaneosly observed in patients with genuine Primary Generalized Epilepsy. The Electrocortical features of the this response included: S1, S2, PT and W components of individual spike -wave complexes, generalized spike-wave discharges with sleep spindle afterdischarges. These were accompanied by clinical motionless stare, 3/sec eye blinking, lip smacking and fail in responsiveness to simple visual stimuli of patients under a simple responding task. Comparable stimulation of right lemniscal + left CM failed to produce such a response. This work was performed on patients with intractable seizures and CM electrodos for treatment.

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78.10

CORTICAL SPIKE-WAVE COMPLEX RESPONSES TO HUMAN HIPPOCAMPAL HIGH INTENSITY STIMULATION, A.L. Velasco,* M. Velasco,F. Velasco,I.Marquez and B.Rojas.

Unilateral 3/sec,1.0msec and 50-60v(5-6MA) stimulation of the anteromedial portions of either Hippocampal Formation or Parahippocampal Gyrus elicited some feature of the Typical Absence Attacks in patients with Temporal lobe seizures where either depth or subdural electrodes were implanted for the topographic diagnosis of the epileptic foci and/or eloquent cerebral areas.The absence attack features included: S and W components of the individual spike-wave pattern,generalized discharges with no sleep spindle after discharges,3/sec eye blinking and a partial "irresponsiveneses" consisting of delayed reaction time and repetitive responses to visual stimuli of patients under a simple response situation.The fact that Hippocampal stimulation produced typical absence attacks only when large stimulation intensities are used suggests that such "Hippocampal Response" is due to the indirect volume conduction activation of the Thalamic and Reticular Ascending Pathways closely located to the mesial hippocampal area. Although a primary hippocampal response can not be rulled out.

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78.11

MATCHING PURSUIT ANALYSIS OF EPILEPTIC EEG TRANSIENTS B. Shen*, R. Zappulla, H.W. Harper. New Jersey Neuroscience Institute at JFK Medical Center, 65 James Str., NJ 08818

Epileptic EEG transients contain essential information about the seizure mechanism. The initial location of epileptic EEG transients suggests the epileptogenic focus; the spread of epileptic activities mainly reflects synaptic propagation along conducting pathways(Jasper 1969); the termination of seizure has been considered as a result of an active inhibition, possibly by cortical norepinephrine(Efron 1961, Levy and O'Leary, 1965, Chauvel et al., 1982). Traditional analytic techniques that are heuristic or based on the assumption of stationary processes(e.g., Fourier analysis) fail to provide objective, precise time-frequency characterization of the EEGs and are thus sub-optimal for either clinical applications or studies of epilepsy. A recently developed technique, matching pursuit, offers adaptive decompositions of non-stationary signals(Mallat and Zhang, 1993) and has been shown to be effective in hippocampal EEG analysis(Shen, et al., 1995) and event-related-potential studies(Allen, et al., 1995). In this study, we applied matching pursuit analysis to multi-channel EEGs recorded from epileptic patients. The Wigner distribution, the energy distribution of the decomposed signal in the time-frequency domain, revealed a fast transient of frequency increase from 2-4 HZ to 6-8 HZ at the beginning of a seizure, followed by a slow transient of frequency decrease from 6-8 HZ to 3-5 HZ. The spatial distribution of energy at different frequencies provided an intensity map of epileptic activities at any instance; a movie of it offered a clear picture of the onset, the propagation and the termination of a seizure event.

78.12

INTERICTAL PERCEPTUAL IMPAIRMENT IN THE ABSENCE OF EPILEPTIFORM SURFACE EEG ACTIVITY

S. Knecht, L. Osinska, B. Diehl, S. Stodieck, C. Weiller*, H. Henningsen. Depts.of Neurology, Univ. of Münster and Jena, 48129 Münster, 07740 Jena, Germany

Conscious perception is held to depend on the induction of highly coordinated activity in extended cortical cell assemblies. Conversely, in epilepsy there is neuronal dysfunction spreading from a focus. We tested whether neural function remote from an epileptic focus can be disturbed in the absence of clinical or EEG evidence of epileptic activity. Bilateral psychometric forced-choice assessment of somesthetic thresholds in parallel to continuous surface EEG recording was performed in 12 patients with focal lateralized epilepsy but without structural lesions of the somatosensory cortex. Lateralization was evaluated by seizure semiology.

In 7 pts., somesthetic perception on the side with clinical involvement during focal seizures was impaired relative to the unaffected side, as well as to 32 normal controls. In the remaining pts., no side-to-side difference was found.

We suggest that epilepsy-related mechanisms, e.g. continuous covert spiking or enhanced inhibition, can impair remote cortical function even when there is no clinical or surface-EEG evidence of epileptic activity.

78.13

HIPPOCAMPAL DAMAGE IN INTRACTABLE FOCAL EPILEPSY OF CHILDHOOD. J.H. Cross[1], A. Connelly[2], G.D. Jackson[1], C.L. Johnson[3], B.G.R. Neville[1] and D.G. Gadian[2]. [1]Neurosciences and [2]Radiology and Physics Units, Institute of Child Health, and [3]Great Ormond Street Hospital for Children, London WC1N 1EH, UK. (SPON: European Brain and Behaviour Society)

Patients with epilepsy commonly show hippocampal pathology, but debate continues as to the causes of this damage and its relationship to early febrile seizures. Hippocampal pathology can be investigated non-invasively using quantitative MRI techniques, including T2 relaxometry. Thirty-eight children (age range 3-17 years) underwent MRI on a 1.5 Tesla clinical imaging system, using a protocol that included T2 maps through the hippocampi. Based on clinical and/or EEG data, 28 had temporal lobe epilepsy (TLE) and 10 had extratemporal epilepsy. The children with TLE were divided into two subgroups: those with no tumour (n=19), and those with temporal lobe tumour on MRI (n=9). Abnormal hippocampal T2 values were seen in 14 of the 19 TLE cases with no tumour (unilaterally in 9 cases, bilaterally in 5), in 7 of the 9 TLE tumour cases (unilaterally in 2, bilaterally in 5), and in 6 of the 10 extratemporal cases (unilaterally in 3, bilaterally in 3). In the tumour and extratemporal cases, the damage tended to be less severe and less lateralizing than in the nontumour TLE group. These results demonstrate a high rate of hippocampal abnormality, not only in TLE, but also in extratemporal epilepsy where the hippocampus is assumed not to be primarily involved in seizure onset. In the latter cases, and in the tumour cases, the hippocampal damage may therefore be secondary rather than primary, resulting from seizures originating elsewhere in the brain. Such secondary damage could contribute to the cognitive impairments often seen in these children.

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78.14

ASTROCYTIC CHANGES IN HUMAN EPILEPSY: NITRIC OXIDE SYNTHASE-LIKE IMMUNOREACTIVITY. B. Quinn, S. Sharma*, W. Doyle, & L.S. Conklin, Div. Neuropathology, NYU Medical Center, New York, NY 10016.

Although some cases of human epilepsy are attributed to low-grade neoplasms, trauma, cortical maldevelopment, or other lesions, many cases of adult epilepsy are unexplained even after pathologic examination of tissue removed at surgery. Traditionally, 'gliosis', including a subpial form called Chaslin's gliosis, has been one histopathologic change noted. However, increasing understanding of neural-glial interactions and the important role of astrocytes in glutamate uptake and metabolism make more detailed studies of astrocytic involvement important in human epilepsy. Immunohistochemical studies of surgical tissues can provide qualitative data for protein induction in astrocytes, and suggest areas of study for *in vitro* systems such as human astrocyte cultures. To date, we studied 15 adult temporal lobe resections and several control cases from autopsy tissue. We found marked induction of nitric oxide-synthase (NOS)-like immunoreactivity in neocortical astrocytes using a polyclonal antibody to NOS Type III (endothelial NOS: Transduction Labs N30030), but not with a battery of other antibodies to Type I and Type II NOS (neuronal and inducible/macrophage NOS, respectively) from several sources. Best immunohistochemical results were obtained in alcohol-fixed tissues in floating sections after PEG embedment. The results contrast with animal studies reporting NOS II induction (iNOS) after epilepsy. It would seem unlikely that this NOS III antibody is cross-reacting with iNOS, since iNOS antibodies did not label the same cells in our hands. The immunoreactivity did not resemble CD44, GFAP, or S100 astrocyte staining. Additional studies with NOS III *in situ* probes and with additional NOS monoclonal antibodies are indicated. If the strong immunoreactivity does not reflect a NOS isotype, it may represent an inducible astrocytic protein different than GFAP or S100. Sponsored by the American Epilepsy Foundation and the Stanley Foundation.

78.15

GABA TRANSPORT IS REDUCED IN PATIENTS WITH MEDIAL TEMPORAL LOBE SCLEROSIS. Anne Williamson* and Dennis D. Spencer Section of Neurosurgery, Yale University School of Medicine, New Haven, CT

The dentate gyrus of epileptic patients with medial temporal sclerosis (MTLE) exhibit a number of anatomical and physiological changes that are not seen in the hippocampi of patients with extrahippocampal masses (MaTLE). Using intracellular recording techniques in dentate granule cells maintained in slices, we have previously shown that the responses to exogenously applied GABA are prolonged in MTLE tissue relative to those seen in MaTLE. This prolongation appeared to be due to an impairment in the GABA transport system. In the present study, we have used nipecotic acid (NPA) to test further the function of the GABA transport system in MTLE. Brief applications of NPA can induce heterotransport of GABA and can thus be used to assess transporter function.

We compared the effects of GABA and NPA directly by using double barreled drug application pipettes. We found that NPA produced robust depolarizing responses in the MaTLE tissue which were indistinguishable from the responses to GABA in the same cells. The reversal potential for these responses was -54.8 ± 6.0 mV for GABA and -51.6 ± 4.6 for NPA, n= 3. By contrast, in the MTLE tissue, the response to NPA was greatly attenuated relative to the GABA responses. The reversal for the NPA response was -58.0 ± 6.2 mV, n=7. We hypothesize that these depolarizing GABA responses are due to bicarbonate conductance through the $GABA_A$ channels. These effects of NPA were mediated by GABA heteroexchange since they could be blocked by either bicuculline or the GABA transporter type 1 (GAT-1) specific antagonist NO-711 in both types of tissue. These data provide further support for the hypothesis that there is an impairment in the GABA transport system in the dentate gyri of patients with MTLE.

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78.16

MODULATION OF NEURONAL EXCITABILITY IN DEVELOPING HUMAN CORTICAL NEURONS K.L. Altemus*, C. Cepeda, H.C. Cromwell, Z. Li, W. Peacock, N.A. Buchwald, M.S. Levine, Mental Retardation Research Center, Division of Neurosurgery, University of California, Los Angeles, CA 90024.

Our laboratory has examined dopamine (DA) modulation of excitability of developing human neocortical neurons obtained from patients undergoing cortical resection as treatment for catastrophic childhood epilepsy. We have shown that DA enhanced responses mediated by activation of NMDA receptors but attenuated responses induced by glutamate. The present studies had two aims: 1) to assess the role of different DA receptor subtypes in DA-induced modulation, and 2) to determine if human cortical neurons expressed persistent Na^+ currents. Intracellular recordings were obtained from cortical neurons in tissue slices using standard techniques. Subsequent identification of recorded cells revealed that most cells were pyramidal neurons. DA and D_1, D_2 and D_3 receptor agonists produced small decreases in excitatory postsynaptic responses when slices were bathed in standard artificial cerebrospinal fluid. When response components mediated by NMDA receptors were isolated pharmacologically, DA and SKF 38393 (D_1 agonist) enhanced responses. Quinpirole, a D_2 agonist and 7-OH-DPAT, a D_3 agonist, produced more variable effects. Thus, the modulatory actions of DA in human cortex will depend upon the subtypes of both excitatory amino acid receptors and DA receptors activated. To examine expression of persistent Na^+ currents, whole-cell patch clamp recordings were obtained from visually identified pyramidal neurons using infrared videomicroscopy. The majority of cells displayed a tetrodotoxin-sensitive inward current probably corresponding to the persistent Na^+ current. These currents are important because they are potential targets for antiepileptic drugs. Supported by USPHS NS 28383.

78.17

THE SERUM CONTENT OF AUTOANTIBODIES TO FRAGMENTS OF GLUTAMATE RECEPTORS INCREASES IN CHILDREN WITH EPILEPSY. O.V. Globa , E.G. Sorokina, L.G. Gromova, O.I.Maslova, V.G. Pinelis* Dpts of Neurological Deseases and membranology, Inst. of Pediatrics, RAMS, Moscow, 117963, Russia.

Neurotoxicity of glutamate (GLU) is responsible for neuronal damage that results from epilepsy. There are indications that damage of central glutamatergic neurons results in the appearance of autoantibodies (aAb) to fragments of GLU receptors (FGR) which can be detected in serum (S.A. Dambinova,1989). The objective of the present work was to estimate the serum content of aAb in children with epilepsy using the paroxismal activity test (Pat) elaborated by Inst. of Human Brain, RAS (St. Peterburg, Russia). We examined 85 children from 0.2 to 15 years of age with epilepsy. Control group consisted of 20 patients with different deseases of non neurological nature. The principal findings of present study are as follows: (1) The content of aAb to FGR was increased in 80% of patients with epilepsy and was in about two times higher than in control group (1.44 ± 0.05 ng/ml in patients 0.2-1 years and 2.6±0.4 ng/ml in children 1-15 years, 0.7±0.4403 ng/ml and 1.4±0.1 ng/ml in control group accordingly, $p < 0.05$). (2) The level of serum aAb depended on the frequency and type of seazures. (3) There is direct corellation between aAb content and epileptic activity in EEG. (4) Even first episode of seizures accompanied by increase of serum aAb level. (5) In treated patients with positive effect of anticonvulsant drugs the content of aAb to FGR in serum decreased. The results obtained point to high sensitivity of Pat and this test can be used in diagnostic of epilepsy and in the estimation of therapy efficiency. (Supported by RFFI).

79.1

LONG TERM REDUCTION OF INHIBITION IN THE DENTATE GYRUS OF KINDLED RATS

P. Rutecki*, Ü. Sayın, T. Sutula. Dept. of Neuro., Neurosurg., and Neuroscience Training Program, Wm. S. Middleton VA Hosp., Univ. of Wisconsin, Madison, WI 53792

Previous studies have shown that electrical stimulation of limbic pathways that kindled seizures increased inhibition in the dentate gyrus as assessed by paired pulse stimulation of the perforant path *in vivo* (Spiller & Racine, *Brain Res.* 635: 139, 1993), and also increased the frequency of spontaneous miniature IPSC's in granule cells of the dentate gyrus (Buhl et al., *Science* 271: 369, 1996). To investigate changes in inhibition during the different stages of kindling, inhibition was assessed by paired pulse stimulation of the perforant path and recording the response in the dentate gyrus of rat hippocampal slices. The second evoked population spike was expressed as a percentage of the amplitude of the initial response. Paired pulse inhibition (PPI) was investigated after 3 class (cl) V seizures (n=7 rats, 12 slices), 1 week after 30-35 cl V seizures (n=8 rats, 16 slices), 2.5-3 months after 90-120 cl V seizures (n=5 rats, 10 slices), and was compared to age-matched controls. PPI at a 15 ms interpulse interval was increased significantly after 3 cl V seizures (33.1±9.2% vs 73.4±4.5%) and at 1 week after 30-35 cl V seizures (38.8±4.5% vs 71.6±4.8%). A significant loss of PPI occurred at 2.5-3 months after 90-120 cl V seizures (120±6.6% vs 49.9±3.5%). In the early stages of kindling, epileptogenesis was associated with increased PPI. After kindling-induced neuronal loss and mossy fibre sprouting, there was an eventual loss of inhibition in the dentate gyrus which may contribute to a permanent susceptibility for evoked and spontaneous seizures.

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79.2

PARTIAL RECOVERY OF PERFORANT-PATH-EVOKED HYPEREXCITABILITY IN THE DENTATE GYRUS OF FREELY-BEHAVING RATS DURING THE FIRST WEEK AFTER KAINATE TREATMENT

J.L. Bloomquist[1], P.R. Patrylo[1], P. Dou[1], G.M. Rose[2], and F.E. Dudek[1]* [1]Dept. of Anat. and Neurobiol., Colorado State University, Fort Collins, CO 80523 and [2]Dept. of Pharmacology, UCHSC and VAMC, Denver, CO 80220

The kainate-treated rat is an animal model of temporal-lobe epilepsy. After kainate treatment, perforant-path stimulation initially evokes enhanced responses. We tested the hypothesis that perforant-path responses recovered to normal levels after 1 week. Chronic in vivo recordings were performed during a 3-day period before and 1,4, and 7-8 days after kainate treatment. Rats were given either multiple kainate injections (IP; 5 mg/kg per hr; n = 6) for 5-10 hr, with class IV/V seizures elicited for ≥ 3½ hr, or multiple saline injections (n = 3). Prior to kainate treatment, maximal stimulation of the perforant-path evoked a positive field-potential PSP that lasted for 5-13 ms (at ½ maximum amplitude) with 1-2 population spikes (n = 6). One day after kainate treatment, however, single stimuli evoked prolonged field PSPs (duration 16-52 ms; n = 6/6) with 3-5 population spikes (n = 4/6), suggesting that inhibition was depressed. By 4-8 days, the duration of the field PSP and the number of population spikes were decreased in 4 of 6 kainate-treated rats, relative to 1 day after treatment. Five kainate-treated rats that were allowed to survive became epileptic months after treatment. Although further experiments are necessary, these results suggest that there can be a significant restoration of inhibition within 1 week after kainate treatment in rats that will eventually become epileptic.

Supported by NIH grant 16683 to F.E.D.

79.3

BEHAVIORAL AND ELECTROGRAPHIC CHARACTERISTICS OF EPILEPTIC KAINATE-TREATED RATS: SIMILARITIES WITH HUMAN TEMPORAL-LOBE EPILEPSY

P.R. Patrylo*, J.L. Bloomquist, P.S. Buckmaster, and F.E. Dudek Dept. of Anat. and Neurobiol., Colorado State University, Fort Collins, CO 80523

Tissue resected from patients with temporal-lobe epilepsy and the kainate-treated rat model show a qualitatively similar pattern of cell loss in the CA3 area of the hippocampus and the hilus and both show mossy fiber sprouting. We examined whether the kainate model has other characteristics of temporal-lobe epilepsy (e.g., a delay of seizure onset following an initial injury, permanence, and focal hippocampal seizures with secondary generalization). Adult male rats (175-360 g; n = 33) were given multiple injections of kainate (IP; 5 mg/kg) for 5-10 hr, and class IV/V seizures were elicited in all rats for ≥ 3½ hr (approximately 12% mortality). The behavior of 28 rats was then monitored for an average of 6 hr/wk from 1 day after treatment until euthanasia. Chronic in vivo recordings with surface EEG and electrodes in the dentate gyrus were obtained from freely behaving epileptic rats (n = 2) to determine whether electrographic seizure activity occurs in the dentate gyrus without overt behavioral manifestations (i.e., complex-partial seizures). All kainate-treated rats had recurrent, spontaneous, generalized seizures (i.e., either class III/IV/V) that were first observed after a delay of 69.9 ± 31.8 days. Furthermore, 8 of 10 rats, which were monitored for ≥ 200 days post-treatment, continued to have generalized seizures until euthanasia, suggesting a permanent epileptic state. The chronic in vivo recordings showed that epileptic kainate-treated rats (n = 2/2) can have electrographic seizures in the dentate gyrus with or without any overt behavioral manifestations (i.e., generalized and complex-partial seizures, respectively). Although further experiments are necessary, these data suggest that kainate-treated rats, like human temporal-lobe epileptics, show a delay of seizure onset, permanence, and focal limbic seizures.

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79.4

NMDA DEPENDANT SYNAPTIC CURRENTS DO NOT CONTRIBUTE TO PERMANENT SEIZURE SUSCEPTIBILITY AFTER KINDLING

Ü. Sayın, P. A. Rutecki, T. P. Sutula* Depts. of Neuro., Neurosurg., and Neuroscience Training Program, Wm. S. Middleton VA Hosp., Univ. of Wisconsin, Madison, WI 53792.

Kindling is a phenomenon of brain plasticity in which repeated activation of neuronal pathways induces permanent susceptibility to evoked seizures and epilepsy. The NMDA receptor is critical for the induction of kindled seizures. To investigate whether long-term increases in NMDA-dependant synaptic neurotransmission contribute to permanent seizure susceptibility in kindled rats, NMDA-dependant synaptic currents were studied in granule neurons of hippocampal slices using single-electrode voltage-clamp techniques. Kindled rats were sacrificed after 3 class (cl) V seizures (n=5 cells, 5 rats), 1 week after 30-35 cl V seizures (n=6 cells, 6 rats), 2.5-3 months after the last of 90-120 cl V seizures (n=6 cells, 5 rats), and were compared to age matched controls. The charge carried by NMDA receptor activation was calculated as the difference in percent total charge before and after APV (50 µM). Significant increases in the NMDA-dependant component of synaptic transmission were observed after 3 cl V kindled seizures (100%) and 1 week after 30-35 cl V kindled seizures (62%), but no increase was found 2.5-3 months after the last evoked seizure. The NMDA receptor plays a role in the induction of kindling, but does not account for permanent increases in seizure susceptibility in kindled rats.

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79.5

The redox site of NMDAR controls the synaptic plasticity expressed by isolated NMDAR-mediated EPSPs.

J.C. Hirsch*, C. Bernard, H. Gozlan & Y. Ben-Ari. INSERM U29, 123 Bld de Port-Royal. Paris, France.

NMDAR are sensitive to S-H and S-S reagents and in the presence of the oxydizing reagent DTNB, pharmacologicaly isolated NMDAR-mediated field responses recorded in low magnesium (0.1 µM) were decreased by 50% (GOZLAN & al., 1995, Neurobiol.26:360-369). Despite this decrease, both LTP or LTD of NMDA-dependent AMPA-mediated field responses could be induced indicating that the redox site of the NMDAR was not involved (id.; BERNARD & al., 1995, Neurosc.Lett.193:197-200).

We have examined in the CA1 area of the rat hippocampal slices if synaptic plasticity expressed by NMDAR-mediated responses depended upon the redox state of the receptor. In 1.3 mM extracellular magnesium, intracellular recordings showed that DTNB (200 µM) irreversibly decreased (63 +/- 14%; N=15) the pharmacologicaly isolated NMDAR-mediated EPSPs (CNQX: 15µM; bicuculline: 20µM; glycine: 10µM) evoked by stimulation of the Schaffer-commissural pathway. The full response could only be restored by addition of a reducing drug such as TCEP (200 µM). In control slices, LTP (197 +/- 35% increase, N=8) or LTD (77 +/- 4% decrease, N=9) of the isolated NMDAR-mediated EPSPs were induced. In contrast, when the isolated NMDAR EPSPs were mediated by fully oxydized NMDAR (i.e. in the presence of DTNB) neither LTP nor LTD could be obtained. However, returning the NMDAR to a reduced state by addition of TCEP readily restored the ability of inducing LTP or LTD (N=3 & 3, respectively).

In conclusion, the redox site controls synaptic plasticity expressed by NMDAR in non physiological conditions. This property could useful in pathological states such as epilepsy in which NMDAR are overexpressed (see companion poster, Quesada & al.).

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79.6

The redox site of NMDAR controls the epileptiform activity in the kainic lesioned rat hippocampus in vitro.

O. Quesada, C.Bernard, J.C. Hirsch, J.P. Ternaux* & Y. Ben-Ari. INSERM U29, 123 Bld de Port-Royal, Paris, FRANCE.

In the presence of oxydizing drugs pharmacologically isolated NMDAR-mediated EPSPs are irreversibly decreased by 50%; the full response can only be restored by a reducing agent (see companion poster Hirsch & all.). Because epileptiform discharges (ED) result from an overactivation of NMDAR we have investigated whether drugs that selectively act at the redox site of NMDAR could control their expression. Evoked EDs were recorded in the pyramidal cell layer of the CA1 area a week after intracerebroventricular injection of kainate. In this model, CA3 pyramidal cells degenerate and stimulation of the surviving fibres in the stratum radiatum produced multiple population spikes in extracellular recordings and a burst of action potentials in intracellularly recorded pyramidal cells (Turner & Wheal, 1991, J. Neurosc. 11,2786-94).). In seven cells which showed graded bursting activity in relation to the intensity of the stimulus, the oxydizing reagent DTNB (200 µM) decreased to one the number of action potentials overriding the depolarisation. The bursting activity never recovered even following extensive washing (> 60 min) but was readily restored by addition of the reducing agent TCEP (200 µM). APV (40 µM) blocked the late part of the burst leaving a single AMPAR-mediated action potential. When NMDAR-mediated EPSPs were pharmacologically isolated, synaptic stimulation still evoked graded EDs. At sub-threshold intensity for action potential generation, DTNB decreased the NMDAR response to 56+/- 10% of control (N=6) and TCEP reinstated the response to control levels (101 +/- 9%).

Thus manipulation of the redox sites of NMDAR may provide a novel epileptic treatment which does not share the disadvantages resulting from the complete blockade of NMDAR.

Supported by INSERM

79.7

Interneurons are not so dormant after all in various models of temporal lobe epilepsy. C.Bernard, J.C. Hirsch, R. Khazipov, M. Esclapez, B. Zivkovic* & Y. Ben-Ari. INSERM U29, 123 Bld de Port-Royal, Paris, FRANCE.

One week following intracerebroventricular injection of kainate, or two months following intraperitoneal injection of pilocarpine, stimulation of the surviving fibres in the stratum radiatum produced multiple population spikes in extracellular recordings, bursts of action potentials in pyramidal cells (intracellularly recorded) and surviving interneurons (recorded in the cell attached and whole cell configuration) in the CA1 and CA3 areas. Neurons were injected with biocytin and morphologically identified *a posteriori*. Bursts occurred simultaneously in interneurons and pyramidal cells. This evoked epileptiform activity was blocked by NMDA or AMPA receptor antagonists, suggesting that epileptiform activity was glutamatergic, or high concentration of divalent cations, suggesting the involvement of polysynaptic pathway. In CA1 pyramidal cells, fast and slow GABAergic IPSPs could be evoked after perfusion of CNQX and D-APV to block AMPA and NMDA receptor-mediated responses. When CNQX was applied alone, NMDA receptor-mediated EPSPs could be evoked in physiological concentration of Mg2+ (1.3 mM) at resting membrane potential, despite the presence of underlying fast and slow IPSPs. NMDA receptor-mediated graded bursting activity could also be evoked. Together, these results suggest 1) that interneurons are not dormant since they are not disconnected from their glutamatergic afferences, 2) that inhibition is still present in CA1 pyramidal cells but 3) that its ratio with excitation has been modified since NMDA receptor-mediated responses can be measured in contrast to control tissue.

Supported by INSERM

79.8

GRANULE CELL AXON REORGANIZATION AND INTERNEURON LOSS IN THE DENTATE GYRUS OF EPILEPTIC KAINATE-TREATED RATS: A QUANTITATIVE STEREOLOGICAL STUDY. P.S. Buckmaster* and F.E. Dudek. Dept. of Anatomy and Neurobiology, Colorado State University, Fort Collins, CO 80523.

We sought to describe quantitatively the morphological changes that occur in the dentate gyrus with temporal lobe epilepsy. Adult rats were treated systemically with kainate and months later, after displaying spontaneous recurrent seizures, were perfused for immunocytochemistry and Timm's staining. The optical fractionator and Cavalieri methods were used to estimate the numbers of parvalbumin-, CCK-, and somatostatin-immunoreactive neurons in the dentate gyrus and the volume of Timm's-positive regions in the granule cell layer and molecular layer. Age-matched vehicle-treated controls had averages of 3034 parvalbumin-, 1808 CCK-, and 8537 somatostatin-immunoreactive neurons/dentate. Some epileptic rats showed loss of parvalbumin- and CCK-positive neurons, and many showed loss of somatostatin-positive cells (means: 2343, 1311, and 4315 cells/dentate, respectively). The largest difference between epileptic and control rats was in the extent of Timm's staining in the granule cell layer and molecular layer. The volume of the granule cell layer and molecular layer that was Timm's-positive was less than 10% in almost all controls and greater than 10% in all epileptic rats (means: 5% vs. 30%). Axon reorganization and interneuron loss were most severe in the temporal pole -- an area homologous to the part of the hippocampus that is resected to treat patients with temporal lobe epilepsy. Supported by NIH grants NS16683 and NS01778.

79.9

HILAR CELL LOSS AND MOSSY FIBER SPROUTING DUE TO BILATERAL KAINIC ACID LESIONS IN THE CA3 REGION OF RAT HIPPOCAMPUS. **D.N.Chakravarty*, T.L.Babb, C.K.Chung and R.M.Bushey. Depts. of Neurosciences and Neurosurgery, The Cleveland Clinic Foundation, Cleveland, OH 44195.**

Unilateral kainic acid (KA) induced CA3 hippocampal damage in rats and ensuing hilar cell loss results in aberrant mossy fiber sprouting onto granule cell dendrites of the inner molecular layer. The aim of this study was to evaluate mossy fiber sprouting after 0.4 µg/0.2 µl unilateral injections of KA in the CA3 compared to bilateral injections of lower doses of KA. Adult male Sprague-Dawley rats were divided into 3 groups and anesthetized with ketamine/xylazine (85 mg/kg i.p.). Normal saline or KA was stereotaxically infused into the CA3 region of the hippocampus using coordinates from Sherwood and Timiras (1970, 2.5 mm anterior to lambda, 4.5 mm lateral, 5.0 mm ventral from brain surface). Group 1 received unilateral right-sided KA (0.4 µg/0.2 µl) and the same volume of saline in the contralateral CA3. Group 2 received 0.2 µg/0.2 µl of KA in right CA3 and 0.1 µg/0.2 µl in the left. Group 3 received 0.3 µg/0.2 µl of KA in the right CA3 and 0.1 µg/0.2 µl in the left. One month following KA lesions, animals were perfused with 0.9% saline, 0.1% sodium sulphide and 3% glutaraldehyde, brains were removed and sectioned. Timm's and Cresyl violet staining were done. Hilar cell loss and mossy fiber sprouting were observed in all three groups. Mossy fiber sprouting was graded according to an ordinal scale 0-3, where 0= no staining and 3= most intense staining. In group 1, sprouting was observed only unilaterally (KA side) with a score of 2. In groups 2 and 3, hilar cell loss and mossy fiber sprouting were bilateral. In group 2, sprouting had a score of 2 on the right side and 1 on the left. In group 3, the sprouting had a score of 3 in right hippocampus and 2 in the left. This indicates that bilateral KA induced CA3 lesions, even at lower doses of KA will induce mossy fiber sprouting that is at least comparable to unilateral lesions induced by a higher dose of KA. This may be attributed to commissural deafferentation caused by even smaller KA hilar lesions.

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79.10

MK-801 PRETREATMENT PREVENTS THE DEVELOPMENT OF SPONTANEOUS RECURRENT SEIZURES IN THE PILOCARPINE MODEL OF LIMBIC EPILEPSY. R.J. DeLorenzo*, A.C. Rice, M.D. Shumate, and S.B. Churn. Department of Neurology, Medical College of Virginia, Richmond, VA 23298.

Ca^{++} entering neurons through N-methyl D-aspartate (NMDA) channels has been associated with many specific responses such as long-term potentiation, delayed neuronal cell death and hyperexcitability. Here we are assessing the ability of the NMDA channel blocker, MK-801, to affect the development of spontaneous recurrent seizures (SRSs) in the pilocarpine model of limbic epilepsy in rats. EEG monitoring of the animals indicated both the MK-801 pretreated (MP) and pilocarpine alone (PILO) rats exhibited comparable status epilepticus (SE). Diazepam abruptly terminated the SE in the MP rats, whereas the PILO rats exhibited SE for at least 3.6 ± 1hr. Therefore, the MP animals were not administered DZ until 4 hrs post pilocarpine (MP-4). No SRSs were observed in the MP-4 group of animals compared to pilocarpine alone rats in which 78 % of the animals exhibited SRSs. Animals were video monitored for the occurrence of SRSs beginning 2 wks post pilocarpine injection and continuing for 6 wks or until a seizure was observed. In conclusion, MK-801 administered prior to pilocarpine-induced SE blocked the normal development of SRSs observed in this model of epilepsy. Supported by NIH grants RO1-NS23350 and T32-NS07288.

79.11

PROTRACTED EXPOSURE OF PIRIFORM/PERIRHINAL CORTEX SLICES TO 0 Mg2+ INDUCES DIFFERENTIAL PATHOLOGY IN NORMAL VS KINDLED TISSUE. M.E. Kelly*, D.P. Nocent and D.C. McIntyre. Psychology Dept., Carleton Univ., Ottawa, Ont., Canada, K1S 5B6.

Partially relieving the Mg2+ blockade from neurons in many kinds of *in vitro* slice preparations facilitates induction of self-sustaining, recurrent, epileptiform discharges. Recently, sustained seizure activity electrically triggered in low Mg2+ has been suggested as an *in vitro* model of status epilepticus (SE) (Rafiq, et al., J. Neurophysiol., 1995).

In the present report, after many hours of exposure to 0 Mg2+, we examined amygdala-kindled and control tissue to determine whether a protracted seizure experience *in vitro* would induce a pattern of brain damage that was similar to *in vivo* models of SE. Coronal slices of the amygdala/piriform/perirhinal area from either normal or amygdala-kindled rats were exposed to 0 Mg2+ or normal perfusate for 6 or 10 hours. At the appropriate time, slices were removed from the chamber and immersion fixed. Each 400 µm slice was resectioned at 40 µm and examined for argyrophilic neurons using two modifications of the Gallyas silver method. Tissues from control rats protractedly exposed to 0 Mg2+ showed extensive damage throughout layers 2B and 3 of the piriform cortex similar to SE in *in vivo* models. By contrast, the kindled tissue in the piriform cortex was relatively little damaged. This result is similar to our previous *in vivo* demonstration of kindling-based neuroprotection against kainic acid SE. On the other hand, damage to the perirhinal cortex was laminarly more complicated, but again more extensive in control compared to kindled tissues. Tissue exposed to normal medium did not exhibit damage similar to 0 Mg2+.

79.12

PARTIAL NEUROPROTECTIVE EFFECT ON THE HOST CA3/4 SUBFIELDS BY HIPPOCAMPAL FETAL GRAFTS FOLLOWING SEIZURES IN RATS. H. Keyoung[1], E.F. Sperber[2] and M-T. Romero*[1]. [1]Department of Psychology, State University of New York at Binghamton, Binghamton, NY 13902; [2]Albert Einstein College of Medicine, NYC, NY 10461.

Systemic administration of kainic acid (KA) to adult rats produces status epilepticus and subsequent neural degeneration of the CA3/4 pyramidal cell layer of the hippocamus. However, the immature hippocampus (up to 3-4 weeks of age) is protected against neural degeneration.

We used fetal transplantation to explore whether the immature or adult hippocampus, devoid of its main inputs and outputs, was still resistant to neuronal degeneration and whether the presence of the grafted hippocampus in an adult host would have a neuroprotective effect on the host's hippocampus. Embryonic day 22 hippocampal tissue was implanted in the lateral ventricle of adult female rats. Fifteen or 51-61 days following grafting the implanted hosts were injected with KA (15mg/kg, IP). Seizure activity was monitored for at least 2 hrs following the injection and status epilecticus was reduced with sodium pentobarbital a minimum of 3 hrs after KA administration. Five days later the animals were perfused and their brains processed for Timm staining and cresyl violet to visualize the mossy fiber pathway, determine the location of CA3/4 cell group in the grafted tissue, and assess the extent of neural degeneration produced in the host and graft.

Preliminary data indicate that following KA administration the percentage of hosts with complete degeneration of the CA3/4 subfields (40%) is significantly reduced as compared to nongrafted controls (100%). Sixty percent of the hosts showed either unilateral or no damage. There was no apparent degeneration in the graft regardless of age, indicating that input pathways are mediating seizure-induced damage. Further studies are underway to determine whether the protective effect is specific to hippocampal grafts.

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80.1

APP AND APLP2 ARE FOUND IN DIFFERENT INTRACELLULAR COMPARTMENTS IN NTERA2 CELLS, E. Campbell*[1], S.M. Bowes[1], R.C.A. Pearson[2], D. Parkinson[1], P. Francis[2], M-T. Webster[2]. [1]Dept. of Biomedical Sci., Sheffield Hallam University, UK. [2]Dept. of Biomedical Sci., University of Sheffield, UK. [3]UMDS, London, UK.

Altered processing of the amyloid precursor protein (APP) is implicated in the pathogenesis of Alzheimer's disease. APP is very alike in structure to the amyloid precursor-like protein 2 (APLP2). It is therefore likely that they perform similar cellular functions and it was expected that they may follow the same route of subcellular trafficking. NTera2 cells, a human cell line, were selected for this study because of their ability to differentiate into a neuronal phenotype upon treatment with retinoic acid, thus providing a model of APP and APLP2 processing in neurons in the brain.

Indirect immunocytochemistry was performed on NTera2 cells in order to study the relative distributions of APP and APLP2 within the cell, using monoclonal and polyclonal antibodies. APLP2 is concentrated in large vesicles clustered close to the nucleus which appeared to be lysosomes. APP is localized in smaller perinuclear vesicles with a reticular appearance characteristic of the Golgi apparatus. These results suggest that APP and APLP2 have different subcellular distributions and therefore may be adapted to perform similar functions in different compartments. Supported by the Wellcome Trust.

80.2

SUBCELLULAR DISTRIBUTION OF PRESENILIN-1 (PS-1) IN HUMAN AND RAT CEREBRAL CORTEX. M.J. Walsh[1], J.M. Murray[1], J. Shioi[2], N. Tezapsidis[2], N.K. Robakis[2], W. Byne[2*] and G.M. Pasinetti[2,3] [1]Department of Neurology, [2]Psychiatry and [3]Brookdale Center for Molecular Biology, Mount Sinai Medical Center, New York, NY 10029, USA

The distribution of proteins in subcellular fractions as assessed by immunoblotting is often a good indication of their subcellular localization in vivo. In this study we explored the sub-cellular distribution of Presenilin 1 (PS-1) and ß-amyloid precursor protein (ßAPP) in cerebral cortex of human and rat brain. Post-mortem cerebral cortices were fractionated by density gradient centrifugation; immunodistribution was quantified by immunoblotting with polyclonal antibodies against PS-1 or ßAPP. PS-1 affinity purified antibodies were raised against a synthetic peptide of the predicted AA sequence of human PS-1 and characterized against a glutathione S-transferase bacterial fusion protein. Polyclonal ßAPP antibody cross-reacts with both the human and rat ßAPP. Immunoblots of total human and rat cortical homogenates reacted with PS-1 antibodies revealed a major immunolabelled protein band of approximately 55 kDa and a faint band of 110 kDa consistent with PS-1 homodimerization. Moreover, there was 3 fold enrichment for PS-1 in the nuclear and synaptosomal fractions compared with the signal from the total homogenate in both human and rat samples. Immunohistochemistry examined the cell type expression in human cortex and revealed an apparent neuronal PS-1 distribution of signal, as shown in rat cortex (Pasinetti et al., 1996). The same fractions used for PS-1 studies were also examined in parallel immunoblots probed with a polyclonal antibody against ßAPP. Unlike PS-1, ßAPP was found to distribute in all subcellular fractions without dramatic enrichment in any fraction examined and consistent with the widespread distribution of ßAPP throughout the CNS and many cell types. Thus, comparative immunoblot analysis is not indicative of any consistent co-localization of PS-1 and ßAPP in vivo. Supported by NIA grant AG13618 to GMP and AG08200 to NKR.

80.3

EFFECTS OF PRESENILIN 1 AND ITS MUTATIONS ON THE PROCESSING OF AMYLOID PRECURSOR PROTEIN. X. m. Xu*, X. Wu, P. E. Fraser#, J. M. Rommens#, P. H. St George-Hyslop# and P. Gambetti. Division of Neuropathology, Case Western Reserve University, Cleveland, OH 44106 and #Centre for Research into Neurodegenerative Disease, Departments of Medicine and Medical biophysics, University of Toronto, and Division of Neurology, Department of Medicine, The Toronto Hospital, Toronto, Ontario M5S 1A8, Canada.

The majority of the cases with early-onset familial Alzheimer's disease are associated with mutations in presenilin 1 (PS-1) gene. This gene is postulated to encode a seven-transmembrane protein of unknown function. To determine whether PS-1 gene is involved in the processing of the amyloid precursor protein (APP), we have developed an efficient expressing systems of wild type and mutant forms of APP and PS-1 in human neuroblastoma cells. We found that secretion of the soluble form APP (APP_s) by cells co-transfected with wild type APP (APPwt) and wild type PS-1 is slightly reduced compared to that by cells transfected with the APPwt construct and pcDNA3 vector used for PS-1 expression construct. Secretion of APP_s is further reduced in cells co-transfected with the Swedish mutant APP (APPsw) and the PS-1 gene. These results are consistent with the hypothesis that the PS-1 gene has a regulatory effect on APP metabolism. The effect of PS-1 on amyloid β-protein (Aβ) production is under investigation. Supported by NIA grants AG1812, AGNS08155, AG08992 and the Britton Fund.

80.4

CO-EXPRESSION OF WILD TYPE AND FAMILIAL ALZHEIMER'S DISEASE (FAD) LINKED MUTANT PRESENILINS AND THE AMYLOID PROTEIN PRECURSOR (APP) IN A HUMAN NEURONAL CELL SYSTEM. T. E. Golde*, D. G. Cook, M. Forman, P. A. Holloway, V. M-Y. Lee, J. Q Trojanowski and R. W. Doms. Dept. of Pathology and Laboratory Medicine, Univ. of Pennsylvania. Philadelphia, PA 19104-4283

Mutations in the presenilin (PS) genes result in FAD that, except for variation in age of onset, is virtually indistinguishable from FAD caused by mutations in the APP gene. Therefore, mutations in PS may alter APP processing or amyloid ß peptide (Aß) metabolism in a manner that leads to amyloid deposition. To explore the possible interaction between PS protein expression and APP processing in neurons, a Semliki Forest Virus expression system was used to transiently co-express PS and APP in a human neuronal teratocarcinoma cell line (NT2) that can be terminally differentiated with retinoic acid into post-mitotic fully polarized human neurons (NT2N). This system enables high level expression of APP and PS in the same cell, and proteolytic processing of both PS and APP is observed. Using this system multiple aspects of APP processing can be monitored including production of intracellular and secreted Aß which are detected using a sensitive sandwich ELISA capable of distinguishing Aß40 from Aß42. High endogenous APP levels in the NT2N cells make it possible to study the effect of PS expression on Aß production without co-expressing APP. To reproducibly measure intracellular Aß, however, co-expression of Semliki Forest virus transduced APP is necessary. The results of a systematic evaluation of the effects of expression of wild-type and mutant PSs on both intracellular and extracellular Aß production as well as other aspects of APP and PS processing in NT2N cells will be presented.
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80.5

DIFFERENTIAL EFFECTS OF ALZHEIMER'S DISEASE-CAUSING PRESENILIN MUTATIONS ON REGULATED APP PROCESSING. U. Langer[1], M. Mayhaus[1], P.H. St. George-Hyslop[2], J.H. Growdon[3] and R.M. Nitsch[1,3]*. [1]Center for Molecular Neurobiology, University of Hamburg, 20246 Hamburg, Germany; [2]University of Toronto, Toronto, Ontario, Canada; and [3]Department of Neurology, Massachusetts General Hospital, Boston.

Several forms of familial Alzheimer's disease are caused by missense mutations of the presenilin (PS) genes S182 (PS1) or STM2 (PS2). PS1 and PS2 are ubiquitously expressed, homolog genes of unknown function. In order to study whether disease-causing PS mutations interact with regulated APP processing pathways, we stably overexpressed both wild type and mutated PS1 in 293 cells, stimulated endogenous protein kinase C (PKC) activity with phorbol myristate acetate (PMA), and estimated the concentrations of APPs in the conditioned media by dot blotting and densitometry. In cells stably overexpressing the wild-type form of PS1, APPs secretion induced by PMA (10nM) was ~3.5-fold higher than in the untransfected parent cell line, or in control-transfected (pcDNA3) cells. The PS1 mutant L286V within the TM6-TM7 loop (presuming a 7-TMD structure) completely abolished increased APPs secretion. Dose-response curves revealed ~10-fold higher EC_{50} values in the L286V mutants, as well as reductions of the maximal secretory response by approximately 50%. In contrast, mutations in other PS1 domains including A246E between TMD5 and TMD6, H163R within the TM2-TM3 loop and M146L within the second TMD failed to change PMA-induced secretion. Neither wild-type PS1 nor the disease-causing mutants changed the rates of unstimulated, constitutive APPs secretion. These data indicate a role for PS1 in regulated, but not constitutive, APPs secretion, and they suggest that structural integrity of the TM6-TM7 loop domain is important for this function.

80.6

EXPRESSION OF A ~10-kD AMYLOIDOGENIC FRAGMENT OF ßAPP USING A VACCINIA VIRUS T7-EMC COUPLED TRANSCRIPTION-TRANSLATION SYSTEM. J. Daly, D.K. Lahiri*+ and G.J. Kotwal. Department of Microbiology and Immunology, Univ. of Louisville, KY-40292; +Department of Psychiatry, Indiana Univ. School of Medicine, Indianapolis, IN-46202

The amyloid ß-peptide (Aß) that aggregates to form the core of senile plaques in Alzheimer's disease, is generated proteolytically from a family of large integral membrane glycoproteins, ß-amyloid precursor proteins (ßAPP). The cleavage of ßAPP by 'α-secretase' at amino acid Lys^{16} of Aß prevents the formation of amyloidogenic fragments. Cleavages of ßAPP occurring at amino- and carboxyl-termini of the Aß sequence by 'ß-' and 'γ-secretase', respectively, generate an intact ß-peptide which is potentially amyloidogenic.To understand the biosynthesis and cytoplasmic transport of ßAPP, we utilized the novel expression system of the vaccinia virus. DNA encoding Aß and the carboxyl-end of ßAPP was amplified by PCR from ßAPP cDNA. The 1.1 kb amplified DNA was cloned into the multiple cloning site of a vector containing the thymidine kinase gene of the vaccinia virus. The truncated ßAPP gene is placed under the control of a bacteriophage T7 promoter and a leader sequence from the encephalomyocarditis virus (EMC) that enables the cap-independent translation of the mRNA. Correct insertion of the DNA was confirmed by Southern blot analysis. The chimeric plasmid was then used to generate a stable recombinant virus containing the truncated ßAPP gene and was used as a template in an *in vitro* coupled transcription-translation reticulocyte lysate system in the presence of labeled methionine. Analysis of the translation products by SDS-PAGE revealed the presence of ~10 kDa protein equal in size to that predicted from the inserted cDNA. On 2D analysis, two spots of the expected size were observed. Also noted were spots of a higher size, suggestive of aggregation of the expressed protein. This expression system is being used to study the intracellular processing occurring in neuronal cells. Supported by the Humana and Graduate Research Council funds (GJK) and NIH-R01 grant (DKL).

80.7

IS APP IN CULTURED CELLS AND PLATELETS PROCESSED IN MULTIPLE PATHWAYS ?

R. Fukatsu[1]*, K. Tsuzuki[2], Y. Takamaru[3], Y. Hayashi[1], N. Sasaki[1], T. Yoshida[1], N. Fujii[2] and N. Takahata[1] Depts. of [1]Neuropsychiatry, and [2]Microbiology, Sapporo Medical University, School of Medicine, South 1, West 16, Sapporo 060, Japan. [3]Dept. of Neuropsychiatry, Sapporo City General Hospital, Sapporo 062, Japan

The extracellular deposition of about 40 amino acids peptides, amyloid β protein (Aβ) is invariable pathological feature in Alzheimer' disease brain. Aβ is derived from large cell surface protein, amyloid precursor protein (APP) by the proteolysis. It remains central issue to identify the subcellular compartment, or a pathway in which Aβ is generated from APP. In this study, we have examined processing of APP in cultured cells under lysosomotropic agents, and that in platelets before and after thrombin stimulation.

We characterized APP fragments, both potentially amyloidogenic and non-amyloidogenic in cultured U937, and HUT78 cell lines under leupeptin, chloroquine using polyclonal and monoclonal antibodies against synthetic peptides homologous to amino acid sequence of APP regions. APP fragments of 50, and 20 kDa containing full-length Aβ were increased in cultured cells under leupeptin and chloroquine. Subcellular fractionation of these cells showed that these 50 and 20 kDa fragments were produced in compartment closely related to endosomal/lysosomal pathway. 5-6 kDa amyloidogenic fragments were also detected in the same fractions, but these fragments did not apparently increase under lysosomotropic agents. These 50 and 20 kDa fragments were present in platelet lysate and insoluble membrane fractions after thrombin stimulation. 5-6 kDa fragments were also detectable in the same fractions but not in the platelet thrombin releasate.

Our data imply that compartments related to endosomal/lysosomal pathways may be involved in processing and generations of Aβ, and that there may be multiple pathways in which APP could be processed to generate Aβ. Amino acid sequencing of 5-6 kDa fragments are now under way.

80.8

Reversed-Phase HPLC Separations of the ß-Amyloid Peptides.

B. E. Boyes*[1], D. G. Walker[2] 1)Rockland Technologies Inc., Newport, DE 19804, USA., 2) Div. of Neurological Sciences, Dept. of Psychiatry, Univ. of British Columbia, 2255 Wesbrook Mall, Vancouver, B.C. V6T 1W5 Canada.

In the Alzheimer Disease (AD) brain, amyloid precursor polypeptides (APPs) are processed in a fashion which ultimately leads to the deposition of extracellular amyloid plaques. The major peptidic components of the plaques are the ß-amyloid peptides (ßAPs), which extend to the C-terminal 40-43 residue positions. Little is currently known of the N-terminal heterogeneity of the peptides. Chromatographic separations of the amyloid peptides would aid in detailing the heterogeneity of peptides present in tissue, but have been problematic, exhibiting poor resolution and recovery under standard gradient reversed-phase HPLC conditions. Conditions are described which permit highly-efficient HPLC separations of the ßAPs, allowing baseline resolution and high mass recovery. This procedure was applied to examine the *in vitro* proteolysis of synthetic ßAP 1-40, 1-42, and 1-43 peptides by human cathepsins. Cleavage sites were established for the peptides by mass spectral and compositional analysis. Although the sites of cleavage did not differ, the rates of digestion were slower for the longer peptides. This separation has also been applied to measure the levels of resolved ßAP peptides in AD brain tissue, using off-line immunoassays. Limited amounts of peptides extending to the N-terminal aspartate have been observed, raising the possibility that the peptides may be deposited as N-terminal truncated species, or may undergo processing after formation of the plaque.

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80.9

Subcellular localization of Alzheimer Amyloid Precursor γ-secretase cleavage products by electron microscopy

Tobias Hartmann[1], Sophie Bieger[2,3], Pentti J.Tienari[1,4,6], Nobuo Ida[1,5], Colin L. Masters[7], Carlos Dotti[8], Klaus Unsicker[1] and Konrad Beyreuther*[1] [1]ZMBH Univ. Heidelberg, Germany; [2]Dept. Anatomy & Neurobiol., Dalhousie Univ., Halifax, N.S., Canada; [3]Dept. Neurol. Univ. Helsinki, Helsinki, Finnland; [4]Toray Industries, Inc. Sonoyama Otsu Shiga, Japan; [5]EMBL, Heidelberg, Germany; [6]Dept. Anatomy & Cell Biol. Univ. Heidelberg, Germany; [7]Pathology, Univ. Melbourne, Australia.

The Alzheimer Amyloid Precursor Protein (APP) can be cleaved by different proteolytic activities which release the Alzheimer's disease-associated βA4 or related peptides. Although the cleavage sites are well-characterized, none of the proteases involved have thus far been identified. APP γ-secretase is responsible for the final processing of APP that leads to the secretion of the Alzheimer's disease-related βA4 peptides. The major activitiy of APP γ-secretase releases βA4 peptides ending at Val_{40} (βA4 numbering) and Ala_{42}. In order to better characterize this protease, we have investigated its subcellular localization by immuno-electron microscopy using our newly developed monoclonal antibodies directed against the free carboxy-terminal end of βA4 1-40 (mAb G210) and 1-42 (mAb G24). Since these antibodies do not react with full-length APP, only βA4 peptides should be recognized. Our studies with non-transfected and stably transfected COS-7 cells as well as with primary hippocampal neurons have revealed that ßA4 immunoreactivity is restricted to specific cellular localizations. Cell labeling could be blocked almost completely by pretreatment of the cells with APP γ-secretase inhibitors, while the immunoreactivities for the uncleaved APP and the precursor peptides A4CT and p3CT were not reduced. These results further support a direct correlation between the observed immunoreactivity with our βA4 1-40 or 1-42 specific antibodies and the generation of βA4 peptides. The subcellular localization of βA4 provide insights into the mechanisms by which it is produced.

80.10

PROTEOLYTIC PROCESSING AND TURNOVER OF THE TRANSMEMBRANE DOMAIN OF THE AMYLOID PRECURSOR PROTEIN (APP). W. L. Bunnell* and C. G. Glabe. Dept. of Mol. Biol. and Bioch., Univ. of Calif., Irvine, CA. 92717.

The generation of Aβ from APP requires proteolytic cleavage within the transmembrane domain (TMD) of APP by γ-secretase. This intramembrane cleavage as well as degradative proteolytic processes acting upon TMDs are not well characterized. To examine the processing and turnover of the TMD of APP, a 55 residue probe was constructed containing a signal sequence and the APP TMD flanked on each side by unique epitope tags. In vitro translation in the presence of pancreatic microsomes indicates that the probe is efficiently inserted into the membrane in the proper orientation. Pulse-chase experiments with stably-transfected 293 cells indicate that the probe turns over with a half life of ~0.5 hr, similar to the half life of the carboxyl-terminal fragments of APP. Although the cells secrete a 3 kDa P3-like protein in the media, no fragments or degradation intermediates are detected in the cell lysate with antibodies to either epitope. This suggests that the degradation process is highly processive and once a protein is selected for turn-over, it is rapidly degraded. A wide variety of protease inhibitors were tested without success in stabilizing any intermediates. A cysteine protease inhibitor causes a robust increase in the steady-state level of the probe and results in increased secretion of the 3 kDa fragment. 3-methyladenine, an inhibitor of autophagy, has similar effects. Treatment of transfected cells with Brefeldin A blocks secretion of the 3 kDa fragment, while having no effect on the steady-state levels of the probe. These results suggests that TMD degradation and γ-secretase processing are separate processes and that TMD degradation may occur in the RER. Leupeptin, E64 and chloroquine have no effect on the steady-state levels of the probe or on the secretion of the 3 kDa fragment, suggesting that lysosomal hydrolases may not play a critical role in TMD turnover. Supported by NS31230 and AG00538.

80.11

RU486 REGULATES βAPP PROCESSING. F. Lam* and P. B. Reiner, Kinsmen Laboratory of Neurological Research, University of British Columbia, Vancouver, BC, V6T 1Z3

Regulation of β amyloid precursor protein (βAPP) is believed to be an important factor in the pathogenesis of Alzheimer's Disease. βAPP cleavage is regulated by various signal transduction cascades in cells. In the course of studying this phenomenon, we observed that the glucocorticoid and progesterone receptor antagonist RU486 potently increased APP_s secretion from rat PC12 cells.

The effects of RU486 are clearly dose-dependent, with an EC_{50} of about 3.0 nM. RU486 is potent: at the EC_{50}, the increase in APP_s secretion in PC12 cells is ~2.5 fold higher than when treated with 50 ng/mL nerve growth factor. RU486 is known to block transcriptional activation via steroid hormone receptors. However, the ability of RU486 to stimulate APP_s secretion was manifest within 15 minutes of application, suggesting that this effect is not due to an alteration in gene transcription. Several intracellular second messenger cascades converge upon the enzyme MAP kinase; following 15 minutes of exposure to RU486, MAP kinase activity was unchanged, suggesting that RU486 was not altering APP_s secretion via activation of this pathway. Current efforts are directed towards understanding the mechanisms underlying this phenomenon.

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80.12

ON THE MEK OF APP_s REGULATION J. Mills*‡, D.L. Charest† S.L. Pelech† and P.B. Reiner‡. ‡Kinsmen Laboratory of Neurological Research, †Dept. of Medicine, University of British Columbia and Kinetek Biotechnology, Vancouver, B.C., Canada

Secretory processing of β-amyloid precursor protein (βAPP) is subject to regulatory control involving both PKC-dependent and PKC-independent mechanisms as well as activation of a protein-tyrosine kinase. One such signal transduction pathway may involve the sequential activation of MAP kinase kinase (MEK) and mitogen-activated protein kinase (MAP kinase). To test the hypothesis that the MAP kinase cascade is involved in regulation of βAPP processing, MEK was pharmacologically inhibited using the novel synthetic inhibitor PD98059. Western blot analyses demonstrated that PD98059 inhibits both NGF stimulation of APP_s secretion and MAP kinase activation in PC12 cells. Moreover, PD98059 inhibits both phorbol ester stimulation of APP_s secretion and activation of MAP kinase in HEK 293 cell lines. Our data indicate that activation of MAP kinase may be critically implicated in regulation of APP_s secretion.

(Supported by the Alzheimer Society of Canada)

80.13

SECRETION OF MUTATED FORMS OF AMYLOID PRECURSOR PROTEINS. T. Utsuki, W.C. Wallace, J.W. Kusiak* and B. Zhao. Drug Design & Development, Lab. of Cellular & Molecular Biology, and Molecular Neurobiology Unit, Lab. of Biological Chem., GRC/NIA/NIH, Baltimore, MD 21224

Cerebral amyloid-b protein (Aβ) deposits, in the form of senile plaques, are a major pathological feature of Alzheimer's disease (AD). Aβ derives from a glycosylated integral membrane protein, β-amyloid precursor protein (APP), which is expressed in most tissues. Aβ and other components of APP have been reported to possess both neurotoxic and neurotrophic actions depending on factors such as their dose, mode of delivery, the cell type and state and exposure time. We have previously described the use of PC12 cells stably transfected with constructs harboring mutated forms of the APP (mAPP; A692G, E693Q and V717F) to elucidate the role of APP and its processing in AD neuropathology. The presence of these mAPPs elicited apoptosis in the cell culture. In order to determine whether the apoptosis was caused by processing of the mAPP or by the properties of the secreted mAPP, we have characterized their secretion. APP levels were measured in conditioned media by immunoblot analysis using antibody 22C11, which recognizes the amino terminal. Secreted APP levels in wild type, APPA692G, APPE693Q or APPV717F transfected cells were higher than levels in vector transfected cell; 2.2-, 1.6-, 1.8- and 5.5-fold by densitometric analysis, respectively. The secreted APP exhibited a single species of similar molecular weight (about 110 KDa) for each mAPP. We are currently purifying the mAPPs from conditioned media to determine whether the normal biological functions of APP were disrupted by the mutations.

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80.14

EFFECTS OF WORTMANNIN AND BFA ON PROCESSING OF THE AMYLOID PRECURSOR PROTEIN. Suzana Petanceska,[1]* Gopal Thinakaran,[3] Sam Sisodia[3] Paul Greengard[1] and Sam Gandy[1,2]; [1]Lab.of Mol. and Cell. Neurosci., Rockefeller University, 1230 York Avenue; [2]Cornell Univ. Med. Coll., Dept. of Neurol. and Neurosci., 1300 York Avenue, New York , NY 10021, [3]Dept. of Pathol. and the Neuropathol. Lab., Johns Hopkins Univ. Sch. of Med., Baltimore , MD 21205

Brefeldin A (BFA) is a fungal metabolite known to have pleiotropic effects on cellular trafficking, due to action on various cellular compartments from ER to lysosomes; hence BFA can not only prevent proper maturation of various cargo molecules but it can also cause their missorting. Wortmannin is a relatively specific phosphoinositide 3-kinase inhibitor; recently this fungal product was shown to cause missorting of newly synthesized lysosomal enzymes, and to interfere with early stages of endocytosis. We studied the effects of wortmannin and BFA on the formation of processed derivatives of the amyloid precursor protein (APP), in PC12 cells and N2a cells transfected with wild type or Swedish mutant variant of human APP. In our experimental paradigm, wortmannin caused a modest decrease of both sAPPα and amyloid β peptide in the media, and an increase in the levels of these APP derivatives within the cell, raising the possibility that it acts either by causing prolonged covesiculation of full length APP with secretases or by inhibiting fusion of sAPPα and/or Aβ transport vesicles with the plasma membrane. BFA caused retention of full length APP (wt and Sw) within the cell accompanied by a dramatic decrease in secretion of sAPPα and amyloid β peptide. The effects of both drugs can be partially reversed by including phorbol ester (PDBu) in the treatments. The implications of these results for localizing the cellular compartments where α, β and γ secretase cleavages occur are under investigation.

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80.15

IS CATHEPSIN D THE RATE LIMITING ENZYME IN BETA AMYLOID PRECURSOR PROCESSING? E. M. Johnstone*, E. P. Dixon, K. Hui, S. P. Little. Central Nervous System Research Division, Lilly Research Labs. Eli Lilly and Company, Indianapolis, IN 46285

Aspartyl proteases are thought to cause the gamma secretase or carboxyl terminal (C-terminal) cleavage of the beta amyloid precursor protein (APP) to release the beta peptide. The implication of Cathepsin D as a gamma secretase candidate comes largely from studies using synthetic substrates or purified full length APP whereby Cathepsin D cleaves a region of APP which is physiologically predicted to be transmembrane or membrane proximal (Landror, et al, 1994, J.B.C.269:18422, Evin et al, 1995, Biochemistry 54, 14185). We have transfected 293 cells transiently with either wild type or Swedish mutant APP and Cathepsin D cDNAs and shown that under physiological conditions, Cathepsin D generates an APP C-terminal fragment that is larger than the predicted C-terminal fragment if Cathepsin D was gamma secretase. Second, we have treated cells transfected with the Swedish mutant APP with a Z-phe-ala-FMK, a cysteine protease inhibitor, that prevents the maturation of Cathepsin D to the mature active form of the enzyme and the level of amyloid peptide increased. Z-phe-ala-FMK, as well as cell-penetrating HIV protease inhibitors (aspartyl protease inhibitors) cause accumulation of APP C-terminal fragments in transfected cells. We conclude that Cathepsin D is involved with processing of APP under physiological conditions but not in beta peptide production.

80.16

Prevalence of γ-Secretase Cleavage of ßPP in Cultured Smooth Muscle Cells

T. Haske[a], H.M. Wisniewski[b], J. Frackowiak[b], B. Mazur-Kolecka[b], K.S. Kim[b], P. Mehta[b], M.R. Emmerling[a] and H. LeVine III[a]*. [a]The Parke-Davis Pharmaceutical Research Division, Warner-Lambert Company, Ann Arbor, Michigan 48105 and [b]New York State Institute for Basic Research in Developmental Disability, Department of Pathological Neurobiology, 1050 Forest Hill Road, Staten Island, NY 10314

ß-amyloid (Aß) angiopathy in the brains of aged dogs is similar to that found in human brain. Aß deposition is also seen in culture in smooth muscle cells (SMC) obtained from leptomeningeal blood vessels of aged dogs. We used SMC from leptomeningeal vessels to study the possible processing pathways that lead to cleavage of ß-amyloid precursor protein (ßPP). SMC were isolated from the leptomeningeal vessels by enzymatic digestion and grown in DMEM (low glucose) supplemented with 10% heat-inactivated fetal calf serum. Cultures used for the study contained at least 95% myocytes, as determined by immunostaining with antibodies to smooth muscle-specific α-actin and desmin. ß-protein deposits were detected with monoclonal antibodies (mAb) 4G8 and 6E10, respectively. ßPP was detected with mAb 22C11 (N-terminus) and mAb R57 (C-terminus). We show that dog vascular SMC secrete Aß as determined by ELISA. ßPP and its C-terminal fragments from both formic acid-soluble fractions and cell media were characterized by western blotting using various monoclonal and polyclonal antibodies. Our results provide evidence for the existence of extensive proteolytic processing of ßPP, a minor ß-secretase pathway, an unusual α'-secretase pathway and a major γ-secretase pathway. The prevalence of γ-secretase processing may be related to the accumulation of Aß-immunoreactive material.

Supported by Warner-Lambert.

80.17

GLUTAMATE METABOTROPIC RECEPTOR REGULATION OF AMYLOID PRECURSOR PROTEIN SECRETION IN HUMAN NTERA 2 NEURONS (NT2N CELLS). Y.C.Jolly*, V.M-Y. Lee and B.A. Wolf. Dept. Pathol., Univ. Penn. Sch. Med., Philadelphia, PA 19104.

Amyloid precursor protein (APP) can be cleaved by secretases to generate a beta amyloid (Aβ) peptide implicated in the pathogenesis of Alzheimer's disease. This study examines the signaling pathway of APP secretion in human neurons.

Metabotropic glutamate receptor subtypes 1α/β and 5α were identified in the NT2N cells by reverse-transcription-polymerase chain reaction (RT-PCR). Stimulation of these phosphatidylinositol (PI)-linked receptors with glutamate or specific receptor agonists resulted in a dose- and time-dependent increase in APP-s secretion, measured by the immunoprecipitation of APP-s from the media of [^{35}S]methionine-labeled NT2N cells. Glutamate-induced APP-s secretion was maximum at 30 min. However, glutamate caused a decrease in APP synthesis, measured by pulse-chase, suggesting that glutamate regulated the secretion of stored APP rather than newly synthesized APP. Glutamate-induced APP-s secretion required activation of phospholipase C (PLC), which resulted in inositol 1,4,5-trisphosphate (IP_3) and diacylglycerol (DAG) production, as shown by the rapid glutamate-induced accumulation of IP_3. The protein kinase C activator phorbol 12-myristate 13-acetate (PMA), a phorbol ester, as well as 1-oleoyl-2-acetoyl-3-glycerol (OAG), a cell-permeable DAG analog, also stimulated APP-s secretion. These findings suggest that APP-s secretion from NT2N cells can be regulated by activation of the PI-linked metabotropic glutamate receptor signaling pathway.

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80.18

POST-TRANSLATIONAL PROCESSING OF AMYLOID PRECURSOR PROTEIN IN NTERA2 CELLS S.M. Bowes*[1], R.C.A. Pearson[2], D. Parkinson[1] [1]Div. Biomedical Sci., Sheffield Hallam University, S1 1WB, UK and [2]Dept. of Biomedical Sci., The University of Sheffield, S10 2TN, UK

Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterised by the presence of neurofibrillary tangles and senile plaques (SP) in the brains of patients. The principle component of SPs is β-amyloid (βA4), a 40-42 amino acid peptide, cleaved from amyloid precursor protein. Several lines of evidence indicate that the production of βA4 is central to AD pathogenesis. βA4 is found in the CSF of control patients indicating that APP undergoes both amyloidogenic and non-amyloidogenic processing in normal brain. One of the possible steps leading to the deposition of βA4 in AD is that the proportion of APP undergoing amyloidogenic cleavage is increased.

The cell line Ntera2 (NT2) is a human testicular carcinoma cell line which can be induced to differentiate into a neuronal phenotype (NT2N cells). It has been shown that NT2N cells are a good model for studying APP expression and processing. Methods to study the processing of APP in NT2 and NT2N cells have been developed.

To study APP processing monoclonal antibodies were developed to specific epitopes in APP and used to recover secreted APP from the media of NT2 cells by immunoprecipitation. Any APP containing βA4 was removed by serial immunoprecipitation with an antibody recognising an epitope in the first 12 amino acids of βA4, then the remaining APP was recovered by an antibody recognising an epitope immediately N-terminal to βA4. The samples were then analysed by SDS-PAGE and Western blotting. Our results indicate that APP is processed by both amyloidogenic and non-amyloidogenic pathways in NT2 cells.

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80.19

REGULATION OF AMYLOID PRECURSOR PROTEIN METABOLISM IN HUMAN PRIMARY NEURONAL CULTURES. A.C.LeBlanc*, C.G. Goodyer. Depts. Neurol. and Pediatrics, McGill U. and Lady Davis Inst. for Medical Research, Sir Mortimer B. Davis, JGH. Montréal, PQ. H3T 1E2

The amyloid ß peptide (Aβ) is proposed to play a major role in the pathogenesis of Alzheimer's disease. In order to determine if the production of amyloidogenic peptides is cell and/or species-specific, we have been investigating human and rodent amyloid precursor protein (APP) metabolism in primary cultures of the major cerebral cell types associated with the senile plaques of Alzheimer's disease (AD). In the present study, we find that metabolism of APP through amyloidogenic pathways is enhanced in human neurons and astrocytes compared to our previous study of rat APP metabolism [LeBlanc et al., J. Neurochem.66(6), 1996]. Human neurons and astrocytes generate abundant 4 kDa Aβ and five C-terminal fragments. Astrocytes produce an additional C-terminal fragment of 14 kDa. In contrast, microglia produce low levels of C-terminal fragments and undetectable Aβ. Since decreasing the levels of Aβ is a likely therapeutic target of AD, we are presently investigating the regulation of APP metabolism in the human primary neuronal cultures. Supported by NIH RO1 NS31700 to ALB.

DEGENERATIVE DISEASE: ALZHEIMER'S-BETA-AMYLOID—GLIAL INTERACTIONS

81.1

IS β-AMYLOID A CHEMOTACTIC PEPTIDE IN ALZHEIMER'S DISEASE? D. Lorton* and J. Schaller. Sun Health Research Institute, Sun City, AZ 85372.

We propose that Aβ induces microglial functional changes by interacting with formyl chemotactic receptors. Formyl chemotactic peptides have been reported to promote chemotaxis and induce VLA expression and release of cytokines. It appears that Aβ promotes similar functions in cells of monocytic origin. Previous reports demonstrate that Aβ is chemotactic for microglia and macrophages (Davis et al., 1992) and induces the release of IL-1β from a monocyte cell line, THP-1 (Lorton et al., 1996). To test our hypothesis, we used THP-1 cells, to examine the effects of chemotactic peptides on Aβ-induced IL-1β release and determine whether Aβ can induce adhesion of these cells to VCAM, the ligand for VLA.

THP-1 cells were incubated with 125 ng/ml lipopolysaccharide (LPS) and Aβ 1-42 (5 μM) with and without the formyl chemotactic peptide inhibitor *in vitro*. Conditioned media were assayed for levels of IL-1β using an ELISA. Treatment of activated monocytes with Aβ 1-42 resulted in an elevation of IL-1β into the media compared to untreated activated monocytes confirming previous studies. Treatment of activated human monocytes with Aβ 1-42 and either chemotactic peptide blocked the release of IL-1β into the media.

To examine the ability of Aβ 1-42 to induce adhesion of THP-1 monocytes to VCAM, eight chambered coverglass wells were coated with VCAM, the wells washed, and blocked with 3% BSA in phosphate buffered saline. THP-1 cells ($4x10^6$ cells) were added to the wells and incubated with 500 nM Aβ 1-42 or FMLP in the presence and absence of chemotactic peptide inhibitor (500 nM). Control wells contained only untreated THP-1 monocytes. The media was removed and the wells washed. Cells attached to the coverslip were fixed with paraformaldehyde, stained, and counted. Treatment with either Aβ 1-42 or FMLP significantly increased the number of cells bound to VCAM coated coverslips compared to untreated cells. This effect was blocked by addition of chemotactic peptide inhibitor. Together these data provide evidence that Aβ may act via formyl chemotactic receptors to induce functional changes in microglia of Alzheimer's disease.

81.2

MICROGLIAL CELLS BIND AND INTERNALIZE AGGREGATES OF THE ALZHEIMER'S DISEASE AMYLOID ß-PROTEIN BY A SCAVENGER RECEPTOR. D. M. Paresce[1], R. K. H. Liem[1,*] and F. R. Maxfield[3].

[1] Pathology Dept., and [2] Biology Dept., Columbia University, New York, NY 10032, [3] Biochemistry Dept., Cornell University Medical College, New York, NY 10021.

Microglia are immune-system cells associated with senile plaques containing ß-amyloid (Aß) in brain tissue from patients with Alzheimer's disease (AD). Although increased Aß plaque density is one of the hallmarks of AD, whether this is mainly due to increased production or decreased clearance of plaque proteins is unclear. We found that although murine microglia could rapidly bind and internalize microaggregates of Aß peptide (1-42), degradation of the Aß microaggregates was slow. When microglia were pulsed briefly with fluorescent Aß microaggregates, then chased in Aß-free medium, Aß fluorescence was increasingly concentrated in lysosomes with time. However, even after a one week chase, a large proportion of Aß fluorescence persisted in the microglial lysosomes whereas fluorescently labeled α_2Macroglobulin was degraded within 3 hours. Fluorescent Aß microaggregate uptake was reduced by excess unlabeled Aß indicating that binding was saturable. Binding was reduced by co-incubation with excess acetyl-low density lipoprotein, and DiI labeled acetyl-LDL uptake by microglia was blocked by excess Aß suggesting that Aß and Ac-LDL bind to the same receptor. Other type A scavenger receptor ligands (fucoidan and dextran sulfate) reduced Aß binding to microglia. These results show that a type A scavenger receptor plays a significant role in saturable uptake of Aß peptide aggregates by microglia. Using this receptor, microglia may play a role in the clearance and phagocytosis of Aß plaques from the brain. Supported by NIH grants DK27083 and NS34761 to FRM.

81.3

ENDOCYTOSIS OF AMYLOID-BETA PROTEIN BY CULTURED HUMAN MONOCYTES. March D. Ard[1]* and Alan R. Salkind[2]. Depts. of [1]Anatomy and [2]Medicine, Univ. of Mississippi Med. Ctr., Jackson, MS 39216.

Microglia cultured from adult rat CNS accumulate exogenous amyloid β-protein (Aβ) from the medium, as shown by our previous studies. Depending on the composition of the medium, Aβ may accumulate at the cell surface or, with the addition of high density lipoprotein (HDL), in peripheral cytoplasmic vacuoles. HDL contains apolipoproteins E and J, as does CSF lipoprotein; so its ability to facilitate microglial endocytosis of Aβ suggests that lipoprotein may be an important determinant of Aβ clearance in brain.

In the present experiments we have used human peripheral blood monocytes as a model for microglia, to test the effects of HDL on endocytosis of Aβ (amino acids 1-42) by human cells. In culture, the monocytes become active macrophages. They accumulate Aβ both at their surface and intracellularly. Using immunogold labeling and electron microscopy, we find nonfibrillar Aβ accumulated in endosomes with heterogeneous contents. Fibrillar Aβ is phagocytosed into large vacuoles which appear to contain only Aβ fibrils plus fluid. Degradation or possible exocytosis of the fibrils over time remains to be studied.

Electron microscopic evidence shows that, unlike adult rat microglia, human monocytes endocytose Aβ in the presence or absence of added HDL. Monocytes appear to be in a state of activation in culture, having ruffled borders and numerous lipid droplets and endosomes, which are not typical of microglia. Activation may enhance their endocytosis of Aβ directly, or it may cause monocytes to secrete high levels of endogenous lipoproteins. Alternatively, the difference in results may be due to differences in receptors and transport mechanisms between the two cell types.

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81.4

NITRIC OXIDE ALTERS BETA-AMYLOID PRECURSOR PROTEIN LEVELS IN BV-2 MICROGLIA. R.T. Carroll*, B. Hartl, C. Helmer, C. Parker, J. Grimm and B.D. Shivers. Neuroscience Therapeutics, Parke-Davis Pharmaceutical Research, Division of Warner-Lambert, Co., 2800 Plymouth Road, Ann Arbor, MI 48106-1047.

Activated microglia are intimately associated with senile plaques in the brains of individuals with Alzheimer's disease (AD). Upon activation, microglia can induce of nitric oxide synthase (NOS) leading to the production of large quantities of nitric oxide (NO). NO has been shown to be a highly reactive compound with neurotoxic properties and affects the enzymatic activity of a number of proteins. Because of the profound effect of NO on cellular activity, we sought to determine if NO could alter beta-amyloid precursor protein (APP) expression/ processing in activated microglia. For this experiment we used BV-2 mouse microglia which were activated with 4 μg/mL of lipopolysaccharide (LPS) for 18 hrs. Activation of the BV-2 cells caused an increase in APP secretion into the media and a decrease in intracellular APP levels. NOS was also induced and nitrite, a breakdown product of NO, could be detected in abundance (>100 μM) 18 hrs after activation. When NOS activity was inhibited with N-methylarginine, an additional 4-fold increase in secreted APP was observed. This increase corresponded to an equivalent increase in detectable cell-associated APP. These data suggest that NO may be either inhibiting APP synthesis or accelerating its intracellular degradation through proteolysis. Either mechanism could influence beta-amyloid production derived from APP and, hence have profound on the pathogenesis of AD.

Supported by: WarnEr-LambErt Co.

81.5

Aβ- INDUCED SUPEROXIDE ANION PRODUCTION BY CULTURED MICROGLIA. C.Colton*, O. Chernyshev, J. Snell, M. Vitek and D. Gilbert. Georgetown Med. Sch., Washington DC 20007; BNP, NINDS, NIH, Bethesda, MD 20892 and Dept. Neurol., Duke Med. Cntr., Durham, NC 27710

Microglia, the CNS macrophage, have been implicated in the formation of amyloid deposits associated with Alzheimer's disease. Although their exact role is unknown, microglia may serve as a source of reactive oxygen species and thus, alter the redox status of the surrounding environment. We have examined the effect of the amyloidogenic fragment of amyloid precursor protein Aβ (1-40) on superoxide anion production in cultured neonatal hamster microglia and human monocyte-derived macrophages. Treatment with 1 to 25 μM of Aβ(1-40) for 24 hours produced a significant dose dependent increase in non-stimulated superoxide anion production but no change in phorbol ester-stimulated superoxide production. This effect was indistinguishable from the reverse peptide, Aβ (40-1), or Aβ (1-28), a peptide fragment which forms fibrils but is not directly toxic to neurons. The microtubule inhibitor, colchicine, reduced the level of superoxide produced in each case. Treatment with nitroblue tetrazolium demonstrated the deposition of formazan granules within vesicles of microglia treated with each of the 3 peptides. This data suggest that superoxide anion production is enhanced during phagocytosis of these peptides. In addition, the increased superoxide anion production is not associated with an increase in nitric oxide (NO) production since NO was not detected in the macrophage supernatants treated for up to 5 days with each peptide.

81.6

NEUROTOXICITY OF Aß PEPTIDE: CONFOCAL IMAGING OF CELLULAR CHANGES INDUCED BY ß-AMYLOID IN RAT CNS *IN VIVO*.

D.T. Weldon, J.P. Cleary, W.P. Esler, J.R. Ghilardi, E. O'Hare, S.D. Rogers, S.Q. Giraudo*, J.E. Maggio, P.W. Mantyh. Behav Pharm and Mol Neurobiol Labs, VAMC and University of Minnesota, Minneapolis, MN 55417; Dept of Biol Chem and Mol Pharm, Harvard Medical School, Boston, MA 02115.

A key issue in Alzheimer's Disease (AD) is the relative contribution that soluble versus aggregated forms of Aß play in eliciting the reactive gliosis and neurotoxicity that are hallmarks of AD. To address this question, single 10 μl injections of fibrillar $A\beta^{1-40}$ aggregates (≈ 2500 agg/inj, av diam = 20 μm), 10^{-4} M soluble $A\beta^{1-40}$ or saline were made into rat striatum or hippocampus. The cellular changes that occurred in response to these injections were then quantified using specific cell markers for microglia (OX-42), astrocytes (GFAP) and inducible nitric oxide synthase (iNOS) together with confocal microscopy, which allows single and intracellular resolution of cellular changes.

Injection of soluble Aß showed no significant increase in glial proliferation or activation beyond that induced by saline injection. In contrast, by 14 days, injections of Aß aggregates induced a significant increase in the number of OX-42+ microglia when compared to injections of soluble Aß, with microglia observed phagocytizing individual Aß aggregates. Similarly, by 30 days, injections of Aß aggregates induced a significant increase in GFAP+ astrocytes when compared to injections of soluble Aß, with astrocytes observed "walling off" the injection area. Expression of iNOS paralleled these trends and showed a marked increase over soluble Aß injections. These data demonstrate that injections of fibrillar Aß aggregates, but not soluble Aß, induce glial proliferation, activation and iNOS expression, which may mediate the neurotoxicity of Aß in the CNS. These data suggest that injection of aggregated Aß may prove an excellent model system for assessing Aß effects *in vivo* and for evaluating therapies for blocking Aß neurotoxicity.

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81.7

AMYLOID β PEPTIDE AND OTHER INFLAMMATORY MEDIATORS ENHANCE PRODUCTION OF L-GLUTAMATE BY MACROPHAGES.

A. Klegeris, D.G. Walker, E.G. McGeer* and P.L. McGeer
Kinsmen Laboratory of Neurological Research, Department of Psychiatry, University of British Columbia, Vancouver, B.C., Canada, V6T 1Z3.

Immunological mechanisms, including stimulation of brain microglia and activation of complement, as well as NMDA receptor-mediated excitotoxicity have been implicated in the pathogenesis of Alzheimer's disease, while accumulation of amyloid β peptide (Aβ) is one of its main pathological features. We previously reported that activation of macrophages by amyloid β peptide and/or its subfragment (25-35) can be readily detected in several assays including monitoring of the respiratory burst, cell aggregation, NO production and TNF-α secretion. In this study the effect of Aβ and various other substances on rat peritoneal macrophages was studied by their effect on L-glutamate secretion by the cells.

As previously reported by others, it was confirmed that macrophages spontaneously produced significant amounts of L-glutamate (100 μM range) and that this secretion was further enhanced by serum-opsonized zymosan, lipopolysaccharide and phorbol myristate acetate. Human IFN-γ, IL-1β and TNF-α did not have any significant effects. Application of Aβ (1-40) also enhanced the production of glutamate, while Aβ (25-35) did not. The effect of Aβ (1-40) on macrophages was not altered by 'aging' of this peptide in PBS for 4 days at 37 °C.

The data presented support the hypothesis that inflammatory mediators, by acting upon microglia, could increase the concentration of L-glutamate in brain tissue. It is also suggested that Aβ could directly stimulate glutamate secretion by microglia, placing glutamate-sensitive neurons surrounding amyloid plaques at high risk. Both these mechanisms could play important roles in the pathogenesis of neurodegenerative disorders, including Alzheimer's disease.

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

81.8

CHARACTERIZATION AND EVALUATION OF MMGT-16 CULTURE (A TRANSFORMED MURINE MICROGLIAL CELL LINE) AS A MODEL SYSTEM FOR STUDYING MICROGLIA AND EFFECTS OF POLYMERIZED Aß (1-40) ON THE VIABILITY OF MMGT-16 CULTURE

F. Dean Miller[1], Andre Van De Voorde[2], Tony W. Briers[2], Paul A. Hyslop[1*])
Dept. CNS Research, Eli Lilly & Co., Lilly Corporate Center, Indianapolis, IN 46285. 2) Innogenetics, Industriepark Zwijnaarde 7, Box 4, B-9052 Gent, Belgium. Inflammation in the central nervous system (CNS) is thought to play a major role in the development and progression of chronic neurodegenerative diseases. It is also thought to be a major determinant in controlling the extent of neuronal cell damage following acute injury to the CNS. Activated microglia associated with the senile plaque in brains from Alzheimer's disease patients produce and secrete many known mediators of the inflammatory process. In order to further understand the role of microglial activation in the progression of Alzheimer's disease, we have further characterized the microglial cell line MMGT-16 [Briers, et al, J. Neuroimmunol., 52; 153, 1994]. We have confirmed that these cells secrete the appropriate cytokines (IL-1β , IL-1α, TNF-α , and IL-6) when activated by LPS and also display the correct surface marker profile for microglia as measured by immunocytochemical methods. We have also shown that this cell line constitutively produces PGE_2 (2.8 ng/ml ± 0.25 ng/ml) during a 24 hr incubation and the mechanism of this production is under investigation. This cell line however does not secrete any of the above cytokines in the presence of 25 to 50 μM Aß following 6 to 72 hr exposure. Furthermore, exposure of these cells to deposited fibrillar Aß also gave no enhancement of cytokine response. However, microglia exposed to the Aß fibrils maintain their viability following withdrawal of growth media for longer than control as measured by calcein fluorescence [3 day control 2900 ± 81 RFUs, 3 day Aß treated 8490 ± 885 RFUs]. This observation may indicate that microglia interacts with Aß and this mechanism of interaction is under investigation.

(Funded by Eli Lilly and Company)

81.9

IMMUNOLOCALIZATION OF PERLECAN TO MICROGLIA IN VITRO AND IN VIVO. J.D. Miller*, J.A. Cummings , C. Ngo, K. Kimata, T.N. Wight, and A.D. Snow. Dept. of Pathology, Neuropathology Labs Box 356480, University of Washington, Seattle, WA 98195-6480 and Institute for Molecular Medicine, Aichi Medical University, Aichi, Nagakute, Japan 480-11.

The origin of the specific heparan sulfate proteoglycan (PG), perlecan, in beta-amyloid protein (Aß)-containing amyloid deposits in Alzheimer's disease(AD) brain is not known. In the present study indirect immunofluorescence identified possible cell candidates of perlecan production in primary cell cultures and in a rodent model. Double and triple-labelled indirect immunofluorescence was performed on dissociated primary rat septal cultures using specific antibodies for identification of different cell types, and for perlecan core protein. In mixed cultures of both embryonic day18 and post-natal day 2-3, microglia identified by labeling with OX-42 or anti-ED1 were the only cell type also double labeled with a polyclonal antibody against perlecan core protein. Similar immunolabeling of microglia with the anti-perlecan antibody was also observed in purified cultures of post-natal rat microglia. Western blot analyses of microglial cell layer PGs with the perlecan core protein antibody revealed a ~ 400 kDa core protein (suggestive of perlecan) following heparitinase digestion. Other lower M_r bands were also found implicating either degradation of the 400 kDa core protein or the presence of separate and distinct gene products immunologically related to perlecan. Following a 1-week continuous infusion of Aß (1-40) into rodent hippocampus, double and triple-labeled immunofluorescent studies revealed endogenous perlecan accumulation primarily localized to infiltrating microglia within the Aß infusion site. These studies suggest that microglia may represent one source of perlecan (or an immunologically related PG), which may be important for the ongoing accumulation of both perlecan and Aß in the amyloid deposits of AD. Partially supported by NIH AG12953-02 and AG05136.

81.10

MICROGLIAL RESPONSE TO ALZHEIMER β-AMYLOID PROTEIN, B. Tate*, J. Maguire, J. Newton and R. Majocha. Dept. Psychiatry and Human Behavior, Miriam Hosp. and Brown Univ., Providence, RI and McMaster Univ. and Hamilton Civic Hosp., Hamilton, ONT, Canada.

In Alzheimer's Disease, activated microglia are associated with dense β-amyloid deposits. In vitro, β-amyloid can stimulate microglia to become reactive. The in vivo microglial response to β-amyloid peptide 1-40 in mammalian brain was investigated. Rats were given intracranial ventricular (ICV) infusion of β-amyloid for 2-4 weeks. Light microscopic immunohistochemistry documented increased numbers of microglia including reactive glia surrounding amyloid deposits. Staining with antibodies to inducible nitric oxide synthase (iNOS) demonstrated iNOS expression in the area of amyloid deposition. At the ultrastructural level both diffuse and fibrillary amyloid were found within cells. Macrophages with intracellular amyloid were found surrounding blood vessels. The finding of intracellular fibrillary and diffuse amyloid suggests a role for cellular amyloid processing early in senile plaque formation and subsequent neuronal degeneration.

Medtronic Neurological Division

81.11

BRAIN MICROGLIA CONTAIN FRAGMENTS OF AMYLOID β-PROTEIN IN ALZHEIMER'S DISEASE, H. Akiyama[1*], H. Kondo[1], H. Mori[1], F. Kametani[1], K. Ikeda[1], C. Schwab[2], E. G. McGeer[2] and P. L. McGeer[2]. [1]Tokyo Institute of Psychiatry, 2-1-8 Kamikitazawa, Setagaya-ku, Tokyo 156, Japan, and [2]Kinsmen Laboratory of Neurological Research, University of British Columbia, Vancouver, B.C., V6T 1W5, Canada

Microglia which contain granules that are immunopositive for C-terminal fragments of amyloid β-protein(Aβ) appear around extracellular amyloid deposits in both aged control and Alzheimer's disease(AD) subjects. Brain macrophages in some AD patients with cortical ischemic lesions, also contain such granules. Granule containing cells are absent in control brains lacking Aβ deposits. The granules themselves are not recognized by antibodies specific for the N-terminal sequences of Aβ. These include Aβ1(specific for N-terminal Asp^1), R17(Aβ8-17) and 6F/3D(Aβ8-17). As far as the C-terminal regions are concerned, the vast majority are recognized by an antibody specific for the Ala^{42}-Thr^{43} region of Aβ but negative for the Val^{40} region. This might be attributable to the relative paucity of Aβ fragments ending at Val^{40} in extracellular Aβ deposits. Two antibodies 4G8 and 2F recognize N-terminally located epitopes of Aβ(17-24) peptide. Both 4G8 and 2F stain the majority of granules, although a small number which are negative for 4G8 and 2F are positive for E50(Aβ17-31) and βmid(Aβ25-35). These data suggest that the granules are fragments of Aβ which have been cleaved between residues 17 and 24, with the N-terminal regions being degraded. We propose that Aβ is phagocytosed and processed by brain microglia and macrophages, and that the C-terminal fragments are more resistant to proteolysis than the N-terminal fragments.

Supported by grants from the Naito foundation and the Alzheimer Society of B.C.

81.12

LN3 IMMUNOPOSITIVE MICROGLIA ARE PRESENT WITHIN ALL SUBTYPES OF SENILE PLAQUES IN ALZHEIMER'S DISEASE. Brian J. Cummings*[1,2], Shari L. Weinstein[2], Arman Afagh[2] and Carl W. Cotman[2]. [1]Lab for Molecular Neuroscience, McLean Hospital, Harvard Medical School, 115 Mill St., Belmont, MA 02178 and [2]Institute for Brain Aging and Dementia, Irvine, CA.

Senile plaques are the major neuropathological hallmark of Alzheimer's disease (AD), yet it is unclear what role, if any, microglia play in plaque formation and maturation. We have undertaken a series of studies to help elucidate the time-course and contribution of different cellular elements to the development and maturation of senile plaques. For example, APP laden neurons are present within diffuse plaques (Cummings *et al.*, 1992), while GFAP-immunopositive astrocytes are associated predominantly with thioflavine-positive "primitive" and "neuritic" plaques and are rarely observed in thioflavine-negative "diffuse" plaques (Pike *et al.*, 1994). In the present study, tissue sections from mild, moderate and severely demented AD cases were simultaneously triple-labeled for Aß (anti-ß42 and Cy3 fluorochrome), activated microglia (anti-LN3 and DAB peroxidase) and thioflavine S counter-staining to evaluate the relative level of thioflavine/ß-sheet assembly and extent of microglial association within each plaque. One hundred randomly picked plaques per case were selected via Aß immunoreactivity and evaluated by two blind observers on a semi-quantitative scale for (*i*) thioflavine intensity and (*ii*) extent of LN3-positive microglia. Inter-rater reliability for scoring was strong (0.92). Nearly 50% of diffuse plaques contained elevated numbers of LN3-positive microglia relative to the surrounding neuropil. The percentage of plaques containing LN3 positive microglia increased with increasing thioflavine intensity. Chi-square analysis indicates this relation is highly significant ($p<0.0001$). These results suggest that, unlike GFAP-immunopositive astrocytes, a subset of activated microglia (*e.g.* LN3-positive) are present in the initial stages of plaque formation. Alternatively, plaques many form/mature by more than one pathway. (Supported by AG00538 to C.W.C.)

81.13

RAGE IN ALZHEIMER'S DISEASE: A RECEPTOR MEDIATING AMYLOID-BETA PEPTIDE-INDUCED ACTIVATION OF MICROGLIA. Shi-Du Yan, Xi Chen, Jin Fu, Ming Chen, Huajiie Zhu, Lei Zhao, Mariko Nagashima, John Morser, Alex Roher*, David Stern, Ann Marie Schmidt. Columbia University, New York, NY 10032; Sun Health Res. Inst., Sun City, AZ 85372.

The immunoglobulin superfamily molecule RAGE has been previously characterized as a Receptor for Advanced Glycation Endproducts (RAGE) expressed by macrophages. Microglial expression of RAGE is enhanced in affected Alzheimer's brain, by immunostaining for RAGE and the microglial marker CD68. Transfection of COS-1 cells with the RAGE cDNA resulted in specific binding of amyloid-beta (Aß) peptide, Kd ≈25 nM, compared with absence of such binding on mock-transfected controls. Primary cultures of rat microglia and the transformed microglial line of BV-2 cells expressed RAGE; binding of ^{125}I-Aß (1-40) to these cells was dose-dependent (Kd 50 nM), and was prevented by blocking RAGE with anti-RAGE IgG or by adding excess soluble RAGE (extracellular domain). Chemotaxis of BV-2 cells occurred in the presence of an increasing concentration gradient of soluble Aß (1-40 or 25-35); this was prevented by blocking RAGE. Aß adsorbed to the upper surface of the chemotaxis membrane prevented the migration of microglia in response to another stimulus, such as formyl-met-leu-phe. This was due to microglial RAGE engagement of the immobilized Aß, as blocking RAGE allowed the cells to pass through the membranes as in controls coated with albumin or irrelevant peptides. Aß-induced activation of microglia, assessed by activation of NF-kB and induction of tumor necrosis factor-alpha (TNF; the latter at the mRNA and protein level) were both blocked by anti-RAGE IgG. We propose that in Alzheimer's microglia migrate down a concentration gradient of soluble Aß, their motility is arrested when they encounter immobilized Aß, and they undergo sustained activation, due at least in part, to RAGE interaction with Aß. AHAF, NIA

81.14

REGULATION OF β-AMYLOID PRECURSOR PROTEIN (APP) ISOFORM mRNAs AND PROTEINS BY SENILE PLAQUE ASSOCIATED CYTOKINES IN CULTURED NEURAL CELLS. A.J. Patel, A. Jen, C. Wickenden, L.S. Jen and H.A.R. de Silva (SPON: Brain Research Association). MRC Neurodegenerative Disorders Group, Neurological Sciences Research Division, Charing Cross & Westminster Medical School, London W6 8RF, U.K.

Recently we proposed that in Alzheimer's disease the normal balance of metabolic pathways regulating plaque associated cytokines is disrupted. Reduction in cytokines may result in neurons being deprived of neurotrophic factors while an excess may initiate a cascade of interactions between glial cells and APP metabolism thereby facilitating plaque formation. In support of the latter proposal, we show that these cytokines play an important role in the regulation of neural cell specific splicing of APP. More than 90% of APP mRNAs expressed in astrocytes and microglia were of Kunitz inhibitor domain (KPI)-containing APP751 and APP770 isoforms, while neurons expressed mainly the non-KPI-containing APP695 isoform. Purified IgGs against N-terminal (AP-1), KPI (AP-2), β-amyloid (AP-4) and C-terminal (AP-5) stained discrete bands on Western blots of homogenates of neural cells. Levels of KPI-containing proteins were much greater in cultured astrocytes than neurons. In fimbria-fornix lesioned rat hippocampus, labelling of KPI-containing APP proteins was specifically increased in glial fibrillary acidic protein-positive reactive astrocytes. Treatment of cultured neural cells with plaque associated cytokines, basic fibroblast growth factor, transforming growth factor-β1 and interleukin-1β, significantly elevated the expression of APP isoform mRNAs and proteins in age- and neural cell-specific manner. The changes in the regulation of alternative splicing of the APP gene with excess production of the exon 8 containing isoform and no production of the exon 15 containing isoform would modify the properties and tertiary structure of APP protein, which, in turn, may change the patterns of APP trafficking, proteolysis and the production of β-amyloid peptide in the Alzheimer's brain. (Supported by MRC Grant).

81.15

INDUCTION OF IMMEDIATE EARLY GENES BY ß-AMYLOID PEPTIDE IN CULTURED GLIAL CELLS. A. Copani[1*], A. Calogero[2] and F. Nicoletti[2,3]. Institute of Pharmacology, [1]School of Medicine or [3]Pharmacy, Catania University, Italy; [2]I.M.N. "Neuromed", Pozzilli, Italy.

ß-Amyloid peptide (ßAP) induces apoptosis in cultured neurons, and its action is accompanied by a persistent increase in c-jun and an occasional and transient increase in c-fos expression (Anderson et al., J. Neurochem., 1995). Knowing that ßAP induces morphological changes without apparent toxicity in astrocytes, we have studied the expression of immediate early genes (IEGs) in primary cultures of cortical glial cells exposed to ßAP. ßAP induced a substantial expression of Fos and Egr-1 proteins, which peaked between 3 and 6 hours and was no longer visible after 24 hours. The immunostaining was restricted to cell nuclei. In cultures arrested in G_0 and then committed to proliferate, ßAP induced a longer-lasting increase in Egr-1 expression, which was still present after 20 hours. Whether or not the induction of IEGs by ßAP is cell-cycle dependent is under investigation.

81.16

ß-AMYLOID STIMULATES METALLOPROTEINASES, PLASMINOGEN ACTIVATORS, AND THE DEGRADATION OF EXTRACELLULAR MATRIX PROTEIN IN RAT ASTROCYTES. S. Deb*, B. Bing and P. E. Gottschall University of South Florida, Department of Pharmacology and Therapeutics, Tampa, FL 33612-4799

Senile plaques of Alzheimer's disease (AD) are characterized by the presence of ß-amyloid (Aß) at the core and are associated with activated astrocytes and microglia, and dystrophic neurites. Recent studies indicate that components of the extracellular matrix (ECM), especially laminin, fibronectin and heparan sulfate proteoglycan, as well as ECM degrading enzymes, the matrix metalloproteinases (MMPs), are localized in and around senile plaques in AD brain. We have shown that Aß(1-40) induces MMP-9, MMP-2 and MMP-3 production in rat mixed hippocampal and astrocyte cultures. The aim of this study was to further elucidate whether Aß induces the production of ECM-degrading enzymes and if this stimulation of enzyme influences the stability of ECM components. Aß(1-42) was a potent inducer of gelatinase (Glase) activity in rat astrocyte cultures. Also, increased plasminogen activator-like activity (measured by plasminogen-casein zymography) was observed in conditioned medium obtained from Aß(1-40)-treated astrocytes compared to untreated cells. Astrocyte Aß-treated conditioned medium, when immunoblotted for fibronectin using polyclonal anti-rat fibronectin, showed several lower molecular weight products indicative of degradation of the protein. These products were observed to a significant lesser degree in control conditioned medium. The molecular weight of these products were 160, 100, 72, 56 and 47 kDa. Thus, Aß peptides including Aß(1-42) induce Glase and plasminogen activator-like activities in astrocyte cultures. In addition, Aß appeared to stimulate the release of enzymes capable of degrading fibronectin. (supported by NIH AG12160)

81.17

ACTIVATION OF CULTURED ASTROCYTES BY AMYLOID β PEPTIDE. K.T. Akama*, J. Hu, L.-M. Zhou, G.A. Krafft, and L.J. Van Eldik. Depts of Cell and Molecular Biology, and Mol Pharmacology and Biological Chemistry, Northwestern University Medical School, and Northwestern Univ. Institute for Neuroscience, Chicago, IL 60611.

One common feature of many neurodegenerative disorders, including Alzheimer's disease (AD), is the presence of large numbers of activated astrocytes and microglia. Glial activation involves morphological changes (enlargement of the cell soma, hypertrophy of nuclei, and process elaboration) and changes in expression of a variety of proteins. In AD, activated astrocytes surround the neuritic shell of the amyloid plaque, and activated microglia are near the center of the neuritic shell adjacent to the core of amyloid β protein (Aβ). There are a number of stimuli that lead to glial activation, but the relationship between glial activation and disease progression is not known. We evaluated the possibility that Aβ, a primary constituent of plaques, contributes to glial activation by testing the effects of aggregated Aβ peptides on cultures of rat cortical astrocytes. In contrast to its neurotoxic effects, Aβ did not significantly decrease astrocyte viability. Instead, Aβ induced astrocyte activation, as assessed by reactive morphological changes and upregulation of selective glial mRNA and proteins, such as the inflammatory cytokine IL-1β. Aβ 1-42, a major form of amyloid associated with neurotoxicity, activated astrocytes in a time- and dose-dependent manner, whereas Aβ 17-42 (the Aβ form associated with diffuse plaques) had little or no effect. Our data suggest that part of the neurodegenerative cascade in AD involves effects of Aβ on glial cells, perhaps contributing to the progression of toxicity by release of glial factors that exacerbate or attenuate Aβ function (supported in part by NIH grant AG10481).

81.18

Alpha-1-antichymotrypsin secretion from human astrocytoma cells induced by amyloid ß. B. D. Gitter* and J. Hackett. Central Nervous System Research Division, Lilly Research Labs., Lilly Corp. Ctr., Indianapolis, IN 46285.

Neurodegenerative processes in Alzheimer's disease (AD) are thought to be driven in part by the deposition of amyloid ß (Aß), a 39-43 amino acid peptide product resulting from an alternative cleavage of amyloid precursor protein (APP). Aß may promote AD neuropathology directly through its neurotoxic effects or indirectly by activating immune/inflammatory pathways. In support of the latter hypothesis, we have recently shown that Aß potentiates inflammatory cytokine secretion from interleukin-1 (IL-1)-stimulated human astrocytoma cells (Proc. Natl. Acad. Sci., 92, 10738-10741, 1995). α-1-antichymotrypsin (ACT), an acute phase reactant produced by astrocytes and found in close association with compact neuritic plaques, may also be secreted from Aß-stimulated cells. Here we report that Aß can activate human astrocytoma (U-373 MG) cells to secrete ACT. Freshly prepared Aß (2-50 μM) or human IL-1ß (0.01-10 ng/ml) dose-dependently induced an increase in ACT secretion from U-373 MG cells. At the highest concentrations tested, 100% (with Aß) and 340% (with IL-1ß) increases in ACT secretion were observed (determined by Western blotting and densitometric scanning). The effects of combining these treatments (50 μM Aß + 0.01-10 ng/ml IL-1ß) were additive. In addition, significant increases in ACT secretion were not detected from $Aß_{(25-35)}$-treated U-373 MG cells. Since ACT may promote Aß fibrillogenesis and regulate APP processing, increased ACT secretion by Aß-stimulated astrocytes may exacerbate AD neuropathology. This study was fully funded by Lilly Research Laboratories, a division of Eli Lilly & Co.

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

82.1

NEUROPROTECTIVE EFFECTS OF INSULIN-LIKE GROWTH FACTOR I AGAINST TOXICITY INDUCED BY INTERLEUKIN-15, HUMAN AMYLIN AND ß-AMYLOID PEPTIDES IN CULTURED HIPPOCAMPAL NEURONS. S. Doré*, S. Bastianetto, F. Mennicken, S.Kar and R. Quirion. Douglas Hospital Research Centre, McGill University, Montréal, Québec, Canada, H4H 1R3.

Multiple evidence suggests the critical importance of the insulin-like growth factor I (IGF-I) in the maintenance of tissues integrity. IGF-I acts via specific receptors uniquely distributed throughout the brain, being especially concentrated in the hippocampal formation (eg. Quirion *et al.*, this meeting). We recently reported that IGF-I was able to protect against ß amyloid-induced toxicity in primary hippocampal neuronal cultures (Doré *et al.* Soc. Neurosci. Abst 21, 475, 1995). Our objective was to evaluate if this neuroprotective action was unique to Aß toxicity ($Aß_{25-35}$: 30μM; $Aß_{1-42}$:20μM) or applicable to other models such as toxicity induced by human amylin (30μM; rat amylin being ineffective) and interleukin-15 (IL-15: 50ng/ml; Mennicken *et al.*, this meeting). Rat hippocampi were dissected from E19 rat embryos and grown in DMEM for 6 days under serum-free conditions with B-27 as supplement. Neurons were treated with the various peptides for another 6 days in a Neurobasal medium with N-2 supplement with or without IGF-I. The neurotoxicity was determined using the MTT colorimetric assay which is an index of mitochondrial activity. Interestingly, IGF-I (10-100nM) was found to significantly protect the neurons against these various types of toxicity in a time and concentration-dependent manner. IGF-I is thus able to act as a rather global neuroprotective agent. Supported by the MRCC and the Alzheimer Society of Canada.

82.2

PROTECTIVE EFFECTS OF TRANSFORMING GROWTH FACTORS-β AGAINST CYTOTOXICITY OF β-AMYLOID PEPTIDE IN HUMAN POSTMITOTIC NEURONS. Renee F. Ren*, Richard S. Kim and Kathleen C. Flanders. Laboratory of Chemoprevention, National Cancer Institute, NIH, Bethesda, MD 20892

In a previous study in rat hippocampal neurons, we observed that transforming growth factors-β (TGFs-β) can protect cells from neuronal degeneration induced by β-amyloid peptide fragment 25-35 (βAP). This finding could be valuable for protection of neurons in Alzheimer's disease (AD) if this phenomenon also appears in human neurons. Here we report similar protective effects in human postmitotic neurons (hNT neurons). The hNT neurons were generated by treating the human teratocarcinoma cell line NT_2/D1 with retinoic acid for 14 weeks, inducing the expression of markers of terminally differentiated neurons. Cells were treated with 10 ng/ml TGF-β1 for 24 h before being exposed to 10^{-5} M βAP. After 24 h exposure to βAP, morphological examination following MTT incorporation showed that the TGF-β treated cells had a significant increase in cell viability compared to cells not pretreated with TGF-β. Studies using reverse transcription-polymerase chain reaction (RT-PCR) revealed endogenous expression of TGF-βs in hNT cells. Semi-quantitative RT-PCR showed that treatment with βAP resulted in little change in mRNA levels of TGF-β1 and TGF-β3, while TGF-β2 mRNA increased approximately 4-fold. The mRNA level of amyloid precursor protein (hApp 770, 751) was decreased approximately 2-fold. Interestingly, the protective effects of TGF-βs were not found in NT_2/D1 cells from which hNT neurons are derived. Our preliminary results show that TGF-β type II receptor, that is expressed in hNT cells, is not detectable in NT_2/D1 cells, suggesting the protective effects of TGF-β are probably mediated by the TGF- β Type II receptor.

82.3

REDUCTION OF BETA-AMYLOID NEURONAL TOXICITY BY HIGH DENSITY LIPOPROTEIN. Z.S. Farhangrazi[1]*, L.M.T. Canzoniero[1], S. L. Sensi[1], G. Bu[2], L.L. Dugan[1], D. Holtzman[1], D.W. Choi[1]. [1]Center for the Study of Nervous System Injury and Dept. of Neurology, and [2]Edward Malinckrodt Department of Pediatrics and Molecular Biology and Pharmacology, Washington Univ. School of Medicine, St. Louis, MO 63110.

Recently, a major genetic risk for Alzheimer's disease (AD) was identified to be the ε4 allele of apolipoprotein E (apoE). It is thus of interest to examine the ability of lipoproteins to influence the neurotoxicity of beta-amyloid (Aβ). In the CSF, both apoE and some Aβ are found within high density lipoprotein (HDL) particles.

In this preliminary study, we examined the effect of adding HDL upon the toxicity of Aβ (1-42) on cultured murine cortical neurons. Culures exposed to 30 μM Aβ exhibited widespread neuronal degeneration without astrocyte degeneration 48 hr later. We found that inclusion of HDL purified from human serum in the exposure medium markedly reduced Aβ toxicity, in a dose dependent manner, over the range 5-100 μg/ml cholesterol. One possible mechanism for this protective effect is an ability of HDL to associate with Aβ and perhaps reduce the Aβ aggregation required for neurotoxicity. We also found that a significant amount of Aβ associates with HDL using a column assay.

These results support the idea that lipoproteins within the brain may substantially influence the neurotoxic potential of Aβ.
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82.4

ZINC ATTENUATES AMYLOID ß (Aß)1-40 NEUROTOXICITY. K.S. Fuson*, L.N. Boggs, and P.C. May. Lilly Research Laboratories, CNS Research Division, Eli Lilly and Company, Indianapolis, IN 46285.

Amyloid-beta peptide (Aß) is a major component of senile plaques found in Alzheimer's disease (AD) brain regions important for cognition and memory. While both soluble Aß and zinc are found in cerebrospinal fluid (CSF), recent *in vitro* studies have stated that zinc binds to Aß and induces aggregation of the peptide. These *in vitro* Aß-zinc interactions have led some investigators to propose that an altered zinc metabolism in AD patients may contribute to Aß pathology. Using primary fetal rat hippocampal cultures, we examined the effect of zinc on the neurotoxic properties of multiple lots of synthetic Aß 1 - 40 (Bachem). The co-addition of zinc with Aß caused a dose dependent attenuation of Aß neurotoxicity with 50 μM zinc affording complete neuroprotection against 25 - 50 μM Aß. Similar concentrations of zinc conferred no protection against kainate or hydrogen peroxide neurotoxicity, suggesting a specific zinc-Aß peptide interaction. Co-treatment of cultures with the metal chelator o-phenanthroline (o-P) could reverse most of the Aß neuroprotection afforded by zinc. However, the intrinsic neurotoxicity of o-P limited our testing capacity in these hippocampal cultures. Interestingly, the intrinsic neurotoxic properties of o-P could be blocked by co-treating cultures with zinc. May *et al* (accompanying abstract) show that zinc interfered with the cellular deposition of Aß (as measured by thioflavin S fluorescence) under the same conditions used to assess zinc neuroprotection. Thus, zinc may protect against Aß neurotoxicity by interfering with Aß deposition. [Eli Lilly & Co.]

82.5

ZINC INTERFERES WITH Aß(1-40) DEPOSITION ONTO CULTURED NEURONS: CORRELATION WITH NEUROPROTECTION. P.C. May*, L.N Boggs, Z. Zhang, G.J.Drzewiecki, P.A. Hyslop and K.S. Fuson. CNS Div., Lilly Research Labs, Eli Lilly and Co., Indianapolis, IN 46285

Aggregation of Aß appears to be a critical determinant for neurotoxicity and recent studies suggest that Zn can modulate Aß aggregation in vitro. However, in the accompanying abstract (Fuson et. al) we demonstrate that Zn co-treatment affords dose-dependent neuroprotection against Aß toxicity. To explore the mechanism of Zn neuroprotection, we evaluated Aß deposition onto cultured rat hippocampal neurons in the presence and absence of zinc. Primary mixed hippocampal cultures were treated with 25 µM Aß (Bachem ZM605) in N2/DMEM. At various times after treatment, conditioned media was removed for analysis of LDH. Aß remaining adherent to the cultures was quantified by addition of thioflavin-S (ThS) to 0.2% and the fluorescence shift measured in a cytofluorimeter. A significant fraction (> 50%) of aggregated Aß deposited in the culture by 24 hours and maximal deposition occurred at 48 hours. Interestingly, water-lysed wells containing only cell-derived extracellular matrix components, did not support the deposition of Aß at these time points, suggesting some requirement for intact cells. Co-treatment of cultures with 50 µM zinc and 25 µM Aß markedly interfered with the formation of adherent ThS-positive Aß deposits. Control experiments confirmed that Zn did not interfere with detection of pre-formed Aß deposits by ThS fluorescence. These data suggest that Aß deposition precedes overt neurotoxicity by 24-48 hours. Moreover, neuroprotection from Aß by zinc may result from interference with the deposition of ThS-positive Aß aggregates in vitro. [Eli Lilly & Co.]

82.6

Reduction of Aß Fibril Formation and Neurotoxicity by Native or Activated α_2-Macroglobulin

Y. Du[1]*, B. Ni[1], M. Glinn[1], J. W. Horn[2], E. Hamilton-Byrd[1], X. Wu[1], K. Bales[1], X. Liu[1], S. P. Little[1] and S. M. Paul[1,3] [1]Division of CNS, Research, [2]Toxicology, Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN 46285 and [3]Departments of Pharmacology and Toxicology and Psychiatry, IU School of Medicine, Indianapolis, IN 46285

The amyloid β (Aβ) peptide is a normal proteolytic processing product of the amyloid precursor protein (APP). For reasons which are still unclear, the Aβ peptide is deposited in an aggregated fibrillar form in both diffuse and senile plaques in the brains of patients with Alzheimer's disease (AD). The factors responsible for aggregation and fibril formation of soluble Ab present in biological fluids or tissues are poorly understood. We now report that α_2-macroglobulin (α_2M) and α_1-antichymotrypsin (ACT) regulate Aß fibril formation and toxicity. The addition of aged A$\beta_{1\text{-}40}$(25 µM) to cultured fetal rat cortical neurons results in significant cell death following 2-3 days of exposure. However, the addition of native α_2M (≥ 50 µg/ml) significantly reduces Aß toxicity of these neurons compared to parallel cultures exposed to Aß alone. ACT (100 µg/ml) enhances Aß neurotoxicity and α_2M attenuates the toxicity of neurons exposed to Aß and ACT. Fibrils formed by insoluble Aß were examined by electron microscopy and thioflavin-T fluorescence. Incubation of A$\beta_{1\text{-}40}$ alone for 3 d at 37°C in PBS resulted in the formation of 8 nm filaments and increasing thioflavin-T fluorescence. When A$\beta_{1\text{-}40}$ was coincubated with ACT, more fibril formation was observed. Incubation of α_2M with A$\beta_{1\text{-}40}$ led to the formation of fewer fibrils than A$\beta_{1\text{-}40}$ only at 3 d. No protease activity was detected in native α_2M (500 µg/ml). Moreover, trypsin (also expressed in AD brain)-activated α_2M (α_2T) is able to degrade Aß, preventing formation and toxicity. ACT appears to inhibit α_2T-mediated degradation of Aß possibly by decreasing binding of Aß to α_2T. We postulate that α_2M inhibits Aß fibril formation by binding to Aß and that protease-activated α_2M may represent a clearance mechanisms for Aß.

82.7

A SECRETED FORM OF THE AMYLOID PROTEIN PRECURSOR (APP_{751}) ENHANCES MEMORY IN VISUAL DISCRIMINATION AND OPERANT TASKS IN NORMAL AND AMNESTIC MICE. H. Meziane[1], C. Mathis[1]*, J. Clemens[2], S. Little[2], A. Ungerer[1] and S.M. Paul[2]. [1] Lab Ethol & Neurobiol, URA-CNRS 1295, ULP, Strasbourg, France; [2]Lilly Res Lab, Indianapolis, 46285, USA.

In some early-onset cases of Alzheimer disease, abnormal proteolytic processing of the amyloid protein precursor (APP) is believed to lead to β-amyloid peptide accumulation in extracellular neuritic plaques. However, the normal physiological function(s) of APP is/are unknown. Secreted forms of APP (APP_{751} or APP_{695}) have neuroprotective actions in a number of neurodegeneration models. The intracerebroventricular (i.c.v.) administration of anti-APPs antisera induces memory impairment in rats performing a passive avoidance task (Huber et al., 1993). In the present study, the possible memory-enhancing effects of APP_{751} were studied following i.c.v. administration of APP_{751} to mice either alone or following scopolamine administration using both a Go-No Go visual discrimination and operant learning tasks. Administration of scopolamine (3 mg/kg, s.c.) after the first session of training impairs learning during the second and third daily sessions in the visual discrimination task. Administration of APP_{751} (0.05 - 5000 pg, i.c.v.) after the first session of training, dose-dependently blocks learning deficits induced by scopolamine, with the most potent effect at doses of 5 and 50 pg. In addition, APP_{751} (0.5 and 5 pg i.c.v.) has a memory-enhancing effect when administered alone after the first training session. Post-training administration of APP_{751} (5 pg i.c.v.) also reduces learning deficits induced by 3 mg/kg scopolamine in the operant bar-press task. In a traction reflex test, scopolamine (3 mg/kg s.c.) causes motor disturbances when administered 15 min before the first session. These deficits disappeared 24 h later and suggest that memory deficits induced by scopolamine are not related to its motor effects. Furthermore, APP_{751} (5 pg i.c.v.) co-administered with scopolamine does not block these motor deficits, which suggest that APP_{751} has a specific action on memory processes.

82.8

THE NONPSYCHOTROPIC CANNABINOID HU-211 RESCUES HIPOCAMPAL NEURONES FROM β-AMYLOID PEPTIDE INDUCED TOXICITY, Striem S., Lavie V.* and Biegon A. Pharmos Ltd., Kiryat Weizman, Rehovot 76326, Israel.

The synthetic cannabinoid HU-211 that lacks psychotropic effects, has been shown to act as a noncompetitive NMDA-receptor antagonist in vivo and in vitro. In vitro studies revealed that HU-211 possesses an overall broader spectrum of neuron protective activities and mechanisms as compared to other NMDA-receptor antagonists, such as: (a) preventing lipid peroxidation triggered by peroxyl radicals, (b) inhibiting protein oxidation, (c) acting as a reducing agent and (d) protecting neurons from sodium nitroprusside-induced toxicity. In the present study we further characterize its neuroprotective effects and mechanism of action. Primary hipocampal cultures, derived from 18-day old rat embryos, were exposed for 24 hours to various concentrations of the synthetic β-amyloid peptide, β-22-35 (βAPP), either alone or in concert with 3 - 5 µM HU-211. "Aged" (incubated for five days at 37°c) βAPP at conentrations of 5 - 30 µM was toxic to the neurones in a dose-dependent manner (42.1% - 100% toxicity). HU-211 present with all the βAPP concentrations was significantly protective against this toxicity: βAPP at 20 µM was 100% toxic while HU-211 at 5 µM showed 79.7 ± 6.3% rescue. These results are consistent with previous works that suggested that the β-amyloid peptide toxicity is mediated by destabilization of calcium homeostasis. HU-211 may attenuate this toxic effect by blocking the influx of calcium ions into the neurones, although other mechanisms of action are not excluded. β-amyloid protein deposition in the core of the senile plaques is a hallmark of Alzheimer's disease. The present work suggests possible therapeutic implicatiions of HU-211 in Alzheimer's disease.

82.9

THE ACETYL-L-CARNITINE DERIVATIVE ST857 PREVENTS β 25-35 INDUCED-NEUROTOXICITY IN CEREBELLAR GRANULE CELLS. §^A. Scorziello, §O. Meucci, #M. Calvani, and §G. Schettini*. §Chair of Pharmacology, Department of Clinical and Experimental Oncology, University of Genoa, Unit of Neuroscience, Advanced Biotechnology Center (ABC), National Institute for Cancer Research (IST), ^Department of Neuroscience, Section of Pharmacology, University of Neaples "Federico II", #Sigma-Tau Gathered Pharmaceutical Industries, ITALY.

We studied the effect of ST857 on β 25-35-induced neurotoxicity in cerebellar granule cells and the intracellular mechanisms involved. Cells at different stages of maturation in vitro (1 or 6 DIV), were treated with β 25-35 and ST857 in presence of 25 mM KCl in the culture medium, and neuronal viability was assessed by morphological criteria. The treatment for 3 days with the peptide sligthly modified the survival of 1 DIV-treated cerebellar granule cells, which degenerate and die five days later β–amyloid matching. Similarly, a significative neurotoxic effect was observed on 6 DIV treated-cells after 5 days of treatment, while the death occurred within 8 days. Coincubation with β 25-35 and ST857 was able to rescue neurons from β 25-35-induced neurotoxicity.

We also studied the changes in calcium homeostasis following glutamate stimulation, in control and β-amyloid treated single cells, either in presence or in absence of ST857. β 25-35 did not affect basal $[Ca^{2+}]_i$, while modified glutamate-induced $[Ca^{2+}]_i$ increase, causing a sustained plateau phase of $[Ca^{2+}]_i$, that persisted after the removal of the agonist. ST857 pretreatment completely reverted this effect suggesting that, in cerebellar granule cells chronically treated with β 25-35, ST857 could protect the cells by neurotoxic insults of the pepide likely interfering with the intracellular mechanisms involved in the control of calcium homeostasis.

82.10

PROTECTIVE EFFECT OF CATECHIN AGAINST β-AMYLOID TOXICITY IN HIPPOCAMPAL NEURONS AND PC12 CELLS

K. Shin-ya*, T. Kunigami and H. Seto. Institute of Molecular and Cellular Biosciences, The University of Tokyo, Bunkyo-ku, Tokyo 113, Japan

Alzheimer's disease is associated with accumulation of β-amyloid peptide and disappearance of specific neurons. β-Amyloid has been shown to induce neuronal cell death *in vitro*.

In the course of our screening for substances to inhibit the toxicity of β-amyloid peptide, we isolated catechin from a traditional medicinal plant, guarana. To estimate the protective effects of catechin against β-amyloid toxicity, we have employed the system reported by Behl *et al.* (Cell, **77**, 817-827 (1994)). Vitamin E which was reported to show the protective effect against β-amyloid toxicity, was used as a positive control. Catechin and vitamin E protected hippocampal neurons from the β-amyloid toxicity at concentrations of 50 µM and 100 µM, respectively. β-Amyloid peptide decreased MTT reduction in PC12 cells. In this system, catechin completely recovered the MTT reduction rate at a concentration of 50 µM, whereas the effect of vitamin E to the same system was partial.

Since the toxicity of β-amyloid is considered to involve oxygen radicals, we assessed the intracellular oxygen radicals. In hippocampal neurons β-amyloid peptide significantly increased intracellular oxygen radicals, which were suppressed by the addition of catechin and vitamin E. In contrast, β-amyloid did not affect the intracellular oxygen radical level in PC12 cells. Other antioxidants, which were known to scavenge directly intracellular oxygen radicals, did not show protective effects against the β-amyloid toxicity in both hippocampal neurons and PC12 cells.

We next evaluated the function of mitochondria, which acts as the main source of MTT reduction. β-Amyloid markedly suppressed the mitochondrial function in hippocampal neurons. On the other hand it had no effect on the mitochondrial function in PC12 cells, even though it decreased MTT reduction. In addition, catechin and vitamin E did not recover the mitochondrial function in hippocampal neurons. These results indicate that β-amyloid peptide acts on hippocampal neurons and PC12 cells through different mechanisms.

82.11

BETA-AMYLOID TOXICITY IN CULTURED NEURONS IS NOT BLOCKED BY STANDARD ANTIOXIDANTS. B.J.Hamilton*, D.D.McKinley, J.S.Althaus and D.B.Carter. CNS Research, Pharmacia & Upjohn, Inc., Kalamazoo, MI 49001.

Beta-amyloid peptide (Aβ), a primary component of senile plaques, is a predominant neuropathological feature of Alzheimer's disease. Several studies have suggested a direct neurotoxic effect by Aβ. One possible mechanism of action for Aβ toxicity is through the generation of free radicals. To investigate this hypothesis, we utilized an *in vitro* tissue culture cell model system. Using both continuous cell culture and primary cell lines, we demonstrate a cytotoxic response to Aβ (25-35) by the inhibition of cellular redox activity as measured by MTT reduction. This inhibitory response occurred at concentrations of peptide at which no decrease in cell viability was observed, suggesting that this assay is an early indicator of cell damage. The pyrrolopyrimidine, U-104067, shows a weak protective effect against Aβ toxicity in this assay. In contrast to numerous published reports, no other antioxidants, including Vitamin E, acetyl cysteine and other low oxidation potential pyrrolopyrimidines have shown a significant protective effect. The lack of robust efficacy of antioxidant pyrrolopyrimidine compounds in addition to the lack of efficacy of some standard antioxidants in this assay indicates that processes other than those associated with oxidative damage may be involved in beta amyloid toxicity on cultured cells.

82.12

CYTOTOXICITY OF β-AMYLOID PEPTIDE 25-35 ON A CULTURED VASCULAR SMOOTH MUSCLE CELL MONOLAYER AND ATTENUATION BY VITAMIN E AND GARLIC. E. T. Gwebu*, M. Selassie, J. Williams, B. Webb, J. Warden. Department of Chemistry, Oakwood College, Huntsville, Alabama 35896.

A hallmark feature of Alzheimer disease is the presence of insoluble deposits of β-Amyloid peptide (Aβ) on vascular walls and other tissues. Cultured vascular smooth muscle cells accumulate Aβ, (J. Frackowiak et al. Brain Res. 676: 225; '95) and the β-Amyloid protein precursor is bound to the media of blood vessels (M. Kawai et al. Brain Res. 623: 142; '93). Neuronal death induced by the Aβ peptide is prevented by vitamin E (C. Behl et al. Biochem. Biophysic. Res. Commun. 186: 944; '92) The Aβ is toxic to vascular endothelial cells (E. Blanc et al. FASEB J. 10 : Abstract #3911, '96).

The purpose of this study was to determine the extent to which Aβ is cytotoxic to vascular smooth muscle cells (VSMC) and possible attenuation of this toxicity by vitamin E and garlic. A primary culture of vascular smooth muscle cells was prepared and experiments performed at passage 3-4 using "aged" Aβ. The methylthioltetrazolium (MTT) assay was used to determine cell death. In this report, we show that Aβ is toxic to VSMC. This Aβ toxicity is attenuated by the antioxidant, vitamin E and garlic.

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82.13

A PRODUCT(S) FROM A MONOCYTE CELL LINE (RAW 264.7) PROTECTS CULTURED CHOLINERGIC NEURONS FROM β-AMYLOID NEUROTOXICITY. G. M. Jonakait, Y. Wan, G. E. Ringheim, and L. Ni*. Dept. of Biological Sciences, Rutgers University, Newark, NJ 07102 and Hoechst Marion Roussel, Bridgewater, NJ 08807.

In cultures of embryonic septal nuclei with adjacent basal forebrain (SN/BF), treatment with β-amyloid (250 μg/ml) results in a 67.6 ± 5.1% loss of choline acetyltransferase (ChAT) activity, one measure of cholinergic neuronal survival. In previous studies, we have identified a soluble factor(s), obtained from media conditioned by either activated microglia or by a murine macrophage/monocyte cell line RAW 264.7 (RAW-CM), that promotes cholinergic differentiation of undifferentiated precursors (Jonakait et al., *Dev. Biol.*, in press). When included with β-amyloid, RAW-CM restores ChAT activity to control or above-control levels. Neurotrophins (NGF, BDNF, NT3, and NT4) had no such protective effect. Basic FGF (10 ng/ml) was as effective as RAW-CM in restoring ChAT levels.

Anti-oxidants were also tested for their ability to protect against β-amyloid neurotoxicity. Both β-mercaptoethanol (0.00003%) and N-acetylcysteine (1.5 mM) -- even in the presence of neurotoxic concentrations of β-amyloid -- elevated ChAT 2.5-5-fold, suggesting that they, too, protected against β-amyloid neurotoxicity in this population.

82.14

DOMINANT NEUROPROTECTIVE EFFICACY OF α-SECRETASE DERIVED SECRETED-APP MEDIATED BY HEPARIN-BINDING DOMAIN OF Aβ. K. Furukawa*, B. L. Sopher, R.E. Rydel, G.M. Martin, M. Fox, and M.P. Mattson. Sanders-Brown Research Center on Aging, Univ. Kentucky, Lexington KY 40536. Dept. of Pathology, Univ. of Washington, Seattle, WA 98195. Athena Neurosciences, Inc., South San Francisco, CA 94080.

β-amyloid precursor protein (βAPP) is known to be a source of amyloid β peptide (Aβ) and secreted forms of βAPP (sAPP). We have reported that Aβ kills neurons by destabilizing cellular calcium homeostasis (*J. Neurosci.* 12: 376-89) and increasing reactive oxygen species (*J. Neurosci.* 15: 6239-49), and that sAPPs protect neurons by activating K^+ channels and stabilizing cellular calcium homeostasis (*Nature* 379: 74-78). Different sAPPs (sAPPα and sAPPβ) are liberated from βAPP by α- and β–secretases, respectively; βAPP mutations linked to Alzheimer's disease may decrease levels of sAPPα. We now report that sAPPα is 100-fold more potent than sAPPβ in protecting cultured rat hippocampal neurons against excitotoxicity, Aβ toxicity and glucose deprivation. Patch-clamp recording and calcium imaging using fura-2 showed that sAPPαs were also more effective than sAPPβs in activating K^+ channels and attenuating Ca^{2+} increases by glutamate. Structure-activity studies using recombinant sAPPα and sAPPβ fragments and synthetic APP peptides showed that a C-terminal region of sAPP mediates neuroprotection, and that a heparin-binding domain specific for sAPPα is responsible for the increased efficacy of sAPPα. Physiological and pathological conditions that affect βAPP processing are likely to greatly affect the biological activities of the resultant sAPP products (supported by the NIH and the Alzheimer's Association).

82.15

Aβ1-42 NEUROTOXICITY AND THE INFLUENCE OF NMDA RECEPTOR ANTAGONISTS. G.K. Rieke* and K. Jacobson. Dept. of Anat. & Cell Biol., Sch. of Med. & Health Sci., Univ. of North Dakota, Grand Forks, ND 58202.

The neurotoxicity of the beta amyloid protein fragment Aβ1-42 is an issue of intense interest with respect to the neuropathology of Alzheimer's Disease. Neurons in the hippocampus/dentate gyrus (HP/DG) die in response to chronic challenge with Aβ1-42. Does the NMDA receptor which is present at high density on HP/DG neurons, participate in the expression of Aβ1-42 neurotoxicity? Male Sprague Dawley rats anesthetized with pentobarbital had cannulas implanted, under sterile conditions, into the dorsal HP/DG. Test substances (Aβ1-42 [0.22 nmol/uL], AP-5 [0.22 nmol/uL, pH 7.4]) were delivered through guide cannulas or cannulas coupled to Alzet (2004) pumps. Co-injection of AP-5 with Aβ1-42 attenuated the toxicity of the peptide. The calculated volume of the Aβ1-42 lesion was five times larger than the Aβ1-42/AP-5 lesion. Aβ1-42/AP-5 resulted in a limited localized reduction of HP pyramidal cells, while Aβ1-42 alone destroyed HP pyramidal cells and induced apoptosis in many DG granule cells. Other NMDA receptor antagonists (MgCl , AP-7, CGS 19755) are being tested to determine if they have can ameliorate the toxicity of Aβ1-42 similar to AP-5.

Supported by UND SOM Grant to G.K. Rieke.

82.16

POTASSIUM-INDUCED DEPOLARIZATION PROTECTS CULTURED NEURONS FROM β-AMYLOID TOXICITY. C.J. Pike,* R. Balázs and C.W. Cotman. Institute for Brain Aging & Dementia, *Univ. of California, Irvine, CA 92717 USA.*

β-Amyloid protein (Aβ) is implicated as a primary contributor to neurodegeneration associated with Alzheimer's disease (AD). In cultured neurons, fibrillar aggregates of Aβ induce apoptotic cell death. Apoptosis is also observed in vulnerable regions of AD brain. To investigate potential protective strategies, we examined the ability of high $[K^+]_o$ to attenuate Aβ-induced neurotoxicity *in vitro*. Previous *in vitro* studies have characterized a Ca^{++}-dependent pathway by which KCl-mediated depolarization inhibits development-related apoptosis. In 12-16 DIV cultures of rat hippocampal neurons, increasing $[K^+]_o$ from the normal 5 mM to a depolarizing 30 mM resulted in a significant attenuation of cell death induced by both β1-42 and its active fragment β25-35. Interestingly, membrane depolarization induced by high $[K^+]_o$ offered protection against Aβ peptides only following a several hour pretreatment; increasing $[K^+]_o$ immediately prior to Aβ addition did not significantly affect Aβ-induced cell death. Consistent with observations in developmental paradigms of apoptosis, the elevated $[K^+]_o$ protective pathway was mimicked by a Ca^{++} channel agonist [S(-)-Bay K 8644] and attenuated by a blocker of voltage-dependent Ca^{++} channels [R(+)-Bay K 8644] as well as by an inhibitor of Ca^{++}/calmodulin-dependent protein kinase II (KN-62). These data suggest that depolarizing doses of KCl can prevent Aβ-induced neuronal apoptosis by a cellular pathway involving Ca^{++} influx through voltage-sensitive channels followed by stimulation of Ca^{++}/calmodulin-dependent protein kinase activity and perhaps new protein synthesis. Extrapolation of these concepts to the *in vivo* condition would suggest a novel form of use-dependent plasticity whereby depolarization resulting from neural activity may provide neurons with a significant margin of protection against apoptotic insults associated with AD and other age-related neurodegenerative diseases. (Supported by NIH grant #AG12663).

82.17

ß-AMYLOID ACTIVATES PROTEIN SERINE/THREONINE KINASE CASCADE WITH THE INVOLVEMENT OF PROTEIN KINASE Cα. Y. Luo*, D. B. Hawver, T. Sunderland, K. Iwasaki, and B. Wolozin. Section on Geriatric Psychiatry, NIMH, Bethesda, MD 20892-1264.

Alzheimer's ß-amyloid peptide (Aß) is normally present at subnanomolar concentrations in body fluids and in the medium of cultured cells. In vitro experiments have shown that Aß has neurotrophic effects and can promote neuronal adhesion and elongation of axon-like processes. We have recently found that nanomolar doses of Aß can stimulate tyrosine phosphorylation and activate phosphatidylinositol-3-kinase in neuronal cells. [Luo, et al., Brain Res. 681:65-74 (1995); J. Neurochem, in press (1996)]. Here we show evidence that Aß activates a protein kinase C (PKC) involved in the serine/threonine kinase cascade in PC12 cells. Aß-stimulated kinase activities can be detected by anti-phosphothreonine immunoblotting and *in vitro* phosphorylation of S6 peptide. Depletion of PKC by pretreatment of PC12 cells with PMA (1 μM) for 24 hr prevented the increase of *in vitro* S6 phosphorylation by Aß. Additional immunoblotting experiments showed that Aß decreased the cytosolic 80 KDa PKCα and increased translocation of PKCα to the particulate fraction of cell lysates. Aß also increased immunoreactivity to a particulate fraction 50 KDa protein, a presumed proteolytic fragment of PKCα. This Aß-induced translocation of PKCα was enhanced by ionomycin, a calcium ionophore, and blocked by calcium-dependent protease inhibitors, calpain I and II inhibitors (1 μM and 2 μM respectively). These data suggest that Aß may regulate the protein serine/threonine kinase cascade through calcium-dependent PKC pathway.

82.18

sAPP'S NEUROTROPHIC ACTIVITY INVOLVES TWO DISTINCT SIGNALING PATHWAYS I. Mook-Jung, M.W. Jung* & T. Saitoh. Dept of Neurosci, School of Medicine, UCSD, La Jolla, CA, 92093-0624. *Neurosci Lab, Institute for Medical Sciences, Ajou University, Suwon, Korea, 442-749.

Secreted form of amyloid precursor protein (sAPP) functions as a trophic factor for neuronal and non-neuronal cells. Little is known, however, about the intracellular signals that mediate these effects. We previously suggested that sAPP function is mediated through tyrosine phosphorylation. To determine which domain of sAPP induces tyrosine phosphorylation, we tested effects of sAPP and a RERMS domain peptide (APP319-335) known to have neurotrophic activity. Western blot analyses using an antibody against phosphotyrosine showed that 125kD band was increased in a dose-dependent manner by sAPP. The RERMS domain peptide induced tyrosine phosphorylation more strongly than sAPP. Consistent with this result, Aβ1-15 inhibited tyrosine phosphorylation. Thus, Aβ1-15 domain might play an important role in regulating the action of sAPP. Heparin or a heparitinase treatment attenuated the neurotrophic effect as well as tyrosine phosphorylation induced by sAPP. Since Aβ1-15 region contains a consensus heparin binding domain, these results suggest that the Aβ1-15 domain is essential for sAPP action. The present results suggest that sAPP induces tyrosine phosphorylation, and the RERMS domain and Aβ1-15 domain in sAPP activate separate signal transduction pathways. The balance between activities of the two regions might be important for regulation of biological functions of sAPP. Supported by NIH and Alzheimer's Assoc.

82.19

ALZHEIMER'S BETA AMYLOID PRECURSOR PROTEIN (βAPP) AND PROLINE-RICH LIGANDS BIND TO PTB AND WW DOMAINS OF THE FE65 ADAPTOR AND FORM TRI-PARTITE PROTEIN COMPLEXES. K. Ermekova, N. Zambrano#, T. Russo# and M. Sudol* Dept. of Biochem. Mt Sinai School of Med., New York, NY 10029; # Dept. of Biochem. and Med Biotech. Univ. of Naples, Naples 80131, Italy.

FE65 is a new adaptor protein expressed preferentially in the brain. Its structure is modular and contains one WW and two PTB domains. The PTB domains of FE65 bind βAPP [1]. Using expression cloning, we isolated binders to the WW domain of FE65, which may encode activities involved in normal function of the βAPP. From among the potential binders, we selected those that form a complexs with FE65 protein both *in vitro* and in cell lysates. Similarly to SH2, SH3, PH and PTB, the WW domain mediates protein-protein interaction and is present in various signaling molecules in yeast, nematode and mammals [2,3]. The name WW stands for 2 tryptophans (W) present among 4 conserved aromatics in the consensus sequence spaced 20 amino acids (aa) apart. The WW domain is 38 aa long and forms a compact structure composed of a 3 stranded beta-sheet. The structure is distinct from that of the SH3 domain [4]. A cognate ligand (WBP1 protein) for the WW domain of human YAP was isolated and its binding site was mapped to the PPPPY motif with the consensus XPPXY being critical for binding [5]. Recent studies (review [6]) have implicated the WW domain-ligand link in several genetic disorders and biological processes including Liddle's syndrome, muscular dystrophy, limb and kidney development, and retroviral budding. **1**. Fiore, F., et al., (1995) J. Biol. Chem. 270; 30853-56; **2**. Bork, P., & Sudol, M. (1994) TiBS 19, 531-33; **3**. Sudol, M., et al., (1995) J. Biol. Chem. 270, 14733-41; **4**. Collaboration with Oschkinat, H., EMBL, Heidelberg; **5**. Chen, H.I. & Sudol, M. (1995) Proc. Natl. Acad. Sci. USA, 92, 7819-23; **6**. Sudol, M. (1996) TiBS, 21, 161-3. *Supported by grants from NIH, HFSPO, and MDA.*

DEGENERATIVE DISEASE: ALZHEIMER'S—COGNITIVE FUNCTION I

83.1

CORTICAL DEMENTIA OF ALZHEIMER'S DISEASE IS ASSOCIATED WITH DIFFUSE AND CORTICAL ATROPHY AND THE PRESENCE OF AT LEAST ONE APOE4 ALLELE. P.R. Mouton[1,3*], M.E. Calhoun[3], N. Bakhos[2], and J.D. Glass[2]. Departments of Pathology[1] and Neurology[2], and the Neuropathology Laboratory[3], The Johns Hopkins University School of Medicine, Baltimore, MD 21205.

The presence of apoE4 alleles is associated with clinical-pathological findings characteristic of Alzheimer's disease (AD). This prospective study was designed to determine whether a specific pattern of cortical atrophy combined with psychometric testing and apoE genotyping are useful criteria for the diagnosis of AD. Stereology was used to quantify total cortical volumes (V_{ctx}) of formalin-fixed brains from 100 autopsied cases that received longitudinal neuropsychological testing and comprehensive neuropathological studies. Final diagnoses of young and aged controls and cases of definite AD, possible AD, Lewy body variant of AD (AD-LB), Parkinson's disease (PD), Huntington's Disease (HD), and AIDS were made on the basis of clinical and pathological findings. No age-related V_{ctx} loss was seen in controls ranging in age from 45-91 years. ANOVA revealed severe, diffuse reduction in V_{ctx} from frontal to occipital poles in cases of definite AD (25%), HD (24%), and PD (20%) compared to age-matched controls, whereas V_{ctx} for AD-LB and possible AD were not different from controls. Ninety percent of cases with AD and cortical atrophy ($V_{ctx} \leq$ median) have at least one apoE4 allele; cases with normal cognitive function did not show the combination of cortical atrophy and apoE4 allele. Cortical atrophy in AIDS is mild as compared to AD and not associated closely with dementia. These findings support the view that severe, diffuse cortical atrophy provides an important adjunct to the antemortem diagnosis of AD and a logically compelling basis to understand the pathophysiology of cortical dementia. This work was supported by a grant from the NIH (NS 26643).

83.2

ISOFORM-SPECIFIC NEUROTOXICITY OF APOLIPOPROTEIN E: POSSIBLE ROLE OF THE 22 KDA FRAGMENT. M.A. Marques*, M. Tolar and K.A. Crutcher. Department of Neurosurgery, University of Cincinnati College of Medicine, Cincinnati, OH 45267-015.

In vitro toxicity assays demonstrate that the 22 kDa thrombin-cleavage fragment of apolipoprotein E (apoE) is toxic to embryonic chick sympathetic neurons in culture (Crutcher et al, Neurobiol. Aging, in press). Since a similar fragment is present in postmortem human brain tissue (Marques et al., Soc. Neurosci., **21**: 1485, '95) and apoE is susceptible to proteolytic degradation by different cell types in vitro, the present study was undertaken to examine the potential toxicity of apoE when added to primary neurons in culture.

ApoE was isolated from concentrated media of HEK cells transfected with either the E3 or E4 human apoE gene (kindly provided by Dr. M.J. LaDu). ApoE was purified by an affinity heparin column followed by gel filtration chromatography. Addition of either apoE isoform to embryonic chick sympathetic neurons resulted in significant neurotoxicity. In all cases the greatest toxicity was associated with the E4 isoform. An immunoblot of the media exposed to neurons showed the presence of a 22 kDa apoE fragment. Furthermore, an immunoblot of fresh cerebrospinal fluid from both Alzheimer's disease (AD) and control cases demonstrated the presence of a 22 kDa apoE fragment, indicating its normal production in vivo.

These results, demonstrating a potent isoform-specific neurotoxicity of apoE, suggest that the generation of the 22 kDa fragment may be the means by which apoE causes neuronal degeneration. These findings implicate apoE as a candidate for playing a direct role in AD pathogenesis and provide a biological basis for the apoE isoform-specific risk for developing the disease. (Supported by NIH NS31410.)

83.3

LOSS OF THE PRESYNAPTIC VESICLE PROTEIN SYNAPTOPHYSIN IN HIPPOCAMPUS CORRELATES WITH EARLY COGNITIVE DECLINE IN AGED HUMANS. C.-I. Sze[1,4], J.C. Troncoso[1,2,4*], C.H. Kawas[2], P.R. Mouton[1,4], D.L. Price[1,4], and L.J. Martin[1,3,4]. Departments of Pathology[1], Neurology[2], and Neuroscience[3], and the Neuropathology Laboratory[4], The Johns Hopkins University School of Medicine, Baltimore, Maryland, USA.

We tested the hypothesis that regionally specific defects in the levels of the presynaptic vesicle protein synaptophysin (p38) occur in the brains of aged humans with recent onset memory decline and in demented individuals with Alzheimer's disease (AD). Controls with normal and stable cognition, cognitively normal subjects with senile plaque densities diagnostic for possible AD (p-AD), and individuals with a clinical and neuropathological diagnosis of AD were studied. By immunoblotting, p38 and β-tubulin levels were quantified in synaptic membranes of hippocampus, entorhinal cortex, caudate nucleus, and occipital cortex. Average p38 levels were reduced significantly in hippocampus when comparing AD to control (55%, $p < 0.0001$), p-AD to control (25%, $p < 0.005$), and AD to p-AD (30%, $p < 0.05$). When comparing either AD or p-AD to controls, p38 levels in entorhinal cortex, occipital cortex, and caudate nucleus were either unchanged or less significantly altered than in hippocampus. Regional β-tubulin levels were similar in all groups. By univariate analysis of all cases, p38 levels in hippocampus strongly correlated with Mini-Mental State Examination scores ($r=0.83$, $p<0.0001$, Spearman correlation=0.77) and Blessed scores ($r=0.74$, $p<0.001$, Spearman correlation=-0.58). We conclude that synaptic abnormalities in hippocampus correlate significantly with the severity of age-related cognitive/memory deficit and that defects in presynaptic functioning in hippocampus occur as an early abnormality in the progression of memory impairments in AD. This work was supported by a grant from the NIH (AG 07914 and AG 05146).

83.4

COMPARTMENTAL PERIAQUEDUCTAL GRAY PATHOLOGY IN ALZHEIMER'S DISEASE. G.F. Wu[1], A. Solodkin[*2], and G.W. Van Hoesen[2]. Program in Neuroscience[1] and Departments of Anatomy and Neurology[2], University of Iowa, Iowa City, IA 52242.

Recent studies have suggested a discrete functional organization in the midbrain periaqueductal gray (PAG). Although the brainstem has been analyzed in Alzheimer's Disease (AD), data on the PAG is sparse and mentioned usually in the context of dorsal raphe pathology. Since the PAG has close anatomical connections with limbic areas, we evaluated AD pathology in the PAG and correlated it with entorhinal pathology in 30 brains. Fifty micron sections were stained with Thioflavin-S and immunohistochemically labeled with the Alzheimer's-related antibodies Alz-50 and 10D5. Pathology in the PAG was clearly segregated both rostro-caudally and dorso-ventrally. Neuritic plaque (NP) distribution was most concentrated in the dorsolateral, lateral, and ventrolateral areas at rostral and intermediate levels, whereas neurofibrillary tangles (NFTs) were situated preferentially in the caudal ventrolateral and ventral areas. Dorsomedial pathology was consistently low. Furthermore, a significantly high correlation between midbrain and entorhinal cortex density of NFTs, as well as NFT density and the number of years with clinical dementia, was found. The density of NPs did not have the same correlation. These results suggest the existence of a compartmental organization in the human PAG similar to that reported in other species. Also, these data indicate a delayed but parallel development of pathology in the PAG in relation to other limbic areas. The pathological changes in the PAG may contribute significantly to some of the later behavioral changes seen in AD and must be viewed as an adjunct correlate to those previously attributed only to cortical destruction. Supported by NS 14944 and 19632.

83.5

PATHOLOGY OF THE INSULAR CORTEX IN ALZHEIMER'S DISEASE. D.J. Bonthius*, A. Solodkin and G.W. Van Hoesen. Departments of Anatomy, Neurology and Pediatrics, University of Iowa, Iowa City, IA 52242.

The insular cortex plays important roles in a variety of regulatory mechanisms ranging from visceral control and sensation to covert judgements about inner well being. The dementia of Alzheimer's Disease (AD) often includes behavioral dyscontrol not observed in other diseases that affect cognition. This could be related to a loss of the sense of self, and the insula may play an essential role. The pattern of pathology in the insula of 16 patients with AD was examined, and the severity of pathology in the insula was compared with that of the entorhinal cortex (EC), a region known to be involved early in AD and to have reciprocal connections with the insula. Thioflavin S staining revealed that neurofibrillary tangles (NFTs) of the insula are largely confined to layers V and VI, while neuritic plaques (NPs) are distributed throughout the cellular layers and subcortical white matter. In the insula, the density of NFTs, but not of NPs, was highly correlated with the degree of pathology in the EC. However, NFTs were not seen in the insula until pathologic changes in the EC reached a relatively advanced level, with NFTs necessarily present in EC layers II and IV. In the insula, the density of NFTs, but not of NPs, was also highly correlated with years of clinical dementia. No anterior-posterior differences in NFT or NP density were observed. Immunohistochemical staining with Alz-50 revealed labelling of cell bodies in all of the cell layers of the insula, but most densely in layers III and V. The density of Alz-50-labelled cells in the insula was correlated with degree of pathology in the EC. The results demonstrate that the insula is often involved in AD and suggest that some of the behavioral abnormalities in AD may reflect insular pathology. Support: The John Martin Fund for Neuroanatomical Research, NS14944 and NS19632.

83.6

ELEVATED α_1-ANTICHYMOTRYPSIN, BUT NOT a_2-MACROGLOBULIN, IS ASSOCIATED WITH COGNITIVE IMPAIRMENT IN A PROSPECTIVE STUDY OF THE VERY OLD. S.M. Gabriel*, D.B. Marin, P.S. Aisen, M. Lantz, L.D. Altstiel, K. L. Davis, R.C. Mohs, Departments of Psychiatry, Mount Sinai School of Medicine, New York, NY 10029 and Bronx VA Hospital, Bronx, NY 10468.

Considerable research suggests an immunological component to Alzheimer's Disease (AD), the primary neurodegenerative illness of aging. The present prospective study determined the levels of the acute phase proteins, α_1-antichymotrypsin (ACT) and α_2-macroglobulin (MAC), in sera from 94 medically stable, elderly nursing home residents. Protein concentrations were determined by antibody capture enzyme-linked immunosorption assay. There was a negative corellation between mini mental state examination (MMSE) score and ACT ($r=-0.39$, $p<0.0001$), and a positive corellation between clinical dementia rating (CDR) score and ACT ($r=0.37$, $p<0.001$). There was no significant relationship between MAC and these cognitive measures. These data extend previous reports that patients with AD have increased concentrations of ACT in their blood by demonstrating in a diagnostically diverse nursing home population a relationship between serum ACT and cognitive status. Further, increased serum ACT in AD and in patients with compromised mental status may reflect a cerebral acute phase response.

83.7

THE APOLIPOPROTEIN CI A ALLELE SERVES AS A RISK FACTOR FOR ALZHEIMER'S DISEASE. **S.E. Podusio*, M. Neal, K. Herring, S. Shelly. Texas Tech University Health Sciences Center, Department of Neurology, Lubbock, Texas, 79430**

Alzheimer's disease is a degenerative disorder characterized by loss of memory and cognition. The disease is genetically heterogeneous in that genes on chromosomes 21 and 14 have been associated with early onset disease, a gene on chromosome 1 in Volga German kindreds, and a gene on chromosome 19 with late onset disease. The E4 allele of the APOE gene on chromosome 19 is a significant risk factor for Alzheimer's disease (Saunders et al, 1993). Downstream from the gene for APOE is the gene for APOCI, both of which are located within a 45 kb cluster of apolipoprotein genes. The presence of a restriction site in the 5' end of APOCI is present at increased frequency in Alzheimer's patients. It can also be considered to be a risk factor for the disease. The APOCI A allele and the APOE 4 allele are in linkage disequilibrium. The frequency for the APOCI A allele is increased in both early and late onset Alzheimer's patients as well as in familial and sporadic cases. No differences in frequencies were found between male and female patients or in the average age of onset of the disease in the patients with AA or AB phenotypes. However, we did find that males with a higher frequency of the APOCI A allele tend to have an earlier age of onset than females. The odds ratio indicates that the risk of developing the disease is 6x greater for those homozygous for the APOCI AA genotype. Haplotype frequencies indicate a strong association between the two markers and the disease, but there appears to be a more significant association with APOE.

This research was supported by funds from the state of Texas for the DNA bank of Alzheimer's families for genetic research.

83.8

ANGIOTENSIN CONVERTING ENZYME INHIBITORS AND COGNITION IN AN ALZHEIMER'S POPULATION. A. Kashani*[1], C.F. Phelix[1], M.F. Weiner[2]. [1]Univ. of TX. at San Antonio, Division of Life Sciences, 6900 North Loop 1604 West, San Antonio, TX 78249-0662; [2]Univ. of TX Southwestern Med. Sch., 5323 Harry Hines Blvd., Dallas, TX 75235-9070.

Alzheimer's disease (AD), the most common dementing illness of older adults, is characterized by decreased cholinergic activity in brain. Choline acetyltransferase activity is reduced substantially in neocortex, certain forebrain nuclei, and the hippocampal region of AD patients. The reduction of brain cholinergic activity parallels the cognitive decline seen in these patients. By contrast, there is increased activity of angiotensin converting enzyme (ACE) in the brains of these patients suggesting increased synthesis of angiotensin II (AII). Because AII inhibits presynaptic acetylcholine release in rats and humans, AII might aggravate the cholinergic deficit in AD and contribute to the impairment of cognition. For this reason, ACE inhibitors have been considered for use as cognitive enhancers in humans. Short-term studies of ACE inhibitors have shown no effect on cognition in AD. We reviewed the records of 458 AD patients in the database of the Alzheimer's Disease Center at the University of Texas Southwestern Medical Center. Of these 56 patients were hypertensive, 39 patients were on ACE inhibitors and 17 were on other antihypertensive medications. The remaining 402 patients were on no antihypertensive medication. There was a trend toward lower scores on the Blessed Dementia Rating Scale (4.8 ± 2.56) in the ACE group as compared to the non-ACE group (5.81 ± 2.82) and the normotensive group (5.76 ± 3.37) – with no significant difference ($p=0.24$). There was also a trend for the ACE group to have higher Mini-mental State scores (18.31 ± 5.79) than did the non-ACE group (16.65 ± 7.06) or the normotensive group (16.14 ± 6.51) – $p=0.14$. Both findings suggest the possibility that ACE inhibitors might slow the progress of AD, but to demonstrate an effect, a long-term prospective study would be needed. Supported in part by: HL02914-02 - CFP, NIA P30AG12300 - MFW.

83.9

SHORT-TERM TREATMENT WITH CEP-1347 PRODUCES CHRONIC IMPROVEMENT IN DELAYED ALTERNATION PERFORMANCE BY RATS WITH NUCLEUS BASALIS LESIONS. A. DiCamillo, N. Neff*, S. Carswell, C. Murakata#, M. Miller and F. Haun. Cephalon Inc., West Chester, PA, 19380, and #Kyowa-Hakko Kogyo Co., Tokyo, Japan.

The structurally novel indolocarbazole CEP-1347 prevents the death of neurons in nucleus basalis magnocellularis (nbm) following ibotenic acid lesions in adult rats, as well as increasing markers of cholinergic function in these animals. This study examined whether CEP-1347 also improves a behavior sensitive to nbm lesions. Adult Sprague-Dawley rats were first trained to alternate responses in a T-maze to an 88% correct criterion (discrete trial procedure, 1 min delay between runs). The animals then received bilateral ibotenate lesions of the nbm followed 18 h later by administration of CEP-1347 (s.c. 0.1 mg/kg, Q.O.D.) for 12 days. Retention of delayed alternation learning was first tested 24 h following the last injection of CEP-1347. Lesioned rats receiving vehicle-only injections committed 4 times as many errors before reaching pre-operative criterion as did sham operates (15.9 ± 3.7 vs. 3.5 ± 1.1), while rats receiving CEP-1347 made significantly fewer errors (8.6 ± 1.3) than the vehicle controls. To test whether this behavioral improvement reflects a chronic change in cognitive ability, the same rats were re-tested 10-12 weeks after dosing was completed with no further behavioral training during the test-retest interval. Lesion/vehicle rats continued to make significantly more errors (11.1 ± 3.7) than did sham operates (4.6 ± 0.7), while the performance of CEP-1347 rats was identical to that of unlesioned untreated animals (2.75 ± 0.8 errors). To our knowledge this is the first demonstration of a long-term cognitive improvement following short-term delivery of a neurotrophic agent in a model of Alzheimer's disease. Initial analysis of the types of errors committed suggests that the nature of the cognitive improvement by CEP-1347 is attentional, rather than mnemonic.

83.10

LONG-TERM ADMINISTRATION OF IDEBENONE AMELIORATES AGE-RELATED BEHAVIORAL DEFICITS AND PROLONGS LIFE SPAN IN RATS. M. Miyamoto*, M. Ohkura, T. Nakayama and Y. Nagai. Pharmaceutical Res. Lab. I, Takeda Chem. Industries, Ltd., Osaka 532, Japan

Major involvement of oxidative stress in pathogenesis of Alzheimer's disease (AD) and in normal aging has been demonstrated. In the present study, the effects of idebenone, a centrally active antioxidant, on age-related behavioral deficits and life span in rats were examined. Also, effects of idebenone on reduced local cerebral glucose utilization (LCGU) were studied. Fischer 344 aged rats were fed laboratory chows containing idebenone (67 or 200 ppm) commencing at 24 months of age. Young adult rats and aged control rats were given laboratory chows containing no drug. Aged rats showed a marked impairment in the Morris water maze task at 27 months of age as compared with young rats. Long-term administration of idebenone ameliorated the impairment in a dose-dependent manner; the high dose group (5.6 mg/kg/day) had a significantly decreased latency to find the hidden platform and exhibited a significant increase in the number of the platform area in the spatial probe test. Idebenone also attenuated abnormality of circadian rhythm of spontaneous motor activity and drinking behavior, in which both of the low (2.1 mg/kg/day) and high dose groups reversed the reduction in circadian amplitude. Furthermore, the long-term treatment with idebenone prolonged life span. However, food consumption and body weight in idebenone-treated groups did not differ from those of the control group, suggesting that the prolongation of life span is not attributable to the caloric restriction. Also, repeated administration of idebenone (30 mg/kg/day) over 30 days significantly recovered the rate of LCGU, especially in the temporal cortex and hippocampus which play a key role in learning and memory. These effects of idebenone are considered to be closely related to its protective action against oxidative stress and enhancement of cerebral energy metabolism, and strongly suggest that idebenone is useful for the treatment of AD.

83.11

IDEBENONE PROTECTS AGAINST OXIDATIVE STRESS MEDIATED NEURONAL CELL DEATH BY COUPLING WITH THE MITOCHONDRIAL ELECTRON TRANSPORT SYSTEM. K. Hirai, H. Hayako, K. Kato* and M. Miyamoto. Pharmaceutical Research Laboratory I, Takeda Chemical Industries, Ltd., Osaka 532, Japan

We examined the effects of idebenone, a potent antioxidant, on two models of oxidative stress mediated neuronal cell death, glutamate-induced cell death in the N18-RE-105 cell line and amyloid ß-peptide (Aß) 1-40-induced cell death in rat hippocampal neurons. Glutamate-induced neurotoxicity in the N18-RE-105 cell line is mediated by an inhibition of cystine uptake from medium into the cell, which leads to a marked decrease in cellular glutathione levels, exposing the cells to the oxidative stress. The glutamate-induced cell death was partially protected by rotenone (complex I inhibitor) and antimycin A (complex III inhibitor) and enhanced by thenoyltrifluoro-acetate (TTFA: complex II inhibitor). These results suggest that reactive oxygen species (ROS) generated in the mitochondrial electron transport system is important in the neurotoxicity. Idebenone protected against the glutamate-induced cytotoxicity in a concentration-dependent manner (0.1 to 10 µM). The protective effect of idebenone was attenuated by rotenone and TTFA. Idebenone (0.1 µM) protected against Aß1-40-induced neuronal cell death after 4-5 days treatment. The protective effect of idebenone against the Aß1-40-induced toxicity in hippocampal neurons also evaluated by immunostaining with anti-MAP-2 antibodies. After 4 days exposure to 10 µM of Aß1-40, the numbers of neurons were reduced and the survived neurons had apparently declined number of neurites. When idebenone was added to the culture medium with Aß, the numbers of survived neurons were significantly increased and neurites of survived neurons remained long as seen in the control culture. These results suggests that idebenone protects neurons against oxidative stress caused by glutamate and Aß1-40 by coupling with the mitochondrial electron transport system.

83.12

TRANSDERMAL ESTROGEN IMPROVES MEMORY IN WOMEN WITH ALZHEIMER'S DISEASE. S. Asthana*, S. Craft, L.D. Baker, M.A. Raskind, E. Avery, C. Lofgreen, C.W. Wilkinson, S. Falzgraf, R.C. Veith, S.R. Plymate. Geriatric Res., Educ. & Clinical Center (GRECC) & Extended Care, VA Puget Sound Health Care System, American Lake Div., and Univ. of Washington, Seattle, WA 98195.

Estrogen has well documented neuromodulatory, neurotrophic and organizational effects in developing, mature, and aging rodent brain. Recent research findings suggest that estrogen replacement therapy in women with Alzheimer's disease (AD) might reduce their risk of developing AD, and enhance cognition. However, no controlled clinical study has evaluated the potential role of estrogen in augmenting cognition in AD. We report results of a randomized, placebo-controlled, double-blind, parallel group design study that characterizes the effects of transdermal estrogen on cognitive functions of postmenopausal women with AD. Ten subjects with probable AD were randomized into one of two treatment groups. Each group received an 8 week therapy of either 50 µg/day of 17-ß estradiol (n=5) via a transdermal patch (Estraderm™, Ciba-Geigy) or a placebo patch (n=5). Following termination of therapy, subjects were followed for another 5 weeks to study the effects of estrogen withdrawal on cognition. An extensive battery of neuropsychological tests was administered during both estradiol treatment and withdrawal phases to evaluate effects on cognition. Verbal memory as measured by the Buschke Delayed Recall [$F(1, 4)=16$, $p<0.02$) and Paragraph Recall [$F(1, 4)=8.12$, $p<0.05$) significantly improved above baseline with estradiol treatment. The improvement in Buschke test scores was seen within 1 week of starting estradiol and persisted for up to 2 weeks after the treatment was terminated. The improvement in Paragraph Recall test scores was seen in week 5 of treatment. In the placebo group, no significant change ($p>0.15$) above baseline in performance was observed on either test.

The results of this prospective, controlled clinical study demonstrate that treatment with transdermal 17-ß estradiol significantly enhances verbal memory in postmenopausal women with AD. Supported by a grant from the Alzheimer's Association/The National Catholic Soc. of Foresters and by Ciba-Geigy Corporation.

83.13

CORRELATION OF COGNITIVE STATUS WITH α–TOCOPHEROL LEVELS IN PATIENTS WITH ALZHEIMER'S DISEASE. **M. Grundman[*1], M. Sano[2], R. G. Thomas[1], C. Ernesto[1], D. Galasko[1], and L. J. Thal[1]. [1]Dept. of Neurosciences, University of California, San Diego, La Jolla, CA 92093-0949; [2]Dept. of Neurology, Columbia University, NY, NY, 10032.**

Free radicals may be important in the pathogenesis of Alzheimer's Disease (AD). Vitamin E is an antioxidant and might therefore slow cognitive decline in AD. We explored the relationship of cognition to vitamin E levels (α–tocopherol and γ–tocopherol) in Alzheimer's patients with respect to age, gender, body weight and other variables.

Prior to the initiation of a clinical trial, 295 recruited subjects were tested using the Alzheimer's Disease Assessment Scale - Cognitive (ADASCOG) and had serum assayed for α–tocopherol and γ–tocopherol. The subjects consisted of 190 women and 105 men. The mean age of the subjects was 72.9 ± 7.8. The mean score on the ADASCOG was 34.1 ± 9.8. The mean α–tocopherol level was 1.36 ± 0.45. The mean γ–tocopherol level was 0.19 ± .11. The ADASCOG score had a modest but significant correlation with α–tocopherol levels (ρ = -.125, p = .032) but not with γ–tocopherol levels (ρ = -.008, p=.89). Analysis of the results by gender revealed higher α–tocopherol levels in women than men (mean 1.40 ± .48 vs. 1.29 ± .38, p=.04). α–tocopherol levels significantly correlated with the ADASCOG in women (ρ = -.170, p=.019) but not in men (ρ = -.028 p=.78). The correlation remained significant in women after adjusting for age, duration of illness and body weight.

Higher α–tocopherol levels were associated with better scores on the ADASCOG in AD subjects recruited to participate in a clinical trial. The significant correlation in women may be related to their higher α–tocopherol levels. The results suggest a positive relationship between vitamin E intake and cognition in AD patients.
(Supported by AGO-10483)

83.14

HIPPOCAMPAL VOLUME PREDICTS VERBAL AND SPATIAL MEMORY PERFORMANCE IN THE BUSCHKE "CONTROLLED LEARNING" TASK. **Leyla deToledo-Morrell*, B. Dickerson, M.P. Sullivan, C. Spanovic, F. Morrell and D.A. Bennett. Department of Neurological Sciences and the Rush Alzheimer's Disease Center, Rush Medical College, Chicago, IL 60612**

Quantitative magnetic resonance imaging (MRI) techniques provide a unique tool for studying brain-behavior relationships *in vivo*. Atrophy of the hippocampal formation, a region important for the acquisition of new declarative knowledge, has been well documented in Alzheimer's disease (AD) and in individuals with mild cognitive impairment. In the present study, we examined the relation between extent of hippocampal atrophy and memory performance in the "Controlled Learning" task developed by Buschke and his colleagues (Buschke & Grober, *Dev. Neuropsychol.*, 1986,19:287-307). This task has been shown to identify "genuine" memory deficits in aged individuals and in the early stages of AD. 28 subjects were studied with a high resolution MRI protocol as well as the verbal and spatial versions of the Buschke controlled learning task. They consisted of patients with a clinical diagnosis of probable AD (N=14) and those who presented at the clinic with a memory complaint, but did not meet criteria for dementia (N=14). An interactive 3-D reconstruction program was used to compute hippocampal and, as a control, temporal neocortical volume from gapless, 5mm coronal slices taken perpendicular to the long axis of the hippocampus. To correct for individual differences in brain size, volumes of regions of interest were divided by total intracranial volume computed from gapless sagittal slices spanning the entire brain. Separate stepwise regression analyses with age, normalized temporal lobe, right, left and total hippocampal volume as the independent variables showed that total hippocampal volume was the best predictor of free recall and delayed free recall of verbal information ($p<0.002$ and $p<0.0001$ respectively). Recall of the spatial location of verbal items was best predicted by right hippocampal volume ($p<0.001$). Temporal lobe volume did not significantly enter the model in either case. These anatomical/behavioral dissociations strongly suggest that the results reported here cannot be attributed to generalized atrophy. **Supported by NIA grants PO AG9466 & P30 AG10161.**

84.1

IMMUNOHISTOCHEMICAL LOCALIZATION OF PARVALBUMIN AND CALBINDIN D28k IN THE HIPPOCAMPAL FORMATION OF AGED BRAIN WITH ALZHEIMER RELATED NEUROPATHOLOGY. D.M. Armstrong*, K. Mizukami, M.D. Ikonomovic, R. Sheffield and K.G. Baimbridge. Neuroscience Research Center, Medical College of Pennsylvania and Hahnemann University, Pittsburgh PA 15212.

It is our hypothesis that neuronal vulnerability in Alzheimer's disease (AD) is defined, in part, by a balance of excitatory and inhibitory stimulation. Together with our studies of selected glutamate and $GABA_A$ receptor subunits in the AD hippocampus we sought to determine the localization and cytological features of the calcium-binding proteins, parvalbumin (PV) and calbindin D28k (CaBP). Both proteins identify subpopulations of GABAergic neurons thus allowing us to investigate the organization of the inhibitory circuitry in the AD hippocampus. These investigations were performed on hippocampi obtained from 18 subjects presenting a broad range of neuropathology (i.e., Braak stages I-VI). For each subregion of the hippocampus the density (i.e., number per unit area) of immunolabeled neurons was determined. PV-labeled neurons were reduced in polymorphic subregion of the dentate gyrus as well as the CA4, CA1 subfields and subiculum of pathologically severe cases (Braak stages V & VI) compared to mild (I & II) and moderate (III & IV) cases. Moreover, in severe cases the dendrites of remaining PV-positive neurons often appeared shrunken. In contrast, no significant differences were observed in the density of CaBP-labeled cells, in part, because of the marked variability of the severe group. In addition, in many subjects displaying severe AD pathology (stages V & VI) CaBP-positive neurons were characterized by thickened dendrites extending for considerable distances from the soma. In summary, our data support: (1) a differential response for PV- and CaBP-labeled neurons in the hippocampus; (2) the maintenance of at least one subpopulation of GABAergic interneurons even in subjects with severe AD pathology. This work supported by NIH grant AG08206.

84.2

IMMUNOHISTOCHEMICAL LOCALIZATION OF $GABA_A$ RECEPTOR ß2/3 SUBUNITS IN THE HIPPOCAMPAL FORMATION IN AGED BRAIN WITH ALZHEIMER-RELATED NEUROPATHOLOGY. K. Mizukami*, M.D. Ikonomovic, R. Sheffield, R.T. Rubin, D. Grayson, R. Hamilton, D. Warde, and D.M. Armstrong. Neuroscience Research Center, Medical College of Pennsylvania and Hahnemann University, Pittsburgh PA. 15212.

Our work on the role of glutamate in Alzheimer's disease (AD) neuronal degeneration and death provided significant insights into the importance of the GABAergic neurotransmitter system in counteracting the pathologic consequences of excessive glutamate receptor stimulation. It is our hypothesis that neuronal vulnerability is defined, in part, by a delicate balance of excitatory and inhibitory stimulation. As part of our initial investigations into the GABAergic neurotransmitter system in AD we employed immunohistochemical techniques and examined the expression and anatomical distribution of the $GABA_A$ receptor subunits ß2/3 in the hippocampus of nineteen elderly subjects (average age: 81.6 years) presenting a broad range of pathologic severity (i.e., Braak Stage I-VI). Quantitative analysis of eight regions of the hippocampus revealed the highest density of ß2/3 receptor subunits in the inner molecular layer of the dentate gyrus > CA1 > CA2 while the lowest levels were observed in the granular layer of the dentate gyrus ≤ CA4 < CA3 subfields. Despite these regional variations in the density of immunolabeling no significant differences in the density of ß2/3 subunit immunoreactivity was observed among the pathologically mild (Stage I & II), moderate (Stage III & IV), or severe (V & VI) groups, despite significant Nissl-stained cell loss in the CA2 and CA1 subfields as well as the subiculum of severe cases. These data suggest: (1) that within this latter group ß2/3 subunits may be localized to cells not affected by AD pathology (i.e., interneurons); (2) remaining pyramidal neurons may be overexpressing this protein possibly as the result of diminished GABAergic stimulation; (3) a critical role for $GABA_A$ receptor subunits in hippocampal plasticity and in maintaining hippocampal functioning. This work is supported by NIH Grant AG08206.

84.3

TEMPORAL ORDER AND REGIONAL DISTRIBUTION OF NEUROPATHOLOGIC CHANGES IN AD HIPPOCAMPUS: AN IMMUNOCYTOCHEMICAL STUDY USING MAP2, MC1 AND GLUR2/3 ANTIBODIES. M.D. Ikonomovic*, K. Mizukami, R. Sheffield, P. Davies and D.M. Armstrong. Neuroscience Research Center, Medical College of Pennsylvania and Hahnemann University, Pittsburgh, PA 15212.

The results of our previous studies of the hippocampal formation suggested a temporal, if not causal, relationship between the loss and/or reduction of GluR2/3 immunolabeling and the development of neurofibrillary pathology. To confirm these data, we employed double-labeling immunocytochemical techniques and examined the hippocampus of nineteen elderly subjects including controls and those displaying a broad range of Alzheimer's disease pathology (i.e., Braak stages I-VI). Each hippocampus was immunolabeled using antibodies against the AMPA receptor subunit GluR2/3 together with MAP2 (eg. a marker of normal cytoskeleton) or MC1 (eg. a marker of early cytoskeletal alterations involving changes in the conformation of the tau molecule).

In the hippocampus of normal controls and mildly affected AD cases the vast majority of pyramidal neurons within the CA fields display both GluR2/3 and MAP2 immunolabeling. In pathologically more advanced cases the number of GluR2/3-positive neurons are reduced particularly within the CA1 subfield and subiculum. Concomitant with the reduction in GluR2/3 protein is the increase in MC1 immunolabeling. Despite the fact that both proteins are localized within hippocampal pyramidal neurons, in no instance, was there co-localization of GluR2/3 and MC1. These data suggest that the reduction in GluR2/3 immunolabeling precedes the appearance of MC1-positive elements. Moreover, we hypothesize that reduction in GluR2/3 protein contributes to the development of neurofibrillary pathology via a mechanism involving the destablization of intracellular calcium homeostasis. This work is supported by NIH Grant AG08206.

84.4

APP INDUCTION VIA MUSCARINIC RECEPTOR BLOCKADE. L.D. Acevedo,* V. Haroutunian, and W.C. Wallace. Lab of Cell & Mol Biol, GRC, NIA, Baltimore, MD 21224. Bronx VA, Bronx, NY 10468.

Lesions of the nucleus basalis of Meynert in rats cause a marked and persistent elevation in APP secretion into the CSF from cortical target regions. This increased secretion is signaled by elevated APP mRNA synthesis in the cortex. The loss of cholinergic innervation to cortical projection areas which leads to enhanced APP secretion is mimicked by subcutaneous infusion of scopolamine, a muscarinic receptor antagonist. Muscarinic receptor blockade, in the absence of nbM lesions, is therefore sufficient to elevate cortical APP secretion.

We hypothesize that increased APP synthesis *in vivo* is a postsynaptic response to reduced receptor occupancy. We have established *in vitro* cultures of various neuronal cell lines to investigate the mechanism by which APP mRNA induction follows this loss of receptor function. Untransfected SH-SY5Y neuroblastoma and rat PC12 cell cultures produce and secrete APP constitutively into the culture medium, as measured on immunoblot using the 22C11 antibody. Preliminary results suggest that differentiated PC12 cells exposed to nanomolar concentrations of scopolamine exhibit enhanced APP secretion, in a dose-dependent manner. Undifferentiated PC12 cells are not as sensitive to added scopolamine.

These observations may reflect either pre- or post-synaptic responses to disrupted receptor function. To distinguish between these two alternatives, we will administer scopolamine to cells previously exposed to acetylcholine, which more faithfully represents *in vivo* conditions during the nbM lesioning paradigm.

supported by IRP, NIH

84.5

A NOVEL ANIMAL MODEL OF ALZHEIMER'S DISEASE: CHOLINERGIC CYTOTOXICITY INDUCED BY INFLAMMATORY CYTOKINES. G.L. Wenk*, D. Roice and L.M. Baker. Division of Neural Systems, Memory & Aging, University of Arizona, Tucson, AZ 85724.

Chronic inflammatory processes may play a role in the neural mechanisms that underlie basal forebrain (BF) cell loss or dysfunction associated with aging and Alzheimer's Disease (AD). One potential cascade of biochemical steps that might lead to BF cell death is: IL-1 → Prostaglandins → $[Glutamate]_{ext}$ → $NMDA_{rec}$ → Nitric Oxide. The cytokine interleukin-1(IL-1) induces the release of prostaglandins that elevates extracellular glutamate, that activates NMDA receptors to induce the release of the cytotoxin nitric oxide. We investigated whether inflammatory processes are involved in BF cellular dysfunction and death. Lipopolysaccharide (LPS) can induce IL-1 release. In the present study, young (3 mo) and old (24 mo) rats were given single unilateral BF injections. BF injections of IL-1 (0.5 µg), tumor necrosis factor (1.0 µg), LPS (2.0, 20 or 40 µg) or NMDA (1.0 µg), significantly decreased cortical ChAT activity, as compared to the unlesioned side. The effects of LPS were dose-dependent. The combination of 2.0 µg LPS with 1.0 µg NMDA produced a decline in BFC cell number that was greater than the sum of either agent given alone. Immunocytochemical analysis for ChAT-positive cells found that all of the cytokines produced a significant decrease in cholinergic cell number in both young and old rats. These results demonstrate that cytokines, even when administered acutely, are cytotoxic to cholinergic BF cells. The actions of neuroprotectants and chronic exposure will also be presented. Supported by NIH AG10546 and the Alzheimer's Association, IIRG-95-004.

84.6

DISTRIBUTION OF BUTYRYLCHOLINESTERASE IN THE HUMAN FOREBRAIN. S. Darvesh, D. Grantham and D.A. Hopkins*. Depts. of Medicine and Anatomy and Neurobiology, Dalhousie University, Halifax, NS B3H 4H7.

Butyrylcholinesterase (BuChE) is an enzyme closely related to acetylcholinesterase (AChE). In cholinergic neurotransmission, AChE terminates the action of acetylcholine and it has been hypothesized that BuChE plays a similar role. The levels of AChE and BuChE are altered in Alzheimer's disease. Therefore, to obtain baseline data, we compared the distribution of neuronal BuChE and AChE in normal brain using a modified Karnovsky-Roots method.

In the basal forebrain BuChE-positive multipolar neurons were scattered throughout the nucleus basalis of Meynert. There were numerous fusiform neurons in both segments of the globus pallidus. The putamen and ventral striatum had multipolar cells with elaborately-branched processes. In the hippocampal formation there were BuChE-positive granule cells in the dentate gyrus, pyramidal cells in the zona pyramidale and small interneurons in the zona oriens. In the amygdala small multipolar BuChE-positive neurons were found in all the subnuclei of the amygdala. The majority of thalamic nuclei contained numerous BuChE-positive neurons.

The human brain contains populations of BuChE-positive neurons that are distinct from those with AChE. The distribution of BuChE in forebrain structures suggests that it may play a role in neural mechanisms of cognition.

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84.7

DIFFERENTIAL REGULATION OF ADENYLYL CYCLASE ACTIVITY IN SKIN FIBROBLASTS FROM SPORADIC AND FAMILIAL ALZHEIMER'S DISEASE CASES WITH PRESENILIN-1 AND SWEDISH APP670/671 MUTATIONS. M. Vestling*, A. Adem, M. Racchi[1], S. Govoni[1], L. Lannfelt, G. Gibson[2] and R. F. Cowburn, *Karolinska Institute, Div. of Geriatric Medicine, S-141 86 Huddinge, Sweden, 1 Alzheimer's Dept., FBF Hospital, Brescia, Italy, 2 Cornell Univ. Med. Coll. at Burke Medical Res. Inst., White Plains, NY 10605, USA.*

ß-adrenoceptor-stimulated adenylyl cyclase has been shown to be altered in fibroblast cell lines from sporadic Alzheimer's disease patients. From a patophysiological and diagnostic point of view, it is important to elucidate whether disrupted adenylyl cyclase occurs in different (familial and non-familial) subgroups of Alzheimer's disease patients.

In the present study, we compared basal, isoprenaline- and forskolin-stimulated adenylyl cyclase activities in fibroblast cell lines established from sporadic Alzheimer's disease cases (n=7) and age-matched controls (n=7), individuals from a Swedish family carrying the Presenilin-1 163 mutation (n=8), a Swedish/Finnish family carrying the Presenilin-1 146 mutation (n=6) and a Swedish family carrying the APP 670/671 double-mutation (n=14).

Our data showed a *decreased* ß-adrenoceptor-stimulated adenylyl cyclase activity in fibroblasts from sporadic Alzheimer's disease cases when compared to age-matched controls ($p<0.05$). This result is in agreement with that of a previous report (Huang and Gibson, J. Biol. Chem. 268, 14616-14621, 1993).

In contrast, both ß-adrenoceptor- and forskolin-stimulated adenylyl cyclase activities were significantly *increased* in the Presenilin-1 mutation-carrying fibroblasts compared to fibroblasts without the mutation ($p<0.05$, Student's unpaired t-test for the comparison of cell lines from both Presenilin-1 mutation families). No differences were seen between cell lines with and without the Swedish APP 670/671 mutation.

This study suggests that various Alzheimer's disease gene mutations have different consequences for the regulation of adenylyl cyclase mediated signal transduction in this disorder. (Supported by Stiftelsen för Gamla Tjänarinnor and Åke Wibergs Stiftelse, Sweden).

84.8

INDIVIDUAL RESPONSES OF ALZHEIMER PATIENTS DURING TREATMENT WITH THE CHOLINERGIC DRUGS ARECOLINE AND PHYSOSTIGMINE: KINETIC, PHYSIOLOGICAL, AND COGNITIVE MEASURES. K.C. Raffaele,* S. Asthana, A. Berardi, N.H. Greig, M.B. Schapiro, J.V. Haxby, and T.T. Soncrant. LCNB/LNS, National Institute on Aging, Bethesda, MD 20892.

Cholinergic function is consistently decreased in Alzheimer's disease (AD). However, responses of individual AD patients to treatment with cholinergic agents has varied. To determine whether individuals responding to one class of cholinergic agent were more likely to respond to a different class of cholinergic agent, and to evaluate whether positive responses to treatment were associated with specific kinetic profiles or with changes in physiological variables, patients received (during separate inpatient stays) long-term intravenous infusions of escalating doses of physostigmine (a cholinesterase inhibitor; 2, 6, 12, 18, and 25 mg/day) and arecoline (a muscarinic agonist; 1, 4, 16, 28, and 40 mg/day). Before, during, and after drug treatment, plasma drug levels, changes in physiological variables (blood pressure, respiratory rate, temperature, and pulse), and changes in neuropsychological performance (including visuo-spatial construction, verbal fluency, attention, and verbal memory) were measured. Responses for all types of measures varied among patients. Cognitive responses to treatment varied among different cognitive measures and among patients; visuo-spatial performance and verbal memory were most consistently improved for both drugs but not all patients showed improvement, and the dose at which improvement was seen varied. For physiological responses, an increase in pulse was the most consistent change for both drugs and was also most likely to predict an improvement in cognitive performance. Plasma drug levels varied across administered doses and across patients, but given the same administered dose, the plasma level of physostigmine varied more than did that of arecoline. Measurement of multiple parameters in the same individuals will improve our understanding of the interaction of those parameters in determining responses to drug treatment. Funded by NIA (intramural funding).

84.9

SENSITIVITY TO MK-801 NEUROTOXICITY IS INCREASED IN AGED MALE RATS. D. F. Wozniak*, N. B. Farber, A. Nardi, G. Taylor and J. W. Olney. Dept. Psychiat.,Washington Univ. Sch. Med., St. Louis, MO 63110 and Dept. Psychol., Univ. MO-St. Louis, St. Louis, MO 63121.

Blockade of NMDA glutamate receptors by MK-801 or other NMDA antagonists causes vacuolar injury of neurons in the rat posterior cingulate/retrosplenial (PC/RS) cortex. Putatively, the mechanism of this neurotoxic effect may have relevance to Alzheimer's disease. Age and sex are important determinants of sensitivity to this neurotoxic effect in that immature rats prior to puberty are totally insensitive, and during early adulthood, females are more sensitive than males. In the present study, we investigated whether sensitivity of adult rats of either sex changes with increasing age. Male or female adult rats at various ages were treated with 0.5 mg/kg MK-801 and sacrificed 4 hrs later for histological evaluation of the number of vacuolated PC/RS neurons. In the first experiment, Sprague Dawley male rats 24 mos old had significantly larger numbers of injured PC/RS neurons than 4 or 8 month old males. This result was replicated in a second experiment where 20-month old Sprague Dawley male rats had significantly greater numbers of vacuolated PC/RS neurons than 4-month old males. In a third experiment, using female Long Evans rats that were 4, 9, or 24 mos old, the vacuolated neuron counts were not significantly different among groups (all groups showed high sensitivity, an apparent ceiling effect). To rule out strain differences as the basis for absence of an age-related effect in females, we studied 6- and 24-month old Long Evans males and found that the 24-month old males were significantly more sensitive. The results suggest that adult male rats become more sensitive to MK-801 neurotoxicity with increasing age. Whether female rats also become more sensitive with age is not clear from these data because the dose of MK-801 employed produced a likely ceiling effect in females at all ages. Supported by AG11355, DA05072, SDAC DA00290 (NBF), and RSA MH 38894 (JWO).

84.10

Glutamate Transport Deficiency is Associated with Synaptic Pathology in Alzheimer's Disease Shi Li#, Michael Alford, M. Mallory#, Arnold Miller#* and E. Masliah§#. Depts. of Neurosciences# and Pathology§, University of California, San Diego, La Jolla, California 92093.

While the mechanisms of synaptic damage in Alzheimer's disease (AD) are not fully understood, the decreased functioning of glutamate transporters might be involved in neurodegeneration by failing to uptake excess glutamate in the synaptic cleft. When the levels of D- and L-[^{3}H]aspartate binding in midfrontal cortex were correlated with synaptophysin, brain spectrin degradation products and clinical and pathological indicators, AD cases showed a 30-50% decrease in D-[^{3}H]aspartate binding, L-[^{3}H]aspartate binding, and synaptophysin immunoreactivity compared to controls. The decreased aspartate binding activity and synaptophysin immunoreactivity correlated with increased levels of brain spectrin degradation products. Moreover, L-[^{3}H]aspartate binding activity correlated with synaptophysin immunoreactivity. To further understand the molecular mechanisms of decreased glutamate transport activity in AD, we performed RNase protection assays and Western assays in midfrontal cortex of AD and control cases. The preliminary data indicated that the EAAC1 and GLT-1 subtypes expression might be affected in AD. These results suggest that the abnormal functioning of glutamate transport might be involved in the AD synaptic pathogenesis.

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84.11

m2 Imunoreactive Neurons Are Not Reduced In The Basal Forebrain of Patients with Alzheimer's Disease. E J. Mufson*[1], S. Jaffar[1], J. H. Kordower[1] and A.I. Levey[2], [1]Research Center For Brain Repair and Dept. of Neurological Sci., Rush Medical Ctr., Chicago, IL 60612 and [2]Dept. of Neurology, Emory Univ., Atlanta, GA 30322

Some studies suggests that basal forebrain (BF) cholinergic neurons are a source of presynaptic muscarinic receptors in cortical terminal fields. The present study determined the correspondence between neurons containing the m2 muscarinic receptor subtype and cholinergic cell bodies within the aged human BF and evaluated pathologic changes seen in Alzheimer's disease (AD). Tissue immunostained with an antibody against the m2 receptor revealed small and large multipolar neurons scattered within the cholinergic BF continuum. Similar observations were also seen in the cynomologous monkey BF. To determine whether m2 neurons codistributed with cholinergic BF neurons, adjacent sections were stained with an antibody against the low affinity p75NTF receptor, an established marker for these neurons. Neuron counts revealed 10 times as many p75NTR than m2 immunoreactive BF neurons. Co-localization studies indicate that some m2 neurons are cholinergic. Quantitative evaluation of the number of m2 neurons within the BF between normal aged and AD cases failed to reveal a significant difference between groups ($F[1,7]<1$).These observations indicate m2 containing neurons within the primate basal forebrain are primarily non-cholinergic. Further, the lack of a decrease in m2 neurons in AD, suggests that the reduction in cortical "M2" receptors in this disease are not due to degeneration of cortical afferents emanating from cholinergic BF neurons. Supported by AG10668, AG10161, AG09466, AG10130 and NS30454.

84.12

DETERMINING REGIONAL VARIANCES IN GLUTAMATE TRANSPORTER PROTEIN SITES IN ALZHEIMER DISEASE: BINDING VS UPTAKE. H. L. Scott, R. I. Westphalen* and P. R. Dodd
Clinical Research Centre, Royal Brisbane Hospital Research Foundation, Brisbane, Q 4029, AUSTRALIA.

Alzheimer disease (AD) is characterised by area specific neuropathology. Based on the neurotoxic process of excitotoxicity, the present study investigated whether the glutamate uptake system is altered in "affected" areas of AD brain using two methods of transporter analyses: membrane binding and synaptosomal uptake. Other studies have described several forms of the glutamate transporter protein, a variant of which may be inefficient at removing the potential excitotoxant, glutamate, from the synaptic cleft. Previously, we used a range of glutamate transport inhibitors to displace Na^+-dependent, high-affinity D-[^{3}H]aspartate binding to the glutamate uptake site in control and Alzheimer disease cortical tissue[1]. The binding profiles of these inhibitors were found to be significantly different within cortical regions of AD cases, but not in controls. High-affinity synaptosomal uptake inhibition profiles were found to be significantly different within cortical areas of controls but not in AD cases. Comparing rat cortex and cerebellum tissue, a significant difference was found in the binding displacement profile ($p < 0.001$) and uptake inhibition profile ($p < 0.001$), suggesting a variance in glutamate transporter sites between these regions. However, a significant difference was also found between displacement and inhibition profiles within rat brain regions. The differing profiles of binding displacement and uptake inhibition may represent regional variations in glutamate removal systems, depending on the method of glutamate transporter analysis used.

1. H.L.Scott, P.R.Dodd and R.I.Westphalen (1995) *Soc. Neurosci. Abstr.* **21**, 1978.

84.13

REPEATED ADMINISTRATION OF METRIFONATE, A CHOLINESTERASE INHIBITOR, INCREASES LOCAL CEREBRAL GLUCOSE UTILIZATION IN YOUNG AND AGED RATS. M.H.Bassant *, F. Poindessous, Y. Lamour. INSERM U 161, 2, rue d'Alésia, 75014, Paris, France

Inhibitors of acetylcholinesterase (AChE) improve cognitive deficits in some Alzheimer's disease patients. This effect may be related to an increase in local cerebral glucose utilization (LCGU). We have shown previously that metrifonate (MFT, 80mg/kg i.p., single dose), a slow-release AChE inhibitor, increases significantly LCGU in a number of cerebral regions in young and aged rats. In the present experiment, the drug was given repeatedly (oral administration of 120mg/kg, twice daily) over a period of 3 weeks, in order to mimic more closely the clinical situation in the human patient. LCGU was measured in 3 and 28-month-old MFT-treated and vehicle-treated rats, using Sokoloff's autoradiographic 2-deoxyglucose method. Virtually no side effects were observed. In young rats, MFT increased significantly LCGU in 23 of the 54 regions studied. In these regions, the average MFT-induced increase in LCGU was 31% above control. The whole brain mean LCGU increased by 17% ($p< 0.001$). Dramatic effects were observed in the limbic system (cingulate, retrosplenial cortices, medial septum, diagonal band of Broca, dorsal and ventral hippocampus, subiculum, basolateral amygdala), in the thalamus and the substantia nigra.The effects were extremely similar in aged rats. As compared to acute administration, both the number of regions affected and the amplitude of the metabolic activation were larger after chronic treatment. Our results show that the durable inhibition of AChE induced by MFT increases LCGU in young as well as in aged rats in a number of brain regions, including regions involved in learning and memory .

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84.14

TRK RECEPTOR ALTERATIONS IN ALZHEIMER'S DISEASE. B. Connor*[1], D.Young[1], P.Lawlor[1], R.L.M. Faull[2] and M. Dragunow[1].
Depts. of Pharmacology[1] and Anatomy[2], University of Auckland, Auckland, New Zealand.

Recently it has been proposed that neurodegeneration associated with Alzheimer's disease may be due to impaired retrograde transport of nerve growth factor due to deficits in production and/or utilisation of the trkA receptor. The expression of trk receptors in post mortem normal, Huntington's disease and Alzheimer's disease human brains was investigated using immunohistochemistry and *in situ* hybridisation. Hippocampal and temporal lobe sections (50μm) from normal, Huntington's disease and Alzheimer's disease brains were incubated with primary polyclonal antibodies to trkA, trkB (full-length) and trkB (truncated) and the immunostaining visualised using the Extravidin/HRP-diaminobenzidine method. Alzheimer's disease hippocampi displayed an increase in trkA receptor-immunopositive levels in astrocytes around the CA1 region, some of which were associated with β-amyloid-positive plaques. In addition, truncated trkB receptors were found in high levels in senile plaques, while the full-length trkB receptor was expressed in glial-like cells in the hippocampus of Alzheimer's disease brains. Using an oligoprobe directed against trkA, hippocampal and temporal lobe sections (16μm) were processed for *in situ* hybridisation. Results indicated that trkA receptor mRNA levels were also elevated in the Alzheimer's disease hippocampus. The alteration in trkA and trkB receptors in the hippocampus and temporal lobe of the Alzheimer's disease brain and their association with astrocytes and plaques suggests that they may play a functional role in promoting the synthesis of β-amyloid and plaque formation.

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84.15

NITROTYROSINE RESIDUES COLOCALIZE TO NEUROFIBRILLARY TANGLES IN ALZHEIMER'S DISEASE AND ALSC/PDC OF GUAM P.F.Good,*, P. Werner, A. Hsu,. D.P.Perl ,and C.W.Olanow Division of Neuropathology, Mount Sinai School of Medicine, New York, NY 10029

The possibility that oxidative stress produces cellular damage in Alzheimer's disease has received wide attention. Speculations have focused on the coupling of exitotoxic mechanisms initiated by glutamate receptor agonists to the production of reactive oxygen species. While there have been a number of reports demonstrating such phenomena in vitro and in animal models, data on human disease is still in the early stage. Employing antibodies that specifically recognize nitrated tyrosine residues we have demonstrated the presence of nitrated tyrosine colocalized to neurofibrillary tangles in Alzheimer's disease (AD) and in amyotrophic lateral sclerosis/parkinsonism dementia complex (ALS/PDC) of Guam. The nitrated tyrosine residues are colocalized to neurofibrillary tangles occurring in the hippocampus of AD of ALS/PDC, as well as the neurofibrillary tangles within dopaminergic neurons of the substantia nigra in ALS/PDC. Immunolabeling was abolished by preabsorption with antigen and by reduction of tissue sections with sodium dithionite. Nitrated tyrosine residues have been shown to be the product of peroxynitrite attack on tyrosine (Beckman, et al. 1992, Arch. Biochem. Biophysics 298, 438-445). Peroxynitrite is formed in the diffusion rate limited reaction of superoxide ($^{\bullet}O_2^-$) with nitric oxide ($NO^{\bullet}$), derived from either calcium-activated neuronal nitric oxide synthase, (nNOS) or from inducible NOS (iNOS). Nitration of tryosine residues is readily catalysed by iron which has also been localized to neurofibrillary tangles. Evidence that excess superoxide and nitric oxide play a direct role in neuropathological changes in AD provides a potential link of excitotoxicity with oxidative stress in the production of neurofibrillary tangles in AD and ALS/PDC. Supported by grants AG-5138 and AG-2210 from the National Institutes of Health and the Lowenstein Foundation

84.16

MAO INHIBITORS POTENTIATE ACETYLCHOLINESTERASE INHIBITOR EFFECTS ON PASSIVE AVOIDANCE LEARNING IN THE RAT F. Camacho, R.W. Dunn, F.P. Huger, J.T. Winslow*, **Hoechst Marion Roussel, Neuroscience Therapeutic Area, Bridgewater, N.J., 08807**

Cholinomimetic agents have had limited efficacy in treating dementia associated with Alzheimer's Disease. Monoaminergic systems including noradrenergic, dopaminergic and serotonergic substrates have also been implicated in cognitive processes and are significantly affected in Alzheimer's Disease. This study reports the effects of increased monoaminergic and cholinergic neurotransmitter activity produced by drugs which interfere with enzymatic degradation. These effects were examined in a passive avoidance paradigm designed to detect enhanced memory retention in rodents. The acetylcholinesterase inhibitor heptylphysostigmine significantly enhanced retention latencies of a passive avoidance response at moderate doses (1.25-2.5 mg/kg, IP). The non-selective MAO inhibitor tranylcypromine (2.5-20 mg/kg, IP) and the MAOA inhibitor moclobemide (5.0-40.0 mg/kg, IP) did not affect passive avoidance in this paradigm, while a high dose of the MAOB inhibitor deprenyl (40 mg/kg IP) did enhance performance 24 hrs after administration and training. When a sub-threshold dose of heptylphysostigmine (0.625 mg/kg, IP) was co-administered with ineffective doses of moclobemide (5, 10 mg/kg), tranylcypromine (5, 10 mg/kg) or deprenyl (10, 20 mg/kg) the combination of drugs also enhanced retention of the passive avoidance response. These data indicate that cholinergic and monoaminergic substrates may interact in the formation of a long-term memory trace. The specific monoamines associated with this action are not clear from these findings since MAOI's of both the A and B types potentiated heptylphysostigmine. Over all, these results indicate that chemotherapy targeted at multiple neurotransmitter systems may be an efficacious alternative for the treatment of AD.

84.17

SELECTIVE, AGE-RELATED LOSS OF CALBINDIN-D_{28K} FROM BASAL FOREBRAIN CHOLINERGIC NEURONS IN THE MARMOSET. C.-K. Wu* and C. Geula. Harvard Medical School, Boston, MA 02215.

We have reported previously an age-related loss of calbindin-D_{28K} (CB) immunoreactivity from the cholinergic neurons of the human basal forebrain (BFCN). The loss of CB from the BFCN is a potential mechanism for the selective degeneration of these neurons in neurodegenerative diseases of the elderly, particularly Alzheimer's disease. Since the presence of CB is a specific feature of the primate BFCN, we used a primate model for the study of age-related changes in this protein. Two young (2 years) and three old marmosets (8, 9, and 15 years) were processed for CB, choline acetyltransferase (ChAT), high affinity (Trk A) and low-affinity ($p75^{LNGFR}$) nerve growth factor receptor immunohistochemistry in adjacent sections of the basal forebrain. Quantitative assessment revealed loss of CB immunoreactivity from 21% to 41% of BFCN in the old marmosets. ChAT, Trk A and $p75^{LNGFR}$ immunoreactivity, which are co-localized with CB in these cholinergic neurons, displayed no similar age-related loss. Furthermore no age-related loss was observed in CB immunoreactivity within other CB-positive neurons in the forebrain. The similarity between the age-related loss of CB from the BFCN of the human and marmoset indicates that the latter represents an appropriate animal model for investigations of the role of CB in the vulnerability of BFCN to degeneration. (Supported by NIA grant AG10282)

84.18

BUTYRYLCHOLINESTERASE: IS IT IMPORTANT FOR CORTICAL ACETYLCHOLINE REGULATION? E. Giacobini[1]*, PL.Griffini, T.Maggi, G. Mascellani, and R.Mandelli. Research Dept, Mediolanum Farm, Milan, Italy and [1]Dept.of Geriatrics, University Hospitals of Geneva, CH-1226 Thonex-Geneva, Switzerland.

The mammalian brain has been shown to contain two major forms of cholinesterases (ChE), acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE). Using specific substrates and selective inhibitors it has been demostrated that in rat brain, 80% of the ChE activity is AChE and 20 % BuChE (Giacobini and Holmstedt, 1958) and that AChE and BuChE are mainly localized in neurones and glia respectively (Giacobini, 1959). In the cortex of patients suffering by Alzheimer disease (A.D.), AChE activity is decreased to 10-15 % of normal values while BuChE is unchanged or slightly increased (Perry et al., 1978, Davies, 1979, Atack et al., 1987, Giacobini, 1989, Arendt et al., 1992). Since AChE is inhibited by high concentrations of acetylcholine (ACh), while BuChE remains unaffected, it may well be that BuChE may play an important role in the in vivo regulation of synaptic concentrations of ACh in the brain of A.D. patients. This hypothesis may gain support if it were possible to demonstrate that a selective inhibitor of BuChE brings about an increase in extracellular ACh and that this effect can be potentiated by the association with a selective AChE inhibitor. For this purpose we have utilized a sensitive microdialysis method with low femtomole sensitivity without a second ChE inhibitor in the probe using the BuChE selective phenylcarbamate derivative MF 8622 (IC50=6.5 nM). Our results show that MF 8622 (15uM and 50 uM) was able to significantly increase the level of extracellular ACh in a dose dependent manner when infused directly into the cortex via the dialysis probe. The maximal observed extracellular concentration of ACh was 25 nM (from 5 nM base line).

84.19

INCREASED AMYLOID PRECURSOR PROTEIN (APP) SYNTHESIS IN RAT CORTICAL ASTROCYTES BY ADRENERGIC RECEPTORS COUPLED TO CAMP FORMATION. Richard J. Wurtman*, Robert K. K. Lee, Wataru Araki, Youjeong Kim and U. Ingrid Richardson. Dept. Brain & Cognitive Sci., MIT, Cambridge, MA 02139.

We have shown that APP metabolism is accelerated by neurotransmitters and second messengers that activate protein kinase C. We now present evidence that APP synthesis (i.e., mRNA and holoprotein) can be increased by neurotransmitter receptors coupled to a different messenger, cAMP formation. Confluent primary astrocytes treated with serum-free medium containing 50, 100 or 250μM respectively of 8-Bromo-cAMP (8Br-cAMP) for 24h showed a dose-dependent increase in APP mRNA on Northern blots (120%, 150% and 180% of untreated cells). In comparison, β-actin mRNA was decreased to 50% of untreated cells by 8Br-cAMP (250μM). Both L-norepinephrine (NE, 50 or 100μM) and the β–adrenergic agonist isoproterenol (50μM) increased APP mRNA to 180% of untreated cells. The β-adrenergic antagonist propranolol (50μM) reduced the NE-stimulated increase in APP mRNA to baseline levels. N- and C-terminal APP antibodies 22C11 and R37 (from Dr. F. Kametani, Tokyo Inst. Psychiatry) respectively showed that 8Br-cAMP or NE also increased APP holoprotein in cell lysates to 200% of that seen in untreated cells. Both drugs also increased process formation and glial fibrillary acidic protein immunoreactivity in astrocytes. Thus, activation of β-adrenergic receptors coupled to cAMP formation increases both APP mRNA and holoprotein in reactive astrocytes.

The APP gene promotor contains a consensus sequence for a cAMP response element (CRE). The immunosuppressant cyclosporin A (CycA, 10 μM), which is known to inhibit CRE-mediated transcription, blocked the increase in APP protein caused by 8Br-cAMP. This suggests that CycA may be used to prevent increases in APP and, possibly, amyloid formation in Alzheimer's disease.

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84.20

N-Acetylaspartylglutamate, N-acetylaspartate and N-acetylated alpha-linked acidic dipeptidase in human brain and their alterations in Huntington's and Alzheimer's disease. L. A. Passani* °, J.-P. G. Vonsattel° °, R. E. Carter ° and J. T. Coyle °. Lab. Molec. & Develop. Neurosci.° and Lab. Molec. Neuropathol. ° °, Harvard Medical School / MGH, Charlestown, MA 02129.

The dipeptide N-acetylaspartylglutamate (NAAG) may be involved in the process of glutamatergic signaling by both serving as an endogenous receptor ligand and a glutamate protransmitter. NAAG levels and the activity of N-acetylated alpha-linked acidic dipeptidase (NAALADase), an enzyme which hydrolyses NAAG to glutamate and N-acetylaspartate (NAA), are altered in nervous system disorders associated with the dysregulation of glutamatergic neurotransmission such as schizophrenia, seizure disorders, and amyotrophic lateral sclerosis. The processes leading to these alterations are not well understood. In the present investigation, we have extended the study of NAAG and its hydrolytic enzyme to Alzheimer's disease (AD) and Huntington's disease (HD). Since NAAG is localized to neurons especially vulnerable to the degenerative process in AD and HD, we investigated alterations in neuronal and glial density as a possible factor impacting levels and activity. The levels of NAAG, NAA and several amino acids and the activity of NAALADase were determined in eight different brain regions of control, AD and HD brain. Cell density was determined by counting the number of neurons, oligodendrocytes and astrocytes in defined samples of each brain region. The cell density counts were correlated to the NAAG levels and NAALADase activity determined in corresponding samples. Significant reductions in both NAAG levels and enzyme activity were found in AD amygdala, hippocampus, frontal cortex, cerebellar cortex and putamen as well as in HD caudate nucleus, putamen, frontal and temporal cortex and cerebellar cortex. Reductions in neuronal density in brain regions affected by these disorders correlated significant in most regions with the reductions in NAAG, NAA and NAALADase, whereas there were no correlations between NAAG, NAA, NAALADase and glial density. The parallel reductions of NAAG levels and NAALADase activity in these two neurodegenerative disorders suggest a close spatial and/or functional relationship between the two neurochemical parameters in the affected neurons. Therefore, we hypothesize that NAAG and NAALADase may be involved in the pathological processes of HD and AD. This work was supported by NIH Grant MH-572901.

DEGENERATIVE DISEASE: ALZHEIMER'S—NEUROPHARMACOLOGY AND NEUROTRANSMITTERS II

85.1

AN ALPHA$_1$-ADRENOCEPTOR AGONIST, ST 587, AND A PARTIAL GLYCINE-B BINDING SITE AGONIST, D-CYCLOSERINE, STIMULATE SPATIAL BEHAVIOR IN AGED RATS. M. Riekkinen* and P. Riekkinen Jr. Department of Neurology, University of Kuopio, P.O.Box 1627, FIN-70211 Kuopio, Finland.

The present study was designed to investigate the efficacy of single and combined stimulation of alpha$_1$-adrenoceptors and glycine-B binding sites to alleviate age-related water maze (WM) reference (fixed location of the platform) and working (reversal of the platform location) memory defect. We found that in aged rats daily intraperitoneal (i.p.) pretraining treatment with ST 587, an alpha$_1$-adrenoceptor agonist, at 3000 μg/kg, but not at 1000 μg/kg, facilitated reference memory in WM task. However, ST 587 at 3000 μg/kg i.p. did not stimulate working memory. A partial glycine binding site agonist, D-cycloserine (DCS; i.p), at 10 000 μg/kg facilitated WM reference memory performance in aged rats. The subthreshold doses of DCS 1000 or 3000 μg/kg did not increase the therapeutic effect of ST 587 1000 or 3000 μg/kg. Further, a subthreshold dose of ST 587 1000 μg/kg did not enhance the therapeutic effect of DCS 10 000 μg/kg. The present results indicate that activation of alpha$_1$-adrenoceptors and glycine-B binding sites may to some extent alleviate age-related defect of spatial navigation.

Supported by the Finnish Academy of Sciences.

85.2

^{125}I-HUMAN-GALANIN BINDING SITES IN ALZHEIMER'S DISEASE: INCREASES IN HIPPOCAMPAL SUBFIELDS AND A DECREASE IN THE CAUDATE NUCLEUS.

Rodríguez-Puertas Rafael, Nilsson Siv, Pascual Julio*, Pazos Angel, Hökfelt Tomas. Dept. of Neurosci., Karolinska Inst., Stockholm, Sweden. and Dept. of Physiol. and Pharmacol., Univ. of Cantabria, Santander, Spain

Using iodinated human galanin and autoradiography, galanin binding sites were studied in cortical and hippocampal areas and in some brainstem nuclei of 8 patients with senile dementia of the Alzheimer type (SDAT) and of 9 matched control cases. The pattern of distribution showed the highest density in the substantia nigra with a less intense labelling in the hippocampus and cortical regions. In the SDAT cases a significant increase in galanin binding sites was found in some hippocampal areas, a decrease in the caudate nucleus and no significant changes in frontal and entorhinal cortices. These findings suggest that some central galanin systems may be deranged in SDAT.

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85.3

NEUROPEPTIDES, PHOSPHORYLATED TAU AND NEUROPATHOLOGY IN ALZHEIMERS DISEASE G. Bissette*, W. Smith, B. Crain and C. B. Nemeroff. Depts. of Psychiatry and Pathology, Duke Univ. Med. Ctr., Durham, N.C. 27710

In cortical post-mortem brain tissue from Alzheimers disease (AD) patients, the concentrations of the neuropeptide neurotransmitters, corticotropin-releasing factor (CRF) and somatostatin (somatotropin-release inhibiting factor, SRIF), are decreased relative to non-AD control patients. In contrast, the hyperphosphorylated form of the microtubule associated tau protein which is detected by the ALZ-50 antibody and forms the paired helical filaments of the neurofibrillary tangles is increased in the cortex of AD patients. Using punched samples of the frontal (Brodmann's area 10) and temporal (Brodmann's area 21) cortex from patients with neuropathologically confirmed AD (n=91) and non-AD controls with (n=19) and without (n=8) dementia, the concentration of CRF, SRIF and the ALZ-50 reactive tau were measured and correlated with numbers of senile plaques and neurofibrillary tangles as well as the demographic variables of age, sex, duration of disease, post-mortem delay and freezer residence time. Both CRF and SRIF were significantly reduced while the ALZ-50 reactive tau concentrations were increased in AD in both cortical regions relative to the controls. Demented controls had reduced concentrations of both peptides relative to non-demented controls also, but were not significantly different in ALZ-50 reactive tau levels. As expected, the concentration of the ALZ-50 reactive tau was highly correlated with the numbers of neurofibrillary tangles in both regions. Significant correlations were also obtained between CRF and SRIF in both regions in AD and control patients, with SRIF and the tau protein in the controls and for both peptides and plaque counts in the AD patients. These data support the hypothesis that the neuropeptide changes in AD are more associated with the presence of dementia than with the presence of the specific pathognomic structures used to diagnose AD. Supported by NIH grants MH-40524 and AG-05128.

85.4

AUTORADIOGRAPHIC STUDY OF GALANIN BINDING IN THE NORMAL AND ALZHEIMER'S DISEASE BRAIN: EVIDENCE FOR INCREASED RECEPTOR OCCUPANCY IN ALZHEIMER'S DISEASE. J.B. Leverenz*, M.A. Raskind, and M.A. Miller. Depts. Neurology and Psych & Behav. Sci., Univ. of Washington, Seattle, WA 98195

Excess galanin (GAL) may play a role in the memory dysfunction observed in patients with Alzheimer's disease (AD). Previous studies have observed a reduction in the number of GAL receptors in the parahippocampal gyrus (PHG) but not in the basal forebrain (BF) with AD. Since occupancy of these receptors by endogenous GAL may result in an underestimation of total receptor number, GTP pretreatment can be used to induce dissociation of endogenous GAL from its receptor prior to incubation with radio-labeled ligand. We have used this method to evaluate the alterations in total GAL receptor number and receptor occupancy by endogenous GAL with AD. Fresh frozen coronal sections (20 μm) from normal (n=6) and AD (n=6) brains were sampled at the level of the superior frontal gyrus (SFG), BF, hippocampus, PHG, and lateral hypothalamus (LH). Sections were pretreated with or without GTP (10^{-4} M) and then incubated in 0.25 nM ^{125}I-GAL. Non-specific binding was assessed by the addition of excess unlabeled GAL. We observed that the ability of GTP pretreatment to enhance ^{125}I-GAL binding site density was greater in the SFG, hippocampus CA-1, and LH ($p<.05$) of the AD versus normal brain indicating enhanced occupancy of GAL receptors in these regions. Consistent with previous reports, we observed a significant decline in GAL receptor density in the PHG ($p<.05$) which was not reversed by GTP pretreatment. In contrast, GAL receptor number was not reduced in the SFG, BF, LH and hippocampus with AD. Our observation of regionally specific increases in GAL receptor occupancy provides further evidence for excess GAL release in AD. Since the number of GAL receptors in most regions are preserved in AD, this excess in GAL release may contribute to the cognitive deficits observed in this disease. (NIA AG 00503, AG 05136-12; NIH NS33606)

85.5

ALTERATION IN MUSCARINIC ACETYLCHOLINE RECEPTOR SUBTYPES IN DIFFUSE LEWY BODY DISEASE AND ALZHEIMER'S DISEASE K. Shiozaki* and Y. Watanabe, Department of Pharmacology, National Defense Medical College, 3-2 Namiki, Tokorozawa, 359 Japan.

Dementia accompanied by Lewy bodies in the brain stem and cortex has been termed diffuse Lewy body disease(DLBD). Recently, it is reported that DLBD is the second most common cause of dementia in the elderly, after Alzheimer's disease(AD). Severe decrease in choline acetyltransferase activity and increase in muscarinic acetylcholine receptor(mAChR) binding were reported in DLBD cortices. We have generated subtype specific antibodies against mAChR subtypes(m1-m4) and examined their distributions in postmortem brain. Membrane fractions were prepared from postmortem brains which were histopathologically diagnosed as physiological aging(n=3), DLBD(n=7) and AD(n=5). mAChRs were prelabeled with [^{3}H]QNB, then solubilized with 1% digitonin and 0.1% cholate and immunoprecipitated with antisera. The proportions of specifically precipitated mAChR subtypes were estimated as the differences in the amounts of bound [^{3}H]QNB precipitated with specific antibody and those precipitated with non immune serum. Marked changes were observed in preparations of temporal cortex and hippocampus. In these area, [^{3}H]QNB binding sites in DLBD were found to be significantly decreased. The level of immunoprecipitated m1 receptor was found to be significantly decreased in AD compared to physiological aging. The level of m2 was lower in DLBD compared with physiological aging and AD. The level of m4 was also diminished in DLBD.

85.6

BIIP 20 XX: A POTENT AND SELECTIVE A1 ADENOSINE RECEPTOR ANTAGONIST FOR THE TREATMENT OF COGNITIVE DEFICITS, H.A. Ensinger, W.D. Bechtel, W. Gaida, J. Mierau, U. Kuefner-Muehl and M. Wienrich*, Dept. Biological Research and Dept. Chemical Research, Boehringer Ingelheim KG; 55216 Ingelheim, Germany

Alzheimer's patients suffer from extensive neuronal cell loss coherent with reduced synaptic transmission. Antagonism of A_1 adenosine receptors constitutes one possible strategy to activate central neurotransmission.

BIIP 20 XX demonstrates high and selective affinity for the A_1 adenosine receptor subtype in receptor preparations from rat (r), monkey (m) and human receptors expressed in CHO-cells (h). The following affinities could be determined. A_1: K_i=3.0nM (r), K_i=8.2nM (m) and K_i=7.3nM (h). A2: K_i=2640nM (r), K_i=1418nM (m) and K_i=440nM (h). A_3: K_i=2856nM (h).

In functional assays *in vitro* such as electrically stimulated acetylcholine release from rat hippocampal slices a pA_2 value of 8.7 could be determined. BIIP 20 XX also reversed the inhibition of the noradrenaline and serotonin release by CPA (cyclopentyladenosine). In hippocampal slices, the suppression of electrically evoked synaptic transmission by the A_1 adenosine receptor agonist CPA was concentration-dependently antagonized by BIIP 20 XX (pA_2=8.5). Additionally, the activation of human A_1 receptors expressed in CHO cells was measured by the stimulated proton efflux using the cytosensor technology. BIIP 20 XX blocked the agonist CPA effectively (pA_2=8.6). Caffeine, theophylline and DPCPX (dipropylcyclopentylxanthine) were investigated as reference antagonists.

In conclusion, BIIP 20 XX has a high selectivity for the A_1 adenosine receptor from rats, monkey and man. Furthermore, BIIP 20 XX displays a consistently high potency as A_1 antagonist in the rat hippocampal slice preparation and the cytosensor assay using CHO hA_1-cells.

85.7

BIIP 20 XX IS A POTENT AND SELECTIVE ANTAGONIST OF ADENOSINE A_1 RECEPTORS *IN VIVO* AS DEMONSTRATED BY BEHAVIOURAL, EEG AND MICRODIALYSIS STUDIES. A. J. Carter*, W. T. O'Connor, M. J. Carter, W. Gaida, E. Lehr, U. Küfner-Mühl and U. Ungerstedt. Departments of Biological Research and Medicinal Chemistry, Boehringer Ingelheim KG, D-55216 Ingelheim, Germany and Department of Physiology and Pharmacology, Karolinska Institute, S-17177 Stockholm, Sweden.

BIIP 20 XX is a purine derivative which potently and selectively antagonizes adenosine A_1 receptors *in vitro*. In this study, we have investigated the ability of BIIP 20 XX to antagonize adenosine A_1 receptors *in vivo*. Oral administration of BIIP 20 XX failed to influence locomotor activity in mice. However, it antagonized the inhibition of locomotor activity induced by administration of the selective A_1 agonists cyclopentyladenosine (CPA) and dihydroxypropyladenosine (DHPA). Furthermore, oral administration of BIIP 20 XX dose-dependently increased the extracellular levels of acetylcholine in the hippocampus of awake, freely moving rats as measured by microdialysis. These effects were completely blocked when the microdialysis probe was perfused with a Na^+-channel blocker, tetrodotoxin, and strongly attenuated when a Ca^{2+}-free perfusate was used. Furthermore, the effects of BIIP 20 XX were concentration-dependently counteracted by local perfusion of CPA but not by a selective A_2 agonist, CGS 21680. Although BIIP 20 XX had no intrinsic effect on EEG patterns of conscious rabbits, it dose-dependently antagonized the sedative effects of DHPA and attenuated the anticholinergic effects of scopolamine. Finally, BIIP 20 XX produced vigilance-enhancing effects as measured by polysomnography in chronically implanted, freely moving cats. In summary, these results demonstrate that BIIP 20 XX is a potent and selective antagonist of adenosine A_1 receptors *in vivo*. The effects of BIIP 20 XX on central cholinergic transmission demonstrated in this study suggest that this agent may be a novel and powerful approach for the treatment of reduced cholinergic function such as that observed in Alzheimer's disease.

Work funded by Boehringer Ingelheim

85.8

DECREASED EXPRESSION OF mRNA ENCODING NR1 SUBUNIT OF THE NMDA RECEPTOR IN SELECT REGIONS OF ALZHEIMER BRAIN. I. Ulas* and C.W. Cotman. *Institute for Brain Aging and Dementia, University of California, Irvine, CA 92717, USA.*

The aim of this study was to assess whether reduced binding to specific sites of the NMDA receptor complex observed in the hippocampus (HP) and parahippocampal gyrus of patients with Alzheimer's disease (AD, Ulas et al., 1992) is attributable to the altered expression of the gene encoding the obligatory NR1 subunit of the NMDA receptor. Accordingly, we examined the distribution of NR1 mRNA in the HP and adjacent cortical areas of seven AD patients and seven normal, elderly individuals. Densitometric analysis of *in situ* hybridization autoradiograms revealed a 34% (P<0.05) decrease in NR1 mRNA levels in layer III of the entorhinal cortex (EC) in AD brains. Similar deficits were observed in other layers of EC and in dentate gyrus granule cells. Reduced levels of NR1 mRNA were also found in layers II-VI of the perirhinal cortex (40-50% decrease, P<0.02). There were no significant changes in NR1 mRNA expression in the CA1, hilus, or subiculum. However, there was a slight decrease in the number of silver grains overlying individual neurons in the CA1, EC and dentate gyrus granule cell layer. These data suggest that the reduction in the NR1 transcript may reflect either cell loss, occur indirectly in response to abnormalities in glutamatergic input to the HP, or represent alterations in the HP and subicular output. When followed by changes at the NR1 protein level, an aberrant expression of the NR1 gene in AD brain may result in the formation of NMDA receptors with new properties, thus contributing to altered binding to certain receptor recognition sites, abnormal vulnerability of select neuronal populations to excitotoxic cell death, and/or learning and memory deficits.

(Supported by NIA Grant AG-07918)

85.9

ADRENERGIC RECEPTORS IN ALZHEIMER'S DISEASE: INCREASES IN THE CEREBELLA OF AGGRESSIVE OVER NON-AGGRESSIVE PATIENTS. A. Russo-Neustadt* and C.W. Cotman, Institute for Brain Aging and Dementia, University California, Irvine, CA 92697

Behavioral aggression is an important problem in a significant subpopulation of patients with Alzheimer's Disease (AD). Much evidence exists that norepinephrine is important in mediating and modulating aggressive behaviors in limbic regions of the brain. The distribution and concentration of α_2, β_1 and β_2 adrenergic receptors was examined through receptor autoradiography in three brain regions of a group of AD patients who were aggressive and agitated during their disease course. These receptor levels were compared to those in the brains of AD patients without these behavioral complications. Tissue from two regions of frontal cortex (dorsolateral prefrontal cortex and orbitofrontal cortex), hypothalamus (dorsomedial, ventromedial, lateral and posterior nuclei) and cerebellum (cortex from the hemisphere) from 24 patients was processed for receptor autoradiography with [^{125}I]iodopindolol for ß-adrenergic receptors (in conjunction with the subtype-selective antagonists ICI-118,551 and ICI-89,406 to visualize β_1 and β_2 subtypes, respectively), and [^{3}H]bromoxidine ([^{3}H]UK-14,304) for α_2 receptors. Results showed no significant differences in α_2 or ß-adrenergic receptor distribution or density between these groups of AD patients in the frontal cortical areas or hypothalamic nuclei examined. In contrast, the cerebellar cortices of agitated demented patients showed significant increases in adrenergic receptor concentrations over the non-aggressive group, with the most striking differences found in α_2. Cerebellar β_1 and β_2 receptors within the granule cell layer and cortical white matter of the aggressive patients were increased in density approximately 30% over the non-aggressive AD patients. Cerebellar α_2 receptors in the molecular and granule cell layers were increased appoximately 80% in agitated AD patients over the non-agitated subgroup. These results support clinical evidence that adrenergic agents may be helpful in the management of aggression, and also suggest a cerebellar contribution to the modulation of behavior.

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85.10

1-[(3-Fluoro-4-pyridinyl)amino]-3-methyl-1(H)-indol-5-ylmethyl carbamate (P10358) : Pharmacological activity and safety profile of a novel, orally-active acetylcholinesterase inhibitor for Alzheimer's Disease (AD). C. P. Smith*, G. M. Bores, W. Petko, M. Li, D. Selk, D. Rush, J. Winslow,, F. Camacho, R. Fishkin, D. Cunningham, H. Hartman, K. Brooks, L. Davis and H. M. Vargas, Hoechst Marion Roussel, Neuroscience Therapeutic Domain, Bridgewater, N.J. 08807.

P10358 is a potent, reversible acetylcholinesterase inhibitor (AChEI) with central biochemical and behavioral activity after oral or parenteral administration. *Ex vivo* studies showed that P10358 produced significant and dose-dependent inhibition of brain AChE over a range from 0.2 - 20 mg/kg, p.o. At 10 and 20 mg/kg, this compound produced long-lasting hypothermia in mice, another *in vivo* measure of brain cholinomimetic activity. This compound reversed scopolamine-induced passive avoidance deficit in mice (0.03-0.1 mg/kg) and scopolamine-induced deficits in the Morris water maze in rats (1.25 and 2.5 mg/kg, p.o.). It also enhanced performance in rats in both a step-down passive avoidance task (0.62 and 1.25 mg/kg, p.o.) and in a social recognition paradigm (0.32, 0.64, and 1.25 mg/kg). A higher dose (20 mg/kg, p.o.) enhanced striatal homovanillic acid (HVA) levels in rats, an effect consistent with cholinomimetic activity. Hemodynamic studies in the conscious rat showed that there was a 16-fold separation between behaviorally-active doses (1.25 mg/kg) and those which elevated arterial pressure (20 mg/kg). Chemically, P10358 is an N-aminoindole and may not have the hepatotoxic liability associated with aminoacridine structure of THA. Lethality in rats after oral administration did not occur until a dose of 80 mg/kg. P10358 had weak affinity for a variety of other receptors or uptake carriers, and had a pharmacological profile consistent with a single mechanism of action. Based on these studies, P10358 may be a promising therapeutic for the symptomatic treatment of AD. Research funded by HMR, Inc.

85.11

COMPARISON OF THE EFFECTS OF TWO SELECTIVE CHOLINERGIC NEUROTOXINS (AF64A and 192 -IgG Saporin) ON SPATIAL LEARNING IN THE MALE RAT. W.A.Dornan*, M. Easterday, T. Folkers, L. Hickman, J.Johns, M. Kern, J. Litwiller, S. Russell, G. Tinkler. Dept. of Psychology, Illinois Wesleyan University, Bloomington, IL 61702.

Alzheimer's Disease (AD) is caused by a gradual degeneration in specific areas of the brain, most notably the hippocampus and cerebral cortex. Despite this knowledge, however, presently no animal model exists that reliably mimics the profound pathological and behavioral changes that characterize the disease. Moreover, while a considerable amount of evidence has implicated the loss of cholinergic basal forebrain (CBF) input to the hippocampus and cortex as being one of the major neuropathological components characteristic of AD, the exact role the CBF plays in the cognitive deficits observed in AD is still uncertain. One factor that has contributed to this ambiguity is that until recently the lack of a specific cholinergic neurotoxin has hindered attempts to selectively destroy the cholinergic input to the cortex and hippocampus. As a result, there is considerable excitement regarding the introduction of the new cholinergic toxin, 192 IgG saporin as a potential new tool in generating an animal model that mimics the profound memory impairment that characterizes AD. In this study, we compared the effects of multiple injections of 192-IgG saporin into the CBF to intraventricular injections of AF64A on performance of the Morris water maze (MWM) and the a radial arm maze (RAM) task in male rats. Our results revealed a dose-dependent effect of AF64A on three components of the MWM compared to controls. Intraventricular injections of AF64A signficantly affected the performance of a non-cued test, a reversal test, and a discrimination test using the MWM when compared to controls. Multiple injections of 192 IgG saporin into the CBF , however, failed to appreciably effect the retention or acquisition of spatial learning in the RAM or the MWM. These data provide further support for the use of AF64A but not 192 IgG saporin as a viable approach to study the neurochemical mechanisms that underlie the cognitive deficits of AD.

85.12

CORTICAL ASTROCYTES CONTAIN LESS GLUTAMINE SYNTHETASE IN ALZHEIMER'S DISEASE. S.R. Robinson[1], J. Kril[2], G.M. Halliday[3]* & D. Noone[1]. [1]Vision, Touch & Hearing Research Centre, University of Queensland, St. Lucia, QLD, 4072. Australia; [2]Dept. Pathology, University of Sydney, NSW, Australia, 2006; [3]Prince of Wales Medical Research Institute, NSW, Australia, 2031.

Extracellular glutamate concentrations are elevated in Alzheimer's disease (AD; Pomara et al., 1992, *Am. J. Psychiatr.*, **149:** 251-254), suggesting that glutamate may have an excitotoxic role in this disease. Homogenates of AD brain tissue show reduced activity for glutamine synthetase (GS; Le Prince et al., 1995, *Neurochem. Res.*, **20:** 859-862). Since this glial enzyme converts glutamate to glutamine, a reduced expression of GS may underlie the etiology of AD. The present study uses immunohistochemistry to compare the cellular localisation of GS in the entorhinal cortices of normal, non-demented donors (aged 58-84 years), and in individuals who had died with advanced AD (aged 66-76 years). The tissue was fixed in formalin, then cut coronally at 50μm. Sections were incubated with antisera directed against GS (1:500; Chemicon), GFAP (1:2500; Dako) or β-amyloid (1:100; Dako), then processed with streptavidin-HRP-DAB.

Immunolabelling for GS revealed numerous well-labelled cells homogenously distributed through layers 2-6 of the cortex, but they were less frequent in layer 1 and were absent from the white matter. Their dendrites extended to the walls of blood vessels, where they terminated as endfeet. A small proportion of the cells were large and closely resembled GFAP-labelled fibrous astrocytes, but the majority were smaller and had dendrites that were finer, more numerous and more convoluted in their course. They may be protoplasmic astrocytes or grey matter oligodendrocytes. In AD, the frequency of labelled cells was reduced, they had fewer contacts onto blood vessels and the intensity of their immunolabelling was considerably lower. Unlike GFAP-labelled astrocytes, GS-labelled glia showed no reactivity to amyloid plaques. GS is extremely sensitive to inactivation by free radicals (Schor, 1988, *Brain Res.*, **456:** 17-21), so we speculate that the substantially reduced intracellular levels of GS may be due to the elevated levels of free iron present in AD (see Robinson et al., *Alzheimer's Res.*, **1:** 191-196).

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85.13

EFFECTS OF A CHOLINE DEFICIENT DIET ON THE GLUTAMINE SYNTHETASE ACTIVITY IN THE BRAINS OF MALE AND FEMALE SWISS-WEBSTER MICE V. G. Carson*, B.A. LaDuca, H.B. Lee, and T.K. Tran. Chapman University, Orange, CA 92666

Cholinergic neurons are impaired in Alzheimer's Disease (AD). In order to acquire animal models similar to Alzheimer's patients, male and female Swiss-Webster mice were put on a choline deficient diet by substituting N-aminodeanol (NADe) for choline. Some were reversed to a normal diet after six months. It was hypothesized that a choline deficient diet would affect astrocytes, which are the major source of the brain's glutamine synthetase (GS). This enzyme has been shown by Carney et al. (Annals N. Y. Acad. Sci. 738:44-53, 1994) to be depressed in the frontal lobe of AD autopsy material. After behavioral tests were performed on the mice, they were sacrificed at 12 months of age and their brains extracted and stored at -80° C. Distilled water homogenates of tissue samples were analyzed for GS activity per mg protein. The brain homogenates from experimental animals had significantly less GS than those from control or diet reversed mice. This study indicates that there may be a linkage between choline deficiency and the GS enzyme. In addition, this finding strengthens the proposal of Russell et. al. (Pharmacol. Biochem. Behav. 37:811-20, 1990) that the syndrome generated by NADe replacement of choline represents an experimental model of progressive degenerative dementia. (Supported by U. S. Public Health Service Grant MH-17691 to D. J. Jenden and R. W. Russell and Chapman University's Summer Research Grant.)

85.14

CONSEQUENCES OF OXIDATIVE STRESS ON THE LEVELS OF APOLIPOPROTEIN E AND J RELEASED BY CULTURED ASTROCYTES.

C. Ramassamy*, S.Bastianetto, D.Dea, A.Schoofs[a], Y.Christen[b], R.Quirion and J.Poirier. Douglas Hospital Research Centre 6875 Blvd Lasalle H4H 1R3 Montreal, Quebec. a: Centre Europeen de Bioprospective 24 bis rue Jacques Boutrolle BP24 76131 Mont Saint-Aignan Cedex France. b: Institut IPSEN 24 Rue Erlanger Paris. France

Apolipoprotein E (ApoE) genotype and aging are risk factors associated with late onset and sporadic Alzheimer's disease (AD). Age-related oxidative challenges may also be important factors in AD since several indices of oxidative damage, such as lipoperoxidation, were increased in relevant brain regions of AD patients. We have investigated the effects of oxidative stress, induced by hydrogen peroxide (H2O2), on the release of apoE and apolipoprotein J (ApoJ) by cultured cortical astrocytes and hippocampal astrocytes cocultured with neurons from rats. Cells were treated by H2O2 (3mM) for 3 hours in DMEM/F12 medium. The levels of ApoE and ApoJ, measured in culture medium 24h after the end of the treatment by Western blotting, were markedly reduced. In the same conditions, the levels of these apolipoproteins were partially decreased (-≈50%) when hippocampal astrocytes were cocultured with neurons. The pretreatment by a Ginkgo biloba extract (EGb 761), at 10μg/ml, prevented the neurotoxic effect of H2O2 evaluated by the increase of lactate in the culture medium. In these conditions, the neurosteroid dehydroepiandrosterone sulfate (DHEAs) was partially effective. After 3 h exposure to 3mM H2O2 cell viability, as assessed by MTT colorimetric assay, was reduced by 70%. Our results suggest a potential link between processes involved in aging (oxidative stress) and critical proteins involved in the pathogenesis of AD. Furthermore, EGb 761 and DHEAs may be potent agents to protect astrocytes from oxidative injury. (Supported by Centre Europeen de Bioprospective and IPSEN Foundation).

85.15

ASSESSMENT OF CHOLINERGIC NEURONS IN TRANSGENIC MICE OVEREXPRESSING ALZHEIMER AMYLOID PRECURSOR PROTEINS. M. A. Westerman*, L. L. Pundt, S. B. Love, K. K. Hsiao, W. C. Low. Departments of Neurosurgery and Neurology, Grad. Prog. in Neuroscience, Univ. of Minnesota, Mpls., MN 55455.

Transgenic (Tg) mice which overexpress human or mouse Alzheimer amyloid precursor protein (APP) have been shown to die early and manifest behavioral abnormalities which include neophobia and impaired spatial alternation in a Y maze. In one form of clinical Alzheimer's disease there is a reduction of cholinergic neurons in the brain. In the present study, we examined the number of cholinergic cells in the medial septal nucleus (MS) and the horizontal limb of the diagonal band of Broca of Tg mice (HDB). Male Tg positive (n=3) and Tg negative (n=3) mice ranging from three-six months of age were assayed. Anatomically matched sections (20μm) evenly distributed throughout MS and HDB (120μm apart) were stained with an antibody for choline acetyltransferase (ChAT). ChAT positive cells were quantified and assessed for morphological differences. No significant differences were found in the number of ChAT positive cells between Tg positive and Tg negative mice in either MS or HDB. A blinded analysis of morphological differences between groups suggested the presence of densely stained dystrophic processes in Tg positive ChAT neurons.
Arbitrary values were assigned to animals based on specific morphological characteristics of MS ChAT neurons. A value of 2 was given to animals which contained an increased intensity of staining in neuronal fibers and dystrophic processes. Animals with fine, lightly stained neuronal processes were assigned a value of 1. Tg positive mice received an average score of 2.0, while Tg negative mice received an average score of 1.2 ($p<0.001$). The abnormal morphology may be attributable to inefficient transport of molecules along axons to target areas such as the hippocampus. Although the number of cholinergic neurons is not affected by the overexpression of APP, the functional expression of these neurons may be compromised. Supported by PHS grants RO1-NS-24464 and RO1-NS-33249.

85.16

CHOLINERGIC SYSTEMS IN APOPOLIPOPROTEIN E KNOCKOUT MICE

P. Krzywkowski, F. Momoli, J. Rochford, S. Gauthier * and J. Poirier,
Douglas Hospital Research Centre, Department of Psychiatry and Centre for Studies in Aging, McGill University, Montréal, Québec, Canada, H4H 1R3.

Apolipoprotein E (apoE) is a glycoprotein implicated in lipid metabolism. Over the last few years, one of apoE isoforms, apoE4 was shown to be a major risk factor for sporadic and familial Alzheimer's disease (AD). The precise role of apoE in the etiopathology of AD is currently unknown. In view of the selective vulnerability of the cholinergic system in AD (which could be due to its dependence on phospholipids), we decided to study the effect of the absence of apoE on cholinergic activity in apoE knockout mice. Control C57 and apoE knockout mice (2 month-old) were tested behaviorally using the Morris swim maze task. Knockout mice showed a marked deficit in this task. Autoradiographic analysis of cholinergic receptors revealed little or no change in the pirenzepine-M1 binding sites in the medial septal area, the hippocampus or the cortex. In contrast, a decrease in the AF DX 384-M2 binding sites was observed at the middle level of the hippocampus (in the CA1/2 and CA3 fields) and a mild, although non significant, decrease of binding was observed in septal and cortical areas. Furthermore, a significant increase in cytisine-nicotinic binding sites occured in cortex but not in septal and hippocampal areas. Measurment of choline acetyltransferase (ChAT) activity showed a slight but significant decrease in the striatum of the knockout mice, but not in the hippocampus, entorhinal, frontal or parietal cortices nor cerebellum. Studies are currently underway to examine the same parameters in 10 month-old knockout animals.

Taken together, these data show behavioral deficits in apoE knockout mice as early as 2 months of age. These are accompanied by subtle but specific changes in the cholinergic receptors. Although the mechanism underlying these deficits is poorly understood, there may be a close relationship between apoE neurobiology and cholinergic integrity in the CNS. This could very well explain the apoE genotype-dependent alteration of cholinergic function reported in apoE4 AD subjects.

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85.17

ENHANCED VASODILATORY EFFECT OF TACRINE IN THE CORTEX OF BASAL FOREBRAIN LESIONED RATS. P. Peruzzi, J. Seylaz and P. Lacombe*. Laboratoire de Recherches Cérébrovasculaires, CNRS UA 641, Université Paris 7, IFR 6, Faculté Lariboisière-Saint Louis, 10, avenue de Verdun, 75010 PARIS, France.

The cholinesterase inhibitor, tacrine, is presently the most widely used agent for treating Alzheimer disease. The finding in the rat of the remarkable, age-dependent cerebrovasodilatory function of the nucleus basalis of Meynert/substantia innominata (SI) potentiated by cholinesterase inhibitors, is in support of the "cholinergic hypothesis" and substitutive therapy. Since these data suggest that cerebrovascular investigations offer a valuable experimental approach to test this hypothesis, the present study was designed to determine whether tacrine is able to reflect a cerebral circulatory deficit originating in the basal forebrain. Cerebral blood flow (CBF) measurement was performed 1-2 weeks following SI lesion with ibotenic acid, using the tissue sampling [^{14}C]iodoantipyrine technique, in two groups of lesioned rats infused intravenously either with tacrine at 8mg/kg/h or saline. SI lesion resulted in moderate, significant blood flow decreases in the parietal, frontal and occipital cortical areas. In most of the cortical and subcortical regions investigated tacrine induced significant increases in CBF, ranging from 21% (hypothalamus) to 101% (parietal cortex) in the intact hemibrain compared to the control group. The increases in the lesioned hemisphere were 1.9 and 1.5 times greater in the frontal and parietal cortex, respectively, than in the intact hemisphere. This result indicates that tacrine selectively favors the circulation in the cortex, soon after its deafferentation from the SI.

ALZHEIMER'S DISEASE: ApoE

86.1

EFFECTS OF APOLIPOPROTEIN E GENOTYPE ON BRAIN STRUCTURE AND FUNCTION IN ALZHEIMER'S DISEASE. J.L. Eberling*, B.R. Reed, R.M. Krauss, A. Acharya, J. Leung, D.M. Mungas, W.J. Jagust. Lawrence Berkeley Laboratory, University of California, Berkeley, CA 94720, and University of California, Davis, CA 95616. The apolipoprotein E type 4 allele (APOE ε4) is a risk factor for both familial late-onset and sporadic Alzheimer's disease (AD). Positron emission tomography (PET) studies have revealed neocortical reductions in glucose metabolism in at-risk subjects with APOE ε4. We used PET and ^{18}F-fluorodeoxyglucose (FDG) to compare cerebral glucose metabolism in AD patients with and without APOE ε4. Eighteen AD patients were studied with MR followed by 3-D PET during a continuous recognition memory task. Three of the patients were homozygous for the ε4 allele, 9 were heterozygous for ε4, and 6 did not have the ε4 allele. Patients were matched for age, education, gender and dementia severity. Three-dimensional volumes of interest (VOIs), drawn on MR data, were mapped onto the PET data. Atrophy corrected glucose metabolic rates for each VOI were calculated using previously determined rate constants and an operational equation and were normalized to the global metabolic rate for the entire brain. VOIs were grouped together according to structural complexity and differentiation, and included: heteromodal association cortex, unimodal association cortex, primary sensory cortex, and limbic/paralimbic cortex. RESULTS. A repeated measures ANOVA revealed a significant ($p=0.01$) group effect for only the limbic/paralimbic regions. Follow-up one-way ANOVAs and Fisher post-hoc tests showed that the homozygous patients had significantly lower metabolic ratios than heterozygous patients ($p<0.05$) and patients without ε4 ($p = 0.002$) in hippocampus and amygdala. Heterozygous patients had significantly ($p<0.05$) lower metabolic ratios than patients without ε4 in hippocampus. In addition, homozygous patients had significantly smaller hippocampal volumes (normalized to intracranial volume) than patients without ε4. Hippocampal volumes for heterozygous patients were intermediate to homozygous patients and patients without ε4, but not significantly different from either group. These findings show a specific dose-dependent effect of APOE ε4 on hippocampal volume and glucose metabolism in AD patients. (Supported by NIH grants AG07793 and AG10129).

86.2

Neurodegeneration and cognitive impairment in apoE-deficient mice is ameliorated by infusion of recombinant apoE. E. Masliah§#*, W. Samuel#, I. Veinbergs#, M. Mallory#, M. Mante# and T. Saitoh#. Depts. of Neurosciences# and Pathology§, University of California, San Diego, La Jolla, California 92093.

Recent studies suggest that apolipoprotein E (apoE) might play a neurotrophic function in the central nervous system and that altered functioning of this molecule could result in neurodegenerative disorders such as Alzheimer's disease. The main objective of this study was to determine if neurodegenerative and cognitive alterations in apoE-deficient mice are reversible by infusion of recombinant apoE into the lateral ventricles. ApoE-deficient mice treated with either apoE3 or apoE4 showed a significant improvement in their learning capacity in the Morris water maze compared to saline-infused apoE-deficient mice. While this improved performance was associated with restoration of neuronal structure, the poor learning ability of apoE-deficient mice treated with saline correlated with the disrupted synapto-dendritic structure. This study supports the contention that apoE might play a neurotrophic effect *in vivo* and suggests that apoE might have a potential therapeutic role.

This work was supported by NIH grants AG05131, AG10689 and with funding from the Alzheimers Disease and Related Disorders Association, and the Broad Foundation. This work was also partially supported by NIH grant RR04050.

86.3

Effects of Aging on Glial and Neural Markers in Apolipoprotein E-Deficient Mice. C. A. Parker, M. J. Callahan, W.J. Lipinski*, C. L. Bisgaier and L. C. Walker. Neurological and Neurodegenerative Diseases Group, Parke-Davis Pharmaceutical Research, Div. of Warner-Lambert Co., Ann Arbor, MI 48105.

Some neurodegenerative processes appear to be augmented in aged, ApoE deficient ("knockout") mice (Masliah et al., Exp. Neurol. *136*, 107, 1995). Whereas age-related glial changes have been consistently observed, the degree to which neuronal markers are compromised in aged ApoE knockouts remains uncertain (Gandy et al., Soc. Neurosci. Abs. *21*, 5, 1995). We analyzed neuronal and glial markers in ApoE knockout mice (Jackson Labs) ranging from 2 to 20 months of age. One hemisphere from each animal was characterized histochemically and the other by quantitative immunoblots and cholinergic enzyme analysis. Tissue sections and brain homogenate extracts were studied with antibodies against synaptophysin, β-amyloid precursor protein (βAPP), glial fibrillary acidic protein (GFAP) and with probes for microglia. We also measured levels of choline acetyltransferase in whole-hemispheric homogenates. As expected, astrocytic and microglial markers, as well as the strain-specific emergence of clustered granules in the hippocampus, increased with age. Synaptophysin levels showed no consistent changes in aged ApoE knockouts, nor did choline acetyltransferase and βAPP levels change significantly between 2 and 20 months of age. Further work is needed to determine whether neurodegenerative changes are robust enough to qualify ApoE knockouts as reliable models of Alzheimer-like pathological changes. Variability among murine strains and brain regions require particular attention. Supported by Warner-Lambert.

86.4

***APOE* POLYMORPHISM IN ELDERLY EAST AFRICANS**

J.G. Sayi , D.R.D. Premkumar, N. B. Patel, A.S. Bhandari, S. Gatere, W.B. Matuja, R.P. Friedland, E. Koss and R.N. Kalaria (SPON: Society of Neuroscientists of Africa)
Departments of Neurology and Pathology, Case Western Reserve University, Cleveland, Ohio, USA; Neurology Unit, Muhimbili Medical Centre, Dar es Salaam, Tanzania; Department of Medical Physiology, University of Nairobi and Avenue Nursing Home, Nairobi, Kenya.

Recent studies have shown the apolipoprotein E (*APOE*)-ε4 allele to be associated with late-onset familial and sporadic Alzheimer's disease (AD). The frequency of *APOE*-ε4 allele in AD has been reported to be about three times greater than non-demented controls in Western populations. The link between *APOE* polymorphism and dementia remains unknown in developing countries. We examined *APOE* frequencies in non-demented and demented elderly East Africans. Blood samples were obtained from elderly African patients (age range 50-90 years) admitted at two clinical centres in East Africa. The DNA from samples was extracted in Cleveland for *APOE* genotyping. The first group consisted of 150 elderly Tanzanian Africans attending clinic for non-neurological complaints at Muhimbili in Dar es Salaam. *APOE* allele frequencies in this group were found to be 15.7% for ε2, 62.9% for ε3 and 21.3% for ε4. Assessment of *APOE* genotypes in another group of elderly Kenyan patients from Nairobi also revealed high frequencies of the ε4 allele. Our results suggest that elderly East Africans have relatively high *APOE*-ε4 allele frequencies compared to normal aging subjects from Western countries and support the implication that the *APOE*-ε4 allele may not necessarily be a risk factor in indigenous populations of Africa. Supported by WHO-NINDS fellowship and grants from NIH and ADRDA.

86.5

Apolipoprotein E is induced by A β accumulation in transgenic mice

Y. Igeta[1]*, T. Kawarabayashi[1], M. Sato[2], E. Matsubara[1], K. Ishiguro[1], M. Kanai[1], Y. Tomidokoro[1], T. Kobayashi[2], N. Tada[2], K. Okamoto[1], S. Hirai[3] and M. Shoji[1]
[1]Department of Neurology, Gunma University Medical School, 3-39-15 Showamachi, Maebashi, Gunma 371, Japan, [2]Laboratory for Animal Center, Pharma Research and Development Division, Hoechist Japan Limited, 1-3-2, Minamidai, Kawagoe, Saitama 350-11, Japan, [3]Tokyo Metropolitan Hospital, 2-6-1, Musashidai, Fuchu, Tokyo 183, Japan

Apolipoprotein E (ApoE) is a significant risk factor for Alzheimer's disease (AD). The frequency of apoE e4 allele is increased in sporadic and late onset familial AD. Gene dose effect of ApoE4 is also established to accelerate the onset of AD. In vitro study suggests ApoE may accelerate A β aggregation and form stable A β /ApoE complex. However, the precise role of ApoE in A β amyloid formation in vivo is still unclear. Here we show the secondary induction of ApoE by A β accumulation in the pancreas of transgenic mice expressing a signal peptide and 99 residues of carboxyl-terminal fragment of amyloid β protein precursor (β APP). These mice developed A β amyloid fibrils in the pancreas. Immunocytochemically, these A β amyloid fibrils were labeled anti-ApoE antibody. ApoE immunoreactivities were correlated with an age-dependent A β accumulation. Western blotting showed that the amount of apoE was increased in the transgenic pancreas than that in controls. However, the amount of ApoE mRNA in the transgenic liver was not increased compared with controls. These findings suggest that ApoE was locally induced by A β accumulation in the transgenic pancreas.

86.6

NON-Aβ COMPONENT OF ALZHEIMER'S DISEASE AMYLOID (NAC) BINDS ISOFORM SPECIFIC TO APOLIPOPROTEIN E.

O.F.Olesen[1], P.H.Jensen[2], K. Frederiksen*[1] and J.D.Mikkelsen[1]. 1) Dept. of Neurobiology, H. Lundbeck A/S, Ottiliavej 9, DK-2500 Valby-Copenhagen, Denmark and 2) Dept. of Medical Biochemistry, University of Århus, DK-8000 Århus C, Denmark

The non Aβ component of Alzheimer's disease amyloid (NAC) is a newly discovered 35 amino acids peptide that is found closely linked to the beta-amyloid fibrils in senile plaques. NAC derives from a larger precursor protein, termed NACP or α-synuclein, that consists of 140 amino acids and is associated with presynaptic vesicles. We report that NAC, as well as its precursor α-synuclein, binds to Aβ and induces its aggregation into amyloid plaques. We further found that NAC binds to apolipoprotein E (apoE), forming a complex that is resistant to reducing agents and separation by denaturing gel electrophoresis, whereas extreme acidic or basic pH strongly affected the binding. The complex was formed rapidly upon mixing the two molecules, reaching a maximum within the first hour of incubation. ApoE exists in different isoforms of which apoE3 is the most frequent, whereas apoE4 has been linked to late-onset Alzheimer's disease. We therefore examined the binding of NAC to individual isoforms of apoE and found that the complex between NAC and apoE was isoform-specific with respect to apoE. More than twice as much NAC was complexed with apoE4 as compared to apoE3. The isoform specific binding to NAC was maintained over a range of apoE concentrations, including those normally encountered in cerebrospinal fluid. The ability of NAC to interact with Aβ and isoform-specific with apoE indicates that NAC and/or NACP may play an important role in the amyloidogenesis during Alzheimer's disease.

86.7

ALPHA-1-ANTICHYMOTRYPSIN POLYMORPHISM MODULATES THE APOLIPOPROTEIN E ASSOCIATED RISK OF ALZHEIMER'S DISEASE. A. Ueki, M. Otsuka and M. Wtanabe* Department of Neurology, Jichi Medical School, Omiya Medical Center, Omiya City, Japan 330

Apolipoprotein E-ε4 allele (APOE*4) is a major risk factor of Alzheimer's disease (AD). However, there are many cognitively normal elderly who carry APOE*4. IIt is postulated there are other genetic or environmental factors preventing the effects of APOE*4 for developing AD. Among these factors, alpha-1-antichymotrypsin (ACT) signal peptide gene polymorphism is interesting. Kamboh et al. have shown that APOE*4 dosage effect associated with AD risk is significantly modulated by the ACT genotype. To test their hypothesis we examined ACT genotypes in AD and age matched controls, and compared their frequencies between APOE*4 carriers and non-carriers. APOE and ACT were genotyped in 58 AD patients (mean age at onset,73.7; range, 60-86) and 105 healthy controls (mean age, 72.6; range, 60-92). There are two ACT alleles (ACT*A and ACT*T) and three ACT genotypes (*AA, *AT, *TT). The distribution of ACT genotypes in total patients with AD and controls did not differ significantly. The frequencies of *AA, *AT, *TT were 0.179, 0.456, 0.366, in total AD, and 0.106, 0.469, 0.425 in total contrlos, respectively. However, the distribution of ACT genotypes differed significantly when compared between subgroups of AD and controls carring APOE*4. The frequencies of *AA, *AT, *TT in APOE*4 carriers of AD (n=38) were 0.198, 0.556, 0.246, and those in APOE*4 carriers of contrlos (n=17) were 0.059, 0.397, 0.544, respectively. Allele frequency of ACT*A in APOE*4 carriers of controls was signifcantly lower than that in APOE*4 carriers of AD (0.192 vs. 0.438, χ^2= 4.9 .p=0.03). ACT genotypes between non-carrier subgroups of AD and controls did not differ. Although it is uncertain at present that ACT*AA is a promoting factor or ACT*TT is a preventing factor, ACT polymorphism modify the risk of APOE*4 carriers for developing AD or not.

86.8

NEUROTOXIC EFFECTS OF APOE-DERIVED SYNTHETIC PEPTIDES ARE RECEPTOR-MEDIATED.

M.Tolar*, J.A.K. Harmony and K.A.Crutcher. Dept. of Neurosurgery and Dept. of Pharmacology and Cell Biophysics, Univ. of Cincinnati, Cincinnati, OH 45267.

The E_4 isoform of apolipoprotein E (apoE) has been repeatedly shown to be associated with familial and sporadic Alzheimer's disease (AD). Synthetic apoE peptides corresponding to the receptor-binding region of apoE, as well as a 22 kDa thrombin-cleavage fragment of apoE, cause extensive degeneration in vitro when applied to dissociated cultures of chick sympathetic ganglia (Exp. Neurol. 130: 120-126; 1994). The E_4 isoform of the 22 kD fragment is significantly more toxic than the E_3 isoform, suggesting that naturally-occurring apoE fragments may directly contribute to the pathology found in AD. Since apoE can be internalized by the LDL receptor or the LDL receptor-related protein (LRP) acting in cooperation with cell-surface heparan sulfate proteoglycans (HSPGs), the role of these receptors in the toxicity of apoE peptides was evaluated.

Cultures of dissociated embryonic chick sympathetic neurons in 96-well microtiter plates were used to assess both the toxicity of the apoE peptides and the effects of various blocking agents. Peptides were applied at previously determined half-maximal effective concentrations and, after overnight incubation, cell viability was assessed with 5-carboxyfluorescein. The LRP-associated protein (RAP), which has been reported to block the binding of all known ligands to LRP, completely blocked toxicity at a concentration of 100 nM. Pretreatment of the cells with heparinase I, which degrades the glycosaminoglycan moiety of HSPG, as well as sodium chlorate, which prevents sulfation of glycosaminoglycans, were used to assess the role of proteoglycans in peptide neurotoxicity. Heparinase I (20 U/ml) or sodium chlorate (20 mM), or both together, provided partial blockade of toxicity. A polyclonal antibody raised against LRP (kindly provided by Dr. D. Strickland) completely blocked the toxicity, whereas a monoclonal anti-LDL receptor antibody showed no protection. Together with the previously reported blockade of apoE peptide-associated toxicity by heparin and heparan sulfate, these results suggest that the neurotoxic effects of apoE peptides are mediated through LRP in cooperation with cell-surface HSPGs. (Supported by a Research Challenge Grant and NIH grants NS31410 and HL27333.)

86.9

EXPRESSION AND REGULATION OF THE LOW DENSITY LIPOPROTEIN RECEPTOR-2 AND ITS LIGAND APOLIPOPROTEIN J IN RAT BRAIN

D. Michel[1], G. Chatellain[1], G. Brun[1] W.S. Argraves[2] and G.M. Pasinetti[3]* [1]Laboratoire de Biologie Moleculaire, Ecole Normale Superieure, CNRS UMR 49, Lyon[1], France; [2]Medical University of South. Carolina, Department of Cell Biology, Charleston, SC 29425; [3]Department of Psychiatry and Brookdale Center for Molecular Biology of the Mount Sinai Medical School, NY, NY 10029;

This study explored the distribution and co-expression of the low density lipoprotein receptor-related protein-2/megalin/gp330 (LRP-2) and its ligand apolipoprotein J/clusterin (apoJ) in young adult rat brain. Experimental brain lesions known to induce apoJ expression were also used to identify the co-regulation of apoJ/LRP-2 mRNA expression in mechanisms of neuronal death/survival. Radiolabelled RT-PCR of total RNA from dissected brain regions was used to quantify LRP-2 and apoJ mRNA expression. Pairs of LRP-2 primers were designed from the coding region of the rat LRP-2 cDNA (nt 12462-12485 and nt 12714-12737); apoJ primers were previously described (Michel et al., 1995). In control rat, we found that regions previously found to express high level of apoJ mRNA such as ependyma/choroid plexus, retina and olfactory mucosa also express detectable levels of LRP-2 mRNA. However, no detectable LRP-2 signal was found in parietal cortex, hippocampus and striatum, which also are known to constitutively express apoJ mRNA (Pasinetti et al., 1994). In response to kainic acid (KA) excitotoxic lesions that model selective neuronal death, apoJ mRNA signal showed elevation in striatum and hippocampus but not in cerebral cortex. Contrary to apoJ mRNA, the KA mediated induction of LRP-2 mRNA in these brain regions was equivocal. Similarly, deafferentation of the olfactory mucosa that models apoptotic death in the olfactory neuroepithelium induced apoJ mRNA in the absence of evident LRP-2 mRNA elevation. This study indicates that apoJ expression may be regulated independently of its receptor LRP-2 supporting the idea that its role in neurodegeneration is independent of receptor binding. Supported by NIA grant AG13618 to GMP.

86.10

EXPRESSION OF THE LOW DENSITY LIPOPROTEIN RECEPTOR-2 (LRP-2) IN THE ADULT RAT BRAIN FOLLOWING LESIONS.

J.T. Chun*[1], T.E. Morgan[1], G.M. Pasinetti [2], and C.E. Finch.[1] [1]Andrus Gerontology Center and Dept. Biol. Sci., University of Southern California, Los Angeles, CA 90089; [2] Dept. Psychiatry, Mt. Sinai School of Medicine, New York, N.Y. 10029

LRP-2, also known as glycoprotein 330 and megalin, is a member of the LDL receptor family. Recent studies using mouse embryonal carcinoma F9 cells differentiated by treatment with retinoic acid and dibutyryl cAMP indicated that LRP-2 functions as an endocytotic receptor for clusterin, a protein present in aged and Alzheimer disease brains (Kounnas et al., 1995, J. Biol. Chem. 270:13070). In view of the fact that clusterin is a component of senile plaques and binds to amyloid β protein (Aβ), these observations raise the possibility that LRP-2 may play a role in neurodegenerative process involving clusterin and Aβ. To address this issue in an animal model, we induced lesions to the rat hippocampus using kainate and perforant path transection and surveyed for LRP-2 mRNA in the rat brain. Toward this end, pairs of oligonucleotides were selected from both coding (nt 6,439-6,462; 6,942-6,921) and 3'-untranslated regions (nt 14,289-14,311; 14,449-14,425) of the rat LRP-2 mRNA sequence (GENBANK) and used as primers for RT-PCR analysis of the total RNA isolated from the intact and lesion-induced hippocampus. The efficacy of the primers was verified with RNA from kidney and liver, where LRP-2 is expressed at high and low levels, respectively. Using these primers, this ongoing study will examine whether the expression of LRP-2 mRNA is enhanced in the hippocampus following kainate-induced lesion and perforant path transection. (Supported by PHS grant AG13499 to C.E.F.)

86.11

SEQUENCE DETERMINATION OF THE LOW-DENSITY LIPOPROTEIN RECEPTOR GENE IN ALZHEIMER SUBJECTS. M. Danik, D. Dea and J. Poirier*. Douglas Hospital Research Centre, Centre for Studies in Aging, McGill University, Montréal, Québec, Canada.

Apolipoprotein E (apoE) is a glycoprotein involved in lipid transport and metabolism throughout the body. It is a component of several lipoprotein complexes and serves as a ligand for specific cell surface receptors that mediate their intracellular uptake. ApoE is the only known lipoprotein ligand within the brain parenchyma. It is thus believed to play an important role in neuronal plasticity by delivering lipid precursors for the synthesis of cellular membranes and neurotransmitters such as acetylcholine. Moreover apoE-lipid complexes binding to the low-density lipoprotein receptor (LDLR) present on neurons increases during the early phase of brain reinnervation. The apoE4 allele frequency was shown to be significantly increased in both sporadic and familial late onset Alzheimer's disease (AD). Although apoE4 has been postulated to be involved in neurofibrillary tangles and plaque formation, as well as in lipid metabolism dysfunction, its exact role in AD remains to be established. However 30-40% of late-onset AD subjects are known not to carry the ε4 allele. We hypothesized that in these subjects there may be an impairment in lipoprotein uptake due to a defect in the LDLR pathway. We have thus undertaken a screening process for the presence of mutations in each of the 18 exons coding for the human LDLR in several AD and control cases. The method is based on PCR amplification of individual exons followed by solid-phase sequencing using an automatic sequencer. No mutations have been found in either exon 18 or exon 5 which code for part of the internalization domain and apoE binding domain respectively. Data regarding other exons will also be presented and discussed.

(Supported by the Fonds de la Recherche en Santé du Québec and Pharmacia Biotech)

86.12

DENTATE GRANULE CELLS IN APOE-KNOCKOUT MICE SHOW NO CHANGE IN SYNAPTO-DENDRITIC STRUCTURES. J.A. Varner*, M.Thomas, J.Gilbert, D. Schmechel, S. Zhang[2], N. Maeda[2], A.D. Roses, and G. Einstein. Depts. Neurobiology and Neurology, Ctr. for Aging, and Joseph and Kathleen Bryan ADRC, Duke University Medical Center, Durham, NC 27710; [2]Dept. Pathology, UNC, Chapel Hill, NC 27599.

Apolipoprotein E (apoE), a lipid carrier protein involved in the repair and maintenance of neurons, has been implicated in the risk of developing late-onset, sporadic and familial Alzheimer's disease. Its link to the disease process may involve a failure in its normal function. However at present, our understanding of the role apoE plays in maintaining neurons is equivocal; some strains of apoE-knockout mice (KO) show little to no neuropathology while others show changes to synapto-dendritic structures as early as 4 mo of age. In order to further explore these changes, or lack thereof, we studied the dendritic morphology and spine densities of an identified population of neurons (dentate granule cells) in a different strain of APOE KO mice. Tissue was prepared for electron microscopy or intracellular dye-injections. Qualitative examination of electron micrographs through the molecular layer of 15 mo old KO mice revealed no obvious changes to dendritic structure; qualitative analysis of dendritic structure of intracellularly filled dentate granule cells at 3, 6, 12, and 15 mo confirmed this. Quantitative analysis of spine density revealed no significant difference at 3, 6, or 12 mo from controls. These results suggest that apoE's effect on mouse neurons may be dependent on various background strains. In addition, certain cell types may exhibit differential susceptibility to the absence of apoE. Thus the stress of aging or damage may be required to reveal fully apoE's role in maintenance and repair.

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86.13

CO-EXPRESSION OF APOLIPOPROTEIN E AND AMYLOID PRECURSOR PROTEIN FOLLOWING BRAIN INJURY. J. Maguire*[1], D.P. Binsack[2], and C.A. Marotta[2,3], Hamilton Civic Hospitals, McMaster University, Hamilton, Ontario[1], and Departments of Psychiatry and Human Behavior[2] and Neuroscience[3], Brown University, Providence, RI 02912.

We explored the relationship between APP and Apo E at the tissue level by means of a rat brain lesion paradigm. Immunohistochemistry techniques were used to assess the response of APP, Apo E, glial fibrillary acidic protein (GFAP), synaptophysin, and choline acetyl transferase (ChAT). Increased GFAP immunostaining was observed at 48 hours and well-established by 72 hours, while the ChAT activity was weak, and synaptophysin barely detectable, in the area of the wound. Immunoreactivity to APP was demonstrable by 48 hours within cell bodies surrounding the lesion. Apo E staining within cells was more robust than APP. These findings indicate that within the time frame examined, the experimental model revealed the co-expression of modest levels of APP and relatively high levels of Apo E in response to the lesion. The data support the view that APP and Apo E are components of an early and coordinated response to injury in brain.

86.14

OXIDATION OF APOLIPOPROTEIN E BY MYELOPEROXIDASE, AN ENZYME PRESENT IN THE BRAIN B. Leininger-Muller[a], C. Jolivalt[a], R. Drozdz[b], V. Shuvaev[a], P. Bertrand[a], Y. Christen*[c] and G. Siest[a] a, URA CNRS 597, Centre du Médicament, 30 rue Lionnois, 54 000 Nancy, F; b, Uniwersytet Jagiellonski, Collegium Medicum, Katedra Biochemii Klinicznej, 31-501 Krakow, P; c, IPSEN Foundation, 24 rue Erlanger, 75781 Paris, F.

Oxygen free radicals are increased in Alzheimer's disease patients. Among the proteins accumulating as amyloid deposits in the brain, the most studied at present are β-amyloid (Aβ) and apolipoprotein E (apo E) which is a polymorphic protein showing a strong affinity for Aβ and it seems to be isoform dependent. Yet, if the relation with oxidative process has been investigated for β-amyloid, nothing is known about the oxidation of apo E.

We have already obtained preliminary data with *in vitro* chemical oxidation of recombinant apo E and here we show the consequences of the oxidation of recombinant apo E, in presence of free radicals produced by the action of neutrophils secreted myeloperoxidase (H_2O_2 donor oxidoreductase).This heme enzyme takes place in inflammation and we demonstrate its presence in human brain. The *in vitro* incubation of free apo E with that enzymatic system leads to fragmentation of the protein for low concentrations of H_2O_2 and polymerization for higher concentrations. As compared with bovine serum albumin used as a reference protein, apo E shows a higher susceptibility to oxidation in the same conditions of oxidation. Studies with discoïdal complexes show that oxidation of apo E diminishes its ability to interact with phospholipids. Moreover, investigations of plasmonic resonnance measures show that the affinity for Aβ of oxidized apo E containing complexes is modified.

86.15

DEPENDENCE OF CEREBROSPINAL FLUID TAU PROTEIN LEVELS ON APOLIPOPROTEIN E4 ALLELE FREQUENCY IN PATIENTS WITH ALZHEIMER'S DISEASE. S. Golombowski, F. Müller-Spahn, W. Naser, C. Hock*. Dep. of Psychiatry, University of Basel, Switzerland and Boehringer Mannheim Research Lab., Tutzing, Germany.

We determined Apolipoprotein E (ApoE) genotypes in blood samples corresponding to cerebrospinal fluid (CSF) samples of patients with Alzheimer's disease (AD) that were previously assayed for Tau protein levels. CSF Tau was quantitated with an enzyme-linked imunosorbent assay (Innogenetics, Belgium). ApoE4 genotyping was performed with reverse hybridization applying the ApoE4 Genotyping Kit (Innogenetics, Belgium). CSF levels of Tau protein of patients with a diagnosis of probable AD (n=13, 7 male, 5 female, age 66.4 ± 9.8 years, MMS 19.1 ± 4.5) were increased compared with age-matched control subjects (elderly patients with depression, according to ICD10 (F32.0x/1x, F33.0x) and DMS-IIIR (n=10, 2 male, 8 female, age 71.3 ± 9.8, MMS 27.7 ± 1.9) (65.5 ± 12.9 pg/ml (mean ± SEM) and 29 ± 6.3, respectively, $p < 0.03$). CSF Tau protein levels increased with increasing ApoE4 frequency. AD group (mean +/- SEM): no Apo E4 allele: 32.5 ± 9.5, one Apo E4 allele: 56.5 ± 17.2, two ApoE4 alleles: 96.3 ± 24.3 pg/ml. Control group: no Apo E4 allele: 26.8 ± 6.8, one Apo E4 allele: 38.5 ± 20.5 pg/ml Significance in the Spearman Rank Correlation, $z=2.84$, $p=0.04$ and Kruskal-Wallis Test $p= 0.01$ was demonstrated for the Group of AD patients and controls taken together. The AD group alone showed significance in the Spearman Rank Correlation $z= 2.05$ and $p= 0.03$. Our results suggest CSF Tau levels increase with increasing ApoE4 allele frequency. Our data may be in agreement with the hypothesis of a lesser binding of ApoE4 to Tau protein and a presumed less protective effect on microtubule stability.

86.16

CELLULAR MODELS OF TAU ALZHEIMER-TYPE PHOSPHORYLATION: CHARACTERISATION OF TAU PROTEINS AND APOLIPOPROTEIN E IN VARIOUS HUMAN NEUROBLASTOMA CELL LINES. L. DUPONT-WALLOIS, C. SOULIÉ, A. DELACOURTE, P. VERMERSCH , M.C. CHARTIER-HARLIN, M.L. CAILLET-BOUDIN*; INSERM U422, PL. VERDUN. 59045 Lille, France.@@@@

Tau proteins are microtubule-associated proteins mainly found in axons of neurons. In human, six isoforms arise by alternative splicing of a primary transcript originating from a single gene. The fœtal isoform is found in both fœtal and adult brains. The five other isoforms only found in adult brain correspond to the fœtal isoform modified by insertions corresponding to the expression of alternative exons. In Alzheimer's disease, these 6 isoforms are hyperphosphorylated in comparison to the normally phosphorylated Tau proteins and are the major constituents of the PHF (Paired Helical Filaments) present in neurodegenerating neurons. A recent hypothesis suggested that some Apo E alleles could have a preferential protector effect against the phosphorylation of Tau proteins.

The obtention of cellular model of Alzheimer-type Tau phosphorylation requires the endogeneous expression and the characterisation of both Tau proteins and apo E expressed in the used cell lines. In this purpose, we studied three different human neuroblastoma cell lines. The endogenous expression, Tau transcript and Apo E allele identification were determined by RT-PCR and restriction analysis. Western blotting confirmed the intracellular presence of these two components and allows to study the Tau protein location and phosphorylation using phosphorylation-dependent immunoprobes.

These three cellular lines differed from each other by expressing different Tau isoforms or allele apo E. Although the interactions between Tau and apoE remain to be elucitated, ours results show the presence simultaneous of fœtal-type phosphorylated Tau proteins and Apo E in neuronal-type cell models.

Acknowledgement to Dr. J. C. Fruchart and H. Parra to apo E antibody. C. S. is a recipient of Servier grant.

87.1

SENILE PLAQUES SHOW FIBROBLAST GROWTH FACTOR-9 IMMUNOREACTIVITY S. Nakamura, T. Todo, M. Mizuguchi, K. Arima, S. Haga, T. Aizawa, Y. Namba, N. Otsuka, Y. Motoi, Y. Otsuka, A.Ueki, K. Ikeda* Department of Ultrastructure and Histochemistry, Tokyo Institute of Psychiatry, Tokyo 156, Japan and Jichi Medical School, Omiya Medical Center, Omiya, Japan

FGF-9 is a newly identified member of FGF family including acidic FGF (FGF-1) and basic FGF (FGF-2). In the central nervous system FGF-9 is present in neurons (Seo and Noguchi, FEBS Lett, 370:231-235, 1995). Since senile plaques and neurofibrillary tangles of Alzheimer's disease (AD) brain are associated with FGF-2 immunoreactivity and since both FGF-1 and FGF-2 are implicated in the AD pathogenesis, we performed immunohistochemical studies of AD brain tissues by using antibodies to FGF-9.

Three antibodies, W, M and C, were raised in rabbits, respectively, against human recombinant FGF-9 and two synthetic peptides (corresponding to residues, 87-102 and 193-208). Hippocampal sections including occipitotemporal neocortex from 10 AD cases and 10 age-matched controls were cut from formalin-fixed, paraffin embedded blocks, pretreated with formic acid and subjected to immunohistochemistry.

In brain sections two antibodies (W and M) strongly stained dystrophic neurites of senile plaques. In contrast, antibody C mainly stained amyloid core. Neurofibrillary tangles were not immunostained by three antibodies. Studies of the fine localization of immunoreactivity are underway.

87.2

DECREASE OF TrkA mRNA LEVEL IN CHOLINERGIC NEURONS OF THE STRIATUM AND BASAL FOREBRAIN IN ALZHEIMER'S DISEASE. F. Boissière, B. Faucheux, Y. Agid and E. C. Hirsch*. INSERM U289, Hôpital de la Salpêtrière, 47 Bd de l'Hôpital, 75013 Paris, France.

Besides cortical pathology, one of the major characteristics of Alzheimer's disease (AD) is the selective degeneration of cholinergic neurons localized in the basal forebrain and the ventral striatum. However, cholinergic neurons located in the dorsal striatum (caudate nucleus and putamen) or in the mesencephalon are spared. A link between NGF sensitivity and the vulnerability of specific populations of cholinergic neurons has been suspected, since cholinergic neurons which degenerate are sensitive to this neurotrophin. Because level of NGF is not reduced in AD brains, we hypothesized that the lack of trophic support may be due to a deficiency of NGF receptors. Using *in situ* hybridization, we have analyzed the cellular content of TrkA mRNA, encoding for the high affinity NGF receptor, in immunohistochemistry identified cholinergic neurons of the striatum and of the nucleus basalis of Meynert of 6 patients with AD and of 9 control subjects. We have evidenced, in the brain of AD patients, a decreased TrkA mRNA expression in cholinergic neurons of the nucleus basalis of Meynert (-75%, $P<0.001$) and of the ventral striatum (-41%, $P<0.01$) where they degenerate, but also on those of the anterior (-43%, $P<0.01$) and posterior (-51%, $P<0.01$) parts of the putamen, where they are spared. These data, taken in conjunction with our previously published data showing a loss of high affinity NGF binding both in the ventral and in the dorsal striatum, indicate a loss of NGF receptors. Reduced choline acetyltransferase activity and presence of neurofibrillary tangles in remaining striatal cholinergic neurons suggest that the resulting decrease in trophic support may be a primary event leading to cholinergic neuronal loss observed in AD.

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87.3

TrkA, BUT NOT P75^{NTF}, GENE EXPRESSION IS REDUCED WITHIN BASAL FOREBRAIN NEURONS IN ALZHEIMER'S DISEASE. J-M. Li*, J. H. Kordower and E. J. Mufson, Research Center For Brain Repair And Department of Neurological Sciences, Rush Presbyterian-Luke's Medical Center, Chicago, IL 60612.

We have recently reported that nerve growth factor (NGF) is reduced within basal forebrain neurons and accumulates within the cortex in Alzheimer's disease (AD). These findings led to the hypothesis that basal forebrain degeneration in AD results, in part, from impaired binding and/or retrograde transport of this neurotrophin. The present study examined whether there is a defect in gene expression for the high (trkA) and low (p75^{NTF}) affinity receptors in AD using *in situ* hybridization and RT-PCR. TrkA mRNA within individual nucleus basalis neurons was significantly reduced (66%) in AD cases (n=9) relative to age-matched controls (n=8; p<.01). RT-PCR quantitative analyses also demonstrated that trkA mRNA levels decreased markedly in AD. In contrast, p75^{NTF} mRNA was not significantly different in AD and age-matched controls. These data indicate that there is a selective defect in trkA gene expression in patients with AD supporting the hypothesis that the diminished retrograde transport of NGF within AD basal forebrain neurons may be due to impaired synthesis of the trkA receptor. These data also suggest that impaired NGF trophic support at the level of the NGF/trkA complex plays a critical role in the degeneration of this important population of neurons in AD. (Supported by AG09466, AG10161, AG09468).

87.4

GALANIN BASAL FOREBRAIN INTERACTIONS IN ALZHEIMER'S DISEASE: A CONFOCAL MICROSCOPIC ANALYSIS. R. Bowser[1]*, J.H. Kordower[2] and E.J. Mufson[2]. [1]Dept. of Pathology, Univ. of Pittsburgh, Pittsburgh, PA 15213 and [2]Dept. of Neurological Sciences, Rush Presbyterian Med. Ctr., Chicago, IL.

The galanin (GAL) containing fiber system that innervates cholinergic containing basal forebrain (CBF) neurons hypertrophies and hyperinnervates remaining CBF neurons in Alzheimer's disease (AD). It has also been suggested that GAL plays a critical role in the modulation of cholinergic neurotransmission in the normal human CBF. We and others have suggested that galanin may inhibit the production and release of acetylcholine from surviving CBF neurons in AD. To better understand the morphologic relationship/interactions of GAL hyperinnervation upon surviving CBF neurons in AD, we performed a double-label confocal microscopic analysis with antibodies to GAL and p75NTF receptor, an excellent marker of CBF neurons. Confocal microscopic evaluation revealed a hypertrophy of galaninergic fibers in the AD CBF in comparison to aged matched non-demented controls. Hypertrophic GAL positive fibers were seen in direct apposition to cholinergic neuron cell bodies, axons and dendrites. Galaninergic fibers wrapped around cholinergic cell bodies and neuritic processes. These findings support previous observations of an hyperexpression of GAL fibers in AD and provide evidence that galaninergic fibers make synaptic contact with remaining cholinergic neurons within the AD basal forebrain. Electron microscopic analysis is currently in progress to directly demonstrate the presence of synapses between GAL containing fibers and CBF neurons. Support by AG10668, AG10161 & AG09466.

87.5

SYNAPTIC NUMBERS IN THE ANTERIOR CINGULATE CORTEX (AREA 24) IN PATIENTS WITH ALZHEIMER'S DISEASE. D.A. Price, D.L. Sparks* and S.W. Scheff. Center on Aging Univ. Kentucky, Lexington, KY 40536-0230.

Clinical and experimental data clearly indicate that the anterior and posterior cingulate cortex are functionally distinct regions of the limbic system. The limbic system is known to be profoundly affected in Alzheimer's disease (AD). While the cingulate cortex was once thought to be a homogenous brain region, cytoarchitectonic studies have demonstrated that the anterior and posterior cingulate corticies receive distinct thalamic and cortical afferents. Several key cortical and limbic structures have previously demonstrated a significant decrement in connectivity resulting in a loss of synapses in AD as compared to age-matched controls. The posterior cingulate gyrus (area 23) is one of these areas manifesting a significant loss in AD. Because of the different afferent and efferent connections distinguishing the posterior and anterior cingulate areas, it is important to quantitatively assess the anterior cingulate for possible similarities and/or differences in synaptic numbers in AD.

Human brains were obtained at postmortem examination from 10 patients who met the NINDS-NIA criteria for AD and from 10 age-matched controls. These individuals were used previously to study the posterior cingulate. Both lamina III and V of area 24 were assessed and demonstrated a significant loss of synaptic contacts, similar to that found in the posterior cingulate. These results suggest that the mechanism(s) responsible for synaptic loss in AD may not follow strict morphological boundaries and connectivity. Supported by NIA AG 12138

87.6

MORBUS ALZHEIMER: CORTICAL COLOCALIZATION PATTERN OF NICOTINIC RECEPTORS AND PATHOLOGICAL MARKERS. A. Wevers[1]*, L.M. Monteggia[2], A. Maelicke[3], E. Giacobini[4], J.P. Sullivan[2], S. Arneric[2], and H. Schröder[1]. [1]Inst. II für Anatomie, Univ. zu Köln, 50931 Köln, FRG, [2]Abbott, Neurosci. Res., Abbott Park, IL, USA, [3]Inst. für Physiol. Chemie und Pathobiochemie, Univ. Mainz, 55128 Mainz, FRG, [4]Univ. Geneva, 1226 Thonex-Geneva, CH

The cholinergic dysfunction in Alzheimer´s disease (AD) may be related to intra- and extracellular events impairing the expression of the nicotinic acetylcholine receptors (nAChR). To get more insights into the underlying mechanisms, the mRNA expression of the α-bungarotoxin insensitive α4- and the α-bungarotoxin sensitive α7-subunit of the nAChR and the correlation to accumulation of pathological proteins, e.g. hyperphosphorylated tau and β-amyloid has been investigated.

In situ hybridization was performed on autopsy superior frontal gyrus samples of AD and control brains. Hybridization of hapten-labeled riboprobes to nAChR subunits was visualized by an alkaline phosphatase coupled-digoxigenin antibody and incubation with BCIP/ NBT. Colocalization of the pathological protein markers with α4 and α7 mRNA was achieved after hybridization by application of the corresponding monoclonal antibodies followed by an indirect immunoperoxidase technique.

The α4 and α7 transcripts were expressed in various neurons of all cortical layers, but the α4 one was more abundant than the α7 one as reported earlier already for control brains. The colocalization experiments revealed that in AD brains neurons in the vicinity of β-amyloid plaques bore both transcripts, whereas neurons heavily labeled for hyper-phosphorylated tau expressed little to no α4 and α7 mRNA.

These results show, that in AD intracellular rather than extracellular changes could interfere with the α4 and α7 nAChR subunit expression. Ongoing investigations of the compartment-specific codistribution of both subunits on mRNA and protein level with hyperphosphorylated tau will provide further information on possible causes of the disturbed nicotinic binding in AD. *Supported by the Maria Pesch-Stiftung and DFG*

87.7

PHARMACOKINETICS OF THE COMBINED CHOLINERGIC AND ADRENERGIC THERAPEUTIC CANDIDATE P11467 IN THE RAT AND DOG. E.M. DiLeo and D.J. Turk* Hoechst Marion Roussel, Neuroscience Therapeutic Domain, Bridgewater, New Jersey, 08807.

P11467, a combined acetylcholinesterase (AChE) inhibitor and α_2-adrenoceptor antagonist, is undergoing evaluation as a potential therapeutic agent for Alzheimer's Disease. Pharmacokinetic parameters were determined for P11467 following administration in rat and dog. The samples obtained during a 24 hour period were extracted using a solid-phase extraction procedure and analyzed by a sensitive HPLC-UV method. The results indicated that P11467 was rapidly absorbed with estimated plasma half-lives for absorption on the order of 0.40 hours in both species and terminal phase elimination P11467 from the target brain tissue (rat) less than 3.1 hours. The P11467 brain profile also correlated well with the time course of AChE inhibition *ex-vivo* following oral administration in rats. The parent compound was shown to be the major circulating form with maximal levels (C_{max}) in rat plasma of 482, 2728 and 5118 ng/mL and in rat brain of 170, 1100 and 1954 ng/g following 1, 5 and 10 mg/kg doses, respectively. The C_{max} levels in dog plasma were 327, 526, 1083 and 3081 ng/mL following 0.5, 1, 5 and 10 mg/kg doses, respectively. A comparison of the $AUD_{0\text{-}24hrs}$ values in both rat and dog following oral and intravenous administration indicated an absolute bioavailability on the order of 100% in both species. There was a linear increase in both the C_{max} and the $AUD_{0\text{-}24hrs}$ as the dose increased for rat (plasma and brain) and dog (plasma). In addition, the rat brain-to-plasma ratio remained constant at 0.4 across the dose range. The pharmacokinetic profile of this combined AChE inhibitor and α_2-adrenoceptor antagonist indicated that P11467 was orally bioavailable, penetrated the target brain tissue and correlated well with biochemical activity.

Research Support: Hoechst Marion Roussel

87.8

DOPAMINERGIC LOSS CORRELATES WITH HIPPOCAMPAL LEWY BODY DENSITY. A.Y. Wang, M.S. Mega, U. Tomiyasu, H.V. Vinters, A.W. Nakanishi, J.L. Cummings.* Alzheimer's Disease Center, UCLA School of Medicine, Los Angeles, CA 90024.

Objective: To determine if dopaminergic (DA) cell loss in the ventral tegmental area (VTA) is correlated to Lewy body (LB) occurrence in the hippocampus.

Background: DA decline is observed across the spectrum of Lewy body disorders. The VTA is the DA source for the limbic forebrain and D2 autoreceptor immunolabeling shows a terminal distribution in the hippocampus that corresponds to the regional distribution of LBs. If degenerating end terminals of DA cells contribute to LB production in the hippocampus, then the number of hippocampal LBs will be inversely related to the number DA cells in the VTA.

Methods: The study included 25 dementia patients with varying degrees of LBs and AD pathology. Melanin containing DA cell profiles in the VTA were counted on H&E sections of midbrain at the level of the red nucleus; LBs were counted on ubiquitin immuno-stained hippocampal sections. LB and DA cell density per mm2 were derived from profile counts corrected using the method of Abercrombie. Linear correlations among hippocampal LBs and VTA regions were determined by the least square fit method.

Results: Total hippocampal LB density had a linear inverse correlation (R2 = 0.52) with total VTA DA cell density when the extreme counts in the VTA were removed. Regional analysis of LB counts into the three CA fields showed CA3 had the weakest (R2 = 0.13) inverse correlation, while CA1 had the strongest (R2 = 0.68) inverse correlation with VTA DA cell density.

Conclusion: The inverse correlation between hippocampal LBs and VTA DA cell density supports the involvement of DA degeneration in the formation of LBs. (This project was supported by a VA Neuroscience Fellowship and an NIA Alzheimer's Disease Center grant: P30, AG10123).

87.9

CHARACTERISTICS OF ALZHEIMER'S DISEASE PATHOLOGIC CHANGES IN THE OLDEST-OLD. P. Giannakopoulos[1], P.R. Hof[2,3], E. Kövari[1], P.G. Vallet[1], D.P. Perl*[2,4], and C. Bouras[1,2]. [1]Dept of Psychiatry, Univ. of Geneva, HUG, Belle-Idée, 1225 Geneva, Switzerland, Depts of [2]Neurobiology, [3]Geriatrics, and [4]Pathology, Mount Sinai Sch. Med., New York, NY 10029.

Recent studies have revealed that in some very old people, certain neuronal subpopulations are consistently resistant to the degenerative process that occurs during aging and in Alzheimer's disease (AD). To explore further this issue, a quantitative stereological analysis of a large series of nonagenarians and centenarians was performed. Samples from the hippocampal formation and Brodmann's areas 9 and 20 were obtained from 24 non-demented (ND) patients and 19 AD patients, all older than 96 years of age (mean: 98.5 ± 2.0 years; range: 96-105 years). The ND group had mean MMSE of 28.0 ± 1.5 and extended CDR of 0.8 ± 0.2, whereas these values were 20.5 ± 1.5 and 3.0 ± 0.5, respectively, in the AD group. Materials were stained with antibodies to tau and amyloid Aß proteins. Neuronal loss was estimated from Nissl-stained serial sections. Cases with AD had significantly higher NFT densities in the CA1 field of the hippocampus only compared to ND individuals. SP densities in areas 9 and 20, but not in the hippocampus, were higher in demented compared to ND centenarians. In contrast to younger cohorts; neuronal loss was observed in layer II of the entorhinal cortex and layers II-III and V-VI of areas 9 and 20, but not in the CA1 field, in centenarians with AD, and it was not correlated with the development of NFT in any of the areas studied. Moreover, there was a negative correlation between SP and neuron densities in entorhinal cortex and area 20 suggesting that SP formation may parallel neuronal depletion in certain neocortical areas in these cases. The present data reveal a distinct pattern of AD pathology in very old individuals and imply that brain aging patterns may change considerably in this particular age group.

Supported by NIH AG05138 and FNRS.

87.10

OVEREXPRESSION OF β-AMYLOID PRECURSOR PROTEIN (β-APP) IN CEREBELLUM AFTER LESIONING IN THE INFERIOR OLIVE. K. Ito, K. Schluterman, R.D. Skinner, W.S.T. Griffin, T. Walls*. Neurosurgery, U. Ryukyus, Okinawa, Japan; Anatomy, Physiology, and Medicine, U. Ark. Med. Sci., Little Rock, AR 72205.

We have previously used unilateral, ventral electrolytic lesioning of the rat inferior olive to characterize local astrocytic activation (enlargement) responses as well as those in projection targets, *e.g.* in cerebellum. In Alzheimer's disease, such astrocytic activation correlates with growth of dystrophic neurites overexpressing β-APP (J Neuropathol Exp Neurol 55:273; 1996). We sought to identify changes in β-APP expression in cerebellum following lesion of the inferior olive. Lesions were confined to the right pyramid and inferior olive; these were surrounded by activated astrocytes. In the cerebellum of these rats, activated astrocytes were also numerous. Ten days post lesion, tissue β-APP levels were significantly increased ($p<0.05$) in cerebellum, as determined by Western analysis. This suggests that physiological responses to traumatic damage (perhaps in the form of overgrown dystrophic neurites or neuronal somatic β-APP overexpression) occur in projection sites distant from a trauma. These results support our hypothesis (Neurosci Letts 176:133;1994) that diffuse inflammatory responses following head trauma may be an important predisposing factor for the later development of Alzheimer's disease. Supported in part by AG10208 and AG12411.

87.11

OLFACTORY DISCRIMINATION AND REPEATED REVERSAL LEARNING IN YOUNG AND AGED DOGS. E. Head[1]*, H. Callahan[1], V. Balkissoon[1], C. Thirlwell[1], G.E. Adam[2], R.S. Astur[2], F. Barrington[2], A. Koerner[2], M.P. Weisend[2], R.J. Sutherland[2], B.A. Muggenburg[3], B.J. Cummings[4],C.W. Cotman[5] and N.W. Milgram[1]. [1]University of Toronto, Scarborough, Ontario, Canada, M1C 1A4, [2]Dept. of Psychology, UNM, Alb., NM, [3]Inhalation Toxicology Research Institute, Alb., NM, [4]Harvard Med. School, Boston, M.A. and [5]Institute for Brain Aging and Dementia, UCI, Irvine, CA.

Olfactory discrimination, identification and memory deteriorate with age in humans and these deficits are exaggerated in early Alzheimer's Disease. We are unaware of parallel studies in olfaction using animal models of aging. We studied olfactory discrimination (OD) and reversal learning (OR) in pound source dogs of mixed breeds ranging in age from 2 to 12 years. We previously reported that dogs show age dependent cognitive deficits that are correlated with β-amyloid accumulation (Cummings et al, 1996)

We found marked differences between young and old dogs in OD learning, with aged dogs making more errors (mean=121.8 sd=80.3 n=5) than young dogs (mean=44.4 sd=28.4 n=5) in reaching criterion. Two of the 5 aged dogs could not solve the problem in 400 trials. Dogs reaching criterion on the OD task were subsequently tested for OR learning. Three young dogs and one aged dog required more training trials to learn the OR problem, whereas two young dogs and one aged dog learned the OR problem with fewer errors than OD. Two dogs (one aged, one young) were also tested with repeated reversals to measure development of a learning set. Both dogs eventually learned to reverse odors in two or fewer errors. The aged dog however, showed a slower decline in error scores than the young dog. Our results suggest that aged dogs are deficient on an olfactory discrimination task and on repeated reversals. Supported by NIA Grant (AG 12694) to CWC, BJC, BAM and NWM.

87.12

A CANINE MODEL OF DEMENTIA: TESTS OF COGNITIVE FUNCTION IN THE BEAGLE G.E. Adam,*[1] R.J. Sutherland,[1] R.S. Astur,[1] F.M. Barrington,[1] A. Koerner,[1] D. Oakley,[1] J. Schlife,[1] M.P. Weisend,[1] C.W. Cotman,[2] E. Head,[3] N.W. Milgram,[3] & B.A. Muggenburg[4] [1]Univ. of New Mexico, Abq., NM; [2]UC, Irvine; [3]Univ. of Toronto, Scarborough; [4]Inhalation Toxicology Research Institute, Abq., NM

A key to understanding human dementia is the development of an animal model to closely tie behavior to brain abnormalities. The search for a model of dementia led to the discovery that certain dog breeds develop β-amyloid plaques. These plaques, often seen in and around hippocampus, are diffuse, and the canine brains are without neurofibrillary tangles (Cummings et al., 1993). Furthermore, we recently have obtained preliminary evidence of a significant correlation between plaque density and a global index of cognitive dysfunction (Cummings et al., 1995). We have now tested 17 colony-raised beagles (2 - 15 years old) in a version of the Morris water task which has been shown to be hippocampal-dependent in rodents. After 15 training trials (2 per day) and one hidden platform trial (all cues removed from within the pool), the animals were tested for their knowledge of the platform location during a 60 second swim with the platform and cues removed. The proportion of swim path in the correct quadrant declined as the animals' age progressed from very young (2-4 years) to moderately old (8-11 years), but increased for the very old dogs (12+ years). These same beagles are now being trained on an object recognition task (DNMS). Although we expect no age-related differences in acquisition, with increasing delays a pattern of performance decline similar to that seen in the Morris water task would further support our idea that the very oldest dogs are less susceptible to plaque formation and cognitive dysfunction. MRI scans and histological procedures will be conducted at the end of behavioral testing to assess plaques and other pathology. [Supported by grant AG 12694 to CWC, DOE (OHER, contract #DE-AC04-76EV-01013) at ITRI, and SRAC to GEA]

87.13

HUMAN BRAIN TISSUE IN NEUROSCIENCE: A RESOURCE FOR ANALYSIS OF BRAIN-BEHAVIOR CORRELATIONS. S.L. Weinstein*, M. H. Oshita, A.J. Wasserman, B. Choi, R. Kim, M. B. Dick, W. R. Shankle, and C.W. Cotman. Institute for Brain Aging and Dementia, *University of California, Irvine, CA 92717 USA*

The Institute for Brain Aging and Dementia Tissue Repository provides human, canine and feline brain tissue, blood, and CSF for use by other investigators. Available human cases have short *post mortem* intervals (most <4 hours), and represent many variants of dementia: including Alzheimer's disease (AD), Vascular dementia, Down Syndrome, and Lewy Body disease. Cases are all neuropathologically defined, and available across a range of disease severities, allowing for investigation of the sequence and timecourse of neurodegenerative events. Additionally, neuropsychological and neurological assessments have been conducted, providing clinical data which can be used in conjunction with pathological and genetic information to identify relevant correlations. Furthermore, all cases are subjected to a standard battery of immunohistochemical stains. Using a computer-based image analysis program in combination with immunohistochemistry, we provide quantitative analysis of antibody staining. This type of quantitative data can be invaluable when used in conjunction with other variables (*i.e.* cognitive decline, genotyping, etc.). For example, we recently reported that increasing levels of ß-amyloid deposition correlate with severity of cognitive impairment on the mini mental state exam (r=.90, p<.001; Cummings *et al.*, 1995). We also discovered a novel "neurite-only" plaque type whose presence appears to be independent of disease severity (Pike *et al.*, 1995). Additionally, the ApoE isotypes of most cases have been determined, allowing for careful examination of the role of the ApoE gene in AD and other forms of dementia. In conclusion, use of human brain and select animal tissues is a valuable and available resource for investigation of dementia and other brain-behavior correlations.

87.14

MRI-BASED QUANTITATIVE ASSESMENT OF THE HUMAN ENTORHINAL AND PERIRHINAL CORTEX. R. Insausti[1], K. Juottonen[2], H. Soininen[2], K. Partanen[3], P. Vainio[3] and A. Pitkänen*[4]. Dept. Anatomy, Univ. of Navarra, Pamplona, Spain[1]; Dept. Neurology[2], Clin. Radiology[3] and A.I. Virtanen Institute[4] Univ. of Kuopio and Kuopio Univ. Hospital, Kuopio, Finland.

The entorhinal and perirhinal cortex are part of the medial temporal lobe neuronal circuitries involved in memory processing. To investigate the contribution of the entorhinal and perirhinal damage to the memory impairment in neurological diseases such as in Alzheimer's disease and temporal lobe epilepsy, we designed a protocol that can be used to determine the volumes of these cortical areas in coronal MRI images. Histological material for the analysis of the cytoarchitectonic boundaries of the cortical areas was available from 72 autopsies. MRI scans (1.5 T Magnetom, T1-weighted partitions, 2.0 mm thick contiguous slices perpendicular to the axis of anterior-posterior commissure) were performed for 20 normal volunteers (11 women and 9 men, age 21-79 years). In one histological case, a premortem MRI was available. Based on cytoarchitectonic criteria, we determined the location of the entorhinal and perirhinal cortex in the human brain according the sulci and gyri that would best indicate the boundaries of these cortical areas as determined from the histological sections. By using the sulcal and gyral pattern as well as the rostrocaudal level, the outline of the entorhinal and perirhinal cortex was drawn on successive MR images and the volume was calculated. The right entorhinal cortex (1636 $\pm$ 313 mm^3) was larger than the left (1378 $\pm$ 328 mm^3)($p<0.001$). The volume of the right perirhinal cortex (5293 $\pm$ 697 mm^3) was smaller than that on the left (5572 $\pm$ 644 mm^3)($p < 0.042$). The normalized volumes of the entorhinal or perirhinal cortices did not correlate with age. The intraobserver variability was < 8%.

(supported by Kuopio University Hospital)

87.15

VOLUMETRIC MRI ANALYSIS OF THE ENTORHINAL AND PERIRHINAL CORTEX IN ALZHEIMER'S DISEASE. K. Juottonen[2], R. Insausti[1], A. Pitkänen[4], K. Partanen[3], P. Vainio[3], and H. Soininen*[2]. Dept. Anatomy, Univ. of Navarra, Pamplona, Spain[1]; Dept. Neurology[2], Clin. Radiology[3] and A.I. Virtanen Institute[4] Univ. of Kuopio and Kuopio Univ. Hospital, Kuopio, Finland.

The entorhinal and perirhinal cortices are part of the medial temporal lobe memory system that is damaged at the early stages of the Alzheimer's disease. By using our own recently designed protocol, we measured the volumes of the entorhinal and perirhinal cortex on MR images in patients with Alzheimer's disease (AD)(n=10, all fulfilled the NINCDS-ADRDA criteria for probable AD) and in age- and sex-matched normal controls (n=10). MR was performed with a 1.5 Tesla Magnetom and a 3D-technique allowing the reconstruction of 2.0 mm thick contiguous slices perpendicular to the axis of anterior-posterior commissure. The volumes of the right entorhinal cortex (AD 840 $\pm$ 274 mm^3, controls 1659 $\pm$ 374 mm^3), left entorhinal cortex (AD 703 $\pm$ 281 mm^3, controls 1395 $\pm$ 382 mm^3) and left perirhinal cortex (AD 4085 $\pm$ 1312 mm^3, controls 5464 $\pm$ 672 mm^3) were smaller in AD patients than in controls ($p<0.01$). There was significant asymmetries between the volumes of the left and right entorhinal and perirhinal cortices in controls but not in patients with Alzheimer's disease. Our volumetric MRI measurements reveal significant atrophy of the entorhinal and perirhinal cortex in patients with probable AD.

(supported by Kuopio University Hospital)

87.16

LONGITUDINAL EVALUATION OF BRAIN ASYMMETRIES IN RELATION TO DEVELOPMENT OF PRECLINICAL DEMENTIA. S.M. Koger[1]* and J.A. Kaye[2]. [1]Willamette Univ., Salem, OR, 97301 and [2]Dept. of Neurology, Oregon Health Sciences Univ., Portland, OR 97201.

Alzheimer's disease (AD) is characterized by bilateral hippocampal volume loss. Loss of normal hippocampal asymmetry (right>left) may be related to age-associated memory impairment (Soininen, et al, Neurology, 1994) or preclinical dementia. Volumetric analysis of magnetic resonance imaging (MRI) assessed if asymmetrical degeneration of medial temporal and cortical structures predicts development of dementia. Annual MRIs collected on 29 healthy elderly subjects, who were cognitively intact at entry, were compared over time with 12 of the subjects who subsequently manifested cognitive impairment as defined by a Clinical Dementia Rating of $\geq$ 0.5. Repeated measures ANOVA of hippocampal volume showed a nonsignificant (p=.07) diagnostic group by side interaction, suggesting a trend toward less asymmetry in those who declined cognitively. Left parahippocampal volume exceeded right for both groups. Atrophy of temporal and frontal cortices in the cognitively impaired subjects did not develop asymmetrically.

Supported by: Dept. Veterans Affairs, NIH AG 08017 and Alzheimer Research Alliance of OR

87.17

MR VOLUMETRY OF FRONTAL LOBE REGIONS IN ALZHEIMER'S PATIENTS: AN EFFECT OF SEX?, J.A.Y. Hall*, S.E. Black and S. Kohler, Sunnybrook Health Sci. Centre, Toronto, ON, Canada, M4N 3M5

Recent studies suggest that language functions may be more impaired in women than men with Alzheimer's Disease (AD). Using functional MRI, one report demonstrated sex-dependent variation in the activation of the inferior frontal gyrus (IFG) during phonological processing. Given that women demonstrate a pre-morbid advantage over men on some verbal tasks, the relative decline in women with AD is curious. We performed MR volumetry of frontal regions including; the superior frontal gyrus (SFG), middle frontal gyrus (MFG), IFG and inferior pre-central gyrus (PCG). Subjects were 9 males and 9 females who met NINCDS-ADRDA criteria for probable AD and an equal number of control subjects (NC) matched for sex, age and education.

Analysis was performed using ANALYZE software on images obtained from a 1.5 Tesla GE magnet. The volume of each gyrus was calculated by stereology (point counting) using serial 1.3 mm thick coronal sections on a T1 weighted 3D sequence. Data for each gyrus (right, left and total) were analyzed in 2-way ANOVAs (sex by group) using a total brain volume, age and duration of illness as covariates.

No significant effects or interactions were noted for the SFG, MFG or IFG. The total PCG was smaller in women than men [$F(1,27)=5.34$, $p<.02$] and smaller in the AD than NC group [$F(1,27)= 6.45$, $p<.02$], but there was no interaction. The left PCG demonstrated the same effect of sex [$F(1, 27)= 5.10$, $p<.02$] and group [$F(1,27)= 6.50$, $p<.02$] with no interaction. The right PCG showed no main effects or interaction.

The PCG is known to mediate some degree of motor processing for the lips, tongue and facial musculature. Although the interaction did not reach significance, the general direction of the data may suggest that women have a PCG which is smaller and more efficient for processing verbal/language information than men. A relatively small insult to this area may lead to a disproportionate loss of function as is seen in AD women compared to AD men. We are expanding our sample size to test this hypothesis. This project was funded by the Ontario Mental Health Foundation.

87.18

CELLULAR DEATH IN NORMAL AGING AND ALZHEIMER'S BRAINS. W.P.Li[1], H.W.L.Lai[2], M.C.Yu*[3], and D.T.Yew[4]; Dept. of Neurology, First Military Medical Univ., Guangzhou, China[1]; Dept. of Anatomy, Chinese Univ. of Hong Kong, Hong Kong[2,4]; and Dept. of Anatomy, Cell Biology & Injury Sciences, New Jersey Medical School, New Jersey 07103[3].

Seven normal aged brains and four Alzheimer brains (average age: 83 and 88 years old, respectively), obtained at postmortem with consent of patients' families and approval of institutions, were processed for evaluation of apoptosis in different areas of the cortex, using the "TUNEL" hybridization technique. The frontal cortex and hippocampus from Alzheimer's patients showed a higher number of apoptotic cells, with the CA4 field of the hippocampus being the most pronounced region exhibiting this difference. To determine whether the apoptotic cells were neurons or glia, these cells were dually labeled with GFAP antisera specific for astrocytes. Quantitative analysis indicated that the dying astrocytes comprised of 13% of apoptotic cells, suggesting that the majority of apoptotic cells were neurons. The proportion of cholinergic to dopaminergic to GABAergic neurons in the cortex of normal aged and Alzheimer's brains, based on immunohistochemical localization with appropriate antisera, was found to be very similar in these subtypes of neurons in both groups. The results indicate that some degenerative mechanisms may not be unique to Alzheimer's disease.

87.19

Quaternary Carbamates with Blood Brain Barrier Permeability as Potential Drugs for the Treatment of Central Cholinergic Deficiency. E. Heldman, E. Rachaman, R. Adani, I. Rabinovitz, R. Brandeis and G. Amitai, IIBR, Ness Ziona 74100, Israel.

A series of quaternary-lipophylic carbamates were synthesized based on N- alkyl substituted pyridostigmine (PYR), and on conjugates of these compounds with glucosyl residues attached via hydrocarbon spacers. The incorporation of alkyl chains (C_6-C_{12}) renders these quaternary compounds lipophilic resulting in blood-brain barrier permeability. The kinetic parameters for inhibition of purified FBS-AChE by these compounds are: dissociation constants, $K_i = 1.2x10^{-7}$-$2.3x10^{-5}$ M, reactivation rate constants, $k_s = 0.006$-0.059 min^{-1} and bimolecular rate constant, $k_i = 1.5x10^4$-$2.6x10^6 M^{-1} min^{-1}$. Despite of their similar inhibitory potency some of these carbamates are 16-18 fold less toxic than PYR in mice (im). Moreover, PO (PYR-C_8) is 46 fold less toxic (LD_{50} = 230mg/kg, sc) than PYR (5mg/kg, sc) in rats. In vivo blood ChE inhibition by PO and PD (PYR-C_{10}) reaches its peak level within 0.25-6 hours in rats (45% at 20 mg/kg, sc) and sustains at 17-33% even after 24 hours. Significant inhibition of AChE at several brain regions was observed even 24 hours after sc injection in rats. Central activity of PO (20mg/kg, sc) was demonstrated by reversing scopolamine-induced impairment of memory retention in rats using the passive avoidance test. These lipophylic-quaternary carbamates are potential non-toxic long-acting drugs for diseases associated with central cholinergic deficiencies, such as Alzheimer's disease and stroke.

ALZHEIMER'S DISEASE: IMMUNE MECHANISMS

88.1

EXPRESSION OF CD43 IN HUMAN MICROGLIA AND ITS DOWN REGULATION IN ALZHEIMER DISEASE. A. Matsuo[1, 2], K. Terai[1], D. G. Walker[1], S. C. Sung[1]*, E. G. McGeer[1] and P. L. McGeer[1]. Kinsmen Lab. of Neurol. Res., Univ. of British Columbia, 2255 Wesbrook Mall, Vancouver, BC, V6T1Z3, CANADA[1], and Dept. of Neurology, Kyoto Univ., Kyoto, JAPAN[2].

CD43, which is also known as leukosialin or sialophorin, is thought to play an important role in inhibiting leukocyte adhesion, although its exact functions are still uncertain. Prominent expression of CD43 has previously been detected on the surface of T lymphocytes, monocytes, neutrophilis and some B lymphocytes. Since microglia are the brain representatives of the monocytic phagocytic system, we investigated whether microglia also express CD43. We also explored whether this expression changes when microglia are activated as in Alzheimer disease (AD). Abundant CD43-like immunoreactivity was detected in ramified microglia of normal brain. It was also seen in residual leukocytes in capillaries and was faintly detectable on the surface of some normal appearing neurons. In AD brains, the overall expression of CD43 on microglia was markedly lower than in control brains. This was opposite to the change in expression of HLA-DR, which was greatly upregulated due to the reactive state of the microglia. This is the first report of a microglial marker which is more highly expressed in the resting or ramified state compared with the reactive state. Such expression is consistent with theories that CD43 plays as anti-adhesional role, and cleavage occurs during cellular activation. Supported by grants from the Alzheimer Society of B.C. and Jack Brown and Family A.D. Research Fund.

88.2

TISSUE SECTION CULTURE STUDIES OF RAT MICROGLIA ON ALZHEIMER'S TISSUE. S.N. Joshi* and K.A.Crutcher, Department of Neurosurgery, University of Cincinnati, College of Medicine, Cincinnati, OH 45267-0515.

Senile plaques consist of a variety of protein and cellular components. Activated microglia and reactive astrocytes are invariably associated with neuritic senile plaques, suggesting that glial cells may play a role in the production of, or response to, the plaque. Furthermore, some studies have shown that synthetic amyloid peptides induce reactive gliosis and activate microglia. However, since senile plaques are composed of many other elements, the response of glial cells to plaques may involve factors other than amyloid. An alternative approach is to use tissue section culture to determine the response of glial cells to plaques. Tissue sections from AD, human control and rat brains were thaw-mounted onto round coverslips and placed in 4-well plates. The sections were seeded with partially purified microglial cells prepared from mixed cultures of neonatal (P0-P5) rat cortical tissue (separated at 4°C by rotation for 2 hours on an orbital shaker). DiI-labeled acetylated-LDL, which is taken up by scavenger receptors, was used to identify microglial cells on the sections after 4 to 5 days in culture. Initial observations suggest that there are greater numbers of rat microglia on AD tissue sections as compared with the number on human control and rat tissue sections. Co-visualization of plaques, carried out using thioflavin S, did not reveal preferential association of microglial cells with plaques. These preliminary results demonstrate the feasibility of culturing neonatal rat microglial cells on tissue sections of postmortem human brain and suggest that such an approach can be used to examine the interaction of microglial cells with plaques. (Supported by NIH NS31410.)

88.3

NEURITIC PLAQUE EVOLUTION IN ALZHEIMER'S DISEASE (AD) IS ACCOMPANIED BY MICROGLIAL TRANSITION FROM PRIMARY TO ACTIVATED TO PHAGOCYTIC FORMS. J.G. Sheng,* R.E. Mrak, and W.S.T. Griffin. DVAMC; Univ Arkansas Med Sci, Little Rock, AR 72205.

IL-1α^+ microglia are important in neuritic plaque formation in AD as evidenced by their distribution among different plaque types (J Neuropathol Exp Neurol 1995;54:276). To further evaluate this role, we classified and counted plaque-associated and non-plaque-associated IL-1α^+ microglia as primary (resting), activated (enlarged), and phagocytic (enlarged with heterogenous cytoplasmic contents), and we determined the distribution of the plaque-associated microglia among plaque types. Temporal tissue from 11 AD patients (58-88 y) and 7 controls (61-83) was immunoreacted for IL-1α and β-amyloid. In AD, the number of primary, activated, and phagocytic microglia were increased 2.3-, 4.4-, and 8-fold, respectively. Most (84%) primary microglia were not plaque-associated; 13% were in diffuse non-neuritic plaques (DnNP) and 3% in diffuse neuritic plaques (DNP). In contrast, most activated (55%) and phagocytic (91%) microglia were plaque-associated; activated forms were prominent in DNP (39%), with some (8%) in DnNP and dense core neuritic plaques (DCNP), while phagocytic forms were sparse in DnNP (5%) but prominent in DNP (56%) and DCNP (30%). This suggests that microglial transformation from primary, to activated, to phagocytic forms is important in plaque evolution. NIH AG10208, AG12411.

88.4

ACTIVATED AND PHAGOCYTIC IL-1α^+ MICROGLIA, BUT NOT PRIMARY IL-1α^+ MICROGLIA, INCREASE WITH AGE IN NORMAL HUMAN BRAIN. R.E. Mrak,* J.G. Sheng and W.S.T. Griffin. DVAMC; Univ. Arkansas Med. Sci., Little Rock, AR 72205.

Microglia-mediated inflammatory responses have been implicated in the pathogenesis of neuritic plaques in Alzheimer's disease. The strong age association of Alzheimer's disease suggests that events in normal aging promote these responses. We used computerized image analysis to determine the number and immunoreactive cross sectional area of IL-1α^+ microglia in temporal lobe of 20 neurologically normal patients, ages 2-83 yrs. IL-1α^+ microglia were characterized as primary (resting), activated (enlarged), and phagocytic (enlarged with heterogenous cytoplasmic contents). The number of activated and phagocytic IL-1α^+ microglia, but not primary IL-1α^+ microglia, increased with age ($r = 0.5$ $p = 0.02$, $r = 0.5$ $p = 0.007$, $r = 0.39$ $p = 0.09$; respectively). The total number and cross sectional area of IL-1α^+ microglia also correlated with age ($r = 0.5$ $p = 0.017$, $r = 0.6$ $p = 0.006$; respectively). The number of activated and phagocytic IL-1α^+ microglia in patients > 60 yrs was greater than that in patients < 60 yrs ($p < 0.001$). These age-related changes in activated and phagocytic, but not in primary, IL-1α^+ microglia suggest that activation of IL-1α^+ microglia in the course of normal aging underlies the age-related risk for Alzheimer's disease. This work was supported in part by NIH AG10208 and AG12411.

88.5

MICROGLIAL ACTIVATION BY PERIPHERAL AND CENTRAL LIPOPOLYSACCHARIDE IN RAT. W. J. Lipinski, M. J. Callahan, C. A. Parker, C.A. Raby*, K. Spiegel and L. C. Walker. Parke-Davis Pharm. Res., Div. of Warner-Lambert Co., Ann Arbor, MI 48105.

In response to injury or disease, microglia become activated and release factors that can be cytotoxic to neurons. In chronic neurodegenerative processes such as Alzheimer's disease, microglia may remain activated and chronically produce factors that have neurotoxic potential. In this study, lipopolysaccharide (LPS) was administered to rats centrally and peripherally, and microglial activation was assessed using parallel Western blot and immunohistochemical techniques. Histologically, acute intraperitoneal injection of LPS resulted in a 2-3 fold increase in lectin-stained microglia that peaked 24 hrs after injection, and was only slightly reduced after 48 hrs. Quantitative immunoblot using antibody OX-42 did not detect this increase in microglia. Chronic intracerebroventricular (ICV) infusion of LPS for either 7 or 14 days resulted in a large increase in activated microglia that was readily evident by lectin immunostaining and OX-42 immunoblot. Additionally, chronic central infusion of LPS caused a dose-dependent increase in the β-amyloid precursor protein and activation of complement in brain. No change in βAPP or complement was seen following acute peripheral LPS administration. To the extent that activated microglia may play a pathogenic role in some neurodegenerative diseases, these findings suggest that LPS-treated rats are a useful model for studying chronic inflammation in the CNS. Supported by Warner-Lambert.

88.6

ASSOCIATIONS OF IL-1α^+ MICROGLIA AND S100β^+ ASTROCYTES WITH NEUROFIBRILLARY TANGLE (NFT)-BEARING NEURONS IN ALZHEIMER'S DISEASE (AD). W.S.T. Griffin,* J.G. Sheng, and R.E. Mrak. Dept. VA Med Ctr; Univ. Arkansas Med. Sci., Little Rock, AR 72205.

IL-1α^+ microglia and S100β^+ astrocytes are important in the formation of NFT-bearing neurites in AD plaques (J Neuropathol Exp Neurol 1995;54:276 and 1996;55:273). To evaluate the role of these cells in the pathogenesis of neuronal NFT's, we used dual-label immunohistochemistry to quantify IL-1α^+ microglia or S100β^+ astrocytes associated with four different stages of NFT formation. Using a monoclonal, phosphatase-independent Tau-2 antibody, neurons were classified as stage 0 (some, but only diffuse or finely granular, cytoplasmic Tau-2 immunoreactivity), stage 1 (early, delicate fibrillar or rod shaped Tau-2$^+$ tangles), stage 2 (mature Tau-2$^+$ tangles filling the neuronal soma, often with dislocation or pyknosis of the nucleus) or stage 3 (extracellular Tau-2$^+$ tangles--"ghost tangles"). The percentages of stage 0, 1, 2, and 3 neurons with associated IL-1α^+ microglia were 48, 56, 67, and 92%, respectively; and the percentages with associated S100β^+ astrocytes were 21, 37, 55, and 92%, respectively. These results show that NFT formation in neurons is accompanied by early and progressive association with IL-1α^+ microglia and S100β^+ astrocytes, and suggest a pathogenic role for IL-1 and S100β in NFT formation. Supported in part by NIH AG10208 and AG12411.

88.7

ASTROCYTE INVOLVEMENT IN SENILE PLAQUE DEVELOPMENT: STUDIES IN NON-ALZHEIMER TISSUES. L.S. Conklin and B. Quinn*, Division of Neuropathology, NYU Medical Center, New York, NY 10016.

Although amyloid plaques are a hallmark of Alzheimer's disease, their exact origin remains obscure, as are factors defining their exact topologic shape and spatial distribution. Amyloid deposits are seen not only scattered amongst neurons in the familiar neocortical pattern, but also in the glia limitans or outer part of Layer 1, particularly in occipital cortex; in broad penumbras around cortical vessels (in selected cases); and in diffuse broad areas, particularly in mesial temporal cortex (H.M. Wisniewski et al., Acta Neuropathol 78:337, 1989). Amyloid plaques contain many proteins such as heparan sulfate proteoglycans, perlecan, decoran, laminin, apolipoprotein E and others, which may be co-factors in amyloid fibril formation. Using astrocytic markers such as GFAP and CD44, we have noted "smudge-like" zones of staining in normal or epileptic cortex which intermingled with well-defined cellular astrocytic staining for these markers. The immunohistochemical patterns suggest that astrocytes, including protoplasmic astrocytes, can leave behind smudge- or 'plaque'-like zones of glial proteins in human neocortical neuropil. The size and topologic distribution of these zones was reminiscent of the pattern of amyloid plaque formation in Alzheimer's disease. Although speculative, the histochemical data suggests the hypothesis that astrocytes might leave behind zones of neuropil proteins which could provide seeding areas for a much later cascade of amyloid plaque development in individuals vulnerable to Alzheimer's disease. Postmortem Alzheimer neocortex was co-stained for CD44 and amyloid plaques using the sensitive Reusche technique. Silver-positive senile plaques almost always showed broad penumbras or annuli of CD44 staining, and at least occasionally seemed to coincide with the location of large protoplasmic astrocytes. GFAP immunostaining was more likely to show fibrous astrocytes at the perimeter of plaques. Sponsored by the NYU Alzheimer Center and the Stanley Foundation.

88.8

A ROLE FOR INTERLEUKIN-1ß IN IRON REGULATION OF ASTROCYTOMA CELLS. J. Hu* and J. Connor. Dept. of Neuroscience & Anatomy, M.S. Hershey Medical Center, Penn State University, Hershey PA, 17033

Previously, we have demonstrated that iron accumulates in Alzheimer's and Parkinson's diseased brains in those regions undergoing the most severe histopathological change. Ferritin, the iron storage protein, does not increase concomitantly with the increase in iron thereby increasing the possibility for iron induced oxidative damage. The goal of this research is to eludcidate the mechanism by which iron can accumulate in brain tissue without inducing a ferritin increase. The iron regulatory protein (IRP) is responsible for the coordinate regulation of ferritin and the transferrin receptor. This protein acts on an iron responsive element (IRE) present on the transcripts of ferritin (5' UTR) and the transferrin recptor (3' UTR). Cytokines, as part of the inflammatory response, are known to stimulate iron uptake and ferritin synthesis in a number of cell types. In this study, the effect of IL-1ß on the expression of the IRP in an astrocytoma (SW1088) cell line is determined. In the astrocytoma cells, IL-1ß decreased IRP binding activity after 1 day of exposure, but after 2 days exposure IRP binding activity increased and remained elevated at 3 days post exposure. Ferritin protein levels intially increased and then decreased in a manner consistent with increased IRP binding activity. Treatment of cell lysates with a reducing agent revealed the amount of available IRP for binding after 1 day exposure to IL-1ß was similar to control whereas the level of IRP appears increased by 2 days postexposure. These data suggest inflammatory agents known to be released in the brain in a number of neurodegenerative diseases may disrupt cellular iron homeostasis which would lead to decreased metabolic activity and increased susceptibility to oxidative damage.
Supported by IIRG-94-122 (Alzheimer's Association)

88.9

IL-1β ALTERS THE MORPHOLOGY OF S-100 IMMUNOPOSITIVE GLIA IN CULTURES OF ADULT HUMAN TEMPORAL LOBE. D.L. Davies* and F.A. Boop. Depts. of Anatomy and Neurosurgery, Univ. of Arkansas for Med. Sci., Little Rock, AR 72205.

Microglial derived IL-1β has been implicated in several neuropathologic conditions including head trauma, Alzheimer's disease and microbial infections. As part of an endeavor to assess the influence of microglial activation on glial-neuronal interactions, recombinant human IL-1β was administered to secondary cultures of either gray or white matter from surgically resected adult human temporal lobe. In these cultures, vimentin immunopositive epithelioid cells constitute the predominate cell population. However, a subpopulation of cells was immunopositive for GFAP and S-100 protein; generally, these cells had a complex arbor of processes some of which were long (*ca.* 600 µm). Beginning at 6 days *in vitro*, cells were exposed to IL-1β (4.0, 0.8, 0.2 ng/ml) in the culture medium for 72 hrs. Exposure to 4.0 or 0.8 ng/ml of IL-1β increased the number of S-100 immunopositive cells. The majority of these cells were epithelioid and had numerous cytoplasmic vacuoles. Whereas S-100 immunopositive cells in the control cultures retained an extensive arbor of processes. Qualitative differences in GFAP immunopositive cells were not discerned and the responses of gray and white matter cultures were similar. Since neurotrophic and mitogenic activities have been attributed to S-100 protein (Marshak, 1990, *Prog. Brain Res.* 86:169), increased expression of S-100 protein may have a role in the pathogenesis of disease states associated with microglial activation.
Supported by: UAMS Research Council and NIH PO1 AG12411.

88.10

INTERLEUKIN-6 mRNA IS ELEVATED IN ALZHEIMER DISEASE BRAIN. C.Zarow*, K.E.Schlueter, Q.Zhang. Department of Neurology, University of Southern California, Rancho Los Amigos Medical Center, Downey, CA 90242. Interleukin-6 (IL-6) is a cytokine regulator of acute phase proteins produced by microglia and astrocytes under the influence of IL1 and TNF. Increased levels of IL-6 have been reported in Alzheimer disease (AD) brain, but it is not known whether regulation is at the transcriptional or translational level. Using polymerase chain reaction, the levels of mRNA for IL-6 were investigated in 8 AD and 7 non-AD cases. Non-AD cases included one normal control and cases with diffuse Lewy body disease, vascular dementia, PSP, cortical basal ganglionic degeneration, and Pick's disease, neurodegenerative diseases also associated with reactive astrocytes and microglia. Total RNA was extracted from tissue obtained at autopsy from two regions of the temporal lobe: superior temporal gyrus, Brodmann area 22, and the hippocampus. Post-mortem delays averaged 4.8 ± 1.8 h. cDNA was prepared by reverse-transcription from total RNA using random hexamer primers. 20 ng of the resulting cDNA was amplified in a thermal cycler. Amplification products were resolved by electrophoresis in 2% high resolution 3:1 agarose gels. Bands were quantified from ethidium bromide stained gels using videodensitometry software (One-D Scan, Scanalytics). IL6 mRNA was detectable almost exclusively in AD (7 AD and 1 non-AD case in area 22 and 3 AD cases in the hippocampus) despite evidence of neurodegeneration in the non-AD cases. These data provide evidence that the reported elevation of IL6 protein in AD brains is specific for AD and is due to the regulation of IL6 at the level of transcription. (NIH AG-11126 and Alzheimer Association IIRG-94-048)

88.11

CSF OLIGOCLONAL IMMUNOGLOBULIN SYNTHESIS IN A SUBSET OF PATIENTS WITH ALZHEIMER'S DISEASE.

H. Hampel*[1], H.U. Kötter[2], D.A. Körschenhausen[3], and H.-J. Möller[2]. [1]National Institutes of Health, National Institute on Aging, Laboratory of Neurosciences, Bldg. 10/6C414, Bethesda, MD, USA; [2]Psychiatric Hospital, Ludwig-Maximilian University, Nussbaumstr.7, 80336 Munich, Germany.

Oligoclonal bands (OCB) indicate the activation of the humoral immune response and can act as byproducts of CNS inflammation. To investigate whether humoral immunactivation is present in AD, we studied the frequency of OCB in serum and cerebrospinal fluid (CSF). 51 AD subjects [21 EO, 30 LO] were compared to patients with Vascular Dementia (VD; n=11), Major Depression (MD; n=29), Multiple Sclerosis (MS; n=38) and healthy age-matched controls. The presence of OCB was investigated by polyacrylamide Gel isoelectric focusing (IEF). For quantitative intrathecal IgG measurement the IgG-index was used. An elevated IgG-index was found in 82% of MS, 6 % of AD, 9% in controls, and none in VD and MD patients. OCB could be traced in 97% of patients with MS, 20 % in AD, and not in VD, MD and control subjects. The frequency was signficantly elevated between MS and all other groups *($p < 0.0001$)* and in AD compared to MD *($p < 0.03$)* and VD *($p < 0.05$)* patients. There was no correlation with the age of dementia onset and the degree of cognitive impairment. Both the results of IEF and IgG-index seem to be influenced by the presence of blood-brain-barrier (BBB) dysfunction. Furthermore the combined use of quantitative and qualitative IgG detection methods increased the diagnostical sensitivity in AD but not in MS. In conclusion our findings indicate a possible humoral CNS immunactivation in a subset of patients with AD.

88.12

CYCLOOXYGENASE-2 (COX-2) A DEVELOPMENTALLY REGULATED GENE THAT SHOWS REGIONAL INDUCTION TO EXCITOTOXIC LESIONS IN RAT BRAIN. A MODEL FOR ALZHEIMERS' S DISEASE

G. Tocco[1], J. Freire-Moar[2], S. Schreiber[1*], M. Baudry[1], P.S. Aisen[3] and G.M. Pasinetti[3] [1]Hedco Neuroscience Program, USC, Los Angeles, CA 90089; [2]Department of Inflammation Pharmacology, Roche Bioscience, Palo Alto, CA 94303; [3]Department of Psychiatry and Brookdale Center for Molecular Biology, The Mount Sinai School of Medicine, New York, NY, 10029-6574

There is evidence that cyclooxygenase (COX)-2, a key enzyme in prostaglandin biosynthesis, may also control mechanisms of apoptotic cell death in peripheral cells. In this study we explored the developmental expression and regulation of COX-2 mRNA in rat brain during responses to kainic acid (KA)-induced seizures that model select hippocampal neuron death/survival in Alzheimer's disease (AD). We found that COX-2 mRNA is developmentally regulated selectively in the same brain limbic structures where COX-2 is induced by KA-induced seizures. During response to KA-induced seizures in adult rat brain, COX-2 mRNA induction paralleled temporally and overlapped anatomically the appearance of cellular morphological features of apoptosis in subsets of cells of the pyramidal neuron layer of the hippocampal formation, amygdaloid complex and pyriform cortex. However, COX-2 mRNA also showed elevation in neurons of the granule cell layer of the dentate gyrus which are unaffected by KA treatment. Our study indicates that COX-2 is a marker of apoptosis in subsets of neurons of limbic pathways that show degeneration during AD. The involvement of COX-2 in apoptotic neurodegeneration may explain the putative neuroprotective effect of non steroidal anti-inflammatory drugs in AD. This work was supported by NIA grant AG13618 to GMP.

88.13

LOCALIZATION OF COMPLEMENT C5b-9 (MEMBRANE ATTACK COMPLEX) IN DYSTROPHIC NEURITES IN ALZHEIMER'S DISEASE.

W.C. Benzing,* J.R. Wujek, N.F. Veloso and K.R. Brunden. Discovery Research Group, Gliatech Inc., Cleveland, OH 44122.

Production of the terminal complement complex, C5b-9 (membrane-attack complex), may play an important role in the neuropathology of Alzheimer's disease (AD). Clear demonstration of membrane-attack complex (MAC) within senile plaque dystrophic neurites (DN) would suggest complement activation may play an important role in neuritic damage in AD. The presence of MAC in DN, however, has been variably reported. One reason for these disparate findings could be due to fixation differences across studies. Thus, the present investigation assessed whether the immunohistochemical detection of MAC within DN was fixation-sensitive. Frozen samples of AD (n=7) and aged normal control (n=4) frontal cortex or amygdala were cut at 15μm on a cryostat and fixed on slides for 15 min in either 4% paraformaldehyde, acetone, methanol, Carnoy's fixative or Histochoice (Amresco). In addition, temporal pole samples from 3 other AD cases were immersion fixed in 4% paraformaldehyde for 48 hrs and cut at 40 μm on a freezing sledge microtome. All tissue was then immunostained for MAC (Dako clone aE11 and Quidel A239), Aß (4G8 and Sigma A8326), and PHF-tau (AT8, Innogenetics). Both the Dako and Quidel antisera revealed MAC within plaque DN when the tissue was fixed using acetone, methanol or Histochoice. In contrast, only the Quidel antibody revealed MAC immunostaining in the paraformaldehyde-fixed tissue. These results 1) suggest that the reported variablity in detecting MAC within plaque DN may have been due to the choice of antisera and fixation conditions and 2) support the hypothesis that complement activation and consequent MAC formation may lead to the neuritic pathology that is characteristic of AD. Support: Gliatech Inc. and Janssen Pharmaceutica.

88.14

EXPRESSION OF LEUKEMIA INHIBITORY FACTOR (LIF) mRNA IN ALZHEIMER'S DISEASE BRAIN.

R. Lemke‡, R.A. Gadient* and P.H. Patterson. Biology Division, Caltech, Pasadena, CA 91125 and ‡Paul-Flechsig Inst. for Brain Res., Univ.Leipzig, Germany.

The neuropoietic cytokine leukemia inhibitory factor (LIF) is a potent inflammatory mediator, and it is induced in injury models such as peripheral nerve transsection and cortical stab wound. LIF may therefore play an important role in the neurodegenerative disorders that involve an inflammatory component e.g., Alzheimer's disease (AD). We studied LIF mRNA expression in AD and control brains by *in situ* hybridization, paying particular attention to the hippocampus and frontal (A9) and temporal (A22) cortices. In normal brain, LIF mRNA is predominantly expressed in neurons such as hippocampal hilar interneurons, pyramidal cells of hippocampus and cortex and granular cells of the dentate gyrus. Double-labeling with the neuronal markers MAP2 and NF200 confirm this identification. Neuronal LIF mRNA levels are reduced in AD brain as compared to controls. In addition, the staining intensity of MAP2 was strongly diminished in AD, reflecting a significant loss of neurons. A hypothesis currently under investigation is, whether activated glial cells, known to be important in CNS inflammation, may also express LIF mRNA.

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88.15

OXIDATIVE DAMAGE IN AD LEUKOCYTES.

P. Mecocci*, A.Cherubini, R. Cecchetti, U. Senin. Dept. of Gerontology and Geriatrics, Perugia School of Medicine, Perugia (Italy).

Several studies have recently shown the usefulness of 8-hydroxy-2'-deoxyguanosine (8OHdG) as a marker of oxidative stress in DNA. Since the level of this molecule was found to be higher in nuclear and mitochondrial DNA of AD brain, the hypothesis that AD DNA is more sensitive to oxidative stress is conceivable. In this study we measured 8OHdG in leukocytes from 12 patients suffering from AD (mean age 73.3 ± 5.6) and 12 normal subjects (mean age 75.8 ± 6.4). After separation of leukocytes, DNA was extracted and digested to nucleoside level. Analysis was performed by means of HPLC with electrochemical and UV detection to reveal 8OHdG and deoxyguanosine (dG) respectively. Results, expressed as ratio of 8OHdG per 10^5 dG molecules, showed a statistically significant higher level in AD leukocytes compared to controls (4.6 ± 1.4 vs 2.7 ± 0.8; $p<0.001$). Treatment of cells with increasing amount of H_2O_2 slightly increased 8OHdG levels both in AD and control leukocytes but differences were not significant. From these results we may hypothesize that in AD leukocytes DNA is more sensitive to oxidative stress. The reason for this susceptibility is not clear but it could be related to a deficient repair or to a reduced activity of antioxidant systems.

88.16

C3a CONCENTRATIONS IN CSF FROM SUBJECTS WITH ALZHEIMER'S DISEASE, PARKINSON'S DISEASE, AND NORMAL CONTROLS.

P.A. LeWitt*, D.A. Loeffler, C.M. Brickman, P.L. Juneau, M.F. Perry, and N. Pomara, Clin. Neurosci. Pgm., Sinai Hosp., Detroit, MI 48235, and Div. Geriatric Psych., Nathan S. Kline Inst. for Psychiatric Res., Orangeburg, NY 10962

Complement protein C3a concentrations were measured C3a in CSF from patients with probable AD (n = 19), Parkinson's disease (PD) (n = 5), normal aged controls (n = 9), and normal young controls (n = 10). AD and aged normal C3a concentrations (means ± SE: 365.93 ± 40.47 and 427.57 ± 92.28 ng/ml, respectively) significantly increased vs. young controls (102.24 ± 18.24). C3a in PD CSF also increased two-fold vs. young controls (226.59 ± 51.12). These concentrations are similar to C3a levels in serum, in which C3a also increases with age. While C3a content in CSF may, in part, reflect serum levels (due to the low MW [9 kd] of C3a), similar C3a concentrations in CSF and blood suggest the possibility of significant local synthesis as well. Conclusion: CSF C3a appears to increase with age, but does not provide a marker for AD. (Supported by National Parkinson Foundation and Mental Illness Research Association)

88.17

A NFκB/REL SITE IN THE APP GENE REGULATORY REGION IS RESPONSIVE TO INTERLEUKIN-1 AND GLUTAMATE. M. Grilli*, F. Goffi, M. Memo & P.F. Spano. Div. Pharm., Dept. Biom. Sci. & Biotec., Un. of Brescia, Medical School, 25124 Brescia, Italy.

We originally reported that members of the family of transcription factors NF-κB/Rel can specifically recognize two identical sequences, referred to as APPκB sites, which are present in the 5' regulatory region of the APP gene (Grilli et al., JBC, 270, 26774-26777,1995). Now we show that the APPκB sites interact specifically with a complex which contains one of the subunit of the family, the so called p50 protein, and that they behave as positive modulators of gene transcription in cells of neural origin. Additionally, the nuclear complex specifically binding to the APPκB sites is constitutively expressed in primary neurons from rat cerebellum and is upregulated in response to the inflammatory cytokine Interleukin-1β (IL-1β) and the excitatory aminoacid glutamate. We believe that these evidences are potentially relevant for understanding the neuropathology associated with Alzheimer's Disease (AD). Although distinct, two of the major pathogenetic pathways which are likely to contribute to the neuropathology associated with AD (inflammatory cytokines and excitatory aminoacids), share a common step represented by the activation of a NF-κB/Rel activity.

The work was partially supported by grants from Regione Lombardia and Centro Nazionale delle Ricerche (CNR)

PARKINSON'S DISEASE: PHARMACOLOGY AND THERAPY

89.1

COMPENSATORY EFFECTS OF GLUTAMATERGIC INPUTS TO THE SNc IN MPTP-TREATED MONKEYS C.E. Gross*, E. Bezard, T. Boraud and B. Bioulac CNRS UMR 5543, Université de Bordeaux II, 33076 Bordeaux Cedex France.

It is well known that Parkinson's disease results from a progressive loss of dopaminergic neurons, but that initial symptoms are only slightly perceptible due to compensatory effects. The substantia nigra pars compacta (SNc) receives glutamatergic inputs from several structures. These inputs may play an important role in this compensation.

In order to test this hypothesis, we injected kynurenic acid (10 µl at 5µl/min; broad spectrum glutamatergic antagonist) through an injection cannula into the SNc of two monkeys rendered parkinsonian by the daily injections of low doses of MPTP (i.v., 0.2 mg/kg/day) until the first clinical signs of the disease appeared. This method allowed us to obtain progressive SNc neuronal death (10-15 days). The effects of this temporary daily blockage of glutamatergic inputs were scored on a clinical rating scale.

Until the first appearance of irreversible clinical signs (1< score <5), the injections of kynurenic acid did not provoke any motor abnormalities. Between the first clinical signs and full parkinsonism (score > 20), however, this injections drastically increased parkinsonism.

These results would confirm the postulate that SNc afferent glutamatergic pathways play an essential role in compensation. What must now be elucidated is the relative influence of each glutamatergic input to the SNc.

CNRS, FRM

89.2

IN SITU HYBRIDIZATION OF PREPRODYNORPHIN mRNA IN THE CAUDATE-PUTAMEN OF MPTP MONKEYS: EFFECT OF CHRONIC TREATMENT WITH L-DOPA AND WITH THE D2 AGONIST U91356A. M. Goulet[1-2]*, M. Morissette[4], P.Falardeau[1-3], P.J. Bédard[4] and T. Di Paolo[1-2]. [1]Sch. of Pharmacy, Laval Univ., and [2]Dept of Mol. Endo. CHUL, Québec, G1V 4G2, [3]Centre de recherche du CHUL, Québec, G1V 4G2, [4]Dept of Pharmacology, Fac. of Medecine, Laval Univ., Québec, G1K 7P4, and Neurobiology Res. Center, Enfant-Jésus Hospital; CANADA.

Dopamine (DA) regulation of the relative levels of preprodynorphin (PPD) mRNA in the striatum of monkeys was analyzed by *in situ* hybridization histochemistry using a specific monkey cDNA. DA exerts its postsynaptic actions through interaction with two major populations of neurons, pallidal(GPe)- and nigral(GPi)-projecting neurons showing a segregation of peptide and receptor expression, in that the majority of the former express D2 receptor and enkephalin, whereas the majority of the latter express D1 receptor, dynorphin and substance P. Susceptibility to develop dyskinesias may be related to relative changes of pallidal- versus nigral-stimulation. *Macaca fascicularis* monkeys were rendered parkinsonian with administration of the toxin MPTP. Three of these animals received pulsatile administration of L-DOPA whereas U91356A was administered using a pulsatile (n=3) or continuous (n=3) schedule for one month. Untreated MPTP (n=4) as well as naive control (n=3) animals were also studied. Animals treated in a pulsatile mode with U91356A showed progressive sensitization and had dyskinesias whereas those receiving U91356A continuously developed behavioral tolerance and no dyskinesia. PPD mRNA was slightly increased by MPTP treatment when compared to control animals. L-DOPA treatment did not correct the MPTP induced increase PPD mRNA, whereas U91356A whether continuous or pulsatile corrected the lesion induced change. The failure of L-DOPA to alter levels of PPD mRNA compared to U91356A treatments reflects important D1 excitatory tone. The mode of chronic U91356A administration (continuous vs. pulsatile) did not affect differently PPD mRNA expression. Therefore, the different behavioral outcome observed in these animals is not directly related with different PPD mRNA expression. (Parkinson Foundation)

89.3

REGULATION BY CHRONIC TREATMENT WITH THE LONG-ACTING DOPAMINE D2-LIKE AGONIST CABERGOLINE OF DOPAMINE D2 mRNA AND RECEPTOR LEVELS IN STRIATUM OF PARKINSONIAN DRUG-NAIVE MONKEYS. M. Morissette[4], M. Goulet[1-2], R. Grondin[4], P. Falardeau[1-3], D. Levesque[2-4]*, P.J. Bédard[4] and T. Di Paolo[1-2]. [1]Sch. of Pharmacy, Laval Univ., and [2]Dept of Molecular Endocrinology CHUL, Québec, G1V 4G2, [3]Centre de recherche du CHUL, Québec, G1V 4G2, [4]Dept of Pharmacology, Faculty of Medecine, Laval Univ., Québec, G1K 7P4, and Neurobiology Res. Center, Enfant-Jésus Hospital; CANADA.

The effects of chronic continuous stimulation of D2-like receptor with the long-acting D2-like dopamine agonist, cabergoline, on D2 mRNA and receptor levels in the striatum were examined in drug-naive MPTP parkinsonian monkeys. Cabergoline was administered s.c. at 0.25 mg/Kg every 48 h during a month to 3 drug-naive ovariectomized female *macaca fascicularis* monkeys rendered parkinsonian by MPTP. Untreated MPTP as well as naive control animals were also studied. The parkinsonian features in response to cabergoline were initially greatly improved, but decreased somewhat thereafter. However, a good relief of parkinsonian features was maintained until the end of the study. We also observed in the first days, transient dyskinesias in 2/3 monkeys. After a drug washout period of 3 days, the cabergoline-treated MPTP monkeys returned to their initial akinetic state. Density of D2 antagonist sites was examined by autoradiography of [^{3}H]Spiperone and *in situ* hybridization histochemistry was performed using a human specific cDNA for D2 mRNA levels. A significant increase in D2 receptor density in the caudal putamen (+20%, $p<0.05$) was accompagnied by an increase in D2 mRNA (+12% in medial caudal putamen, $p<0.05$) in MPTP-monkeys compared to control animals. Cabergoline treatment significantly decreased by half striatal D2 receptor density versus untreated MPTP monkeys. However, no change in D2 mRNA in middle and caudal striatum was observed, and only a slight tendency to decrease was measured in rostral striatum. The present results suggest that the cabergoline-induced D2 receptor decrease could occur via an increased receptor internalisation or degradation. Hence, the cabergoline-induced D2 receptor decrease may be implicated in behavioral partial tolerance, but our results do not support its decrease via alteration of D2 mRNA. (Parkinson Foundation)

89.4

β-CARBOLINE INDUCED NIGROSTRIATAL NEUROTOXICITY VIA N-METHYLATION IN C57BLACK MICE. K. Matsubara*, Y. Kobayashi, T. Gonda, T. Idzu and K. Kimura Neurosci. & Sense Organs, Shimane Med. Univ., Izumo 693, Japan

Several classes of heterocyclic molecules structurally related to MPTP have been advanced as possible natural toxins underlying the nigrostriatal degeneration. Indoleamine-related β-carbolines (βCs) are structural analogs of MPTP. Simple-βC, 2-methylated βC and 9-methylated βC were administered intraperitoneally twice per day for 7 days to C57/Black mice. Motor function was evaluated 48 h after the last injection. The mice were decapitated 72 h after the last injection, and catechol- and indole-amines were analyzed in various brain regions. Brain concentrations of βCs were evaluated using in vivo microdialysis technique. Both sub-chronic treatments of simple-βC and 2-methylated βC induced movement disorder and reduced dopamine content in the nigra and striatum regions. Although 2-methylated βC is a cationgenic form, a part of that administered penetrated blood-brain-barrier into brain possibly due to forming neutral base. 9-Methylated βC potently induced movement dysfunction, but reduction of dopamine and its metabolites was not specific to nigrostriatal pathway. Also, 9-methyl βC reduced 5-HT and 5-HIAA in many regions. Since simple- and 2-methylated βCs themselves are very weak toxins, 2,9-dimethylated βC formed in the brain would be an active neurotoxic metabolite of βCs. The 2,9-dimethylated βC mirrors to MPP^+, that is an active metabolite of MPTP. From the present results, simple-βC would be a neurotoxin precursor undertaken methylation of 2[β] and 9[indole] nitrogens of its structure sequentially. The 2,9-dimethylated βC lost selective toxicity to nigrostriatal neurons at relatively higher concentrations, however.

89.5

β-CARBOLINE-N-METHYLTRANSFERASE ACTIVITY IN HUMAN BRAIN TISSUE FROM CONTROL AND PARKINSON'S DISEASE (PD) SUBJECTS. D.A. Gearhart*, E.J. Neafsey, and M.A. Collins. Neuroscience Program and Departments of Cell Biology, Neurobiology & Anatomy; and Molecular and Cellular Biochemistry, Loyola University Medical Center, Maywood, IL 60153.

Brain N-methyltransferases catalyze the N-methylation of β-carbolines at the 2N- (pyrido) and 9N- (indole) nitrogens; resultant compounds are structural and functional analogs of the parkinsonian-producing neurotoxin, MPP^+. We have hypothesized that N-methylated β-carbolinium compounds may contribute to the neuropathogenesis of PD. We measured human brain 2N- and 9N-methyltransferase activity (reported as mean pmoles/hr/mg protein ± SEM) in the pellet and supernatant following centrifugation at 10,000g for 30 minutes.

	SUBSTANTIA NIGRA			
	2-Methylation Activity		9-Methylation Activity	
Group (n)	Pellet	Supernat.	Pellet	Supernat.
Control (5)	14.5±10.1	34.4±12.7	12.5±8.3	19.0±8.0
PD (6)	6.1±2.8	39.7±18.0	5.4±2.4	15.1±5.7
	PUTAMEN			
Control (7)	2.0±0.8	16.1±9.2	5.1±1.7	14.9±7.4
PD (7)	3.9±1.2	15.4±5.3	4.1±1.6	14.2±5.4
	FRONTAL CORTEX			
Control (8)	6.8±1.8	13.8±4.5	8.0±1.4	12.5±4.0
PD (7)	8.9±1.2	17.4±3.5	11.1±1.0	*39.0±7.9

The data confirm the presence in human brain of 2N-methylating activity and demonstrate for the first time indole 9N-methylating activity. Specific activity was generally higher in the supernatant, implying cytosolic localization. Levels were similar in Control and PD brains, except that 9N-methylation activity was significantly elevated in frontal cortex in PD (* $p < 0.02$). (NIH NS23891)

89.6

RIBOZYME-MEDIATED DOWN REGULATION OF EXPRESSION OF TYROSINE HYDROXYLASE AND DOPAMINE TRANSPORTER GENES. *Leonidas A Phylactou*, Matthew JA Wood. Department of Human Anatomy, Oxford University, Oxford OX1 3QX, UK.*

Hammerhead ribozymes are enzymes comprised of RNA that catalyse site-specific RNA cleavage in a base-specific way. They are at present being developed for gene therapy and protein function studies as inhibitors of gene expression. Tyrosine hydroxylase (TH) and dopamine transporter (DAT) are key molecules for the synthesis and regulation of neurotransmission of dopamine (DA). DA levels are found to be reduced in patients with Parkinson's disease (PD).

Hammerhead ribozymes have been designed and synthesised for the specific inhibition of production of tyrosine hydroxylase (TH) and dopamine transporter (DAT). The cleaving ability of ribozymes was tested in the presence of their targets *in vitro*. Both ribozymes were cloned into a plasmid containing a prokaryotic promoter. Similarly, inactive ribozymes, lacking catalytic activity were also constructed. Production of ribozymes was carried out by *in vitro* transcription using the T7 promoter. The target RNA was synthesised form total RNA extracted from rat brain followed by two rounds of amplification to introduce a T7 promoter. Each labelled RNA target was incubated with its specific ribozyme at both 50°C and 37°C in a neutral pH environment and in the presence of magnesium ions. Both ribozymes were shown to successfully cleave their targets. On the other hand, no cleavage products were detected with the inactive ribozymes. Adenoviruses will be the means of ribozyme delivery inside rat progenital adrenal cells and animals. Preliminary results show that adenoviruses can efficiently transport reporter genes into the *substantia nigra* when injected into the caudate nucleus of rat brains.

The above results suggest that hammerhead ribozymes can be used for the specific inhibition of TH and DAT production in the brain indicating their potential for therapeutic application in PD. (supported by MRC ROPA grant)

89.7

LONG-TERM POTENTIATION OF THE DOPAMINE-INDUCED RESPONSES IN RAT MIDBRAIN DOPAMINERGIC CELLS. N. B. Mercuri*, M. Scarponi, A. Bonci , and G. Bernardi.Clinica Neurologica, Università di Roma "Tor Vergata", 00173 Rome, Italy and IRCCS S.Lucia, 00179 Rome, Italy.

The inhibition of monoamine oxidase (MAO) A and B potentiated the depression of the firing rate and the hyperpolarization of the membrane potential caused by dopamine (DA) (10 - 30 μM) on rat midbrain dopaminergic cells, intracellularly recorded in vitro. The inhibition of MAO enzymes was obtained by superfusing the slices with pargyline (10 - 100 μM) for 20 - 120 min. The cellular responses to DA, which were due to the activation of somato-dendritic D2/3 autoreceptors, were prolonged and did not completely wash after the pharmacological blockade of both types (A and B) of MAO. On the contrary, the amphetamine-induced inhibition and hyperpolarization of the cells were slight prolonged by MAO inhibition. The actions of amphetamine on the dopaminergic cells were were due to an increased release of dopamine in the extracellular space. The present data suggest that the inhibition of MAO activity in the brain causes a long-term potentiation of the responses induced by exogenous DA but not of those induced by amphetamine-released DA. This increased DA action could suggest a therapeutic approach that use MAO inhibitors and DA precursors in DA-deficient psychiatric disorders and in Parkinson's disease.

Funding source: IRCCS S.Lucia, via Ardeatina 306, 00179 Rome, Italy.

89.8

THE NICOTINIC RECEPTOR AGONIST SIB-1508Y POTENTIATES L-DOPA RESPONSES IN PARKINSONIAN MONKEYS. A. Pope-Coleman*, G. Kenneth Lloyd[1] and J.S. Schneider. Depts. of Anat. and Neurobiol. and Neurology, MCP and Hahnemann University, Philadelphia, PA 19102 and [1]SIBIA Neurosciences, Inc. La Jolla, CA 92037

Neuronal nicotinic acetylcholine receptor (NAChR) agonists may be useful for the treatment of Parkinson's disease (PD) and may protect nigrostriatal dopamine (DA) neurons from degeneration. There have been reports on the usefulness of nicotine as a therapeutic agent in PD patients; nicotine may effect PD symptoms by enhancing DA function in striatal and limbic areas and ACh function in the hippocampus, thalamus and frontal cortex. SIB-1508Y is a subtype-selective NAChR agonist which releases DA *in vitro* and *in vivo* and exhibits activity in rodent models of PD (Soc. Neurosci. Abstr., 1995; 21:11). In the present study, SIB-1508Y was evaluated on parkinsonian symptoms and L-DOPA responses in monkeys. Two cynomolgus monkeys, previously made parkinsonian by chronic administration of MPTP, were evaluated with a behavioral/motor rating scale and a timed motor test that involved retrieval of food pellets from 16 wells recessed in a Plexiglass board. SIB-1508Y, at doses of 8-12 mg/kg p.o., or at 2 mg/kg i.m. caused mild improvement in Parkinsonian symptoms in both monkeys. Both animals responded well to L-DOPA, beginning at doses of 15 mg/kg i.m. The combination of an ineffective dose of SIB-1508Y (1 mg/kg, i.m.) and L-DOPA caused a significant shift of the L-DOPA dose response curve. Significant anti-Parkinson effects were observed with L-DOPA doses as low as 5.0 mg/kg, when administered together with SIB-1508Y. There was no additional improvement in motor ratings, but enhanced reward retrieval performance when SIB 1508Y was added to maximally effective doses of L-DOPA. These data show the NAChR agonist SIB-1508Y to have mild anti-parkinsonian effects as monotherapy and to significantly potentiate the therapeutic responses to L-DOPA in Parkinsonian monkeys. Thus, NAChR agonists may play a significant role in the future therapy of PD. Supported by SIBIA Neurosciences, Inc.

89.9

BRAIN [18F]6-FLUORO-L-M-TYROSINE METABOLISM STUDIED BY IN VIVO MICRODIALYSIS. S. Jordan*, J.L. Eberling, K. Bankiewicz, W.J. Jagust. Center for Functional Imaging, Lawrence Berkeley Laboratory, University of California, Berkeley, CA 94720: Somatix Therapy Corp. Alameda, CA 94501.

[18F]6-fluoro-L-m-tyrosine (FMT) is a Positron Emission Tomography (PET) tracer of central dopa decarboxylase activity. The FMT signal is preferentially retained in dopaminergic brain structures primarily in the form of its major metabolite [18F]6-fluoro-3-hydroxyphenylacetic acid (FPAC). The mechanism of intraneuronal FMT signal retention is currently uncertain. However, FPAC binds with high affinity to the vesicular amine transporter which suggests its possible uptake and capture inside presynaptic cytoplasmic vesicles. In vivo brain microdialysis was used to monitor striatal FPAC accumulation in control and reserpinized rats injected with FMT (25 mg/kg, i.v) and then 120 min later with D-amphetamine (2.5 mg/kg, i.p). Dialysate samples were collected every 20 min for two hours after FMT injection and for one hour after D-amphetamine injection and they were analyzed for their FPAC content by HPLC with electrochemical detection. In both control and reserpinized rats FPAC levels were highest during the first 20 min sampling period after which they remained almost stable prior to the D-amphetamine injection which produced a small but significant rise in striatal FPAC in the control animals only. This D-amphetamine stimulated release of FPAC from a reserpine sensitive pool suggests the FMT signal in PET is associated, at least in part, with presynaptic dopaminergic vesicles. Furthermore, these data support the recent hypothesis that D-amphetamine stimulates dopamine release by interacting with vesicular as well as cytoplasmic dopamine stores.

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89.10

U95666A IS A HIGH INTRINSIC ACTIVITY AGONIST WITH SELECTIVITY FOR THE D2 DOPAMINE RECEPTOR. M.E.Lajiness, C.L.Chio, P.J.K.D. Schreur* and R.M.Huff, CNS Diseases Research, Pharmacia & Upjohn, Kalamazoo, MI 49001

Parkinson's Disease is characterized by progressive degeneration of dopaminergic neurons. Levodopa is currently the most effective therapy for controlling symptoms of the disease. However, long term treatment leads to loss of efficacy and serious side effects. Direct acting dopamine receptor agonists used in addition to or instead of L-DOPA cause a high incidence of psychiatric side effects and have limited efficacy compared to L-DOPA. The discoveries of three related but distinct members of the D2-like dopamine receptor family, (D_2, D_3 and D_4), provided an opportunity to develop dopamine agonists with selectivity for only one of the subtypes, which may enhance efficacy and lessen side effects. U95666A was found to have high selectivity for D_2 receptors over all other dopamine receptor subtypes. In several D_2 receptor expressing- CHO cell-based assays U95666A was a potent, highly efficacious agonist. U95666A does not activate D_3 or D_4 receptors, consistent with the selectivity determined in radioligand binding studies. The EC_{50} values for U95666A stimulation of various second messengers range between 17±4.8 nM in inhibition of forskolin-stimulated cAMP accumulation to 112±34 nM in potentiation of arachidonic acid release. U95666A stimulated mitogenesis with an EC_{50} of 32±6.4 nM, and stimulated extracellular acidification with an EC_{50} of 103±8.7 nM. In all measurements, U95666A had a high level of intrinsic activity. These results indicate that U95666A is a high intrinsic activity agonist with selectivity for D_2 dopamine receptors. Acknowledgements to M.W.Smith, C.B.S., Pharmacia and Upjohn, for receptor binding data.

89.11

A NOVEL THERAPY FOR PARKINSON'S DISEASE USING A GENETICALLY-MODIFIED HUMAN ASTROCYTE LINE. Gal Yadid*, Naomie Fitoussi, Eugene Major# and Carlo Tornatore#, Dept. of Life Science, Bar Ilan University ISRAEL and #Molecular Therapeutic section NINDS, USA.

Parkinson's disease is caused by degeneration of nigrostriatal dopaminergic neurons. A potential therapeutic approach for treating PD that utilizes a rat model for the disease and transplantation of a genetically-modified immortalized astrocyte cells (SVG-TH) that stably expresses tyrosine hydroxylase (TH) has being examined. SVG-TH cells were grafted into the striatum of 6-hydroxydopamine-lesioned (6OH-DA) rats and the *in situ* production of l-dopa, dopamine (DA) and its metabolites, dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), determined by using a microdialysis technique coupled to a HPLC apparatus. Our result indicate that DA levels in extracellular space of 6OH-DA rats were only 3% of those observed in normal animals. Upon transplantation of the SVG-TH cells into the striatum of the parkinsonian rats, the levels of DA reached those of the normal rats. This recovery appear to correlate with a significant improvement of their behavioral deficit. The levels of DA metabolites in parkinsonian rats were low even after transplantation of the SVG-TH cells probably because of the damage to dopaminergic neurons integrity. No change in 5-hydroxyindolacetic acid (5-HIAA), the main serotonin metabolite, levels between all treatment groups indicate that we selectively lesioned the dopaminergic system. Application of NSD-1015 via the microdialysis probe enabled us to examine TH activity in vivo. Experiments using SVG-TH cells as a xenograft drug delivery system may evaluate the therapeutic potential for PD of this unique method for *in situ* supply of l-dopa on a behavioral, cellular, and molecular level.

This study was supported by the National Parkinson's Foundation to G.Y.

89.12

FUNCTIONAL AND ANATOMICAL RECOVERY OF THE NIGROSTRIATAL CIRCUITRY BY CONCURRENT INTRASTRIATAL AND INTRANIGRAL GRAFTS OF FETAL NIGRAL CELLS. M. Hong* and I. Mendez. Neural Transplantation Laboratory, Department of Neurosurgery, Faculty of Medicine, Dalhousie University, Halifax, Nova Scotia, Canada B3H 4H7

It is well known that grafts of fetal nigral tissue survive and grow in the dopamine (DA) depleted adult striatum. Although such grafts produce reversal of behavioural and biochemical deficits in the rat model of Parkinson's disease (PD), little is known about the restoration of the nigrostriatal circuitry following transplantation. To further study the functional and anatomical recovery produced by neural transplantation, we have employed a novel micrografting technique to stereotaxically implant fetal (E14) nigral dopaminergic cell suspensions into both the striatum and substantia nigra of female Wistar rats (200-225 g) bearing unilateral 6-hydroxydopamine (6-OHDA) lesions of the nigrostriatal dopaminergic pathway. Analysis of functional recovery was carried out using a video activity monitor to assess animal locomotor activity and analysis of anatomical recovery was made using tyrosine hydroxylase (TH) immunohistochemistry. A pre-graft challenge with amphetamine (5 mg/kg i.p.) induced asymmetric turning behaviour towards the lesioned side: 566.2 ± 84.5 ipsiversive rotations vs 0.7 ± 0.4 contraversive rotations over a 60 minute observation period (mean ± SEM, n=6). In contrast, a post-graft challenge at 6 weeks with amphetamine failed to induce asymmetric turning behaviour. Immunohistochemical analysis using an antibody against TH revealed a large number of TH-positive cell bodies in the grafts both in the striatum and substantia nigra. A dense network of dopaminergic processes were also found within the grafts and a large number of axons projected from the nigral graft toward the striatal graft and viscera resulting in recontruction of the nigrostriatal pathway. This study for the first time demonstrates both functional and anatomical restoration of the nigrostriatal pathway by concurrent fetal nigral grafts in the striatum and nigra in the 6-OHDA rat model of PD.

[Supported by The Parkinson Foundation of Canada]

89.13

MESENCEPHALIC DOPAMINERGIC NEURONAL CELL LINES FROM TRANSGENIC MICE: NEUROTROPHIC REGULATION AND *IN VITRO* MODEL OF PARKINSON'S DISEASE. J.H. SON*, S.H. CHO, B. CONTI, D.H. PARK, J.W. JAHNG, C. TINTI, C.H. PENG and T.H. JOH. Laboratory of Molecular Neurobiology, Cornell University Medical College at the W.M. Burke Medical Research Institute, White Plains, N.Y. 10605, U.S.A.

The potential implication of neurotrophic factors (NTF) in the etiology of Parkinson's disease (PD) has suggested their use as therapeutic agents in PD, where selective substantia nigra (SN) dopamine (DA) neuronal death occurs. Rational therapy requires detailed understanding of the functional role(s) of specific NTF in both the normal- and patho-physiology of the SN-DA cells. NTF has been tested primarily as to whether they can promote the survival and differentiation of DA neurons in mixed cultures of ventral mesencephalon derived from E15 - E16 rat embryos. The culture studies have often resulted in the confounding data primarily due to the paucity of DA cells in heterogeneous fetal mesencephalic cultures. In addition, the short survival rate and an inability of genetic manipulation have been major obstacles for the mechanistic study of the NTF. Therefore, we have developed *in vitro* model systems: A culture system of conditionally immortalized homogeneous DA neuronal cells lines produced from mesencephalic SN. Cell lines were established from transgenic mice expressing thermolabile SV40-TagtsA58 in DA neurons using our central catecholamine neuron-specific 9 kb promoter region of the tyrosine hydroxylase (TH) gene (Mol. Brain Res. 27, 281 '94). Our SN-DA cell lines showed distinct DA phenotype: expression of TH and AADC enzymes for DA synthesis and target-specific neurite projection to striatum, but not to cortex in explant cultures. Moreover, NTF such as BDNF and GDNF retarded apoptotic SN-DA cell death during differentiation at nonpermissive temperature and enhanced cell survival after MPP^+ insult. (Supported in part by NIH grant MH24285)

89.14

SHORT-TERM SURVIVAL OF NEONATAL SUBVENTRICULAR ZONE PROGENITOR CELLS TRANSPLANTED INTO THE STRIATUM OF ADULT RATS. R. Betarbet*[1,2], T. Zigova[1], R.A.E. Bakay[2], R.M. Lindsay[3], S.J.Wiegand[3] and M.B. Luskin[1]. [1]Depts. of Anatomy and Cell Biol. & [2]Neurosurg., Emory Univ. Sch. of Med., Atlanta, GA 30322 and [3]Regeneron Pharmaceuticals, Inc. Tarrytown. NY 10591.

We have previously shown that the anterior part of the neonatal subventricular zone (SVZa) generates cells that migrate to the olfactory bulb and differentiate into GABAergic and dopaminergic neurons. The SVZa cells, when transplanted into the neonatal striatum, retain their neuronal phenotype as well as their ability to migrate (*Cell Transplantation* **5**:165, 1996). In this study we examined the short-term behavior and phenotype of dissociated, BrdU-labeled SVZa cells transplanted stereotaxically into the striatum of adult rats. Three days after transplantation most SVZa cells were immunoreactive for TuJ1, an antibody which recognizes neuron-specific class III β-tubulin. Within the adult striatum only the transplanted SVZa cells stained intensely for TuJ1. TuJ1(+) cells were also identified within 50 - 250 μm of the transplant, suggesting that these cells had migrated from their site of implantation. Very few of the transplanted cells were GFAP(+). However, the transplant contained numerous GABA(+) cells, as well as a few tyrosine hydroxylase(+) cells. The ability of the SVZa cells to disperse and differentiate into neurons following transplantion into an adult striatum makes them potential candidates for cell replacement therapy in several neurodegenerative disorders. Supported by NIH, National Parkinson Foundation and Regeneron Pharmaceuticals, Inc.

89.15

HIBERNATED MESENCEPHALIC NEURONS UNDERGO APOPTOSIS-INDUCED CELL DEATH; REVERSAL WITH TROPHIC FACTORS, P.M. Carvey,*P. Thajeb, Z.D. Ling and E.D. Potter. Neuropharmacology Research Laboratories, Rush Medical School, Chicago, IL 60612

We have previously demonstrated that harvested fetal rostral mesencephalic tegmentum (RMT) cells remain viable for up to 5 days *in vitro* in a high K^+ hibernation media (HM) and that the survival rate of tyrosine hydroxylase immunoreactive (THir) neurons can be enhanced by supplementing the HM with trophic factors. We have also shown that several factors including pH, storage density, temperature and method of dissociation dramatically influence survival. We now show that apoptosis-induced cell death initiated during hibernation is a major cause of reduced survival. E15 rat RMT was harvested and dissociated using mechanical trituration and stored at 4°C (pH 7.4) at a density of 1 million cells/ml HM. Using these optimal parameters, the number of apoptotic figures (Klenow technique) was determined 6, 24 and 48 hours following storage in HM alone, or HM supplemented with 8% human placental cord serum (HPCS), GDNF (10 ug/ml), or BDNF (10 ug/ml). Apoptotic cell counts were 33% in HM, 28% in BDNF, 19% in HPCS and 16% in GDNF supplemented media. Interestingly, the apoptotic rates were highest at 6 hours but did not change dramatically thereafter. Although HPCS and GDNF significantly reduced the apoptotic rate, all three trophic supplements yielded similar THir neuron survival rates when the hibernated cells were cultured for 72 hours. These results suggest that hibernation conditions dramatically influence cell survival. Moreover, it appears that apoptosis is initiated soon after the cells are harvested and can be prevented by supplementing the media with trophic factors. By reducing apoptosis during hibernation, the survival rate of transplanted DA neurons might be increased. (Supported by NINDS 1RO1NS33174, GDNF supplied by AMGEN, Inc.)

89.16

CYTOKINES INDUCE TRANSFORMATION OF MESENCEPHALIC PROGENITOR CELLS TO DA NEURONS E.D. Potter*, Z.D. Ling and P.M. Carvey, Neuropharmacology Res. Labs., Rush Med. School, Chicago, IL 60612

We previously demonstrated that progenitor cells isolated from fetal rostral mesencephalic tegmentum (RMT) can be maintained indefinitely in proliferation media with EGF and converted to DA neurons when co-cultured with freshly harvested RMT. The factor(s) required for this conversion remain unknown. Various cytokines have recently been shown to influence the development of neurons so we examined several different cytokines for their ability to convert progenitor cells to tyrosine hydroxylase immunoreactive (THir) cells. Interleukin-1 (IL-1) α and β both induced the conversion of progenitor cells to the THir phenotype in a dose-dependent fashion whereas 13 other cytokines studied were without effect. This conversion only occurred when the progenitor cells were co-cultured with striatal cells suggesting that extracellular matrix proteins are required for the conversion process. The number of progenitor cells converted to THir cells could be further increased when a cocktail containing GDNF, leukemia inhibitory factor (LIF), interleukin 11 (IL-11) and IL-1α were added. This cytokine cocktail not only yielded a progenitor cell transformation rate exceeding 20%, but also produced cells whose morphology was similar to typical DA neurons. Interestingly, the gp130 protein is involved in both IL-11 and LIF signaling while the intracellular domain of the IL-1 receptor is homologous with the gp130 protein suggesting the involvement of gp130 signal transduction cascade in the phenotypic conversion process. These results suggest that cytokines may be involved in the conversion of progenitor cells to DA neurons. Success in this regard would yield a DA enriched source of cells for laboratory study as well as in transplantation for Parkinson's disease. (Supported by the Blowitz-Ridgeway Foundation, GDNF supplied by AMGEN, Inc.).

89.17

PRAMIPEXOLE INCREASES MESENCEPHALIC-DERIVED TROPHIC ACTIVITY IN TISSUE CULTURE AND IN VIVO. Z.D. Ling* and P.M. Carvey. Rush Medical School, Chicago, IL 60612

We previously demonstrated that pramipexole (PPX) prevented the levodopa-induced loss of dopamine (DA) neurons in rostral mesencephalic tegmentum (RMT) cultures. In addition, defined media from PPX-treated RMT cultures enhanced the growth of freshly-harvested RMT while conditioned-media from parietal cultures incubated with PPX was without effect. We hypothesized that PPX's neuroprotectant effect against levodopa toxicity could be due to increased production of a RMT-derived trophic activity since the effect was heat-labile. We have now examined this phenomenon *in vivo*. Rats (8/group) were treated with 0.3 or 3.0 mg/kg PPX, 0.5 mg/kg pergolide (PERG) or vehicle, daily for 24 days. After 4 drug-free days the animals were sacrificed. The brains were mounted on a cryostat and 800 μ coronal sections were taken through the mesencephalon. The ventral mesencephalons (nigra and VTA) were dissected from the frozen sections, homogenized in HBSS and centrifuged at 100,000g. A section of the cerebellum was processed similarly. 100 μL of the extracts were added to RMT cultures with defined media. After 72 hours, the cultures were fixed and stained and the number of tyrosine hydroxylase immunoreactive (THir) cells was assessed. Extracts from animals treated with PPX increased the number of THir neurons in a dose-dependent fashion ($F=5.67$; $p < 0.001$) while PERG was without effect. RMTs in cerebellar extracts were unaffected by drug treatment and exhibited reduced growth relative to RMTs incubated in mesencephalic extracts. These data suggest that a trophic activity capable of supporting the growth of DA neurons is normally present in the adult rat and that PPX increases its production. PPX may increase the production of this activity as a result of its full intrinsic activity at D_2 receptors or as a result of its preferential affinity for D_3 receptors. (Supported by Pharmacia & Upjohn)

89.18

THE DOPAMINE AGONIST PRAMIPEXOLE BLOCKS THE OXIDATIVE TOXICITY OF L-DOPA IN CULTURE. J.S.Althaus, G.J.Fici and P.F. VonVoigtlander*. CNS Diseases Research, Pharmacia & Upjohn, Inc., Kalamazoo, MI 49001.

L-dopa is a standard treatment for Parkinson's disease (PD). Unfortunately, autoxidation of L-dopa has the potential to contribute to neuronal loss in PD. This raises questions about the long-term therapeutic benefits of L-dopa. Pramipexole (PPx) is a potent and selective dopamine D_2/D_3 agonist recently submitted to the FDA as a treatment for PD. We found that PPx could oxidize within a biologically relevant range and may possibly act as an antioxidant as well as a dopamine agonist. We developed a cellular model of L-dopa toxicity and tested PPx's effects on this toxicity. L-dopa decreased cerebellar granule cell viability with a TC_{50} of approximately 50 μM after 24 hrs. The mechanism of L-dopa toxicity appears to be based on the generation of superoxide and/or hydrogen peroxide because SOD and catalase were completely protective. PPx and its inactive receptor binding enantiomer blocked L-dopa toxicity equally with an EC_{50} of approximately 1 μM and 70% protection at 10 μM. PPx at 10 μM also decreased the rate of cellular glutathione depletion observed with L-dopa incubation. PPx neuroprotection seen in this cellular model of oxidative stress seems to be independent of dopamine receptor binding and could be due to antioxidant properties of the drug.

89.19

DOPAMINE D_2 AND D_3 RECEPTOR SUBTYPES MEDIATE EXCITATORY AND INHIBITORY RESPONSES RESPECTIVELY IN RAT SNPR. W.E.Hoffmann and M.F.Piercey*, Pharmacia & Upjohn, Kalamazoo, MI 49001.

Postsynaptic effects of dopamine (DA) agonists in caudate nucleus can be monitored in substantia nigra pars reticulata (SNPR), a major output for striatal efferent pathways. The effects of D_2 and D_3 agonists and antagonists were evaluated for effects on SNPR neuronal firing rates, using standard microelectrode techniques for extracellular recordings. The majority of SNPR cells responding to 0.3 to 3 mg/kg i.v. U-91356A, a D_2-preferring DA agonist (Piercey *et al.*, Clin. Neuropharmacol. 18(1):S34-S42, 1995), were stimulated (28 of 43 neurons). Haloperidol (0.1-0.3 mg/kg i.v.), a D_2 antagonist (Sokoloff *et al.*, Nature 347:146, 1990), was able to completely reverse U-91356's stimulatory effects on SNPR neuronal firing rates (ED_{50} for antagonism = 183 μg/kg). Conversely, 1-3 mg/kg i.v. U-99194A, a D_3 antagonist (Waters *et al.*, J. Neural Transm. 94:11, 1993) tended to accentuate rather than antagonize cell firing stimulated by U-91356A. By itself, U-99194A did not affect SNPR firing rates. The predominant response to 1-10 mg/kg pramipexole, a D_3-preferring agonist (Piercey *et al.*, ibid), was inhibition (31 of 63 neurons). U-99194A, 1-3 mg/kg, fully antagonized pramipexole-induced inhibitions in all cells tested. Haloperidol, in doses up to 0.3 mg/kg, was unable to significantly antagonize inhibitory responses after higher cumulative doses (≥3 mg/kg) of pramipexole, and was only partially efficacious when given with high doses (0.3 mg/kg) in antagonizing lower doses of pramipexole. It is concluded that the stimulatory and inhibitory effects of DA agonists in SNPR are mediated through the DA D_2 and D_3 receptor subtypes, respectively.

89.20

ROLE OF ASTROGLIAL ENVIRONMENT IN SELECTIVITY OF DOPAMINERGIC LESION IN PARKINSON'S DISEASE P. Damier[1*], Y. Agid[1], A.M. Graybiel[2] and E.C. Hirsch[1]. [1]INSERM U289, Hôpital de la Salpêtrière, Paris, France Dept; [2] Brain & Cogn. Sci., MIT, Cambridge, MA 02139.

Parkinson's disease (PD) is characterized by a massive degeneration of dopaminergic neurons in the substantia nigra (SN). However, loss of dopaminergic is heterogenous. We recently showed that patterns of calbindin D_{28K}-immunostaining can be used as a template to subdivide the SN. We defined the SN pars compacta (SNpc), a zone overlapped by calbindin-positive neuropile, and the SN pars dorsalis (SNpd), dorsal to that area. Moreover in the SNpc, we used the heterogeneity of calbindin immunostaining to differentiate dopaminergic neurons included within calbindin-positive neuropile (named nigral matrice) and those located in five different calbindin-poorly stained pockets (named nigrosomes). We calculated degree of cell loss in these different groups in PD, and in particular, we found that loss of dopaminergic neurons was significantly higher in nigrosomes (95% ± 5) than in matrice (80% ± 9) and the SNpd (57% ± 8). To investigate reasons of such heterogeneity, we analyzed density of astroglial cells in the different groups of the SN.

Glial fibrillary acidic protein (GFAP) immunohistochemistry was performed at three transverse levels of a control human midbrain. To calculate density of astroglial cells in the different groups of the SN, we plotted GFAP-positive cells in 10 microscopic fields (150 x 195 μm) randomly distributed by an image analysis system on each anatomical region.

Density of astroglial cells was different from one group to another. The density was lower in more affected groups in PD (101 ± 19 GFAP-positive cells/mm^2 in nigrosomes) than in less affected groups (265 ± 35 in the matrice and 282 ± 43 in the SNpd).

Our results suggest that astroglial environment could play a main role in protection of dopaminergic neurons against PD degenerative process, and may explain lesion selectivity.

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PARKINSON'S DISEASE: NEUROTOXICITY

90.1

PATHOLOGICAL GLIAL-NEURONAL INTERACTION IN PARKINSON'S DISEASE. H.M. Schipper*, L. Bernier and G. Bernatchez. Lady Davis Institute, Jewish Gen. Hosp, and Dept. of Neurology & Neurosurgery, McGill Univ., Montreal, Canada

The mechanisms responsible for the accumulation of redox-active brain iron in normal senescence and Parkinson's disease are poorly understood. In rat primary astrocyte cultures, we demonstrated that dopamine (0.1-1 μM) rapidly induces heme oxygenase-1 (HO-1), followed by sequestration of non-transferrin bound, non-heme ^{55}Fe by the mitochondrial compartment. Conversely, dopamine appears to suppress mitochondrial trapping of transferrin-bound iron in these cells. Unlike dopamine, L-DOPA and norepinephrine had no effect on HO-1 expression and mitochondrial iron sequestration in cultured astroglia. In Parkinson's disease, dopamine released from dying nigrostriatal neurons may stimulate up-regulation of HO-1 in nearby astroglia which, in turn, results in the intracellular accumulation of heme-derived free ferrous iron and carbon monoxide. The latter are known mitochondrial toxins which may promote sequestration of redox-active iron within this subcellular compartment. Mitochondrial ferrous iron may promote non-enzymatic oxidation of dopamine or MPTP to neurotoxic semiquinones (Schipper et al. J Neurosci 11:2170, 1991) or MPP^+ (DiMonte et al. Glia 15:203, 1995), respectively, thereby establishing a vicious cycle of pathological glial-neuronal interactions in this disease.

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90.2

CYTOSKELETAL CHANGES IN CULTURED EMBRYONIC MESENCEPHALIC NEURONS BEFORE CELL DEATH INDUCED BY QUISQUALIC ACID. D. Casper*[1], A. Pidel[1], D.L. Gross[2], S. Yung[2], A. Prikhozhan[2], B.M. Riederer[3], I.M. Johnston[1], and A.J. Berman[1], [1]Department of Neurological Surgery, The Montefiore Medical Center, The Bronx, NY, 10467, [2]Fishberg Research Center for Neurobiology, The Mount Sinai School of Medicine, NY, 10028 and [3]Institut d'Anatomie, University of Lausanne, Switzerland.

We have previously demonstrated that dopaminergic neurons degenerate over several days in response to the non-NMDA glutamate agonist quisqualic acid (QA), and can serve as a model for slow neurotoxicity. In this report we have characterized this process at the cytoskeletal level. Cultures of dissociated embryonic rat mesencephalon were established in serum-containing or chemically defined medium. After several days in culture, the medium was replaced with defined medium ± 5 μM QA and incubated for various times. Cultures were then fixed and double-label fluorescence immunocytochemistry was performed with antibodies to the neuronal cytoskeleton in conjunction with antibodies to tyrosine hydroxylase, which identified the dopaminergic neurons in these cultures. Results demonstrate that neurons in this culture system are heterogeneous with respect to the expression of various cytoskeletal epitopes, particularly in the neurofilament triplet. Treatment with QA produced morphological changes which at first resembled neuronal growth and differentiation, followed by degenerative changes. This was reflected in changes in the distribution of some of cytoskeletal epitopes, such as that defined by antibodies to the microtubule-associated protein tau. These observations lead to the suggestion that QA stimulation produces a trophic-like response which precedes cell death in culture.

Supported in part by the Parkinson's Disease Foundation.

90.3

SUBSTANTIA NIGRA DOPAMINE NEURONS FROM MICE HOMOZYGOUS FOR A TRANSGENIC KNOCKOUT OF THE P53 GENE RESIST MPTP NEUROTOXICITY. Patricia A. Trimmer*, Trisha A. Smith, Anthony B. Jung, and James P. Bennett, Jr. Departments of Neurology, Psychiatry and Pharmacology, University of Virginia Health Sciences Center, Charlottesville, VA 22908.

The underlying mechanisms responsible for death of dopamine (DA) neurons in Parkinson's disease (PD) remain unclear. Systemic treatment with N-methyl, 4 phenyl, 1,2,3,6-tetrahydropyridine (MPTP) causes loss of DA neurons, and N-methylpyridinium (MPP+) induces programmed cell death (apoptosis) in DA neurons in culture. The p53 growth control gene is expressed in other systems during apoptosis, and may control the onset of cell death by decreasing the activity of the anti-apoptosis factors bcl-2 and bcl-x. In peripheral models p53 expression is necessary for apoptosis to occur. We tested the hypothesis that expression of the p53 gene is necessary for death of DA neurons in MPTP neurotoxicity.

Male black mice homozygous for a transgenic knockout of the p53 gene (p53 -/-)and wild-type controls (wt) were obtained from Taconic Laboratories. Animals received MPTP at 24 mg/kg s.c. b.i.d. for 2 days (total MPTP dose=96 mg/kg). Animals were sacrificed at 2 or 3 weeks after starting MPTP or before receiving MPTP. Forebrains were frozen on dry ice and cut into 20 micron sections and incubated with ^{3}H-mazindol to measure dopamine transporter (DAT) density. Brainstems were fixed and stained for tyrosine hydroxylase (TH). Quantitative autoradiography and cell counting were used to measure total striatal DAT (^{3}H-mazindol binding) and DA neuron number, respectively.

P53(-/-) and wt had similar baseline numbers of DA neurons and DAT density. MPTP treatment caused 30-35% loss of nigral DA neurons in wt but no loss in p53(-/-). Striatal DAT density was reduced comparably in both groups. Expression of p53 appears to be necessary for subacute MPTP toxicity to nigral neurons. Since both MPTP and spontaneous PD are associated with loss of mitochondrial complex I activity, manipulation of growth control gene function may alter DA neuronal death in idiopathic PD.

90.4

EFFECT OF PROTEIN KINASE C (PKC) ACTIVATION ON MICROGLIA-MEDIATED CATECHOLAMINE NEUROTOXICITY. M.K.McMillian*, P.J.Vainio, M.Tömwall and R.K.Tuominen. Department of Pharmacology and Toxicology, Inst. Biomedicine, University of Helsinki, Finland.

Microglia activation by lipopolysaccharide (LPS) in mixed neuronal/glial mesencephalic cultures selectively kills tyrosine hydroxylase-immunopositive (TH+) neurons and thus decreases [^{3}H]dopamine (^{3}H-DA) uptake. Opsonized zymosan A (OZ), a yeast protein which is phagocytosed by macrophages, reportedly activates microglia differently than LPS, and may produce a different type of neurotoxicity. In our studies, OZ always reduced ^{3}H-DA uptake more effectively than LPS. The OZ neurotoxicity requires microglial activation and persists for at least a week. OZ activates microglial and macrophage PKC and phospholipase D (PLD) while LPS does not. Treatment with the PKC activator phorbol myristate acetate (PMA, 10^{-8}-10^{-6}M) resulted in decreased TH+ neuron number and ^{3}H-DA uptake. PMA potentiated LPS-induced, but not OZ-induced neurotoxicity and production of the active toxin, nitric oxide. Massive PKC translocation and PLD activation responses to PMA were observed in enriched microglia. However, PMA was most effective at reducing ^{3}H-DA uptake early after plating neurons, and in enriched high-density neuronal cultures. PMA may stimulate differentiation and inhibit proliferation of the cultured TH+ neurons, in addition to activating microglia. OZ had no effect on ^{3}H-DA uptake in cortical neurons which become TH+ when co-cultured with blood vessels and membranes. Interestingly, bovine adrenal medullary (BAM) cells after PMA treatment became sensitive to the neurotoxic effect of OZ, presumably mediated via bovine macrophage activation. These PMA-treated BAM cells may provide a useful BIOCHEMICAL model for catecholamine neurotoxicity, since most BAM cells are catecholaminergic, in constrast to the small percent of mesencephalic neurons which are dopaminergic.

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90.5

LEVODOPA ACCUMULATION IN NONDOPAMINERGIC BRAIN REGIONS: POTENTIAL FOR OXIDATIVE STRESS DUE TO AUTOXIDATION? D.A. Loeffler*, P.A. LeWitt, P.L. Juneau, D.M. Camp, and K. Hyland. Clin. Neurosci. Program, Sinai Hosp., Detroit, MI 48235 and Baylor Research Inst., Dallas, TX 75226.

Treatment of Parkinson's disease with levodopa (LD) may result in oxidant stress by deamination of dopamine (DA) or LD autoxidation. We studied LD distribution and DA metabolism in DAergic (striatum, ventral midbrain [VMB]) and non-DAergic (frontal cortex [FC], cerebellum) brain regions. Rats (n = 5/group) received i.p. carbidopa (2.5 mg/kg), 30 min later LD (100 mg/kg), then sacrifice 1 hr later. LD concentrations increased in all regions by > 60-fold vs. vehicle-treated controls. DA production (after subtraction of endogenous levels) was highest in striatum (DA/LD ratio = 1.51), moderate in VMB (0.88), and low in FC (0.10) and cerebellum (0.04). DA deamination increased > 10-fold vs. controls in all regions; DOPAC/DA ratios were: striatum 1.39, VMB 3.67, FC 3.46, and cerebellum 7.25. Conclusion: LD accumulation and DA deamination, potential sources of oxidative stress, increase in DAergic and non-DAergic brain regions after LD treatment. (Supported by National Parkinson Foundation)

90.6

RAPID INDUCTION OF TRANSCRIPTION FACTORS NF-κB AND AP-1 IN BASAL GANGLIA FOLLOWING MPTP TREATMENT IN MICE. C. Fonck*, Y. Rong and M. Baudry. Neurobiology Program, University of Southern California, Los Angeles, CA 90089-2520.

Damage to dopaminergic neurons in the nigrostriatal pathway caused by systemic injection of 1-methyl 1-4-phenyl-1,2,3,6 -tetrahydropyridine (MPTP) has been proposed as a model to study Parkinson's disease. It has been suggested that the deleterious effects of MPTP result from free radical formation within dopaminergic neurons of the substantia nigra. The present study examined changes in the activation of two transcription factors generally associated with oxidative stress, NF-κB and AP-1, in the substantia nigra and the striatum of adult male mice as a result of MPTP treatment. The levels of NF-κB and AP-1 were evaluated by means of a gel shift assay, at various time points after subcutaneous injection of MPTP (35 mg/kg). A significant induction of NF-κB was observed in the substantia nigra 6 hours after MPTP treatment. By 24 hours after MPTP injection, the level of NF-κB in substantia nigra was similar to that of the control group. AP-1 binding activity also doubled in the substantia nigra three hours after MPTP injection and returned to control levels 24 hours after drug treatment. Surprisingly, NF-κB and AP-1 activities showed a two and a four fold increase respectively in the striatum 3, 6 and 9 hours after MPTP treatment. Levels of NF-κB and AP-1 binding activity 24 hours after MPTP injection were still significantly higher than in the control group. Changes in the activation of NF-κB and AP-1 in the substantia nigra are consistent with the hypothesis that oxidative stress is involved in MPTP induced toxicity to dopaminergic neurons. The induction of AP-1 in the striatum also indicates that MPTP treatment might produce a significant activation of the nigro-striatal system. The activation of NF-κB in the striatum suggests that an oxidative stress response also takes place in this structure.

This work was supported by grants from Eukarion Inc. and NIA.

90.7

NEUROPROTECTIVE EFFECTS OF THE NOVEL BRAIN-PENETRATING PYRROLOPYRIMIDINE ANTIOXIDANTS U-101033E AND U-104067F AGAINST POST-ISCHEMIC DEGENERATION OF NIGROSTRIATAL NEURONS. P.K.Andrus*, T.J.Fleck, J.A.Oostveen and E.D.Hall, Pharmacia & Upjohn, Inc., Kalamazoo, MI 49001

A 10-min period of bilateral carotid occlusion (BCO)-induced forebrain ischemia in gerbils triggers a delayed retrograde degeneration of 35-40% of dopaminergic nigrostriatal (NS) neurons. The mechanism involved in nigral cell death is as yet unknown, but evidence suggests that oxidative stress may play a key role. In the present study, we examined whether the novel brain-penetrating lipid antioxidant pyrrolopyrimidine U-101033E and its aromatized analog, U-104067F, could attenuate dopaminergic neurodegeneration in this model. Gerbils were dosed with U-101033E (1.5, 5 or 15 mg/kg p.o., b.i.d.) or U-104067F (5 or 15 mg/kg p.o. b.i.d.) for 27 days beginning on the day of the 10-min ischemic insult. Preservation of NS neurons was assessed by tyrosine hydroxylase immunohistochemistry at 28 days. In vehicle (40% hydroxypropyl-β-cyclodextrin)-treated animals, there was a 42% loss of NS neurons. In contrast, gerbils that received 5 or 15 mg/kg U-101033E had only a 23% or 28% loss of NS neurons, respectively ($p<0.002$ vs vehicle). U-104067F showed little effect at sparing neurons at the 10 mg/kg dose, but did significantly attenuate neuronal loss to only 20% at the 30 mg/kg dose level ($p<0.01$ vs vehicle). The results show that both pyrrolopyrimidines (U-101033E and U-104067F) significantly attenuate the post-ischemic loss of NS dopaminergic neurons, implicating a lipid peroxidative mechanism in this model.

90.8

GLUTAMATE AND DOPAMINE TRANSPORT: SENSITIVITY TO REACTIVE OXYGEN SPECIES, DOPAMINE OXIDATION, AND SULFHYDRYL MODIFICATION. S.B. Berman*, M.J. Zigmond, and T.G. Hastings. Departments of Neuroscience and Neurology, Pittsburgh, PA 15260

Dopamine (DA) oxidizes to form reactive oxygen species (ROS) and DA quinones. Both ROS and DA quinones can modify cysteinyl residues in proteins, and we have shown that DA oxidation products can affect the function of at least one cysteine-dependent protein, the DA transporter (Berman et al, 1996). We have now examined the effect of DA oxidation on glutamate transporters, which contain a conserved cysteinyl residue and demonstrate ROS-induced inhibition in astrocytes (Volterra et al, 1994). We compared the effects of ROS and DA oxidation on glutamate and DA transport in rat striatum by preincubating synaptosomes, washing, and then measuring uptake using ^{3}H-DA (250 nM) or ^{3}H-glutamate (10 μM). The SH-modifying reagent, N-ethylmaleimide (10-500 μM) inhibited the transport of DA (-55 to 100%) and glutamate (-20 to 70%), as did xanthine (500 μM) plus xanthine oxidase (25 mU/ml) (DA, -70%; glutamate, -86%). Preincubation with ascorbate (0.85 mM) under proxidative conditions also inhibited both DA (-67%) and glutamate (-73%) uptake, whereas ascorbate plus the iron chelator DETAPAC (1 mM), which can prevent the proxidant activity of ascorbate, had no effect. Preincubation with DA (100 μM) under conditions where 10% of the DA oxidized (as estimated by aminochrome formation) inhibited both DA uptake (-58%) and glutamate uptake (-24%). Finally, DA oxidation was enhanced by tyrosinase, which enzymatically forms DA quinone. Five min preincubation with DA (100 μM) plus increasing amounts of tyrosinase (to oxidize 0.2 to 90% of the DA) led to a concentration-dependent inhibition of both DA and glutamate uptake. We conclude that glutamate transport, like DA transport, can be modified by ROS and other DA oxidation products. This modification may play a role in several neurotoxic processes, including ischemia and methamphetamine exposure. (Supported in part by USPHS grants NS19608 and GM08208).

90.9

EARLY INDICES OF OXIDATIVE STRESS: IMMEDIATE RESPONSE TO NEUROTOXIC LEVELS OF EXOGENOUS DOPAMINE. A.D. Rabinovic, M.J. Zigmond, and T. G. Hastings*. Departments of Neuroscience and Neurology, University of Pittsburgh, Pittsburgh, PA 15260.

Dopamine (DA) may contribute to the neurodegenerative process in Parkinson's disease, based upon the ability of DA to form reactive oxygen species and DA quinones. We have previously shown in rats that an intrastriatal injection of DA results in both biochemical evidence of DA oxidation at 24 h and selective damage to dopaminergic terminals at 7 d (Hastings et al., 1996). In this study, we examined early biochemical changes following intrastriatal DA (0.4 μmol/2 ml/20 min.). Using microdialysis, we observed that the peak DA concentration in the region of selective damage at 1-2 h following injection, was reduced 10^2-10^3-fold from the injected concentration to micromolar levels. DA levels returned to baseline levels within 6 h. Endogenous levels of GSH were decreased significantly in striatal tissue at 4 h (-24%) and 8 h (-11%) after injections, but had returned to control values by 24 h. Protein-bound cysteinyl-DA and cysteinyl-DOPAC levels were increased significantly above controls at 2 h (47-fold and 37-fold, respectively) and 24 h (21-fold and 16-fold, respectively). In an effort to measure DA-quinone formation in the extracellular fluid following intrastriatal DA, cysteine was utilized as a quinone trapping agent and perfused through a dialysis probe, a technique validated in vitro by incubation of DA with tyrosinase to form DA quinone and, subsequently, cysteinyl-DA. In vivo an intrastriatal injection of DA resulted in the formation of cysteinyl-DA which represent 0.2% of the peak DA level. These findings suggest that oxidative responses to exogenous DA occur immediately following DA administration. These studies may provide information as to the role of DA in the mechanism of neurodegeneration in Parkinson's disease. (Supported by USPHS grants NS 19608 and AG03705).

90.10

METHAMPHETAMINE-INDUCED NEUROTOXICITY IS ASSOCIATED WITH THE FORMATION OF CYSTEINYL-DOPAMINE. M.J. LaVoie*, M.J. Zigmond, and T.G. Hastings. Depts. of Neuroscience and Neurology, Univ. of Pittsburgh, Pgh, PA, USA

Oxidative stress and free radical formation have been proposed to play a role in Parkinson's disease and the neurotoxicity associated with methamphetamine (METH) exposure. Under oxidative conditions, dopamine (DA) may contribute to these neurotoxic events by forming free radicals and reactive quinones. DA quinones bind covalently to cysteinyl residues in proteins, potentially inactivating critical protein functions. We have shown that exogenous DA injected into striatum causes a selective loss of DA terminals that is blocked by antioxidants and correlated with the level of cysteinyl-catechols (Hastings et al, 1996). In this study, we sought to determine whether neurotoxic doses of METH would result in oxidation of endogenous DA. METH (15 mg/kg, s.c.) or saline was administered to male Sprague-Dawley rats (325-375g) every 2 hours, for a total of 4 injections. Protein-bound cysteinyl-DA was increased by METH at 2 hr (+350%; 0.7 ± 0.19 nmol/g tissue; n=9), and remained increased at 4 hr (+260%; n=6) and 8 hr (+260%; n=2). No increase in protein-bound cysteinyl-DOPAC was observed, possibly due to the large decrease in tissue DOPAC (-80%; n=11). In the acid-soluble tissue fraction, the glutathione-DA conjugate was present at 2, 4, and 8 hr (0.06 ± 0.016 nmol/g tissue, n=11) after METH, while absent in control tissue. At 7 days, identically-treated animals showed a 55% loss of DA and a 60% loss of serotonin (n=4). Animals exposed to the same dose of METH at 5°C did not exhibit either long-term monoamine depletion or increased levels of DA oxidation. These results suggest a role for DA oxidation in the process of METH-induced toxicity (Supported in part by USPHS grant NH19608).

90.11

PEROXYNITRITE-MEDIATED MODIFICATIONS OF TYROSINE HYDROXYLASE IN PC12 CELLS. J. Ara, H. Ischiropoulos, J. Horwitz*. Dept. of Pharmacology, Medical College of Pennsylvania, Philadelphia, PA 19129; Institute for Environmental Medicine, University of Pennsylvania, Philadelphia, PA 19104

The pathology of neurodegenerative diseases is associated with excess production of reactive species. Nitric oxide and superoxide are free radicals that react at near-diffusion limited rate to form peroxynitrite, a relatively long-lived and highly reactive species. In previous studies, using PC12 cells, we showed that peroxynitrite decreased tyrosine hydroxylase (TH) activity. This suggested that the enzyme was a target for peroxynitrite-mediated modifications. Part of the toxic effect of peroxynitrite may be mediated by the nitration of tyrosine residues. In order to investigate this issue, homogenates of PC12 cells were treated with peroxynitrite. Nitrated proteins were precipitated with monoclonal antibodies to nitrotyrosine, separated by PAGE and blotted onto nitrocellulose. Western blots of these immunoprecipitates developed with polyclonal antibodies to nitrotyrosine revealed four prominent bands. These data indicate that peroxynitrite nitrates selected proteins. To learn if TH was nitrated, we immunoprecipitated with monoclonal antibodies specific for this enzyme, and analyzed the immunoprecipitate by western blotting. Development of the blot with polyclonal antibodies to nitrotyrosine revealed a band in peroxynitrite treated homogenates that was absent from controls. This band had an Rf corresponding to 60,000 kDa, the subunit molecular weight of TH. Parallel blots developed with polyclonal antibodies to TH revealed a prominent band in both control and peroxynitrite treated homogenates, indicating that TH had been precipitated. These data confirm that TH is nitrated by peroxynitrite. The precise role of nitration in determining the activity of the enzyme remains to be investigated (Supported by the Allegheny-Singer Health Education Foundation).

90.12

GM1 GANGLIOSIDE INFLUENCES THE REGULATION OF TROPHIC FACTOR TRANSCRIPTS IN THE DOPAMINE DENERVATED MOUSE STRIATUM. J.L. Seeburger*[1], J.E. Springer[2] and J.S. Schneider[1]. [1]Dept. of Neurobiol. and Anat., MCP and Hahnemann Univ., Phila., PA 19102, [2]Dept. of Anat. and Neurobiol., Univ. of Kentucky, Lexington, KY, 40536.

MPTP administration to mice results in loss of dopaminergic (DAergic) neurons of the substantia nigra pars compacta and denervation of their striatal targets. Little is known, however, about the regulation of putative DAergic neurotrophic factors in the lesioned nigrostriatal system. We have previously demonstrated that DA cell loss associated with MPTP toxicity can be partially reversed by ganglioside GM1 administration. Although GM1 appears to be trophic for DAergic neurons, little is known about its mechanism of action or its interaction with other putative DAergic trophic factors. Therefore, we have examined the expression of GDNF, BDNF, NT-3 and basic FGF mRNA in the mouse striatum following MPTP and subsequent GM1 administration. Animals were injected with MPTP (4 injections at 3 hr intervals, 20mg/kg, s.c.) and allowed to survive for 2, 5 or 14 days. Half of these animals were also administered GM1 (30 mg/kg, i.p.) daily beginning 24 hrs. following the last MPTP injection. Trophic factor transcripts were amplified from control (untreated) and lesioned striatal specimens using RT-PCR. Following denervation, GM1 administration increased the striatal expression of both GDNF and bFGF mRNAs, but decreased BDNF mRNA expression throughout the time course. The expression of NT-3 mRNA was not affected. These results suggest that some of the ameliorative properties of GM1 may be associated with a specific pattern of regulation of various DAergic target-derived trophic factors. Supported by NIH grant AG-10280.

90.13

ALTERED BCL-2 AND BAX EXPRESSION IN SUBSTANTIA NIGRA DOPAMINERGIC NEURONS FOLLOWING MPTP EXPOSURE. D.W. Anderson[1], J.C. Reed[2], S. Krajewski[2], and J.S. Schneider[1]*. [1]MCP and Hahnemann Univ., Dept. Anatomy and Neurobiology, Philadelphia, PA 19102-1192., [2]La Jolla Cancer Res. Foundation, La Jolla, CA 92037.

Oxidative stress via inhibition of the mitochondrial complex I enzyme (NADH-dehydrogenase) by the dopamine neurotoxin MPP^+, may initiate altered expression of oxidative response proteins associated with apoptosis. Apoptosis and decreased expression of the anti-apoptotic factor Bcl-2, a known anti-oxidant, has been shown to occur *in vitro* in several cell lines in the presence of MPP^+. The present studies were conducted to elucidate any regulatory effects produced by MPP^+ with regard to apoptotic effector factors *in vivo*. The expression of Bcl-2 and its homologue the pro-apoptotic factor Bax, was assessed in the substantia nigra in young (7-8 weeks) and aged (12 months) C57Bl6 mice at both mRNA and protein levels using RT-PCR and immunohistochemical techniques, respectively. Some mice were administered MPTP at a dose that does not cause significant dopaminergic cell death (single injection at 30 mg/kg). In young MPTP-treated mice, there was a maximal up-regulation of both Bcl-2 and Bax at 12 hours post injection and a return to normal levels within 24 hours. A concomitant up-regulation of both Bcl-2 and Bax protein was detected immunohistochemically. The non-pathogenicity of the MPTP regime was identified via preservation of tyrosine hydroxylase immunohistochemical staining, which showed no identifiable loss of expression in nigral neurons. Comparable results have been obtained in the aged mice. Further characterization of the Bcl-2:Bax heterodimerization ratio, thought to be associated with control of apoptotic events, may indicate a potential pre-apoptotic regulatory event. These results are potentially indicative of an MPTP-induced oxidative stress response involving an apoptotic death mechanism which may also be involved in neuronal cell death associated with toxic concentrations of MPTP. The further characterization of MPTP-induced apoptotic cell death through end-labelling techniques and quantification of Bcl-2 and Bax regulation is presently being studied. Supported by NIH grant AG10280.

90.14

NEUROPROTECTIVE PROPERTIES OF NITRIC OXIDE (NO) AND *S*-NITROSOTHIOLS IN THE BRAIN *IN VIVO*. P. Rauhala, A. M.-Y. Lin, A. Khaldi, I. Sziraki & C.C. Chiueh*. Unit on Neurotoxicology & Neuroprotection, Laboratory of Clinical Science, NIMH, NIH, 10/3D-41, Bethesda, MD 20892-1264

Hydroxyl (OH) and nitric oxide (NO) free radicals have been proposed to be involved in neurodegeneration. However, the pathophysiological role of NO has been controversial; it is not clear whether NO is foe or friend to brain neurons. Our *in vivo* data indicates that in contrast to OH generators (ferrous citrate and SNP), NO and NO donors (*S*-nitrosothiols) do not cause injury in the rat nigrostriatal system (*Synapse*, 23:58-60, 1996).

NO and *S*-nitrosothiols, in fact, protected nigrostriatal dopamine neurons against iron-induced oxidative stress/injury, evidently by their suppression of acute lipid peroxidation in the substantia nigra and prevention of chronic dopamine depletion in the striatum. Such *in vivo* neuroprotective properties of *S*-nitrosoglutathione were absent in experiments using light-exposed, NO-exhausted agent. Furthermore, the present *in vitro* studies demonstrated that NO and S-nitrosothiols inhibited iron-induced OH generation and lipid peroxidation, suggesting that these unique antioxidant properties of NO may mediate its neuroprotective effects against OH-induced brain injury.

It is concluded that exogenous NO is not toxic to brain neurons *in vivo*. NO may protect brain neurons against iron-catalyzed oxidative injury through annihilation of OH and/or lipid radicals. (supported by NIMH IRP: Z01-MH-02648-04 LCS)

90.15

DIFFERENTIAL EFFECTS OF NITRIC OXIDE DONORS ON APOPTOSIS IN CHP212 CELLS AS DETERMINED BY ANNEXIN V BINDING D. Terwel (1), L.J.M. Nieland (2), B. Schutte (2), C.P.M. Reutelingsperger (3), F.C.S. Ramaekers (2), H.W.M. Steinbusch (1) European Graduate School for Neuroscience, (1)Dept. of Psychiatry and Neuropsychology, (2)Dept. of Molecular Cell Biology, (3)Dept. of Biochemistry, University of Maastricht, The Netherlands. (Spon: ENA)

Cell death by exposure to NO has been implicated in neurodegenerative diseases, such as Parkinson's and Alzheimer's diseases. In the present study it was investigated whether NO causes either apoptosis or necrosis using a neuronal cell line (CHP212) as a model. Apoptosis and necrosis were assessed by the simultaneous determination of annexin V binding and propidium iodide (PI) uptake using flow cytometric analysis. Annexin V binds to phosphatidylserine residues exposed at the surface of apoptotic cells. The effects of two NO donors (SNAP and SNP) were compared to those of olomoucine, a general inducer of apoptosis. Early apoptosis, as evidenced by the binding of annexin V and the exclusion of PI by the cells, was maximal after 4, 8 and 16 h for cells exposed to olomoucine, SNAP and SNP, respectively. Cells that had taken up PI were of the late apoptotic or secondary necrotic type based on the fragmented and condensed appearance of their chromatin. The morphology of the cells during apoptosis induced by SNAP and SNP was indistinguishable from that induced by olomoucine. SNAP caused a maximum of approximately 20% apoptosis at a dose of 0.25 mM; SNP caused approximately 40% apoptosis at a dose of 2 mM. The difference between the potencies of SNAP and SNP could be related to the fact that SNAP generates $NO^{\bullet}$, whereas SNP generates NO^{+}. However, the presence in the incubation medium of ascorbate, which converts NO^{+} into $NO^{\bullet}$, did not decrease the apoptotic fraction, but rather increased it. In conclusion, SNAP and SNP appear to be able to induce apoptosis in CHP212 cells with a different time dependency and potency, probably related to their different half-lifes. Further studies into the role of NO in neuronal apoptosis will be carried out using primary dopaminergic cells and animal models of Parkinson's disease. (This work was supported by the Prinses Beatrix Fund)

90.16

DNA FRAGMENTATION IN DOPAMINE CELLS INDUCED BY N-METHYL-(*R*)SALSOLINOL, AN ENDOGENOUS NEUROTOXIN W. Maruyama*[1,2], Gen Sobue[1] and M. Naoi[3] [1]Dept. Neurol., Nagoya Univ. Sch. Med., Nagoya and [2]National Inst. Longevity Sci., Obu, [3]Dept. Biosci., Nagoya Inst. Technol., Nagoya, Japan.

There are increasing results suggesting that endogenous neurotoxins may induce Parkinson's disease (PD) after a long term of accumulation. N-Methyl-(*R*)salsolinol [NM(*R*)Sal], a neurotoxin candidate, was found to increase in CSF from untreated patients with PD. Recently, apoptosis has been proposed to be a mechanism of the neuronal death in the neurodegenerative diseases. An animal model of PD was prepared by the administration of NM(*R*)Sal in the rat striatum. The histochemical analysis showed dopamine neuronal loss without tissue reaction in the substantia nigra, indicating apoptotic cell death. The effect of NM(*R*)Sal and related compounds on DNA was systematically examined using single cell electrophoresis (the comet assay). Among the dopamine-derived isoquinolines, NM(*R*)Sal was found to be the most potent to induce DNA fragmentation followed by NM(*S*)Sal. Both the metabolic precursor and the oxidized product of NM(*R*)Sal, (*R*)salsolinol and 1,2-dimethyl-6,7-dihydroxyisoquinolinium ion did not damage DNA significantly. Protein synthesis was required for DNA fragmentation by NM(*R*)Sal, since cyclohexamide reduced the damage. NM(*R*)Sal is oxidized and produces hydroxyl radical simultaneously. Radical scavengers and catalase reduced DNA damage indicating that radical production by NM(*R*)Sal is essential for the damage. Sustained high levels of NM(*R*)Sal in the brain might account for apoptotic cell death of dopamine neurons in PD.

90.17

COPPER SUPPLEMENTATION BLOCKS 1 - METHYL - 4 - PHENYLPYRIDINIUM NEUROTOXICITY IN MICE. **P. Rojas*, R. Alvarez and C. Ríos. Dept. of Neurochemistry, National Institute of Neurology and Neurosurgery,Insurgentes Sur 3877,México city 14269.**

1-methyl-4-phenylpyridinium (MPP+) is the major metabolite of 1-methyl-4-phenyl-1, 2, 3, 6 - tetrahydropyridine (MPTP) a drug which induces a parkinson-like damage in several animal species. It is now accepted that MPP+ is responsible of the neurotoxic effects of MPTP in dopaminergic nigrostriatal pathway. On the other hand, it has been reported that diethyldithiocarbamate (DDC) is a metal chelating agent with high affinity by copper that increases MPTP neurotoxicity.Copper defficiency produces impairment of the many processes dependent of the metal. We reported that MPTP decreases copper content (50%) in corpus striatum, suggesting and important role of copper in dopaminergic neurotoxicity of MPTP. In this study we evaluated the possible role of copper in MPP+ toxicity. Adult male NIH mice (25-30 g) were injected with copper sulphate (2.5 mg/Kg). Later , mice were injected into the right lateral ventricle with MPP+ and 24h after they were killed by decapitation. Striata were dissected out and dopamine concentration was analyzed by HPLC. MPP+ reduced dopamine concentration (40%) and copper pretreatment blocked its toxicity. An additional group was treated with MPP+ and cytochrome C oxidase activity was assayed 24 later. Activity of this copper dependent enzyme decreased (27.5%) in striatum. These results suggest that copper may be important in MPP+ neurotoxicity. This work was supported in part by CONACyT grant 0044P-M9506.

90.18

DOWNREGULATION OF GLUTATHIONE PEROXIDASE LEADS TO INCREASED OXIDATIVE DAMAGE IN MOUSE BRAIN

D. Jiang , D.G. Hom, Q. Wei, J.K. Andersen*, Division of Neurogerontology, Andrus Gerontology Center, University of Southern California, Los Angeles, CA 90089

Glutathione peroxidase(GSHPx) is a free-radical scavenging enzyme which can limit the production of free radicals in the brain. Animal models in which the levels of the GSHPx have been altered by either genetic over- or under-expression were used to explore whether variations in levels of this enzyme offered different levels of protection against neuronal damage. A transgenic mouse line in which GSHPx was over-expressed at levels 2.7 fold higher than normal was created in our lab. A knock-out mouse line with reduced levels of GSHPx was acquired from Dr. Ye-Shih Ho (Wayne State, Detroit). Hydroperoxide levels were tested by analyzing levels of DCF fluorescence and free-radical damage was assessed by examine the levels of protein and lipid oxidation as measured by carbonyl content and MDA levels in the brains of transgenics, heterozygous & homozygous knock-out mice, and normal controls. We found a significant increasing levels of free radicals in both heterozygous and homozygous knock-outs, and decreased levels in the transgenics compared to normal controls. In addition, the hetero- and homozygous knock-outs showed an increased level of both carbonyl content and MDA compared to wild type, while there was no significant difference between the transgenics and normal controls. These results suggest that reduced levels of GSHPx result in more susceptibility to free-radical damage. Elevation of GSHPx appears to have no effect on the level of free-radical damage even though hydroperoxide levels were lowered in the brain. We plan to examine the susceptibility of these mice to MPTP as well as to other neurotoxins which are postulated to elicit their effects through generation of free radicals.

90.19

N-METHYL-(*R*)SALSOLINOL AS A CANDIDATE NEUROTOXIN TO INDUCE PARKINSON'S DISEASE M. Naoi[1]* and W. Maruyama[2] [1]Dept. Biosciences, Nagoya Inst. Technol., Nagoya, Japan and [2]National Inst., Longevity Sci., Obu, Japan,

A series of naturally-occurring dopamine-derived catechol isoquinolines have been examined for neurotoxicity to dopamine neurons. *In vivo* experiments proved that only 1(*R*),2(N)-dimethyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline [(*R*)N-methyl-salsolinol, (*R*)NMSal] could induce parkinsonism to rats with reduction in dopamine and tyrosine hydroxylase activity after injection in the striatum. By histochemical observation dopamine neurons in the substantia nigra were selectively depleted. The mechanism of the selective cell death was clarified as follows. Only (*R*)NMSal was found to be taken up to dopaminergic neuroblastoma SH-SY5Y cells by dopamine transport system. Oxidation of (*R*)NMSal generates hydroxyl radical and cytotoxic 1,2-dimethyl-6,7-dihydroxyisoquinolinium ion (1,2-DMDHIQ^{+}), which inhibited oxidative phosphorylation in cells more markedly than NMSal and 1-methyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline [salsolinol, Sal]. On the other hand, (*R*)NMSal induced DNA fragmentation in SH-SY5Y cells through its oxidation. The dopaminergic neurotoxicity was further suggested by the increase in (*R*)NMSal level in CSF from parkinsonian patients.

The enantio-selective occurrence of (*R*)Sal and (*R*)NMSal was confirmed in the human brain, suggesting its enzymatic synthesis *in situ*. A salsolinol synthase was purified from human brain, which catalyzes the synthesis from dopamine and acetaldehyde. An N-methyltransferase was detected in human brain and the activity was the highest in the striatum. These results suggest that these enzymes may be endogenous factors involving in the pathogenesis of Parkinson's disease.

91.1

DIFFERENTIAL EXPRESSION OF GLUTAMATE RECEPTOR SUBUNIT mRNA IN DOPAMINE NEURONS OF HUMAN SUBSTANTIA NIGRA.

T.J. Counihan, G.B. Landwehrmeyer, A.B. Young, and J.B. Penney*, Jr. Dept. of Neurology, Mass. Gen. Hosp., Boston, MA 02114.

Not all dopaminergic neurons are equally vulnerable to the neurodegenerative process in Parkinson's disease(PD), and the mechanism for this selective vulnerability remains unknown. Evidence is accumilating that glutamate-mediated excitotoxity plays a significant role in the degeneration of neurons in PD. We compared the levels of messenger RNA expression for N-Methyl-D-Aspartate(NMDA) and metabotropic(mGluR) glutamate receptor subtypes in both vulnerable and non-vulnerable dopamine neurons in human midbrain using *in situ* hybridization histochemistry. We found that NMDAR1 (76±7 grains/1000sq μ), NMDAR2A (20±12 grains/1000sq μ), NMDAR2B (79±9 grains/1000sq μ), NMDAR2C (71±8 grains/1000sq μ), NMDAR2D (73±8 grains/1000sq μ), mGluR1 (41±5 grains/1000sq μ) and mGluR5 (112±11 grains/1000sq μ) are all significantly expressed by pars compacta neurons. We also found mGluR1 labeling of dopamine neurons in the ventral tegmental area to be more intense (115±7 grains/1000sq μ) than in other nigral subregions, using double label *in situ hybridization.*

Activation of NMDA receptors is known to be toxic to dopamine neurons in culture, and there is evidence that co-activation of NMDA and mGluR 1 is necessary for "NMDA" toxicity. Differential expression of NMDA and metabotropic receptor genes in nigral dopamine neurons may help explain the selective vulnerability of some of these neurons in PD.

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91.2

ULTRASTRUCTURE OF LEWY NEURITES IN PARKINSON'S DISEASE AND LEWY BODY DEMENTIA

WP Gai*, WW Blessing, PC Blumbergs. Departments of Physiology and Medicine, Centre for Neuroscience, Flinders University, SA 5042, Australia.

Lewy bodies are found in multiple brain regions affected in Parkinson's disease (PD) and Lewy body dementia (LBD). Lewy neurites are ubiquitin-positive degenerating neural processes also found in multiple brain regions affected in PD and LBD, yet the relationship between Lewy bodies and Lewy neurites is not known (Gai et al, Brain 118: 1447, 1995).

The present study examines on the ultrastructure of Lewy neurites in perfusion fixed brains from one LBD and two PD cases with postmortem delay less than 10 hours. The brainstem, hippocampus, hypothalamus, amygdala, cingulate and insular cortices were serially sectioned (50 μm) and immunostained with an antibody to ubiquitin (Dako). Reaction products were visualized with DAB or a silver enhancement kit (Amersham). Portions of sections rich in Lewy neurites were dissected out, fixed with glutaraldehyde, and processed for electron microscopic observation.

Lewy neurites (2 to 30 μm) consisted of amorphous material, granular or vesicular profile and filaments (10-30 nm). The filaments were loosely and randomly arranged, thus distinct from the pared helical filaments found in the Alzheimer's dystrophic neurites. In most Lewy neurites, the amorphous material and granular profiles were intermingled with filaments, whereas in some neurites the amorphous material formed a condensed central core surrounded with radiating filaments (10 nm), resembling classical Lewy bodies. Lewy neurites wrapped in myelin sheaths were not found in the brain regions examined, and in optimal sections some Lewy neurites appeared to form postsynaptic density with axonal terminals. Our results indicate Lewy neurites share morphological similarity with Lewy bodies, and most Lewy neurites are derived from dendrites.

91.3

ISOLATION OF CORTICAL LEWY BODIES FROM DIFFUSE LEWY BODY DISEASE BRAINS.

W.D. Hill*, R. Peloquin, M.M. Tompkins, and B.J. Balin§. Dept. of Cellular Biology and Anatomy, Medical College of Georgia, Augusta, GA 30912. Dept. of Pathology, Hahnemann Univ. Philadelphia, PA 19102§

Lewy bodies are the pathological hallmarks of Lewy body associated disorders including Parkinson's disease and diffuse Lewy body disease. These filamentous neuronal inclusions characteristically label with antibodies to ubiquitin and with antibodies to neurofilament (NF) triplet proteins but not with antibodies to tau or A68 epitopes. Taking advantage of these immunohistochemical staining properties we have utilized Fluorescent Activated Cell Sorting to isolated highly purified populations of cortical Lewy bodies.

Homogenates of cingulate cortex from Diffuse Lewy body disease cases were centrifuged over sucrose gradients. Material pelleting below a 1.5 M sucrose pad was enriched for lewy bodies, but also contained some neurofibrillary tangles, and neuronal processes. This pellet was resuspended and labeled with a rabbit polyclonal antibody to ubiquitin and the mouse IgM Alz50. The pellets were washed and secondary antibodies conjugated with phycoerythrin (anti-rabbit) or FITC (anti-mouse) were used to localize the primary antibodies. An Epics Elite7500 flow cytometer was used to sort the material by size and by single or dual label. The Lewy body fraction contained particles that were single labeled (by anti-ubiquitin/anti-rabbit-PE) and were approximately 5-15μm in diameter. Neurofibrillary tangles were dual labeled and dystrophic neurites were larger than Lewy bodies and outside the size window.

Ultrastructural analysis, both negative staining and resin embedded thin section, showed spherical objects with morphology corresponding to cortical Lewy bodies.

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91.4

DENSITY OF [^{125}I]-TRANSFERRIN BINDING SITES ON MELANIZED NEURONS OF THE SUBSTANTIA NIGRA AND THE VENTRAL TEGMENTAL AREA IN CONTROL SUBJECTS AND PATIENTS WITH PARKINSON'S DISEASE.

B.A. Faucheux[1]*, J.-J. Hauw[2], Y. Agid[1] and E.C. Hirsch[1]. [1]INSERM U289 and [2]INSERM U360, Hôpital de la Salpêtrière, Paris, F-75013, France.

The neurodegenerative process leading to the massive loss of melanized dopaminergic neurons that occurs in the substantia nigra, pars compacta, of patients with Parkinson's disease may be linked to oxidative stress. Increased iron levels measured in the surviving neurons of the substantia nigra, pars compacta, indicate a mechanism by which highly reactive hydroxyl radicals may be generated, as iron is a transition metal that catalyzes the formation of hydroxyl radicals from hydrogen peroxide. Since iron may augment hyperoxidation phenomena and thus participate in the neuronal death process, we studied pathways for iron to gain access to neurons. By quantitative autoradiography, using an image analysis system, we analyzed the specific binding of [^{125}I]-transferrin at the cellular level, on surviving melanized neurons located in the substantia nigra pars compacta and in the ventro-tegmental area of sections from twelve control and four parkinsonian midbrains. Mean densities of specific [^{125}I]-transferrin binding sites on melanized neurons were reduced in parkinsonian patients compared to control subjects, the difference being statistically significant for the ventral tier of the substantia nigra, pars compacta, and ventral tegmental area. These data obtained at the cellular level provide an explanation for some of the changes in radiolabeled transferrin binding previously reported for measurements performed at the regional level. The absence of increase in a population of neurons accumulating iron probably reflects the post-transcriptional gene regulation of the number of transferrin receptors that depends upon iron content in eukaryote cells. These findings suggest that alternative pathways may be involved in the accumulation of iron within melanized midbrain neurons in Parkinson's disease.

Supported by INSERM, Association Claude Bernard, Fondation de France grant 91-5716, and National Parkinson Foundation.

91.5

CALCIUM REGULATORY DIFFERENCES IN MITOCHONDRIALLY TRANSFORMED CELLS FROM PARKINSON'S PATIENTS.

J.P. Sheehan, J.B. Tuttle, and ____ _ J.A. Jane*. Departments of Neurological Surgery, Urology, and Neuroscience, University of Virginia, Charlottesville, VA 22908.

Decreased biochemical activity of the electron transport chain complex I and selective neurotoxin induced neuronal cell death have linked Parkinson's disease (PD) to defects in mitochondrial DNA (mtDNA). The potential functional consequences of mtDNA defects and role in the pathogenesis of PD are not defined. Mitochondrially transformed cells (i.e. cybrids) were created by fusing platelets from either PD patients or disease-free, age-matched controls (C) to a cell line devoid of mtDNA.

Fura-2 based fluorescence image cytometry was performed with PD and C cybrids. Basal cytosolic calcium concentrations for the PD and C cybrids were similar at 124±8 and 124±4 nM, respectively. Also, basal ATP levels as determined by weak anion-exchange HPLC were similar. However, following equivalent cytosolic calcium peaks induced by carbachol, a cholinergic agonist known to increase intracellular calcium and stimulate mitochondrial calcium uptake, the rate of calcium recovery was reduced in the PD cybrids by 49% relative to controls ($P<0.015$). In the presence of MPP+, calcium recovery rates for both PD and C cybrids were reduced, but the statistically significant difference in recovery rates between MPP+ exposed PD and C cybrids persisted ($P<0.016$). Finally, when CCCP released mitochondrial calcium stores, PD cybrids had less calcium sequestered in their mitochondria than controls ($P<0.0001$).

These results suggest that mtDNA from Parkinson's disease patients has a subtle impact upon cellular metabolism. Basal energetics and cytosolic calcium values are normal, but PD mtDNA results in a diminished capacity to buffer calcium transients. This effect may hinder nigral neurons' ability to resist neurotoxic phenomena.

91.6

MITOCHONDRIAL DNA SCREENING IN PARKINSONIAN PATIENTS.

R.H. Haas+, X. Hua+, R.K. Naviaux,+ C.W. Shults*+• Univ. Cal., San Diego, *VAMC, San Diego CA 92161

Our group recently reported that patients with early, untreated Parkinson's disease (PD) have reduced activity of complex I of the mitochondrial electron transport chain in platelets when compared to spouses and age/sex matched controls (Ann Neurol 1995;37:714-722). One possible explanation for this reduction in the activity of complex I could be mutation(s) in one of the seven mitochondrial genes that encode for components of complex I. Using denaturing gradient gel electrophoresis (DGGE), we have identified migrational variants in the ND6 gene in four young patients with extrapyramidal features. We have used DGGE to screen two overlapping fragments of the ND6 gene (14,128 - 14,542; 14,439 - 14,884) for migrational variants in the platelet mitochondrial DNA from the 18 PD subjects, 18 age/sex matched controls and 13 spouses who participated in our original study. We found migrational variants in 1 of our spouses and 2 of age/sex matched control subjects but in none of our PD subjects. Mutations in the segments of ND6 studied do not appear to explain the reduction in complex I activity found in these patients. Studies of the ND1 gene are in progress. This work was supported by a grant from the Blowitz-Ridgeway Fndn. and a Center of Excellence Award from the National Parkinson Fndn. to UCSD.

91.7

[^{3}H]DIHYDROROTENONE BINDING TO PLATELET COMPLEX I IN PATIENTS WITH PARKINSON'S DISEASE

F. Blandini*[1], G. Nappi[1], J.T. Greenamyre[2]

Neurological Institute "C. Mondino", University of Pavia, Italy[1]: Department of Neurology, Emory University, Atlanta, GA 30322, USA[2]

Reduced activity of complex I (NADH:ubiquinone oxidoreductase), the proximal component of mitochondrial electron transport chain (ETC), has been consistently reported in the substantia nigra of patients with Parkinson's disease (PD). A "peripheral" deficit of complex I has also been described in platelets of PD patients. However, the issue is controversial since unchanged activity of platelet complex I has been reported in these patients, as well. [^{3}H]dihydrorotenone([^{3}H]DHR), an analogue of the pesticide rotenone, binds with high affinity to the "rotenone site" (putatively the ND-1 gene product) of complex I. An autoradiographic method previously developed showed that, in the brain, [^{3}H]DHR binding to complex I can provide important information, since it is influenced by the same functional modifications which affect the activity of other two components of the ETC, complexes II and IV. We modified this method to study [^{3}H]DHR binding to complex I in intact human platelets. [^{3}H]DHR binding was saturable, specific and highly reproducible; also, it was inhibited by MPP^+ (1-methyl-4-phenylpyridine) in a concentration-dependent manner. Subsequently, we studied [^{3}H]DHR binding to platelet complex I in 16 PD patients and 16 age-matched controls. We also compared the inhibitory effect of MPP^+ on [^{3}H]DHR specific binding in isolated platelets from both populations. PD patients and controls showed similar levels of [^{3}H]DHR specific binding as well as of MPP^+-induced inhibition. A direct correlation between MPP^+-induced inhibition of [^{3}H]DHR specific binding and daily intake of carbidopa/levodopa was found, which may be related to drug-related changes in the transport of MPP^+ into platelets. Although the existence of qualitative alterations cannot be ruled out, our findings show that, outside the central nervous system, no quantitative alterations of complex I or increased susceptibility to MPP^+ are present in PD patients.

Supported by a Mallinckrodt Scholar Award and PHS grant AG11755 (JTG)

91.8

FINGER TAPPING PERFORMANCE IN YOUNG, AGED AND PARKINSONIAN SUBJECTS: TEMPORAL ANALYSIS OF RESPONSE PATTERNS.

L.D. McCullough*, M.S. Cousins, M. Barwick, A. Nawab, C. Corrow, J.D. Salamone. U. of Connecticut Medical Center, Farmington, CT, and Dept. of Psychology, U. of Connecticut, Storrs, CT 06269-1020.

Finger tapping has been used as a test for the assessment of age-related and pathological deficits in motor control. Typically, the clinical assessment is performed with mechanical counters and the only data recorded are total number of responses. In the present study, a computerized analysis of finger tapping was performed so that measurements of response initiation time (time from offset of one response to onset of the next) and duration were obtained for each key press. Each subject was tested on both hands (five 10-sec trials each), and they all were assessed for performance of both a single telegraph key press and a two-finger alternation test. The most robust age-related effects were obtained on the two finger alternation test. Young subjects generally press at much higher rates with two fingers as compared to one finger, but older control subjects showed significant reductions compared to younger subjects in the rate of pressing on the two-finger test. Compared to age-matched controls, Parkinson's disease patients showed significant impairments in finger tapping on both the one- and two-finger tests. Analyses of the temporal patterns of responding showed that parkinsonian patients had significant increases in both response initiation times and response durations. In addition, parkinsonian patients made significantly more perseverative errors on the alternation task. Computer-controlled finger tapping tests could be useful for the assessment and early detection of parkinsonian symptoms as well as the evaluation of therapeutic drugs or transplants.

91.9

PET MEASUREMENTS OF L-DOPA ACTIVATION OF HUMAN MIDBRAIN.

M. Stambuk*, J.S. Perlmutter, K.J. Black, L. McGee-Minnich. Departments of Radiology, Neurology & Psychiatry. Washington University School of Medicine, St. Louis, MO 63110

Parkinson's disease develops after degeneration of nigrostriatal neurons and subsequent striatal depletion of dopamine. L-dopa initially ameliorates symptoms but the benefit may diminish with decreasing duration of action from each dose and the advent of dopa-induced dyskinesias. To further elucidate the mechanism of action of l-dopa, we used the Siemens 953b PET scanner and bolus injection of [15O]-water to measure dopa-induced rCBF changes in 26 patients with PD (17 men; mean age=62 yrs) and no other neuropsychiatric disease. All patients had normal brain MRIs. Nineteen had not taken dopaminergic meds; the remaining 7 did not take meds for at least 24 hrs prior to the PETs. UPDRS ratings were done prior to each PET study. Once in the scanner, part of the UPDRS motor subscale and plasma l-dopa levels were measured repeatedly. Blood flow was measured twice; oral l-dopa 150mg/carbidopa 37.5 mg given (2 hrs after carbidopa 200 mg p.o.); and 75 mins later, blood flow measurements were repeated twice. L-dopa plasma levels were measured at least 4 times after oral administration. Ratings revealed benefit in each subject and plasma l-dopa increased without change in dopamine. Images were placed in stereotactic space and half of the scans before and half after l-dopa were used to make a composite image of all the paired (before and after l-dopa) subtraction images. Candidate responses were found by searching this image with an automatic peak identification routine. Responses were tested for significance by comparison of the regional flows before and after l-dopa with the remaining data. L-dopa produced an 11.7% increase in posterior midbrain ($p < .00005$), a 6.3% increase in right amygdala ($p = .0020$) and a 5.7% increase in left putamen ($p = .0034$). The midbrain response probably reflects a change in striatonigral neuronal activity and we now demonstrate marked asymmetries in dopaminergic pathways to putamen and amygdala. (support: NINDS NS31001, NS32318, the Dana Foundation & the Greater St. Louis Chapter of APDA).

91.10

Tryptophan Metabolites and Serotonin Synthesis in Akinetic Type of Parkinson's Disease

R. P. Iacono, S. M. Kuniyoshi, J.R. Ahlman, Y.J. Li*, G. Maeda

Defective serotonin release has been implicated in Parkinson's disease(PD) concurrent with the discovery of dopamine deficiency. A significant correlation of serotonin(5HT) metabolites with akinetic, but not hyperkinetic, symptoms of PD have been found. We have analyzed the ventricular cerebrospinal fluid (vCSF) for serotonin precursors and metabolites in PD patients undergoing pallidotomy. Twenty patients were categorized into two groups based on predominance of symptoms, hyperkinetic (type A) vs. hypokinetic (type B) and response to L-dopa therapy. Their vCSF concentration of tryptophan metabolites was analyzed with an isocratic HPLC technique. The akinetic subtype manifest a significantly increased ($P < .05$) level of 5hydroxytryptophan (5HTP) (683.7 ± 333.31 ng/ml) and an associated significantly decreased ($P<.05$) vCSF concentration of 5HT (.53 ± 0.23 ng/ml) when compared to that of type A (5HTP 7.52 ± 1.35 ng/ml; 5HT 1.62 ± .39 ng/ml). The type A patients concentration of 5HTP remained in the upper level of non PD controls reported in other series. The relative decrease in 5HT combined with the significantly elevated level of 5HTP implicates an aromatic amino acid decarboxylase (AADC) inefficiency present in the B type of PD. The putative blockade of 5HT synthesis at the 5HTP/AADC level in a specific well defined subgroup of PD patients is a new finding. The biochemical basis of the clinically distinct akinetic form of PD, is contrasted with the classic tremor dominant type of PD. The pathophysiologic relevance of the 5HT deficiency likely involves the compensatory and potentially protective role of 5HT in the dopa deficient and hyperglutaminergic state of Parkinson's disease.

91.11

LOCALIZATION OF MOTOR THALAMUS: ARE STEREOTACTIC COORDINATES GOOD ENOUGH?

H. Richard*, L. B. Tamas, K. A. Sigvardt, C. M. Gray, E. A. Franz. Pacific Neurosciences Institute, 637 Glorietta Blvd., Lafayette CA 94549 and Center for Neurosciences, UC-Davis, Davis CA 95616.

Electrophysiological studies mapping the nucleus ventralis intermedius (Vim) in primates, including humans, have relied on stereotactic coordinates as the frame of reference. We have studied the Vim of 25 patients undergoing stereotactic thalamotomy by recording neuronal responses related to limb movement and to tactile stimulation. In every case, a clear border between motor and sensory regions was found. This border was, in most cases, different from that predicted from standard atlases. We conclude that this "physiological" border may be a better reference point from which to map "physiological" responses in the motor thalamus.

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91.12

OROFACIAL NONVERBAL CONTROL IN PATIENTS WITH PARKINSON'S DISEASE.

M. Gentil, S. Perrin, P. Pollak, C. Feuerstein*, A.L. Benabid, INSERM U 318, CHU, 38043 Grenoble (France).

Force has been considered as one of the likely variables controlled by the nervous system in producing motor behavior (Stein, Behav Brain Sci, 4: 535-578, 1982). The objective of the present study was to characterize the motor control of idiopathic Parkinson's disease patients and normal subjects during orofacial nonverbal tasks. We examined the fine force control associated with speech-like force magnitudes in 3 articulatory organs : upper lip, lower lip, and tongue. A single-dimensional force transducer (Neuro Logic, Bloomington) was used for sampling anterior tongue force toward the alveolar ridge. A load-sensitive cantilever adjustable in two dimensions (Neuro Logic, Bloomington) was used for sampling at midline compression forces generated by the upper and lower lips. These forces were produced in presence of visual feedback. The relationship among several parameters of the ramp-and-hold force contraction and target force level was quantified in 14 parkinsonian patients (7 males and 7 females) and 14 age-matched normal subjects (7 males and 7 females). Parkinsonian patients were assessed with the Unified Parkinson's disease rating scale (UPDRS) and their speech was classified in stage 2 (moderately impaired). All patients were evaluated in OFF of L-dopa. The target force levels used in the present study included 0.25, 0.5, 1 and 2 newtons. For each structure, 10 consecutive contractions were obtained at each of the 4 target force levels which were randomized. Some variables considered of importance to central nervous system programming and control were measured : reaction time, peak force during the ramp phase, overshoot or undershoot of this peak, peak rate of force change, hold phase variability. The results demonstrated an abnormal isometric control force of the articulatory organs in patients with Parkinson's disease. So, measurements of orofacial force control provide useful insights into the fundamental motor control problem of any individual with dysarthria. Moreover, differences were observed between lip and tongue motor performance, consequently independent assessment of articulatory subsystem functions may turn out to be useful.

91.13

AMELIORATIVE AND ADVERSE EFFECTS PRODUCED BY COACTIVATION OF D_1 AND D_2 RECEPTORS IN MPTP-INDUCED PARKINSONIAN MONKEYS,

S. Kuno[1]*, T. Akai[2], M. Ozawa[2], M. Yamaguchi[2], E. Mizuta[1]. [1]Utano Natl. Hosp. Kyoto 616, [2]Res. Dept. Nihon Schering, Osaka 532, Japan.

L-DOPA is effective in treatment of parkinsonian patients but may produce adverse effects such as dyskinesia and psychosis. It is not known whether both D_1 and D_2 subtypes of the dopamine receptor are equally responsible for the side effects. We examined the effects of D_1 receptor agonist (SKF 82958) or D_2 agonist (quinpirole) alone or those of their joint administration in MPTP-induced parkinsonian cynomolgus monkeys. The parkinsonian symptoms (tremor, bradykinesia and rigidity) and the adverse effects (hyperactivity, aggresiveness and dyskinrsia) were evaluated independently using different behavioral criteria. The parkinsonian symptoms were ameliorated either by the D_1 agonist or the D_2 agonist. D_1 or D_2 receptor agonist produced little adverse effects, but joint administration of the two agonists or apomorphine, a non-selective dopamine agonist induced marked dyskinesia and hyperactivity or aggressive behavior. Thus, the adverse side effects manifested by dopamine agonists require coactivation of the D_1 and D_2 receptor.

91.14

VESICULAR MONOAMINE TRANSPORTER (VMAT2) IMMUNOREACTIVITY IS REDUCED IN PARKINSON'S DISEASED STRIATUM

GW Miller*, NR Nash, JT Perez, JK Staley, DC Mash, DB Rye, AI Levey. Dept. of Neurology, Emory University School of Medicine, Atlanta, GA

Ligand binding studies have suggested that VMAT2, which is responsible for transporting monoamines from the cytoplasm into synaptic and dense core vesicles, is a reliable marker of monoaminergic terminals in the striatum. Here we report the production of fusion protein antibodies to human VMAT2 protein and their use in assessing damage to monoaminergic terminals in PD. The large intraluminal loop of VMAT2 (VMAT2-loop; a.a. 46-131) was amplified from a human substantia nigra cDNA library using the polymerase chain reaction. The loop fragment was ligated to pGEX2T vector and transformed into BL-21 (DE3) cells. Fusion protein consisting of VMAT2-loop fused to glutathione-S-transferase was isolated and used to immunize rabbits for antibody production. Polyclonal antibodies to human VMAT2-loop were purified by affinity chromatography and used to assess human VMAT2 protein via immunohistochemistry. VMAT2-loop immunoreactivity was strong in monoaminergic terminal fields in the caudate, putamen, and nucleus accumbens. Immunoreactive fibers projecting to the striatum displayed puncta and were dense throughout the striatum and cell bodies in monoaminergic nuclei. In PD brain VMAT2-loop immunoreactivity was drastically reduced in the caudate and putamen, and to a lessor degree in the nucleus accumbens. This pattern was similar to that of the plasma membrane dopamine transporter, a marker of presynaptic dopamine terminals. Therefore, VMAT2 appears to be a reliable marker of monoaminergic terminal loss in PD. These results suggest that antibodies to human VMAT2-loop protein will be useful in post-mortem assessment of monoaminergic systems in PD and other neurodegenerative disorders, as well as in aging and drug abuse. Supported by NS-31937, NS-09930, NIDA-09484, Emory CNS, and National Parkinson Foundation.

91.15

PALLIDOTOMY LENGTHENS THE MAGNETIC CORTICAL STIMULATION SILENT PERIOD IN PARKINSON'S DISEASE.

MS Young[1,2], WJ Triggs[1,2]*, D Bowers[2], M Greer[2] and WA Friedman[3]. [1]Human Motor Physiology Laboratory, Departments of [2]Neurology and [3]Neurological Surgery, University of Florida, Gainesville, FL 32610.

We compared the duration of the electromyographic silent period elicited in abductor pollicis brevis with transcranial magnetic stimulation (TMS) in 7 patients (5 men) with Parkinson's Disease (Hoehn & Yahr grade 3) 65 (53—74) years of age before and 88 (37—107) days after unilateral pallidotomy (PAL). Medications were unchanged before and after PAL. We used TMS 200, 150, 120 and 100 % motor evoked potential (MEP) thresholds. Repeated measures ANOVA using PAL (pre and post) and recording site (ipsilateral and contralateral to PAL) as factors showed a significant main effect of PAL on the duration of the silent period elicited with TMS 200 % MEP threshold (prePAL 170 ± 55 vs. postPAL 217 ± 44 msec; $p < 0.02$). We found no main effects or interactions in silent periods elicited at lesser stimulus intensities, and no effects or interactions in MEP thresholds. Our results suggest that PAL improves the function of cortical motor inhibitory circuits in Parkinson's Disease.

Supported by the National Parkinson Foundation.

91.16

UPRIGHT MOBILITY IN PARKINSON'S DISEASE: BACKWARD WALKING IS THE MOST DIFFICULT.

A. Behrman*, K. Light, S. Flynn, D. Bowers, W. Triggs, and W. Friedman. Depts. of Physical Therapy, Neurology, and Neurosurgery, Univ. of Florida, Gainesville, FL 32610.

Individuals with Parkinson's disease (PD) typically demonstrate difficulty in forward walking by a slower speed with small, shuffling steps. Clinical observations indicate that other upright mobility movements appear to be even more difficult for these individuals. The control of upright mobility differs according to the requirements for programming and planning, feedback, balance, pattern generation, and force. The purpose of this study was to explore the relative difficulty of backward walking, stair-climbing, and walking with a figure-eight turn by studying the ratios of these timed movements to the speed of forward ambulation 20' between subjects with PD and subjects without PD. Eleven individuals with PD on medication (M = 67 years) and 11 age- and gender-matched controls participated in this study. Subjects with PD had a mean Hoehn and Yahr of 3.6 and a mean motor UPDR of 42. Each upright mobility task was timed with a stopwatch to 0.1 s for both self-selected and maximum speeds. Ratios were compared between groups with student *t* tests. The ratios of forward walking/figure-eight walking, for both self-selected and maximum speeds, were not found different. All other group comparisons on ratios were significantly different at $p < .01$. The individuals with PD demonstrated that backward walking at maximum speed and backward walking at self-selected speeds were the most difficult, followed by stair-climbing at maximum speed, then self-selected speed. These results suggest that individuals with PD have the greatest difficulty generating an open-loop pattern that is not well practiced and where visual cuing is limited. Individuals with PD also demonstrated problems with force production.

91.17

Selective Chemo-Pallidectomy in Rhesus Maccaca.

Marc-Etienne Corthésy*, Nitin Gogate, Paul F. Morrison[1], Russel R. Lonser, Alex C. Cummins, Robert L. Dedrick[1], Edward H.Oldfield.

Surgical Neurology Branch, NINDS, and [1]Biomedical Engineering and Instrumentation Program, National Center for Research Resources, National Institutes of Health, 9000 Rockville Pike, Bethesda, MD 20892

Latero-ventral pallidotomy has been reconsidered recently in the context of Parkinson's disease. The innocuity of classical neurosurgical techniques is limited by the anatomical situation of the globus pallidum, which is wedged between the optic tract ventrally and the internal capsule medially. We report here application of the high flow microinfusion technique to carry out a selective neuronal pallidectomy. Eight monkeys were used, out of which 4 were rendered hemiparkinsonian by intracarotid injection of MPTP and 4 were rendered fully parkinsonian by intravenous injection of MPTP. After stabilization of the parkinsonian syndrome, 2 full and 2 hemiparkinsonian monkeys were infused with 5 µl PBS and served as controls. The other animals were infused with 250 nmol solution of quinolinic acid in 5 µl PBS (pH corrected to 7.4) at a rate of 0.1µl/min, using a 10 µl Hamilton syringe with a 32 Ga needle reinforced proximally with 28 and 22 Ga tubing.
Clinical assessment of the monkeys showed a clear improvement of the brady-akinetic signs and of the tremor. Rigidity, as assessed under anaesthesia, was also reduced as early as the end of the procedure. Histological evaluation revealed a 3 to 5 mm diameter area of neuronal loss in the posterolateral aspects of the pars medialis of the pallidum and no sign of axonal or myelin lesion in the optic tracts and the internal capsule.

91.18

SINGLE UNIT RECORDINGS OF SUBTHALAMIC NUCLEUS AND PARS RETICULATA OF SUBSTANTIA NIGRA IN AKINETO-RIGID PARKINSONIAN PATIENTS.

A. Benazzouz, B. Piallat, P. Pollak, P. Limousin, D.M. Gao, P. Krack and A.L. Benabid*. Lab. de Neurobiophysique, INSERM U.318, CHU 38043 Grenoble - France.

We have shown that high frequency stimulation (HFS) of subthalamic nucleus (STN) induces a spectacular improvement of akinesia and rigidity. Neuronal properties of STN human parkinsonian patients are not known. We studied 15 STN of 8 akineto-rigid parkinsonian patients during surgical implantation of chronic stimulating electrodes. We have recorded the neuronal activity in pars reticulata of substantia nigra (SNr) which is just below STN in the same trajectory. A set of 5 electrodes was lowered down to the targets. Using microelectrodes, it was possible to record as well as to stimulate the investigated area. STN neurons (n=32) exhibited two different extracellular spike waveforms and differences in their basal firing rates. The majority of recorded cells are characterized by spikes with a mainly negative waveform and tonic regular firing rate. The frequency of discharge is between 15 and 70 Hz (mean±SD = 35.2±18.8 spikes/sec). Some STN cells exhibited biphasic waveforms with a mixture of bursts and isolated spikes. These neurons present a low frequency of discharge (11.1±2.3 spikes/sec) and respond to passive movements of the wrist by an increase of activity. At the target level, intraoperative HFS induces reversal of akinesia and rigidity. SNr recorded cells (n=28) are characterized by biphasic, symetrical and large amplitude spikes. Their discharge pattern is irregular and the frequency is between 10 and 50 Hz (28.7±14.9 spikes/sec). In SNr cells, there was no response to passive or active movements. These results show that STN neurons present an hyperactivity in akineto-rigid parkinsonian patients comparable to that recorded in MPTP-treated monkey. Correlation between single unit microrecordings and intraoperative positive effects of stimulation on akinesia and rigidity lead us to define with precision the localization of sites that yields maximal efficacy.

91.19

COORDINATION OF FINGERTIP FORCES DURING OBJECT RELEASE IN PARKINSON'S DISEASE. A.M. Gordon*. Department of Movement Science, Teachers College, Columbia University, New York, NY 10027.

In the present study we examined fingertip forces during the replacement and release of an object on a table surface in subjects with Parkinson's disease (PD, stage 1-3) ON and OFF medication and age-matched control subjects. Subjects held an instrumented object between the index finger and thumb (precision grip) level with a marker 5 cm over a table surface while the employed grip (normal) forces and load (tangential) forces were measured. Upon verbal instruction, they replaced the object to the table and released it from their grasp. Subjects were asked to perform the task at a comfortable pace (no instructions regarding speed or accuracy were given). Both groups of subjects employed similar levels of grip force and began to decrease their grip force in an anticipatory fashion before the moment of table contact. The rates at which the grip forces decreased following table contact were similar in both groups, and were modified according to the objects weight (faster rates of force release were seen for a 900 g object compared to a 300 g object). The time from initiation of replacement to table contact was similar in both groups, as was the total duration of isometric force decrease and latency between release of the index finger and thumb. None of the parameters measured were affected by dopaminergic medication. These results contradict previous reports of deficits in the ability to terminate motor activity when asked to do so as fast as possible and suggest that natural, well-practiced, self-paced tasks are less impaired by Parkinson's disease than tasks which impose time constraints.

91.20

DETERMINATION OF DOPAMINE-RELEASING PROTEIN (DARP) IN CEREBRO SPINAL FLUID OF PATIENTS WITH NEUROLOGICAL AND NON-NEUROLOGICAL DISORDERS. L. Verdugo-Díaz[1], C. Morgado-Valle[2], G. Solís-Maldonado[3], R. Drucker-Colín[1,2], S. Kuhanathan[4], V.D. Ramirez[4*] [1]Depto de Fisiología, Fac. de Medicina, UNAM. [2]Instituto de Fisiología Celular, UNAM. [3]Servicio de Neurocirugía, Hospital Central Sur de Alta Expecialidad PEMEX, México. [4]Molecular & Integrative Physiology, University of Illinois, Urbana, USA.

In this study we determined the levels of DARP in the cerebro spinal fluid (CSF) of patients having a wide variety of neurological disorders. CSF from patients (2 ml) was obtained by a lumbar puncture and immediately snap frozen in liquid nitrogen. DARP level was determined by an ELISA technique using monoclonal anti-DARP antibodies. A synthetic peptide corresponding to the first 36aa of the N-terminal of DARP was used as standard. A total of 25 non neurological patients versus 71 patients with a wide variety of neurological disorders categorized as malformations, tumors, demyelination, infections, and degeneration were tested. The relative concentrations of DARP were 12.06 ± 5.51 ng/ml in patients with non-neurological diseases (mean ± SEM, n=25), and decreased in patients with demyelinating disorders (7.49 ± 2.53 ng/ml, n=14) and in Parkinson's patients (4.64 ± 1.91 ng/ml, n=3). In the patients with malformations, concentration of DARP was higher (25.66 ± 6.41 ng/ml, n=17) and it is similar to control in patients with tumors (14.32 ± 5.97 ng/ml, n=18) and infections (10.16 ± 3.52 ng/ml, n=17). In addition, in several samples DARP was identified by Western blot using monoclonal antibodies against DARP at different dilutions (1:20 to 1:1000). Under reducing conditions, most samples showed two distinct bands of 50-60 kDa. For example, a patient suffering of syringomyelia with high DARP values by ELISA showed a strong positive Western blot. In contrast, in 3 PD patients, the Western blot signal was feebled in two and moderately strong in one. These data suggest that changes in concentration of DARP may be involved in certain neurological disorders with highest values in patients with malformations of the nervous system and low levels in patients with degenerative diseases.

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DEGENERATIVE DISEASE: OTHER—MOVEMENT DISORDERS

92.1

"EVIDENCE FOR A CORRELATION BETWEEN THE DEGREE OF DNA FRAGMENTATION IN HUNTINGTON'S DISEASED STRIATUM AND THE NUMBER OF CAG REPEATS IN THE HUNTINGTON'S DISEASE (IT15) gene". N.J. Butterworth*, L. Williams, P. Lawlor, D. Love, R.L.M. Faull and M. Dragunow. Dept of Pharmacology, Univ. of Auckland, Auckland, New Zealand.

Huntington's disease (HD) is an autosomal dominant neurodegenerative disorder characterised by the selective loss of striatal GABAergic efferent projection neurons. The chemical diagnosis of HD correlates with an expansion of a CAG triplet codon repeat located on the 5' end of the HD gene (IT15). We have recently shown evidence of DNA fragmentation in neurons in HD striatum, suggesting that neuronal degeneration in HD may occur through apoptosis, which is characterised by DNA fragmentation and may be the mechanism underlying neuronal degeneration in a number of other neurological diseases. In this study we investigated the relationship between the degree of DNA fragmentation (using the TUNEL *in situ* end labelling of DNA method) and the number of CAG repeats in the Huntington's disease gene in striatal tissue from HD brains. We used frozen and fixed tissue from 16 HD postmortem HD brains (graded 0 to 3 on the Vonsattel classification, postmortem delay ranging from 4.5-41 hours), control sections were taken from age and postmortem delay matched, neurologically normal striatal tissue. Our results show a significant positive correlation between the number of CAG repeats and the degree of DNA fragmentation. These results suggest that expanded CAG repeats may lead to neuronal degeneration in HD through an apoptotic mechanism.

N.Butterworth is a New Zealand Neurological Foundation, W. B. Miller scholar. This work was funded by the New Zealand Neurological Foundation and the New Zealand Health Research Council.

92.2

ALTERED NEURONAL EXPRESSION AND INTRACELLULAR TRAFFICKING OF HUNTINGTIN IN THE HUNTINGTON'S DISEASE BRAIN. E. Sapp, C. Schwarz, K. Chase, P. Bhide, A.B. Young, J. Penney, J.P.Vonsattel, N. Aronin, and M. DiFiglia.* Massachusetts General Hospital, Boston MA 02114, & Univ. of Massachusetts Med. Ctr., Worcester, MA 01655.

We examined the immunohistochemical localization of huntingtin, the protein product of the HD gene, in HD brain (N=14) with an antibody that detects both wild-type and mutant huntingtins by immunoblot. In control and HD brain, huntingtin localized to neurons and in the striatum distributed in a patch\matrix-like pattern. Neuronal expression was markedly reduced in the striatum and in both segments of the globus pallidus in HD patients with low grade pathology. Cortical and striatal neurons with normal-looking morphology showed significant accumulation of huntingtin to an endosomal\lysosomal-like compartment in perikarya, dendrites, and axons, particularly in juvenile and low grade cases. Ultrastructural study revealed the labeled structures to be tubulovesicular and multivesicular bodies, which are organelles of the endocytic pathway leading to lysosomes. Some immunoreactive dendrites in cortex were beaded and fragmented similar to neurons undergoing hypoxic/ischemic and excitotoxic injury. Huntingtin was concentrated in the perinuclear cytoplasm and reduced or absent in processes of degenerating neurons. Some neurons had staining in the nucleus; other neurons were devoid of huntingtin expression. A few cortical neurons in 2 of 8 controls had increased endosomal\lysosomal and marked perinuclear accumulation of the wild-type protein. Results demonstrate early changes in the neuronal expression and intracellular sorting of huntingtin in HD affected brain regions. The expanded polyglutamine region in mutant huntingtin may disrupt sorting signals that regulate the intracellular trafficking of the protein and ultimately cause energy failure and cell death. Supported by NS 16367 to MD and NS31579 to NA.

92.3

WILD-TYPE AND MUTANT HUNTINGTIN LOCALIZE TO THE GOLGI COMPLEX AND TO VESICLES IN THE PERIPHERAL CYTOPLASM IN FIBROBLASTS OF CONTROL AND HD PATIENTS. J. Velier, C. Schwarz, C. Young, J. Fallon, B. Hyman, E.J. Martin*, S. Hughes, R. Vallee, N. Aronin and M. DiFiglia. Massachusetts General Hospital, Boston MA 02114, Univ. of Mass. Med. Ctr. Worcester, MA 01655 and Worcester Fnd. Exper. Bio., Shrewsbury, MA

A CAG repeat expansion in the Huntington's disease (HD) gene results in the expression of a mutant huntingtin protein in the brain (Aronin et al., 1995, Neuron). We compared the localization of wild-type and mutant huntingtin in fibroblasts from a control and HD patients who were heterozygote (juvenile onset), and homozygote gene carriers. Immunofluorescence was performed with an anti-peptide antibody to amino acids 2527-2547 in huntingtin and the results analyzed with confocal microscopy. In normal and HD fibroblasts huntingtin associated with tubulovesicular membranes in the perinuclear region and with vesicles widely scattered throughout the cytoplasm. In control fibroblasts, double labeling for huntingtin and a membrane-associated protein of the Golgi complex (58kD), showed that both proteins almost completely overlapped. Treatment with Brefeldin A, a drug that disrupts the Golgi complex by inhibiting the association of coat proteins with Golgi membranes, dispersed huntingtin and 58kd localization to the cytosol. Results demonstrate that wild-type and mutant huntingtin are localized to the same subcellular compartments. Findings support previous speculation that huntingtin plays a role in vesicle transport (DiFiglia et al., 1995, Neuron) and suggest that the protein functions at the level of the Golgi apparatus and in the transport or recycling of vesicles. Supported by grant NS16367 to MD, NS31579 to NA and GM47434 to RV.

92.4

REACTIVE GLIOSIS IN HUNTINGTON'S DISEASE AS DEMONSTRATED WITH [^{3}H] PK11195 BINDING. J.A. Kotzuk[4], M.W. Becher[1,4], A.H. Sharp*[2], C.A. Ross[2,3], M.V. Wagster[1,4,5]. Departments of [1]Pathology, [2]Psychiatry & Behavioral Sciences, and [3]Neuroscience, and the [4]Neuropathololgy Laboratory, The Johns Hopkins University School of Medicine, and [5]Department of Psychology, Johns Hopkins University, Baltimore, MD 21205.

The characteristic neuronal loss and atrophy of the striatum in Huntington's disease (HD) is accompanied by a reactive astrocytosis. In an effort to label and quantify these astrocytic changes, we examined the caudate, dorsal frontal cortex, and temporal pole in cases of HD (Vonsattel grades 2, 3, and 4) and age-matched controls, using the peripheral-type benzodiazepine receptor ligand [^{3}H] PK11195, which has been shown to be a marker for gliosis and indicator of neuronal damage in the CNS (Dubois et al., *Brain Res.* 445:77-90, 1988). Using *in vitro* receptor autoradiography, we incubated tissue sections in 1 nM [^{3}H] PK11195. Glial cell types were visualized in adjacent sections by immunocytochemical staining. [^{3}H] PK11195 binding concentration was increased in all grades of HD when compared to controls in all areas studied. There was little difference in binding between grades 2, 3, and 4 in cortical areas. In caudate, mean binding concentrations in grade 2 and 3 cases were increased compared to controls. Grade 4 cases showed increased [^{3}H] PK11195 binding compared to controls and to grades 2 and 3: control mean = 130.7 fmol/mg tissue standard; grade 2 = 285.7; grade 3 = 279.9; grade 4 = 389.1. These data indicate that reactive gliosis occurs early and abundantly in HD and, in caudate, continues to increase as the disease progresses. [^{3}H] PK11195 binding is an effective tool to measure gliosis quantitatively in neurodegenerative disease.

This work was supported by grants from NINDS.

92.5

IMMUNOHISTOCHEMICAL LOCALIZATION OF MARKERS FOR OXIDATIVE INJURY IN HUNTINGTON'S DISEASE. R.J. Ferrante*, N.W. Kowall, S.M. Hersch, R.H. Brown, M.F. Beal. Bedford VAMC, Boston University School of Medicine, Emory University, and Mass. General Hospital.

There is evidence to suggest that oxidative stress may play a role in the neuronal death observed in Huntington's disease (HD). DNA strand breaks and lipofuscin accumulation are increased in the striatum and cortex of HD patients. MRI spectroscopy shows increased lactate concentrations *in vivo*, consistent with impaired oxidative phosphorylation. Impaired oxidative phosphorylation generates reactive oxygen species which can alter proteins, lipids, and DNA. In the present study we examined immunohistochemical markers for oxidative stress, using antisera against heme oxygenase (HO), 8-hydroxy-2-deoxyguanosine (8-OHDG), 3-nitrotyrosine (3NT), and malondialdehyde (MDA) in postmortem tissue samples of striatum and neocortex from 12 varying grade (0-4) HD subjects and 4 normal controls. 8-OHDG and MDA are markers of oxidative damage to DNA and lipids, 3NT is a marker of peroxynitrite mediated protein nitration, and heme oxygenase is induced by oxidative stress. The general distribution of HO, 8-OHDG, 3NT, and MDA was similar. Control brains showed only scattered, weakly stained, striatal and cortical neurons with increased immunoreactivity (IR) in the striatal matrix zone. The distribution and extent of oxidative injury was enhanced in HD and paralleled the pattern of neuronal degeneration observed in the disorder. In low grade HD cases, HO, 8-OHDG, 3NT, and MDA-IR was greater in the dorsal striatum than the ventral striatum. In more severe grades, IR increased throughout the striatum. In the most severe grades, striatal IR was reduced, consistent with neuronal loss. A gradual increase in neuronal HO, 8-OHDG, 3NT, and MDA-IR occurred in HD cortex with increasing grade of severity. These observations suggest that energy failure and subsequent oxidative injury may contribute to the pathogenesis of HD. Supported by NIH grants AG12922 and NS-01624, the Huntington's Disease Society of America, and the Department of Veteran Affairs.

92.6

MITOCHONDRIAL CALCIUM HOMEOSTASIS IN HUNTINGTON'S DISEASE FIBROBLASTS. C-A Gutekunst*, T-I Peng, WL Whaley, B Rock, SM Hersch and JT Greenamyre. Departments of Neurology, Psychiatry & Dermatology, Emory University School of Medicine, Atlanta GA 30322

The HD gene product, huntingtin, in its expanded form, binds to and inactivates glyceraldehyde phosphate dehydrogenase, a key enzyme in glycolysis. A selective complex II/III defect has also been reported in HD caudate. Moreover, mitochondrial toxins model the pathology and neurologic symptoms of HD. Thus, regional and cellular differences in mitochondrial function, or suceptibility to oxidative damage, may determine selective neuronal degeneration. We hypothesize that the metabolic defects that lead to neuronal death in HD occur in non-neuronal cells, whether or not they cause cytotoxicity. Because studies of the relationship between the HD mutation and metabolic processes require the use of living cells, we are using cultured fibroblasts from control and HD patients and laser scanning confocal microscopy to investigate cytosolic and mitochondrial Ca^{2+} fluxes, mitochondrial membrane potential and free radical production. Our preliminary results indicate that we are able to reliably follow ionomycin-induced cytosolic Ca^{2+} fluxes in human fibroblasts with Fluo-3. In addition, we have used Rhod-2, a calcium indicator which selectively labels mitochondria and nucleoli. In response to ionomycin, there was a robust response in both mitochondria and nucleoli. Challenges with ionophores and metabolic inhibitors may reveal latent defects caused by the HD mutation and lead to a better understanding of the relationship between the huntingtin expansion and neurodegeneration. *Supported by a Markey Fellowship from Emory University (CAG), the Emory Alzheimer's Disease Core Center (TIP) and a Mallinckrodt Scholar Award (JTG).*

92.7

ALZHEIMER'S DISEASE CHANGES IN HUNTINGTON'S DISEASE. M.W. Becher[1,5], J.A. Kotzuk[5], M.V. Wagster[1,5], N.G. Ranen[2], C.A. Kitt[7*], D.L. Price[1,3-5], C.A. Ross[2,4,6]. Departments of [1]Pathology (Neuropathology Division), [2]Psychiatry & Behavioral Sciences, [3]Neurology, and [4]Neuroscience, and the [5]Neuropathology Laboratory and the [6]Laboratory of Molecular Neurobiology, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205, and [7]NINDS, Bethesda, Maryland 20892.

One of the triad of features of Huntington's disease (HD) is cognitive dysfunction, particularly in mid-late stage cases. The dementia of HD has traditionally been considered "subcortical" both in origin and nature, yet this issue remains incompletely characterized. Neocortical changes, such as loss of neurons and changes in excitatory amino acid receptors, can be demonstrated in HD brains, and recent postmortem studies indicate that there are volumetric decreases in HD neocortex. We examined archival HD brain tissue from our Baltimore HD Project Tissue Bank for the presence of concomitant Alzheimer's disease (AD)-type changes by modified Bielchowsky silver stains. Subjects ranged from 38 to 93 years of age. The majority (20/31, 65%) of HD cases, including several elderly subjects, had no evidence of neocortical senile plaque (SP) formation or neurofibrillary tangles (NFT). Interestingly, 9/31 (29%) HD patients had some degree of AD-type pathological changes including rare diffuse SP, scattered NFT, focal neuritic SP, and moderate to frequent diffuse SP with only rare neuritic SP. Using CERAD criteria, one patient (3%) each fulfilled the criteria for "Definite AD" and "Probable AD". These results underscore the need to continually seek heterogeneous neuropathological features in neurodegenerative cases despite the establishment of a primary clinicopathological diagnosis.

This work was supported by grants from NINDS.

92.8

NEUROTRANSPLANTATION OF FETAL LATERAL GANGLIONIC EMINENCE TISSUE IN THREE PATIENTS WITH HUNTINGTON'S DISEASE. O.V. Kopyov, S. Jacques*, M. Kurth, L. Philpott, A. Lee, M. Patterson, C. Duma, E. Kogosov, A. Lieberman, and K.S. Eagle.

Neurosciences Institute, Good Samaritan Hospital, Los Angeles, CA 90017.

Huntington's disease (HD) is an autosomal dominant genetic neurodegenerative disease which leads to dementia, involuntary writhing movements (chorea) and eventual death. Currently there are no effective treatments for HD, and patients must live with the movement and mood disorders it creates. Recent success with fetal transplantation for Parkinson's disease (PD) has led to the consideration of fetal transplantation as a treatment for HD. This group has performed many fetal transplantation procedures for PD (Kopyov et al, 1996) and in the last year has performed fetal transplantation for three patients with HD. Tissue was obtained from the lateral ganglionic eminence (LGE), a region which has been shown to develop into striatal tissue, of 9-week fetal cadavers. This tissue was grafted bilaterally to four sites in the caudate nucleus and one site in the putamen. The effects of tissue grafts have been assessed with both pre- and postoperative MRI and PET scans, extensive neurological testing using the UHDRS and Schwab and England scales, and neuropsychological evaluations. All patients have recovered well from the surgery and demonstrate improvement in motor and cognitive deficits. They exhibit no further degeneration as a result of HD. The positive initial outcome and recovery of these patients provides preliminary evidence that the utilization of fetal transplantation in carefully selected cases may prove an effective treatment strategy for HD. While further investigation is required to confirm the potential of treatment of HD with fetal transplantation, the present report demonstrates that fetal transplants do not appear to have negative effects and that they have the potential to significantly improve the lives of HD patients.

92.9

BEHAVIORAL AND HISTOLOGICAL COMPARISONS OF TWO ANIMAL MODELS OF HUNTINGTON'S DISEASE. Jie Dong, Deborah A. Shear, Christopher D. Gundy, Kristi Haik-Creguer, and Gary L. Dunbar*. Brain Research Laboratory, Department of Psychology, Central Michigan University, Mt. Pleasant, MI 48859.

Intrastriatal administration of the *N*-methyl-D-aspartate agonist, quinolinic acid (QA), and the mitochondrial toxin, 3-nitropropionic acid (3-NP), have been used as animal models of Huntington's disease (HD). The aim of this study was to compare these two models on the following behavioral and histological measures: (1) open-field nocturnal activity levels; (2) motor performance on balance-beam and grip-strength tasks; (3) learning ability in a radial-arm-water-maze (RAWM) task; (4) sparing of NADPH-diaphorase neurons; (5) amount of cytochrome oxidase labelling; and (6) loss of Nissl-stained neurons. Adult male Sprague-Dawley rats were given bilateral intrastriatal injections of either 750 nmol 3-NP (n=6), 200 nmol QA (n=8) or phosphate-buffered saline (n=6). Behavioral testing began two weeks postlesion and histological processing began four weeks postlesion. Both 3-NP and QA injections caused significant hyperactivity at two weeks postlesion in the open-field, but only the QA rats showed hyperactivity at four weeks postlesion. Both QA and 3-NP rats showed significant impairment on the balance beam task, while only 3-NP rats differed significantly than controls on the grip-strength task. Finally, both QA and 3-NP rats showed significant impairments in the RAWM task, with 3-NP rat being more severely impaired. Both types of lesions produced significant reductions in CYO staining and in the number of Nissl-stained neurons in the striatum, with only the 3-NP lesions created necrotic cavities in the striatum. Lesions with 3-NP tended to result in more sparing of NADPH-diaphorase-positive neurons in regions adjacent to the necrotic core. Collectively, these results suggest that the QA model mimics some of the earlier behavioral and neuropathological symptoms of HD, while the 3-NP model may mimic more of the later symptoms (or juvenile onset) of HD. *This project was funded by NIH grant I-R15-NS30694-01A3.*

92.10

EXPRESSION OF FULL-LENGTH AND TRUNCATED HUNTINGTON'S DISEASE PROTEIN (HUNTINGTIN) IN NEURONAL CELLS. J.K. Cooper[1], A.H. Sharp[1], Y.P. Goldberg[3], G. Schilling[1], S-H. Li[1], D. Borschelt[2], S. Sisodia[2], M.R. Hayden[3], and C.A. Ross[1*]. Lab. of Molecular Neurobiology[1] and Div. of Neuropathology[2], Johns Hopkins Med. Sch.[1], Balto, MD, USA; Dept. of Mecdical Genetics, Univ. of British Columbia[3], Vancouver, BC, Canada.

Huntington's disease (HD) is caused by expansion of a CAG repeat coding for polyglutamine in the 350 kDa protein product of the HD gene, huntingtin. While normal and expanded huntingtin is widely expressed, HD is characterized by selective neuronal vulnerability in medium spiny neurons of the caudate and putamen, middle and deep layers in cerebral cortex, and other brain regions. The mechanism of cell death is unknown but has been postulated to involve a gain-of-function mutation. So far, attempts to develop a cellular model of HD using non-neuronal cells have been unsuccessful. We are, therefore, seeking to establish a cellular model using the neuroblastoma cell line, Neuro-2a (N2A). Truncated and full-length HD proteins with normal or expanded repeats (44Q) were transiently expressed in N2A cells using the CMV promotor. Western blots with the antibody AP81 indicate that the full-length HD protein is expressed. Immunofluorescence indicates that full-length huntingtin is present in a cytoplasmic pattern similar to that demonstrated in our previous studies of the HD protein in brains of control individuals and HD patients. There appears to be some enrichment in distal neuritic processes. In addition, the N-terminus (cloned from a human brain cDNA library) has been ligated into the prion protein promotor vector, pPrP. To further assess the role of different CAG sized huntingtin expressed in neuronal cells, we are currently developing stable N2A cell lines as well as huntintin constructs with larger size repeats. Expression of huntingtin containing different polyglutamine lengths in N2A cells will be useful in the elucidation of the pathogenesis of HD. (Supported, in part, by the NINDS and the Canadian MRC.)

92.11

ANTEROGRADE AND RETROGRADE TRANSPORT OF HUNTINGTIN IN PERIPHERAL NERVE. J.Block-Galarza, E. Sapp, K.O.Chase, K. Vaughn, R. Vallee, M. DiFiglia and N. Aronin* Univ. Mass. Med. Sch., Worcester, MA 01655, Worc. Fndn. Exper. Biol., Shrewsbury, MA 01545 and Mass. Gen. Hosp., Boston, MA 02114.

Huntingtin is the protein product of the IT15 gene; CAG repeat expansion in this gene is the fundamental genetic defect in Huntington's disease. We and others detected huntingtin in axon fibers and terminals, in association with a subset of vesicles. Here we examined the transport of huntingtin in the sciatic nerve of the rat. After appropriate anesthesia, the sciatic nerve was exposed and light pressure was placed on the nerve for 5 seconds. The wound site was sutured and the animals were allowed to survive for 6, 12, 24 and 48 h. The contralateral sciatic nerve was sham-operated and served as the control in each case. The animals were decapitated and the sciatic nerves were rapidly removed for immunohistochemistry (immunofluorescence and bright field) or extraction for SDS-PAGE and immunoblot. Results showed that huntingtin accumulated both proximally and distally to the pressure lesion, with increasing intensity from 6 to 12 to 24h. Dynein also accumulated in the same time frame both proximally and distally; SNAP-25 was concentrated only proximally and neurofilament did not change at either site compared to controls. Immunoblot for huntingtin confirmed the immunohistochemical results. We conclude that huntingtin in sciatic nerves is transported bidirectionally. Anterogradely huntingtin moves by fast axonal flow. Thus, huntingtin is recycled in mammalian peripheral nerve. Supported by NIH NS31579 (NA), GM47434 (RV) and NIH NS16367 (MD).

92.12

IT15 EXPRESSION AFTER NEUROTROPHIN AND ANTISENSE TREATMENT: IN VIVO AND IN VITRO STUDIES. N.S.K. Haque*, P. Borghesani, C.J. Le Blanc and O. Isacson. Neuroregeneration Laboratory, McLean Hospital and Program in Neuroscience, Harvard Medical School, Belmont, MA 02178

The mutation of the IT15 gene responsible for Huntington's disease (HD) on chromosome 4 p16.3 results in an increase in the length of a polyglutamic acid tract in the 348kD Huntingtin protein. There is considerable evidence to suggest that susceptibility to excitotoxic damage is a factor involved in the specific neuronal loss observed during HD however, the relationship between this pathology and the HD mutation remains obscure. Moreover, the normal function of this protein has yet to be elucidated. To examine whether reduced IT15 expression affects susceptibility to glutamate receptor mediated toxicity in neurons, an antisense RNA approach was undertaken to modify striatal IT15 gene expression in vivo. Repeated direct injections of an antisense molecule designed to block translation of mouse IT15 mRNA however, did not significantly reduce striatal IT15 protein levels as assessed by immunostaining and Western blotting. IT15 is a large protein and there is evidence to suggest that it has a half-life greater than 72 hours. Thus, in another attempt to answer the same question of the role of IT15 in excitotoxicity, we cloned IT15 antisense cDNA into the pCMVb vector (Clontech) and transiently transfected the "neuron-like" NTera-2 cells (Stratagene). These cells were then subjected to NMDA-mediated toxicity and these results will be presented. Certain growth factors have been shown to protect neurons from excitotoxic insult. Data from experiments examining expression of Huntingtin in vivo after exposure to various neurotrophins will also be shown. (N.S.K. Haque is a Wills Foundation Fellow. This work was also supported by NS30064).

92.13

EXPERIMENTAL THERAPEUTICS IN A MODEL OF HUNTINGTON'S DISEASE. J Timothy Greenamyre*, Eric Lai and Monica Garcia-Osuna. Departments of Neurology and Pharmacology, Emory University, Atlanta, GA 30322

The HD gene product, huntingtin, in its expanded form, binds to and inactivates glyceraldehyde phosphate dehydrogenase, a key enzyme in glycolysis. A selective complex II/III defect has also been reported in HD caudate. We used striatal complex II inhibition by malonate as a model of HD. We report that 2 compounds in clinical development, remacemide hydrochloride and OPC-14117, are protective in this model. Remacemide is a low-potency NMDA receptor channel blocker; its desglycine metabolite has an affinity of ~1 μM. OPC-14117 is an antioxidant compound. Both drugs have been used in small clinical trials of tolerability in HD. Sprague-Dawley rats received an intrastriatal injection of malonate (1μmole/2 μL) with or without systemic remacemide or OPC-14117. Remacemide was administered i.p. 30 min before and 150 min after malonate; OPC-14117 was administered as a single ip injection at a dose of 300 mg/kg 30 min before malonate infusion. Three days later, rats were euthanized. Slide-mounted brain sections were stained for cytochrome oxidase and lesion volumes were reconstructed using an MCID image analysis system. Remacemide, at a dose of 20 mg/kg, reduced lesion volume from 27.7 to 19.6 mm^3 ($p < 0.02$). In a separate experiment, remacemide at a dose of 30 mg/kg reduced lesion volume from 43.4 to 27.8 mm^3 ($p < 0.02$). OPC-14117 reduced lesion volume from 25.7 to 7.8 mm^3 ($p < 0.002$). To the extent that metabolic defects contribute to the pathogenesis of HD, our results suggest these compounds can be expected to be neuroprotective. *Supported by a Mallinckrodt Scholar Award, Astra Arcus USA and Otsuka America.*

92.14

IDENTIFICATION OF NOVEL TRINUCLEOTIDE REPEAT CONTAINING GENES AND ASSOCIATION WITH NEURODEGENERATIVE DISEASE.
A. M. Gormley, P. R. Maycox, R. James, B. W. Crook, G. W. Roberts, D. Howlett* and J. Price. Dept. of Molecular Neuropathology, SmithKline Beecham, Harlow, Essex, CM19 5AW, UK.

At least eight neurodegenerative diseases are known to be associated with genes that contain expanded trinucleotide repeat (TNR) regions. Five of these diseases contain a CAG expansion in the coding region of the respective gene which is translated into expanded polyglutamine in the native protein. One example is Huntington's disease. In the normal population, the Huntingtin gene is polymorphic, containing between 9 and 36 repeats. In contrast, patients with the disease have from 37 to 120 repeats. One characteristic of TNR diseases is the phenomenon of anticipation, whereby the disease appears earlier in successive generations and in a more severe form. It is now known that the expansion is a dynamic mutation that increases in length through generations and the length correlates with the age of onset and severity of the disease

We wish to identify novel candidate genes for trinucleotide expansion. To this end we have screened various genome databases for expressed sequences containing at least seven CAG repeats. PCR primers were then designed so that the chromosomal localisation of candidate sequences could be determined using template DNA prepared from monochromosomal hybrid cell lines. We have compiled a set of novel genes that can be screened against diseases showing the appropriate chromosomal linkage and genetic characteristics, e.g. anticipation. We are screening our set of localised genes, for CAG expansion, against DNA obtained from patients with candidate neurodegenerative diseases. One such example is spinal cerebellar ataxia type 2 for which the locus has been identified but the gene is unknown.
This work was funded by SmithKline Beecham Pharmaceuticals.

92.15

NEURONAL DEATH IN CULTURED MURINE CELLS FOLLOWING GAPDH INHIBITION. C.T. Sheline* and D.W. Choi. Center for the Study of Nervous System Injury and Dept. of Neurology, Washington Univ. School of Medicine, St. Louis, MO 63110.

It has recently been reported that the mutant polyglutamine-containing proteins responsible for Huntington's disease and several other trinucleotide-repeat diseases bind strongly to glyceraldehyde 3-phosphate dehydrogenase (GAPDH), a key enzyme in glycolysis (Burke *et al., Nat. Med.* 2:347, 1996), raising the interesting possibility that neuronal death in these diseases may be triggered by reduced glycolysis and consequent cellular energy shortages.

We established a murine central neuronal cell culture model of GAPDH inhibition. Mixed cortical cell cultures (DIV 12-14) containing both neurons and glia, or near pure neuronal cultures, were exposed to the GAPDH inhibitor, alpha chlorohydrin for 48 hr. Concentrations of 1-20 mM drug induced concentration-dependent neuronal death, and somewhat less prominent glial death, with near complete cell death at 20 mM drug. The high concentrations of inhibitor required to induce cell death may reflect limited metabolism to the active compound chlorolactaldehyde; 0.5 mM concentrations have been found previously to be needed to inhibit 80% of GAPDH activity in sperm cells (Stevenson and Jones, *J. Reprod. Fert.* 74:157, 1985).

With this model established, we tested the hypothesis that supplying the short chain fatty acid palmitic acid, a precursor for acetyl-CoA synthesis permitting NADH synthesis in the absence of glycolysis, would permit cortical cells to survive GAPDH inhibition. Repeated addition of 300-500 μM palmitic acid to the bathing medium resulted in a 40% reduction in neuronal and glial cell death. This observation raises a possibility that administration of short chain fatty acids might constitute a therapeutic strategy for diseases associated with GAPDH inhibition.

Supported by NIH NINDS grant NS 30337.

92.16

AMPA RECEPTOR SUBUNIT mRNAs IN PARKINSON'S DISEASE, PROGRESSIVE SUPRANUCLEAR PALSY, HUNTINGTON'S DISEASE AND AMYOTROPHIC LATERAL SCLEROSIS. M. Tomiyama[1], J.M. Palacios[2], R. Cortés[1], G. Mengod[1]*. [1]Dept. Neurochem., IIBB/CSIC, Barcelona, Spain.

Using in situ hybridization we have investigated mRNA transcripts for flip/flop variants of AMPA receptor subunits in the striatum from patients with Parkinson's disease (PD), progressive supranuclear palsy (PSP) and Huntington's disease (HD), and in the spinal cord from patients with amyotrophic lateral sclerosis (ALS), to elucidate the relevance of AMPA receptor-mediated excitotoxicity and/or to help identify specific therapeutic targets to the diseases. Although both PD and PSP share in common a degeneration of the nigrostriatal dopamine neurons, the transcripts were preserved in PD, whereas they were increased in PSP, especially the flip variants. These findings suggest that the involvement of AMPA receptors is entirely different between PD and PSP. Transcripts for GluR-A_{flip} and GluR-C_{flip} were severely decreased throughout the HD striatum. Dorsal regions of the HD striatum exhibited lower hybridization signals for GluR-B_{flop} and GluR-D_{flop} when compared to controls. In ALS, significant decreases of the mRNA levels were observed for flop forms mainly in the ventral horn. The alterations of the transcripts in HD and ALS did not appear to result from AMPA receptor-mediated neurotoxicity, suggesting that these diseases cannot be explained only by AMPA receptor activation.

[2] Permanent address: Research Institute, Laboratorios Almirall, Barcelona.

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92.17

NEURONATIN-β IN THE ADULT HUMAN BRAIN: SELECTIVE AND ABUNDANT EXPRESSION IN THE CAUDATE AND PUTAMEN. Dexian Dou* and Rajiv Joseph. Department of Neurology, K-11, Laboratory of Molecular Neuroscience, Henry Ford Hospital, Detroit, MI.

Originally isolated from neonatal rat brain, *neuronatin* mRNA exhibits a brain-specific expression pattern (*BBRC 1994; 201: 1227-1234*). There are two alternatively spliced forms, α and β (*Brain Res 1995; 690: 92-95*). The α-form contains three exons, whereas, the β-form contains only exons 1 and 3 (*Genomics, in press*). *Neuronatin* protein is highly conserved in mammals, and the human *neuronatin* gene is located on chromosome 20q11.2-12 (*Brain Res, in press*). Although, both the α and β isoforms are abundantly expressed during embryogenesis, only traces of *neuronatin* mRNA were detected in the adult brain studied as a whole. The present studies evaluated the expression of both isoforms in different regions of the adult brain. The results indicate that *neuronatin-β* is the predominant mRNA species that is expressed in the adult human brain, and its expression is selectively abundant in the caudate nucleus and putamen, findings that may have relevance in **Huntington's disease** and other human diseases that selectively involve these structures. These results, for the first time, implicates *neuronatin* in the function of the mature brain. As opposed to the adult brain, there is an abundance of expression of *neuronatin-α* in the human fetal brain. Conceivably, the middle exon, present only in the α-form, may be involved in the regulation of neuronal growth during embryogenesis, and the β-form, which does not contain the middle exon, may help maintain the postmitotic state of the mature brain. Supported by grants from the NIH (NS-01521) and American Heart Association-National (92-132) (to R.J.).

92.18

DECREASED [18F]SPIPERONE BINDING IN PUTAMEN IN IDIOPATHIC DYSTONIA. J.S. Perlmutter*, M. Stambuk, J. Markham, K.J. Black, L. McGee-Minnich, S. M. Moerlein. Departments of Radiology, Neurology & Psychiatry. Washington University School of Medicine, St. Louis, MO 63110

Dystonia may be associated with abnormalities of dopaminergic pathways since it may occur as the first manifestation of Parkinson's disease or after an initial dose or chronic treatment with a dopamine antagonist. Although a genetic etiology has been identified for childhood onset generalized dystonia the etiology of adult-onset focal dystonias remains unclear. We quantified [18F]spiperone binding in putamen using PET in 21 patients with idiopathic focal dystonias (16 women; mean age = 56) including 13 with Meige syndrome (blepharospasm and lower facial dystonia) and 8 patients with hand cramp as well as 13 normals (7 women; mean age = 53). No patient had dystonia in other body parts and no subjects had other neurological or psychiatric abnormalities. Each subject also had an MRI of the brain. PET studies were done with the Siemens 953b in the 2d mode with 31 simultaneous slices (3.4 mm center-to-center slice separation). Reconstructed transverse resolution was 7 mm FWHM and axial resolution was about 4 mm. Blood flow and blood volume were measured using [15O]-labeled water and carbon monoxide, respectively. Then, 3 to 5 mCi of [F18]spiperone were injected i.v. and sequential PET scans were collected for 3 hrs. For each patient, the location of the center of the putamen was identified using a stereotactic method and the region of interest extended over 3 adjacent slices. Data for left & right putamen were averaged and analyzed using a 3-compartment model to calculate the combined forward rate constant (CFRC; the product of the association rate and the maximum number of available binding sites). We found a statistically significant reduction in dystonics (.020 ± .069 vs .028 ± .138; $p < .05$). These data demonstrate a reduction of [18F]spiperone binding in putamen in patients with focal idiopathic dystonia, and strongly suggest an abnormality of dopaminergic receptor binding. (support: NINDS NS31001, NS32318, the Dana Foundation, the BEB Foundation & the Greater St. Louis Chapter of APDA).

92.19

LYMPHOBLAST GTP CYCLOHYDROLASE I ACTIVITY IN DOPA-RESPONSIVE DYSTONIA. R.A. Levine[1,2,3]*, L. Bezin[1], M.D. Flam[1], M. Neystat[4], J.S. Fryburg[5], W.G. Wilson[5], E.M. Bebin[7], J.M. Trugman[6], T.G. Nygaard[4]. [1]Neurology Labs, Henry Ford Hospital; [2]Dpt of Psychiatry, Wayne State U.; [3]Detroit VAMC, Detroit, MI.Detroit, MI; [4]Columbia U. New York, NY; [5]Dpt of Pediatrics and [6]Neurology, Scholl of Med., U. of Virginia, Charlottesville, VA; [7]Dpt of Pediatrics, School of Med., U. of Alabama, Birmingham, AL.

Dopa-responsive dystonia (DRD) is a dominant inherited disorder associated with mutations in the gene for GTP cyclohydrolase I (GTP-CH). GTP-CH is the rate-limiting enzyme in the synthesis for the tyrosine hydroxylase (TH) cofactor, tetrahydrobiopterin. TH catalyzes the conversion of tyrosine to Dopa and is the rate-limiting enzyme in catecholamine biosynthesis. We assessed the value of the GTP-CH assay for predicting DRD by assaying activity in lymphoblastoid cell lines established from DRD and non-DRD members of a 4 generation family. We found GTP-CH activity to be 20-50% of normal in affected family members and carriers (identified by genetic mutation data). The most severely affected family member had no detectible GTP-CH activity; her father (not exhibiting DRD nor carrying the mutation) had transmitted a non-conservative mutation in exon 6 of the GTP-CH gene and had an activity level for GTP-CH consistent with that seen in carriers from the girl's maternal relatives. Assay for GTP-CH activity in lymphoblastoid cell lines appears to be a sensitive marker for mutations in the GTP-CH gene and can be used as a diagnostic marker for DRD.

92.20

STUDY OF SERUM AND CSF NEUROTRANSMITTER AMINOACIDS IN YOUNG SUBJECTS WITH WRITER'S CRAMP. A. Haque*, I. Kabir, Z. Ahmed, MA Hannan and HKM Yusuf. Deptt.of Neurology, IPGMR, and Deptt of Biochemistry, University of Dhaka

Youngmen specially students are frequently attending the department of neurology , IPGMR, Dhaka, with features of writer's cramp. Clinical examinations revealed mild tremor and slight wasting of small muscles of hand. Personal and family history was negative. Serum T_3, T_4, ceruloplasmin, urinary copper level, X-ray neck and cervical myelogram including MRI in some cases and skin smear for AFB were normal. EMG and NCV studies were nonconclusive. Tranquilisers, and antiparkinsonian drugs were of no benefit. Seven of these subjects with mean age of 21± 3 years consented to lumbar puncture. CSF and serum from these patients were collected. Aspartate, glutamate, GABA and glycine levels were measured by HPLC and were compared with findings of CSF and serum collected from 10 control subjects. The result shows significantly high ($p < .001$) level of aspartate in patients serum (141± 31 nmol/ ml) compared to 29 ± 31 nmol/ml in control. The level of CSF glutamate in control was apparently higher. Difference in GABA and Glycine levels were not significant. High level of aspartate may suggest some motorneurone disease.

92.21

HUNTINGTON'S DISEASE: IDENTIFICATION OF PROTEINS THAT INTERACT WITH HUNTINGTIN. P.W. Faber, C. Dompé, L.M. Carlée, G.T. Barnes, J.F. Gusella* and M.E. Macdonald*. Molecular Neurogenetics Unit, Massachusetts General Hospital East, Bldg 149, Charlestown 02129.

Huntington's Disease (HD) is an inherited autosomal dominant neurodegenerative disorder characterized by uncontrolled eye movements, general motor impairment, psychiatric disorders and dementia. The underlying feature of HD is the specific loss of neurons in the basal ganglia. Genetically, HD is caused by an expanded CAG-repeat in 4p16.3. This CAG-repeat is translated into a polyglutamine stretch near the amino terminus of a novel 350 kDa protein of unknown function, termed huntingtin. Both the normal and HD isoforms of huntingtin are widely expressed in the cytoplasm of cells in many tissues. Also, its expression pattern in brain does not closely match the primary target areas of the disease. Thus the expanded polyglutamine stretch in huntingtin leads to a gain of function mutation whose mode of action remains to be established although a dominant-negative scenario, in which the mutant protein inactivates the normal isoform has been ruled out as HD knock-out mice proved to be embryonic lethal.

To obtain more knowledge about the biochemical pathways involving both the normal and HD isoforms of huntingtin we have employed yeast two-hybrid technology the identify their cellular protein partners. Using matched sets of yeast two hybrid baits from the huntingtin N-terminus with either 21or 60 glutamines we have so far identified three independent interactors. Although none interacts exclusively with one bait, quantitative differences exist. Also, two interactors share a common protein motive, defining the region of interaction. These results as well as the additional characterization of these interactors will be presented.

This work was funded by grants from the Human Science Frontier Program, the Hereditatry Disease Foundation and NIH grant NS16367.

93.1

ROLE OF VOLTAGE-GATED CALCIUM CHANNELS IN TRAUMATIC SPINAL CORD WHITE MATTER INJURY. S.K. Agrawal* and M.G. Fehlings. *Playfair Neuroscience Unit, The Toronto Hospital Research Institute, University of Toronto, Toronto, Ontario, Canada M5T 2S8*

Recent work has suggested the presence of voltage-gated Ca^{2+} channels in CNS white matter and a potential role in the pathophysiology of anoxic injury. To examine the relevance of these findings to traumatic spinal cord injury, the effect of zero calcium and the inorganic voltage-gated Ca^{2+} channel antagonist Co^{2+} were studied in an *in vitro* model of spinal cord injury. A 30mm length of dorsal column was isolated from the spinal cord of adult rats, pinned in an in vitro recording chamber and injured with a modified clip calibrated to deliver a 2g force for 15 sec.

A strip of dorsal column from adult rat spinal cord was pinned in a recording chamber which was perfused with oxygenated Ringer. Compound action potentials were recorded extracellularly from microelectrodes. Injury was accomplished by compressing the dorsal column strip *in vitro* with a 2 g clip for 15 sec. The CAP decreased to 71.4 ± 2.0 % of control ($p<0.05$) after clip compression injury. Removal of extracellular Ca^{2+} ($[Ca^{2+}]_e$) and replacement with 1.5mM EGTA promoted significantly greater recovery of CAP amplitude (86.3 ± 7.6 % of control; $p< 0.05$) after injury. Partial blockade of voltage-gated Ca^{2+} channels with a low concentration of cobalt (20μM) also conferred neuroprotection (CAP amplitude 82.8 ± 1.2% of control; $p<0.05$). Higher concentrations of Co^{2+} (2mM and 200 μM) irreversibly reduced CAP amplitude in uninjured dorsal column segments suggesting a neurotoxic effect. The specific subtypes of Ca^{2+} channel blockers are presently under investigation.

In summary, reduction of $[Ca^{2+}]_e$ confers neuroprotection after spinal cord injury *in vitro*. The injurious effects of Ca^{2+} in traumatically injured CNS white matter appear to be partially mediated by voltage-gated Ca^{2+} channels. (*Supported by Medical Research Council of Canada*).

93.2

EFFECT OF THE SODIUM CHANNEL BLOCKER QX-314 ON RECOVERY AFTER SPINAL CORD INJURY IN VIVO. M.G. Fehlings* and S.K. Agrawal, *Playfair Neuroscience Unit, The Toronto Hospital Research Institute, University of Toronto, Toronto, Ontario Canada M5T 2S8*

There is evidence that intracellular sodium $[Na^+]_i$ entry potentiates spinal cord injury (SCI) and hypoxic/ischemic cell death in vitro by causing cytotoxic cell edema, intracellular acidosis and gating of Ca^{2+} entry by reverse activation of the Na^+-Ca^{2+} exchanger. Recently we obtained evidence suggesting that Na^+ channels may participate in the pathophysiology of spinal cord white matter in vitro (Agrawal and Fehlings 1996 J. Neurosci. 16:545-552). In the present study, we examined the role of Na^+ in mediating traumatic injury to spinal cord in vivo.

To test this hypothesis experiments were performed in a randomized block design. We examined the effect of QX-314, a potent Na^+ channel blocker on recovery after SCI in vivo. QX-314 (2.0 and 10nmol) or placebo (saline) was microinjected (2μL in 10min) into the injury site beginning at 15 min after SCI. Injury was performed by compression of the spinal cord at C7-T1 for 1 min with a modified aneurysm clip exerting a closing force of 35g. Neurological function was assessed one day after injury and weekly thereafter until six weeks by the inclined plane method and by the modified Tarlov technique. After six weeks of injury the origin of descending axons at the injury site was determined by retrograde labelling with Fluorogold (FG).

There was significant improvement in the counts of retrogradely labeled neurons in the red nucleus ($p<0.02$), and a trend toward increased cell counts in the sensorimotor cortex ($p<0.08$) and vestibular nuclei ($p<0.09$) as compared to control vehicle treated group. However, there was no improvement in clinical neurological function (inclined plane or Tarlov scores).

Our results suggest that the Na^+ channel blocker QX-314 partially preserves the integrity of descending motor axons after SCI. The effects were insufficient to result in improved clinical function. Further studies are under way with other dose/drug combinations. (*Supported by MRC of Canada*).

93.3

PROGRESSIVE DEGRADATION OF MAG IN SPINAL CORD INJURY (SCI). N.L. Banik*, D.C. Matzelle, G.G. Wilford and E.L. Hogan. Neurology Dept, Med Univ SC, Charleston, SC 29425

We previously correlated degradation of myelin and cytoskeleton proteins with axon and myelin degeneration in SCI implying a role for proteinases in SCI tissue degeneration. Examining whether myelin-associated glycoprotein (MAG) is degraded in spinal cord trauma, we determined the extent of its degradation (as a measurement of calpain activity) in lesion and control cord. SCI was induced in rats by weight-drop technique (40g-cm). Lesion and control cords were processed at defined time intervals following injury and analyzed by SDS-PAGE and immunoblotting in association with chemiluminescence using MAG antibody. The extent of MAG breakdown was calculated using PDI software after the gels were scanned. A small amount of MAG, 13% and 21%, was degraded at 30 min and 1 hr, respectively, while a significant breakdown of this protein (32%) was evident at 4 hr and a substantial amount (64%) was lost at 72 hr following trauma compared to the sham. The breakdown of MAG in SCI may be calpain mediated since MAG is degraded by purified calpain in vitro and calpain activity, content and immunocytochemical staining also increase following SCI. These results suggest that calpain, which degrades myelin and axonal proteins, plays an important role in the loss of membrane structural integrity leading to membrane dissolution and axonal degeneration in SCI. To maintain the axon-myelin structural unit and cell function in SCI, using calpain inhibitors may protect cells and preserve membrane by preventing protein degradation and restoring motor function. Supported by NIH-NINDS NS-31622 and PVA SCRF-1238.

93.4

SODIUM CHANNEL BLOCKERS PROTECT AGAINST TRAUMATIC NEURONAL INJURY IN THE ADULT RAT SPINAL CORD. S.M. Douglas*, K.L. Panizzon and R.A. Wallis. Spinal Cord Injury Lab., Sepulveda VA Medical Center. and UCLA School of Medicine, Los Angeles, CA. 90024

We investigated whether sodium channel blockade would reverse the effects of traumatic injury to the adult rat spinal cord. Prior studies suggest that sodium channel blockers are neuroprotective against hypoxic injury. In addition, sodium channel blockers help prevent neurotransmitter release which plays a significant role in the evolution of traumatic neuronal injury. Therefore, we evaluated the effect of the sodium channels, lamotrigine and tetrodotoxin (TTX), against traumatic neuronal injury in adult spinal cord slices. To induce trauma, slices were placed in a specialized chamber filled with artificial cerebral spinal fluid. This fluid was then percussed and changes in evoked response were monitored. Slices given trauma alone demonstrated severe neuronal injury with dorsal horn evoked response recovering after 60 mins. to a mean of only 15% ± 4 of initial amplitude. Treatment with 100 μM lamotrigine begun after trauma, provided significant protection, yielding dorsal horn evoked response recovery of 92% ± 5. Similarly, treatment with 1 μM TTX provided protection, yielding dorsal horn evoked response recovery of 87% ± 3, while paired, unmedicated slices recovered to only 13% ± 3 ($p<0.05$). These findings indicate that the sodium channel blockers, lamotrigine and TTX, are protective against traumatic injury to dorsal horn neurons of the spinal cord. They further suggest that sodium channel opening plays a harmful role during the evolution of traumatic spinal cord injury. They additionally suggest that sodium channel blockers may be potential therapeutic agents in the treatment of spinal cord injury.

Supported by a VA Brain Trauma Fellowship and the VA Research Service.

93.5

STRESS INDUCTION OF NEURONAL PRODUCTION IN THE BRAINS OF YOUNG AND ADULT RING DOVES. M.-F. Cheng* and J. Cao. Institute of Animal Behavior/Psychology, Rutgers University, Newark, NJ 07102

In the present study, we asked whether in the avian system, where newly generated subependymal zone (SZ) cells continue to migrate, differentiate and are incorporated into the adult brain, we may find stress-induced neurogenesis following electrolytic lesions in the parenchyma. Adult male ring doves received bilateral electrolytic lesions in the ventromedial nucleus (VMN), followed by a single i.m. injection of [3H]thymidine. Birds were allowed to survive for 24 hr then killed for histological studies. Hu protein, a conserved marker for neuronal phenotype before mitotic cells initiate parenchymal migration (Barami et al., J. Neurobiol. 1995), was used to identify neuronal precursor cells among SZ cells. Using West's stereological methods for neuron counting, we found a significantly greater number of Hu+/newly generated ([3H]thymidine labeled) cells in the VMN-lesioned than in the sham-lesioned brains. In a second experiment, VMN-lesioned birds were similarly treated but were killed at varying intervals. We found that with longer intervals the number of Hu+/newly generated cells increased in brain parenchyma while those in the SZ decreased. This suggests that Hu+/newly generated cells observed in the parenchyma migrated from the SZ. Supported by Hoechst-Celanese Award.

93.6

TEMPORAL PATTERNS OF HEAT-SHOCK PROTEIN 70 EXPRESSION FOLLOWING EXCITOTOXIC VS PENETRATING CORTICAL INJURY. S.A. Dutcher, D.B Michael, F.G. Diaz, and P.D. Walker* Departments of Anatomy & Cell Biology, and Neurosurgery, Wayne State University School of Medicine, Detroit, MI 48201.

It has been repeatedly observed that tissue regions subjected to stress and/or injury undergo a biosynthetic activation of heat shock proteins. In the damaged brain, heat-shock proteins may participate in the processing and stabilization of proteins critical to the survival of neurons and glial cells at the site of injury. In particular, the increased expression of constitutive and inducible members of the heat shock protein 70 (HSP70) family may serve as useful indicators of cells at risk following brain injury. The present study sought to compare the temporal pattern of HSP70 gene expression induced in excitotoxic vs penetrating cortical brain injury. Adult male SD rats (175-200g) received stereotaxic-guided stab wounds or small injections of quinolinic acid (QA, 240mM in 0.2 µl) into the frontal cortex. Animals were sacrificed at 1, 3, 6, 12 and 24 hours following surgery. Total RNA was isolated from the cortical site of injury and Northern analysis was used to measure HSP70 mRNA levels along with other injury-inducible mRNA species. Within the QA-injected cortex, HSP70 mRNA levels were significantly increased by 3 hours and peaked in expression at 12 hours before returning towards contralateral uninjected control levels by 24 hours. This pattern of HSP70 mRNA expression overlapped with immediate early gene activation and the induction of glial fibrillary acidic protein. The penetrating injury produced a similar, but less obvious, pattern of HSP70 mRNA. These results support the hypothesis that HSP70 gene induction may serve as a useful marker of damaged tissue following CNS injury. Supported by Fund for Medical Research and Education and NS30550.

93.7

DOWNREGULATION OF THE ASTROCYTE GLUTAMATE TRANSPORTER GLT-1 mRNA IN BRAIN TRAUMA.

M.D. Norenberg*, W.D. Dietrich, W. Singer, R. Zhao, B. Zhou, R. Busto, M.D.Ginsberg. Departments of Pathology and Neurology, University of Miami School of Medicine, and V.A. Medical Center, Miami, FL 33101.

An excitotoxic mechanism is believed to play a role in traumatic brain injury (TBI). To examine whether a defect in glial glutamate uptake contributes to TBI, we performed an *in situ* hybridization study using an antisense riboprobe to the glial glutamate transporter GLT-1 in a parasagittal fluid percussion (FP) model of brain injury. Fasted Sprague-Dawley rats were anesthetized with 70% nitrous oxide, 1% halothane and 30% oxygen and injured with a FP pulse (1.6-1.9 atm). At 2, 4, 6, 24 and 72 hrs after TBI, brains were processed for *in situ* hybridization (n=4 in each group). In an additional 24 hr group, rats were pretreated with MK-801 (1 mg/kg). At 6 hrs after TBI, mild decreases in the expression of GLT-1 mRNA were seen in histopathologically vulnerable regions. By 24 hrs, moderate reductions in GLT-1 mRNA were detected throughout the ipsilateral hemisphere. The hybridization signal returned to normal at 3 days except in vulnerable cortical areas. Pre-treatment with MK-801 blocked the reduced expression of GLT-1 mRNA. These data indicate that TBI leads to a diffuse downregulation of the glial glutamate transporter. This may result in failure to remove extracellular glutamate and possibly contribute to excitotoxic injury and abnormal glutamatergic neurotransmission. The ability of MK-801 to inhibit the remote downregulation of GLT-1 mRNA suggests that spreading depression may play a role in this pathophysiological consequence of TBI. (Supported by NIH grant NS30291 and the Department of Veterans Affairs.)

93.8

DISCRIMINATION OF POST-TRAUMATIC MEMORY VS. LEARNING IN RATS USING THE MORRIS WATER MAZE. R.F. Cody, M.M. Haglund*[1], M.S. Grady, D.O. Maris, Department of Neurological Surgery, University of Washington, Seattle, WA 98104, Duke University[1], Durham, NC 27710.

Traumatic brain injury (TBI) has been shown to cause impairment of spatial abilities, such as performance in the Morris water maze (MWM). We were interested in whether memory of a previously-learned task could be differentiated from acquisition of a new task following TBI. Using a standard lateral fluid percussion injury (FPI) model, we administered moderate FPI or sham injury randomly to 32 adult male Long-Evans rats. Half of these rats had been pre-trained in the Morris Water Maze (MWM) to find a hidden platform 1 week prior to injury/sham, while the other half were naive. We then tested the rats at 2 weeks post-injury/sham in the MWM for retention of spatial memory (pre-trained groups) or learning of the spatial task (naive groups). Rats were re-tested in the MWM at 4, 8, and 16 weeks post-injury/sham.

Rats in the injured/naive group performed poorly in the MWM compared to the injured/pre-trained, sham/pre-trained, or sham/naive groups at 2 and 4 weeks, but were equal to the other groups by weeks 8 and 16. No significant differences were observed among either sham group or the injured/pre-trained group at any time point. This study suggests that spatial learning may be discriminated from spatial memory following TBI, and that prior training may help ameliorate post-traumatic MWM performance deficits following TBI. (Supported by NIH P 50 NS30305).

93.9

ADAPTATION OF THE FLUID PERCUSSION INJURY MODEL TO THE MOUSE, W.S. Carbonell, D.O. Maris, R.F. Cody, T.D. McCall, R.H. Schmidt[1]*, M.S. Grady, Depts. of Neurological Surgery, Univ. of WA Sch. of Med., Seattle, WA 98104 and University of UT Med. Ctr.[1], Salt Lake City, UT 84132.

Manipulation of the mouse genome causes functional consequences. Insight into the molecular mechanisms of cell-cell interactions and cell metabolism due to a broad spectrum of genes is thus provided. Many of these genes are important in the normal development of the CNS and maintenance of its connections. Recovery from traumatic brain injury (TBI) may rely on many of the same mechanisms operant in development such as axon guidance, synaptogenesis, and neuronal viability. The fluid percussion injury (FPI) model when applied to these transgenic mice may be a particularly useful experimental tool in evaluating specific sequelae of TBI. In the present study we establish FPI as a viable model of TBI in the mouse by characterizing acute response to injury and cognitive and histopathologic effects.

Male C57BL/6 mice (25-30 g.) were anesthetized and injury cannulas were secured to a parasagittal craniectomy site. After a 24 hour surgical recovery period we performed a sham treatment (n=8) or FPI (under halothane, n=8) of 3.5 atmospheres. Acute neurological evaluation of the injured animals revealed occasional transient apnea (lasting 20 seconds or less) and delayed righting and paw and tail flexion reflexes as compared to controls. Deficits in cognition, specifically spatial memory, were assessed over post-injury days 5 through 7 using the Morris water maze (MWM). After the post-injury day 7 MWM testing, the mice were transcardially perfused and brains collected for histopathologic analysis. Gross analysis before sectioning revealed subdural hemorrhage in the vicinity of the injury site extending caudally. Cresyl violet stains showed mild cortical and subcortical tissue loss directly beneath the site of the fluid pulse. GFAP immunocytochemistry demonstrated extensive reactive gliosis in the hemisphere ipsilateral to injury. Other histological techniques performed include silver degeneration stain and TUNEL.

This new FPI model in the mouse makes possible numerous potential studies of neurological, behavioral, and histopathologic sequelae of TBI in transgenic and wild-type mice. As an added advantage, the mouse FPI model as described is immediately employable in labs already using the FPI rat model with no modification to the pre-existing FPI apparatus. Supported by NIH grant NS30305.

93.10

QUANTITATION OF RAT HIPPOCAMPAL CELLS USING THE OPTICAL VOLUME FRACTIONATOR: A COMPARISON OF MIDLINE TO LATERAL FLUID PERCUSSION INJURY. M.S. Grady*, J.S. Charleston, D.O. Maris. Depts. of Neurological Surgery and Pathology[1], Univ. of WA Sch. of Med., Seattle, WA 98104.

Fluid percussion injury (FPI), an experimental model of traumatic brain injury, causes spatial memory dysfunction in rats regardless of injury location (midline vs. lateral). Standard histological analysis of the brains post-injury show hippocampal neuronal loss after lateral injury, but not after midline FPI. We have used the Optical Volume Fractionator (OVF) stereology procedure to estimate volume changes and quantify neuronal loss/glial proliferation within specific subregions of the hippocampus after midline or lateral FPI. The OVF method is an unbiased cell counting procedure which combines cell numerical density estimates (from the optical disector) with volume estimates (generated by point counting and the fractionator stereology method) to produce an estimate of the absolute cell number for each subregion of the hippocampus. The method is capable of detecting subtle changes in cell populations within defined structures.

Twelve adult male Sprague-Dawley rats were randomly divided into 3 groups (n=4/group): midline injury, lateral injury, and naive. A single fluid percussion pulse of 4 atmospheres was delivered to anesthetized rats in the injured groups. Rats were transcardially perfused at 14 days post-injury with 4% paraformaldehyde and the brains were removed for processing and analysis. Rat brains were cut into 1 mm slabs in the coronal plane. All individual slabs were embedded in glycomethacrylate and serial sectioned at 20 or 30 μm. Optical disectors were systematically placed across every 12th section containing the hippocampus and the volumes of hippocampal subregions were estimated by point counting on these same sections.

The results confirmed that the greatest loss of volume and neurons in the hippocampus corresponded with lateral FPI. However, subtle changes, not readily apparent with routine neuropathological examination, were also noted in the neurons and glial cell populations of the hippocampus following central FPI. The implications of the observed cell population changes are discussed in relation to the observed functional deficits associated with both lateral and central FPI.
Supported by NIH grant NS30305.

93.11

Dynorphin Expression in Dorsal Horn Neurons Following Traumatic Spinal Cord Injury T. Tachibana*[1,2], A.Arakawa[1], M.Maruo[1], K.Miki[2], A.Tokunaga[2] and K. Noguchi[2]. [1]Dept. of Orthopedic surgery and [2]Dept. of Anatomy and Neuroscience, Hyogo College of Medicine, Hyogo 663, Japan

It has been suggested that the endogenous opioid dynorphin contributes to secondary tissue damage following spinal cord injury. This study was designed to examine the expression of preprodynorphin (PPD) mRNA at cellular level, and clarify the spatial and temopral properties of the expression in the injuried spinal cord.

After deep anesthesia with sodium pentobarbital (50 mg/kg i.p.), male Sprague-Dawley rats were subjected to traumatic spinal cord injury at T13 spinal segment using the weight drop method. Control animals were subjected to laminectomy. These animals were evaluated on their ability to maintain position on an inclined plane and sacrificed 24h after surgery. Coronal sections were cut through the injuried segment and processed for *in situ* hybridization. Neurons exibiting the increase in the level of PPD mRNA were concentrated in the superficial laminae and the neck of dorsal horn within several spinal segments. The number of neurons expressing PPD mRNA were 5.6 ± 0.8 per section in sham oparated animals (the avarage maximam angles is 64.5 ± 1.1°), 12.2 ± 1.3 in moderate paraplegic animals (55.5 ± 9.7°), and 16.5 ± 2.0 in severe paraplegic animals (38.0 ± 3.7°). Number of neurons expressing PPD mRNA was correlated siginificantly with motor disfunction ($p < 0.05$). This finding suggests that *de novo* expression of dynorphin in dorsal horn neurons may have a important role in pathomechanisms of neurological dysfunction after spinal cord injury. (Supported by Hyogo College of Medicine)

93.12

MODERATE FLUID-PERCUSSIVE BRAIN INJURY IN THE RAT: SENSORIMOTOR AND COGNITIVE EFFECTS, AND THE INFLUENCE OF POST-INSULT AMPHETAMINE H.M.Bramlett*, E.J.Green and W.D.Dietrich, Depts. of Psychology and Neurology, University of Miami, Coral Gables, Florida 33124.

Previous studies assessing the behavioral consequences of traumatic brain injury (TBI) in animals have demonstrated that those insults disrupt previously learned information. Much less attention has been given to anterograde learning deficits, which are observed clinically, or to cue-dependent learning, for which the hippocampus is critical. The current experiments were designed to address these issues by assessing the influence of TBI on the acquisition of both place and cue learning in the water maze (WM). An additional goal was to examine the effects of post-insult amphetamine on sensorimotor and cognitive behavior following TBI.

Anesthetized animals with a modified syringe hub implanted above the right parietal cortex were subjected to fluid percussive (FP) injury (1.8-2.2 atm) at normothermic brain temperature (37°C). Shams underwent all surgical procedures, but did not receive any FP injury. Seventy-two hours after surgery animals received either 4 mg/kg i.p. amphetamine (group TBI-A, n=7; group Sham-A, n=6) or vehicle (group TBI-V, n=7; group Sham-V, n=6). WM and sensorimotor (forelimb placing) testing began 48 hrs post-TBI and continued every 3 days for 6 weeks, and once during each of weeks 7 and 8. TBI animals exhibited WM place acquisition deficits until approximately 3 weeks post-injury ($p<0.002$). Sporadic deficits in cued learning were also observed in TBI animals ($p<0.002$). No group differences in WM swim speed were observed. TBI was associated with an impairment in contralateral vibrissae ($p<0.008$) and forelimb ($p<0.003$) placing, which persisted for approximately 3-4 weeks. Amphetamine did not significantly attenuate any of the deficits. The results of these experiments indicate that there is anterograde and retrograde amnesia in this model of TBI, and that WM deficits result, in part, from extra-hippocampal damage. The lack of evidence for facilitation of recovery by amphetamine in these experiments contrasts with the results of other studies in which such effects have been observed. (NS 30291)

93.13

PATTERNS OF BDNF, NGF, and HSP-70 GENE EXPRESSION FOLLOWING FLUID-PERCUSSION BRAIN INJURY: ANALYSIS BY IN SITU HYBRIDIZATION. M.D. Ginsberg*, J.T. Singer, W. Zhao, W.D. Dietrich, O.F. Alonso, J.Y. Loor-Estades, R. Busto. Neurotrauma and CVD Research Centers, Univ. of Miami School of Medicine, Miami, FL.

Temporal patterns of gene expression were surveyed at 30 min, 2 h, 6 h, 24 h, 3 days, and 30 days following moderate fluid-percussion injury in halothane-anesthetized Wistar rats. In situ hybridization was carried out on coronal sections at 12 levels of the neuraxis using ^{35}S-labeled antisense riboprobes to the neurotrophins nerve growth factor (NGF) and brain-drived neurotrophic factor (BDNF); and the inducible heat-shock protein HSP-70 (n=5 rats per time point). BDNF and NGF were not constitutively expressed in sham rats. In FPI rats, both BDNF and NGF expression appeared at 30 min, peaked at 2-6 h, but was also present at lower levels at 6h - 3 d. For BDNF, dentate gyrus was most heavily labeled, as well as sectors CA3-4 of dorsal and ventral hippocampus, in most cases bilaterally, and in some unilaterally. By contrast, NGF expression was in most cases ipsilateral to trauma (or else ipsilateral >> contralateral), and involved dentate >> CA3-4. By 24 h-30 d, however, contralateral NGF labeling exceeded ipsilateral, representing a secondary injury response. HSP-70 expression, by contrast, was localized largely to the ipsilateral cortical impact site, highest at 2-6 h, and declining at 24 h. Expression of BDNF and NGF in hippocampal structures indicates that these regions are directly or secondarily affected by traumatic stress; contralateral expression suggests a transneuronally mediated effect of trauma.

93.14

EXTRACELLULAR RELEASE OF SEROTONIN FOLLOWING FLUID-PERCUSSION BRAIN INJURY IN RATS. R. Busto*, W.D. Dietrich, O. Alonso, M.Y.-T. Globus, M.D. Ginsberg, Neurotrauma Research Center, Dept. of Neurology, Univ. of Miami School of Medicine, Miami, FL

Serotonin has been implicated in the pathobiology of CNS trauma. Using microdialysis techniques, we performed measurements of serotonin release within the primary somatosensory cortex of rats subjected to moderate fluid-percussion (F-P) brain injury. Twenty-four hours prior to TBI, a F-P interface was positioned parasagittally over the right cerebral cortex. On the second day, fasted rats were anesthetized with 70% nitrous oxide, 1% halothane and 30% oxygen. Under controlled physiological conditions and normothermic brain temperature (37-37.5°C), rats were injured (n = 6) with a F-P pulse ranging from 1.8-2.0 atm. Following trauma, brain temperature was maintained for 4 hr at 37°C during the microdialysis sampling period. Sham trauma animals (n = 7) were treated in an identical manner. TBI induced an immediate elevation in extracellular levels of serotonin (3.5-fold, $p < 0.001$, ANOVA) compared to sham-operated controls. The extracellular levels of serotonin remained significantly higher than baseline during the first 90 min sampling period ($p < 0.05$). In parallel to the increase in serotonin levels after TBI, a significant 71% decrease in extracellular 5-hydroxyindoleacetic acid (5-HIAA) levels was observed during the first 10 min after TBI. The present data indicate that TBI is followed by a prompt but sustained elevation in extracellular serotonin within cortical regions adjacent to the impact site. These neurochemical results implicate serotonin in the pathophysiology of TBI.

NEUROMUSCULAR DISEASES I

94.1

THE MOTOR SYNDROME OF CHOREA: LESSONS FROM THE CLINIC. L. Zhang, H. Mitsumoto* and R.S. Burns. Dept. of Neurology, Cleveland Clinic Foundation, Cleveland, OH 44195.

Chorea, one of the basic types of abnormal movements in man, is attributed to basal ganglia dysfunction, in particular, increased striatal dopaminergic activity. Our experience in a large number of patients where haloperidol suppressed the chorea is consistent with this view. A recent case of severe, intractable chorea suggests that the neural substratum of chorea is more complex. An 11 yr old girl with ataxic cerebral palsy first developed chorea after a varicella infection which lasted 3-4 mo and was controlled with diazepam. One year later, she developed severe chorea ('choreic storm') leading to rhabdomyolysis, myoglobinuria and acute renal failure. The control of her chorea became the focus of her management. She was unresponsive to dopaminergic (haloperidol, reserpine), GABAergic (baclofen, valproate, gabapentin), glutamatergic (amantadine) and cholinergic (trihexyphenidyl) agents as well as to benzodiazepines (diazepam, clonazepam). A surgical approach using a ventral medial pallidotomy was considered. Late in the hospital course, a T-2 weighted MRI brain scan showed an increased signal in the globus pallidus bilaterally. The pharmacological response profile in this case suggests that no one neurotransmitter pathway comprising the related motor circuits played a dominant functional role and that more complex changes in the balance of activity in multiple neurotransmitter pathways or in parallel circuits were involved. Study supported by the Neurology Department at the Cleveland Clinic Foundation.

94.2

INCREASING NUMBERS OF MUTANT MITOCHONDRIA MAY DETERMINE COURSE IN KEARNS-SAYRE SYNDROME. E.J. Fine*, G.D. Vladutiu, C. Warner, R. Heffner, VA Medical Center and School of Medicine and Biomedical Sciences, State University of NY at Buffalo, Buffalo, NY 14215, L-J.C. Wong, Children's Hospital, Los Angeles, CA 90027.

We sought to determine, if increased numbers of mutant mitochondria relate to clinical expression in Kearns-Sayre syndrome (KSS). A man experienced right (R) ptosis at age 12, left (L) ptosis at 14, corrected by plastic surgery at age 16. External ophthalmoplegia without limb weakness appeared at age 22 and became total at age 26. Single fiber electromyography (EMG)s was normal. Conventional EMG demonstrated rare short duration polyphasic potentials (PP). R deltoid muscle (DM), R quadriceps (Q) biopsy showed a few central nuclei, but no ragged red fibers (RRF) at 25. Beginning at 26 he had persistent increased serum creatine phosphokinase, dysphagia, dysarthria and weakness only in deltoid muscles (DM). EMGs demonstrated myopathic changes only in DM. At age 27 L DM and L Q biopsies revealed only 1 RRF in right r DM. All oxidative enzyme levels were normal except succinic dehydrogenase and citric synthetase activity which were 49% and 58% of normal in L DM. PCR of mitochondrial DNA (mtDNA) from L DM showed 4.97 Kb deletion with junctional nucleotides at 8469:13447. No mutant mtdna was detected in leukocytes or hair follicles. Auditory thresholds rose to abnormal from age 27-28. We interpret increasing weakness, CPK elevation, appearance of RRF are due to increased mutant mitochondria. We propose increasing mutant mitochondria determine clinical course in KSS.

VA Medical Center, Buffalo Muscular Dystrophy Association

94.3

EXPRESSION OF MATRIX METALLOPROTEINASES AND THEIR INHIBITORS IN THE DELTOID AND PSOAS MUSCLES OF ALS PATIENTS. I. G. Lin, G. P. Lim, M. J. Cullen, R. D. Atkinson and Z. A. Tokes*. Univ. Southern Calif. School of Medicine, Los Angeles, CA 90033.

Increased expression of matrix metalloproteinase MMP-9 (92 kDa, gelatinase B) is observed in Alzheimer (AD) hippocampus and in amyotrophic lateral sclerosis (ALS) frontal and occipital cortices, and cervical, thoracic, and lumbar regions of the spinal cord (Backstrom et al., *J. of Neurochemistry*, 1992; Lim et al., *J. of Neurochemistry*, 1996). The expression of MMP-2 (70 kDa, gelatinase A) is unchanged in AD and decreases in ALS motor cortex. MMP-2 is localized in astrocytes, while MMP-9 is synthesized in the neurons. Neuron-derived MMP-9 may alter synaptic architecture and contribute to neurodegeneration. To test this hypothesis, the expression of matrix metalloproteinases and their tissue inhibitors (TIMP-1, and -2) was studied in deltoid and psoas muscles of ALS and control patients. Zymography using gelatin as substrate, reverse zymography, and Western blots in combination with enhanced chemi-luminescence were used to measure the expression of these enzymes and their inhibitors, and immunohistochemistry was used to establish their cellular location. Elevated levels of high molecular mass complexes of MMP-9 (110 kDa) and three molecular forms of MMP-2 (60, 56, and 49 kDa) were observed in ALS muscles. MMP-9 appeared to localize at the cell surface in structures resembling the neuromuscular junction. TIMP-1 expression was lower in ALS muscles than in control specimens whereas no significant changes were seen in TIMP-2 levels. These results are consistent with the hypothesis that an MMP-9/TIMP-1 imbalance may contribute to the neuromuscular degeneration in ALS patients. Supported by the ALS Association, and NIA R01-AG09681.

94.4

INCREASED LEVELS OF HYDROGEN PEROXIDE IN CELL LINES DERIVED FROM SIXTEEN FALS PATIENTS WITH DIFFERENT CuZnSOD MUTATIONS. M.S. Ahmed, W-Y. Hung, D. Heintz, P.E. Hockberger, T.A. Skimina and T. Siddique*. Depts. of Neurology & Physiology, Northwestern Univ. Med. Scchool, Chicago, IL 60611

We used fluorescence spectroscopy and digital imaging of 2',7'-dicholorofluorescin (DCFH) and hydroethidine to monitor oxygen free radicals in lymphoblast cell lines derived from patients with familial amyotrophic lateral sclerosis (FALS) which have mutations in CuZnSOD, sporadic ALS (SALS) and controls (spouses of ALS patients). We also tested the influence of antioxidants and inhibitors of antioxidant enzymes on the oxidation of these probes. The results showed that the oxidation of hydroethidine (specific for superoxide anion) in all groups studied were comparable despite 45% decreased CuZnSOD activity in FALS mutants. FALS mutants, however, showed significant increased of DCF fluorescence compared with SALS and normal controls. Further, cells incubated in vitamin C, catalase, or the flavonoid quercetin displayed significant decrease of DCF fluorescence in all groups. The inhibitor of catalase, 3-amino-1,2,4-Triazole, resulted in ten-fold increase of fluorescence in all groups. L-nitroarginine (inhibitor of nitric oxide synthase) and vitamin E had no effect on the oxidation DCFH. These studies suggest an increase level of hydrogen peroxide (H_2O_2) in cells derived from FALS resulting from either (i) increased production of H_2O_2 and/or (ii) increased accessibility of H_2O_2 to DCFH.

This research was funded in part by NIH (NS-31248 and NS-21442), Les Turner ALS Foundation, and the Muscular Dystrophy Association.

94.5

DEGENERATION OF SPINAL INTERNEURONS IN ADDITION TO MOTONEURONS IN A TRANSGENIC MOUSE MODEL FOR ALS.

D. Jaarsma[1], J. Kennis[1], D. Troost[2], J.M.B.V. De Jong[3], J.C. Holstege[1,*] [1]Dept. Anatomy, Erasmus Univ. Rotterdam, 3000DR; [2]Depts. Pathology & [3]Exp. Neurology, AMC, Amsterdam, The Netherlands.

SOD-1 mutations, as found in a subset of cases of familial ALS, cause severe progressive motoneuron disease when expressed in transgenic mice. We have studied the distribution of neuronal degeneration in a line of transgenic mice carrying human SOD-1 with a gly^{93}→ala mutation produced by Gurney et al. (Science 264:1772, Jackson Laboratories) using a silver impregnation procedure specific for degenerating neurons and their processes (Nadler and Evenson, Meth. Enzymol. 103:393-400). Parallel sections were stained immunocytochemically for choline acetyltransferase (ChAT) and calretinin (CR) to determine loss of motoneurons and CR-positive neurons in lamina VI-VIII, respectively. In animals sacrified before the onset of clinical disease (at 5-6 months of age) no argyrophilic structures were observed in spite of the presence of vacuoles in dendrites and axons in spinal and bulbar motoneurons. Transgenic mice perfused 0-5 weeks following the onset of clinical signs (6-8 months of age) showed an increasing amount of argyrophilia in the brainstem reticular formation and the spinal cord white and grey matter. Argyrophilia was never observed in telencephalic and diencephalic areas. The density of argyrophilia correlated with the severity of paralysis and loss of ChAT-positive motoneurons, but argyrophilic motoneurons were rarely seen. Substantial degeneration of spinal interneurons was suggested by the presence of argyrophilia in the ventral and lateral funiculi, the presence of medium-sized argyrophilic neurons in lamina VI-VIII, and by the loss of CR-positive neurons in lamina VI-VIII. So far our results also show that interneuronal degeneration occurs after or simultaneously with motoneuron loss, but not before. (Funding: Prinses Beatrix Fonds and FGG-EUR-ANA-11-02-11)

94.6

CLINICAL AND HISTOMETRIC EFFECTS OF GLIAL-CELL-LINE-DERIVED NEUROTROPHIC FACTOR (GDNF) ON WOBBLER MOUSE MND.

B. Klinkosz*, Q. Yan, T. Ishiyama, E. Pioro and H. Mitsumoto. Dept. of Neurology & Neuroscience, Cleveland Clinic Foundation, Cleveland OH 44195, and Amgen Inc., Thousand Oaks, CA 91320.

GDNF is a potent motor neuron protecting factor. The effects of this neurotrophic factor were tested on wobbler mouse MND. Following the clinical diagnosis, affected animals received subcutaneous injections (6 times/week) of 20 mg/kg GDNF (n=11) or vehicle solution (n=10), in a blinded fashion for 4 weeks. Disease progression was evaluated weekly. After the treatment, animals were perfused, and C5&C6 spinal cord and ventral roots were studied. One mouse died in the GDNF-treated group. Body weight gain was not different between the two groups. Grip strength in GDNF-treated animals was maintained for the first 2 weeks, and thereafter slowly reduced, whereas vehicle-treated animals steadily lost their grip strength. Grip strength at week 4 was 265% better in the GDNF than in the vehicle-treated animals ($P<0.002$). Running speed was also better in GDNF-treated animals at week 4 ($P<0.0005$ compared to control). Paw position and walking pattern abnormalities deteriorated only modestly in the GDNF-treated animals. Locomotion activity was also greater in the GDNF-treated mice than that in the vehicle-treated mice ($P<0.02$ from week 3). In-vivo biceps twitch tension was 50% greater in GDNF- than vehicle-treated group ($P<0.05$). The total number of myelinated nerve fibers at C5 ventral root level was significantly greater in the GDNF group (674.1 ± 88.6), compared to that in vehicle animals (567.8 ± 65.4) ($P< 0.01$). In GDNF-treated animals, myelinated fibers of all sizes increased. GDNF significantly reduced the number of acute axonal degeneration (myelin ovoid) compared to vehicle-treatment ($P<0.005$). GDNF did not prevent motor neuron vacuolar degeneration. Our study showed that GDNF significantly reduced the rate of motor dysfunction and loss of motor axons in wobbler mouse MND.

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94.7

BIOLOGICAL EFFECT OF MOTONEURONOTROPHIC FACTOR ON WOBBLER MICE WITH MOTONEURON DISEASE.

RMW Chau*, XY Wu and SSW Chan. Dept of Anatomy, Faculty of Medicine, The University of Hong Kong, Hong Kong.

Motoneuronotrophic factor1(MNTF1) identified by a "protein band-fishing by cells" method promotes the survival and growth of isolated spinal motoneurons in vitro and the rescue and axonal regeneration of axotomized motoneurons in vivo. Monoclonal antibody(Mab) for MNTF1 and idiotypic antibody(Id-ab) for its putative receptor used for their localization revealed that immunopositive stainings for Mab and Id-ab were localized in the cytoplasm and at the peripheral, respectively, of the soma of anterior horn motoneurons of the cervical and lumbar spinal cords and in those muscles of the fore- and hind-limbs of wobbler mice. As neuromuscular degeneration progressed from stage 1 to 4, the number of stained motoneurons and fibers and the relative intensities in them were reduced very significantly, especially in the cervical spinal cord and muscles of the forelimb. MNTF1 (35μg/kg) in cut-out gels were implanted once dorsally inbetween the trapezius and rhomboid major muscles around the vertebral column at C7-T2 level of wobbler mice at stage 1 of the motoneuron disease. The general health conditions and motor activities were improved noticeably within 2 weeks after MNTF1 treatment when compared to untreated control groups. The pathologic conditions of the forelimb muscles in paw contraction and elbow and shoulder joint movements of these treated wobbler mice were arrested at a grade 1 level in stage 1 of the motoneuron disease at 7 months posttreatment when compared to untreated control groups with degenerating neuromuscular activities and death after 3 months of age. Under LM, anterior horn motoneurons of the treated group appeared almost normal with a few small vacuoles existed in the cytoplasm when compared to the normal motoneurons of their littermates; however, those motoneurons of the untreated groups appeared fewer and smaller with many large vacuoles but fewer Nissl's granules in their cytoplasm,especially in the cervical spinal cord. These results indicated that the target-derived MNTF1arrests further pathologic development of the motoneuron disease in treated wobbler mice. Supported by HKU & GF grants.

94.8

FALS-RELATED *SOD-1* MUTATIONS INCREASE INTRACELLULAR FREE RADICAL CONCENTRATIONS AND CELL MORTALITY IN DIFFERENTIATED PC12 CELLS.

J. Lee*, V.P. Bindokas, R.P. Roos[1], R.J. Miller and G.D. Ghadge[1]. Dept. Pharmacol. and Physiol. Sci. and [1]Dept. of Neurology, University of Chicago, Chicago, IL 60637.

We are studying the mechanism of selective motoneuronal death associated with FALS and ALS. We sought to devise a convenient cell culture model for examining potential mechanisms of neurodegeneration in FALS and potential therapuetic approaches. Human *SOD1* WT or mutant (A4V, V148G) genes were delivered by adenovirus into differentiated PC12 cells. Mock and AdlacZ infections were used as controls. The same number of PC12 cells were initially plated on each coverslip and cells were differentiated by applying NGF. Western blot analyses confirmed expression of WT and mutant SODs. Cell death was analyzed 5 days after infection by staining with fluorescein diacetate and propidium iodide. Infection with AdlacZ and AdWT SOD1 did not produce significant mortality compared to mock-infected cells. However, approximately 50% cell mortality was observed with both mutant forms of SOD. The toxic effects of mutant SODs were ameliorated by metal chelators. The copper chelator TEPA (50 μM) had no toxicity in AdlacZ and mock-infected or AdWT SOD1-infected cultures, and fully protected the cells from death following infection with AdSODV148G or AdSODA4V. Similar protective effects were obtained with DDC, catechol, and 1,10-phenanthroline. Intracellular superoxide concentrations under basal conditions and following stimulation with FCCP and H_2O_2 were monitored using hydroethidine fluorescence. Transfection with either of the mutant SODs produced significantly increased superoxide production above levels found in mock-, *lacZ*-, and WT-transfected cells. These data suggest mutations in *SOD1* result in disturbance of cellular free radical content and that this may be related to increased cell mortality. (This work was supported by PHS grants DA-02575, DA-02121, MH-40165 and NS-33502 to R.J.M.).

94.9

PROGRESSIVE MOTOR DEFICITS IN SOD1 DEFICIENT MICE

A.G. Reaume, J.A. Gruner, E.K. Hoffman, D.S. Howland*, R. W. Scott. Dept. of Molecular Biology, Cephalon, Inc. West Chester, PA 19380

Since the discovery that mutations in the superoxide dismutase-1 (SOD-1) gene are associated with familial forms of amyotrophic lateral sclerosis (FALS) a great deal of attention has focussed on the role of SOD-1 in the maintenance of motor neurons. Previously we have shown that mice completely lacking SOD-1 do not develop overt muscle paralysis with age as is characteristic of mice that express an FALS mutant form of the SOD-1 gene (Reaume et al (1996) Nature Genet. 13:). We also have shown that 4 month old SOD-1 deficient animals are more vulnerable to cell death associated with facial motor nerve axotomy. Running wheel activity, stride length, and grip strength tests were used to compare the SOD1 mutant mice to their wild-type counterparts. Deficits were found associated with aged animals. In addition, the same tests were used to examine both wild-type and SOD-1 mutant mice that either had been allowed to run on a wheel for over a 1 year period or had had received no exercise. Finally, a significant reduction in sciatic nerve conduction velocity was found in SOD knockout animals between 5 and 7 months in age. The results are consistent we our previous hypothesis that although lack of Cu/Zn SOD function does not appear to cause FALS it influences the outcome of neurons exposed to stress and may have an maintenance role in motor neuron function.

94.10

OXIDATIVE STRESS, CALCIUM DYSREGULATION AND SUSCEPTIBILITY TO EXCITOTOXICITY IN SPINAL CORD NEURONS FROM MICE EXPRESSING SOD1 MUTATIONS.

O. Bar-Peled*, P. Wong‡, J. J. Vornov, and J.D. Rothstein. Johns Hopkins University, Dept. of Neurology and Neuropathology‡, Baltimore, MD 21287.

Amyotrophic lateral sclerosis (ALS) is a sporadic and hereditary motor neuron disease. Mutations in superoxide dismutase 1 (SOD1), an enzyme that catalyzes the dismutation of superoxide radical into hydrogen peroxide, causes ALS in approximately 15% of familial cases. Transgenic mice that overexpress the mutant forms of SOD1 (G37R) develop progressive motor neuron disease (*Neuron* 1995, 14:1105). For *in vitro* studies of large motor neurons (LMN) and their response to excitotoxicity, dissociated spinal-cord cultures were prepared from 13 day old transgenic fetuses. Spinal cord cultures were exposed to kainate (25μM) for 20h and LMN were identified by immunoreactivity for non-phosphorylated neurofilament. A two fold increase in kainate-induced LMN cell death was found in G37R cultures as compared to non-transgenic controls. The role of Ca^{+2} in triggering neuronal degeneration was examined using Fura-2. Analysis of intracellular Ca^{+2} in spinal neurons was performed following 10 min exposure to kainate (25μM). At that point, the percent of neurons with Ca^{+2} levels above 200nM was 3 fold higher in G37R cultures as compared to non-transgenic. The possibility that G37R-related oxidative stress is involved in neuronal degeneration was examined by intracellular glutathione (GSH) measurements using monochlorobimane (mBCl, 40μM), a fluorescent indicator for GSH. Cultures were first treated with buthionine sulfoximine (BSO,50μM), an inhibitor of GSH synthesis, thus permitting an accurate measurement of oxidative stress-dependent changes in the levels of GSH. G37R spinal neurons had a marked decrease in GSH levels compared to non-transgenic controls. These results suggest that the G37R mutations produce increased oxidative stress in vivo. Furthermore, these oxidative processes may lead to destabilization of calcium homeostasis, explaining the increased susceptibility to excitotoxicity. (Supported by NIH and MDA)

94.11

Somata and Nuclei of Motoneurons are Enlarged in mnd Mutant Mice. Maria Watson*, Anne Messert & S. Marc Breedlove. Dept. Psychology, U. California, Berkeley, CA 94720; †Wadsworth Ctr., N.Y.State Dept. Health & Dept. Biomed Sci., SUNY, Albany, NY 12201.

Mice homozygous for the motor neuron degeneration (mnd) mutation display delayed neuronal degeneration. In order to investigate the specificity of the process in sub-populations, we examined discrete spinal motor nuclei in homozygous mnd and wild-type male and female mice.

Mice 9-10 months of age were sacrificed and perfused with 4% paraformaldehyde, the spinal cords were removed and post-fixed in buffered formalin. Cords were frozen-sectioned in the coronal plane at 40 µm, and stained with thionin. An observer blind to experimental conditions counted the number of lower lumbar motoneurons in the retrodorsolateral nucleus (RDLN) and the rodent homologue of Onuf's nucleus, the spinal nucleus of the bulbocavernosus (SNB), and measured the size of the somata and nuclei of motoneurons in these pools. As previously reported, there was a sex difference in the number of SNB cells, but not in the number of RDLN cells. We found that homozygous mnd mice did not differ significantly from wild-types in the number of motoneurons in either of these groups. However, the size of RDLN somata and nuclei were significantly larger in mnd mice of either sex ($p<.01$).

RDLN somata:	Males	Females
wild-type:	525.0±26.15 (N=5)	474.3±37.13 (N=5) µm²
mnd mice:	569.4±31.10 (N=6)	640.4±29.84 (N=5)

Enlarged cross-sectional areas of RDLN motoneurons in the mutant mice may reflect a chromatolytic reaction prior to death and/or the appearance of inclusion bodies. The lack of a difference in RDLN number between wild-type and mutant animals may mean that the disorder had not progressed sufficiently by the time of sacrifice to result in disappearance of this set. Other spinal neuronal populations will also be examined. Supported by NS28421 to SMB; NS29110 and the ALS Assoc. to AM.

94.12

INHIBITION OF MYOTONIN-PROTEIN KINASE (MT-PK) CAUSES APOPTOSIS IN MUSCLE CELLS. S. Bhagwati, B. Leung, A. Ghatpande and A. Stelzer*, Department of Neurology, SUNY HSCB, Brooklyn, New York 11203.

The mutation responsible for myotonic dystrophy (DM) has been identified as an aberrantly expanded (CTG)n repeat in the 3' untranslated region of the DM gene. The DM gene encodes a 72 Kd Protein named Mt-PK which is expressed in skeletal and cardiac muscle and brain but nothing is known of the substrate molecule or function of Mt-PK. Knowledge of the function of Mt-PK should provide important insights into the pathogenesis of DM, which remains a puzzle. To determine the function of Mt-PK we introduced a specific antibody to Mt-PK inside cultured human myoblast cells with a lipid carrier, and show that this resulted in apoptosis in 30 to 40% of the cells within 24 hours (unlike control antibodies which had no effect). To confirm the specificity of this result we transfected myoblasts with a C5 propynyl-pyrimidine modified phosphorothiotate anti-sense oligodeoxynucleotide targeted to the translation initiation site of DM mRNA, which resulted in specific reduction of 62 to 74% in the levels of Mt-PK. A large percentage (52 to 73%) of myoblasts transfected with this oligonucleotide (but not control sense or scrambled oligonucleotides) underwent apoptosis within 24 hours. These results provide the first clue to the possible physiological role of Mt-PK and suggest that it may play a role as a cell survival molecule in muscle cells. Future studies should look for the presence of apoptosis in tissues from DM patients. Supported by an NIH grant.

94.13

IDENTIFICATION OF *mdx* MICE SPECIFIC GENES EXPRESSION BY DIFFERENTIAL DISPLAY PCR.

L.NJL. Marlier*[1,2], D. Pierucci[2], O. Porzio[2], M. Minieri[2], G. Silvestri[3], P. Borboni[2] and R. Massa[3].

1: Inst. Exp. Med. (CNR), 2: Dept. of Internal Medicine, 3: Dept. of Neurology, Univ. of Rome "Tor Vergata", Italy;

The mutant *mdx* mouse is a well known model of the human Duchenne Muscular Distrophy. In *mdx* mice, an altered expression of genes other than dystrophin, has been poorly investigated. In this study, the PCR-related procedure named differential display PCR (DD-PCR) was used to identify genes that are differentially expressed especially during the onset of the disease. For this purpose, two weeks old *mdx* mice muscles were processed for RNA extraction and used in a standard DD-PCR assay (GeneHunter) in parallel to muscle RNA prepared from control mice. Expression of numerous genes was up- or down-regulated on displayed gels. So far, we studied 19 fragments, that seem differentially expressed. These fragments were cloned. Among the 42 clones obtained, 12 were sequenced. Four of these sequenced clones had more than 85% homology with sequences known from the GeneBank database. In order to confirm such differential expression, northern blot hybridization studies were performed using 2 weeks (onset time point), 8 weeks and 6 months old *mdx* and control mice RNA. Two genes were clearly down-regulated in all *mdx* mice when compared to normal respective control mice. The two other RNA investigated showed, on the contrary, an almost similar level of expression. Other fragments are currently under analysis to confirm their differential expression. These results would suggest that *mdx* mice related disease might be associated to altered expression of several genes such as ions-channels proteins, docking proteins and/or collagen isoforms expression. This would support the hypothesis of secondary alterations in genes expression with possible implication in the pathophysiology of muscle fiber necrosis and regeneration.

94.14

MUTATIONAL ANALYSIS IN A CONGENITAL MYASTHENIC SYNDROME (CMS) REVEALS A NOVEL ACETYLCHOLINE RECEPTOR (AChR) ϵ SUBUNIT MUTATION K. Ohno, T. Fukudome, S. Nakano, M. Milone, T.E Feasby, G.M Tyce*, and A.G. Engel, Mayo Clinic, Rochester, MN 55905

To determine the basis of a severe CMS present since birth, electrophysiological, ^{125}I-α-bungarotoxin (αBGT) binding, electron microscopy, and molecular genetic studies were carried out. In the propositus, the number of AChR/endplate (EP) was 20% of normal. EP fine structure was preserved but the density of AChR on the junctional folds, determined with peroxidase-labeled αBGT, was greatly attenuated. Miniature EP currents had markedly reduced amplitude and a prolonged monoexponential decay. Direct sequencing of PCR-amplified exons and adjacent intronic regions of α- β, δ, and ϵ-subunit genes revealed a homozygous 20-bp deletion in ϵ exon 10 (ϵ1012del20) that encodes the long cytoplasmic loop of ϵ and predicts 31 missense codons after codon 348 followed by a stop codon. An affected brother was also homozygous for the deletion; the unaffected parents and a normal sibling were heterozygous for the same deletion, indicating that the mutation is recessive. The homozygous ϵ1012del20 mutation can readily account for the severe EP AChR deficiency and the clinical symptoms. Moreover, the prolonged EP currents suggest the presence of γ subunits in EP AChRs. Expression studies are in progress. Supported by NIH Grant NS6277 and an MDA Research Grant.

94.15

4-AMINOPYRIDINE IMPROVES MOTOR UNIT PERFORMANCE IN CANINE MOTOR NEURON DISEASE.

R.F. Waldeck[1*], T.C. Cope[2], L.C. Cork[3] and M.J. Pinter[1], *[1]Medical College of Pennsylvania, Philadelphia, [2]Emory University School of Medicine, [3]Stanford University School of Medicine*

Hereditary Canine Spinal Muscular Atrophy (HCSMA) is an autosomal dominant disorder of motor neurons that shares features with human motor neuron disease. In animals exhibiting the accelerated phenotype (homozygotes), we have demonstrated previously that many motor units exhibit functional deficits that likely reflect underlying deficits in neurotransmission. In this study, we determined whether and to what extent 4-aminopyridine (4AP), which blocks K+ channels, could improve motor unit performance in HCSMA. Systemic 4AP delivered in doses of 1-2 mg/kg increased nerve-evoked whole muscle twitch force and electromyograms (EMG) in parallel. These effects were larger in older homozygous animals that were profoundly weak and possessed failing motor units than in similarly-aged, symptomless HCSMA animals or in one younger homozygote that did not yet possess failing motor units. Direct testing of 4AP on single failing motor units showed that twitch force and EMG could be significantly increased, unit EMG trial-to-trial variability decreased and unit twitch EMG potentiation eliminated. 4AP increased peak forces reached during unit tetanic activation at all frequencies tested, and reduced tetanic force failure at lower frequencies. A comparison between 2 units obtained in a single animal showed that the motor unit exhibiting more force failure during tetanic activation also showed greater 4AP effects. These results indicate that failing motor units in HCSMA possess significant residual functional capabilities that can be demonstrated after 4AP administration. Supported by PHS grant NS31621

94.16

MYOTONIC DYSTROPHY KINASE SHOWS A SPATIALLY AND TEMPORALLY DYNAMIC PATTERN OF EXPRESSION IN CNS DEVELOPMENT. A. Balasubramanyam, D.V. Iyer, C. Beaulieu, A. Potvin, A. Neumeyer, J. Avruch, H.F. Epstein*. Depts of Medicine and Neurology, Baylor College of Medicine, Houston, TX; Dept of Pathology, University of Montreal, QUE; Depts of Neurology and Medicine, Massachusetts General Hospital, Boston, MA.

Myotonic dystrophy is characterized by CNS dysfunction, including varying degrees of mental retardation, cognitive deficiencies, retinal abnormalities and sleep disorders. Myotonic dystrophy protein kinase (DMPK) is the product of the gene linked to the illness. Its regulation and physiological roles have not been specified.

We have performed precise localization studies of DMPK in the central nervous system of the rat at the light and electron microscopic levels. Using a highly-specific antibody against an epitope in the helical domain of DMPK, we find that DMPK expression in the post-natal CNS follows a unique developmental sequence: faintest expression at post-natal day 0 (P0), faint expression at P7, prominent expression in most neuronal cell types with a peak of staining intensity at P14 to P21, followed by restriction of expression to specific subsets of neurons between P21 and P28. The restricted distribution persists into adulthood. The timetable of this dynamic expression correlates with key post-natal maturational events in the CNS of the rat and suggests that DMPK is functionally related to this process.

In the adult CNS, specific staining occurs in particular subsets of cells, e.g., in hippocampus (principally basket cells and oriens/alveus interneurons), cerebellum (Purkinje cells and Golgi neurons, but not granular neurons) and spinal cord (large motoneurons). Electron microscopy reveals that DMPK is localized to the endoplasmic reticulum within somata and in association with microtubules within proximal dendrites. These patterns of development and localization may be useful in elucidating the diverse central nervous system dysfunctions in myotonic dystrophy. [Support: NIH and JDF (AB); MRC Canada (CB)]

94.17

MODULATION OF INTRACELLULAR CALCIUM IN DYSTROPHIC SKELETAL MUSCLE CELLS.

A. C. Passaquin, W.J. Leijendekker, L. Bernheim* and U.T. Rüegg
School of Pharmacy, Lausanne Univ., CH-1015 Lausanne, Switzerland.

Duchenne muscular dystrophy (DMD) patients and *mdx* mice suffer from a genetic disorder characterized by the absence of the cytoskeletal protein dystrophin. The chain of events leading from the absence of dystrophin to muscle degeneration remains unclear. A pathological increase in cytosolic calcium concentrations ($[Ca^{2+}]_c$) has been incriminated as a potential mechanism. We have therefore studied $[Ca^{2+}]_c$ handling (using either $^{45}Ca^{2+}$ influx or fura-2) in skeletal muscle cells of several origins: the C2 cell line, *mdx*-derived primary cultures, myogenic H2K clones, and clones of DMD-derived myogenic cells. Resting $[Ca^{2+}]_c$ were similar in dystrophin-deficient myotubes when compared to normals. Under stress conditions (i.e. high $[Ca^{2+}]_o$ or osmotic shock), $[Ca^{2+}]_c$ as well as $^{45}Ca^{2+}$ influx were increased in both dystrophin-deficient and normal cells but this increase was more prominent in myotubes from *mdx* primary cultures. This gives support to the above hypothesis implicating Ca^{2+} dysregulation as a key feature of the dystrophic phenotype. However, stress imposed on dystrophin-lacking clones (from both mouse and human origin) was without effect, suggesting that exogenous factors present only in primaries could be involved in the Ca^{2+} rise. With the aim of counteracting a pathological increase in $[Ca^{2+}]_c$ by pharmacological means, we have examined several compounds. Among them, α-methylprednisolone, the only drug known to exert a beneficial effect in DMD, the protease inhibitor calpeptin, the blocker of stretch-activated calcium channels Gd^{3+} and the energy supplier creatine proved efficient in lowering the Ca^{2+} increase. We are currently investigating the mechanisms involved in these effects.

Supported by the Swiss National Fund for Scientific Research (32-45004.95) and the Swiss Foundation for Research on Muscular Diseases.

94.18

EXPRESSION PATTERNS OF THE SMN GENE, THE HUMAN SPINAL MUSCULAR ATROPHY DETERMINING GENE, IN THE MAMMALIAN CENTRAL NERVOUS SYSTEM. A. Princivalle, F. Forti, F. Clementi*, M. Zeviani, and G. Battaglia. Neurological Institute "C. Besta", and Dept. of Medical Pharmacology, Univ. of Milano, Italy.

The Survival Motor Neuron gene (SMN) is the proposed gene for human spinal muscular atrophy (Lefebvre et al. 1995). The deduced aminoacid sequence of the human SMN gene is a 32 Kd protein of unknown function, and with no sequence homology with any known protein. In the present work the SMN gene analog was characterized in the rat, and the anatomical expression of the SMN gene was investigated in the central nervous system of different mammals, by means of in situ hybridization (ISH) and immunocytochemistry (ICC) techniques. Using pairs of primers designed on the human sequence, the cDNA corresponding to the SMN ortholog was amplified by RT-PCR on rat liver poly(A)+ RNA. The deduced rat protein is about 90% similar to the human SMN protein product. The rat and human exon 3 sequences were then amplified by PCR technique, and the amplified exons used as templates to obtain radiolabeled antisense riboprobes. Two oligopeptides corresponding to the N-terminus and C-terminus of the human protein were produced, and employed as antigens to raise specific polyclonal antisera in rabbits. The ICC experiments revealed that the protein was mainly expressed in the cytoplasm of neurons in all the examined species, and that was widely but unevenly distributed throughout different cerebral and spinal areas. Strongly immunoreactive neurons were detected in the spinal cord of rats and monkeys, particularly the large lamina IX motorneurons; and in the rat and human cerebral cortex, particularly the pyramidal neurons of the deeper layers. These data were supported by preliminary ISH experiments, that revealed a strong although diffuse signal in the rat spinal gray matter, particularly in the lamina IX of the ventral horn. The SMN expression analysis supports the relevance of this gene for the motorneurons function, and could be of great value in unrevealing the pathogenesis of the related human disease.

Supported by Telethon grant n° 790.

NEUROPSYCHIATRIC DISORDERS I

95.1

EFFECTS OF OLFACTORY BULBECTOMY (OBX) ON FORCED-SWIM TEST (FST) AND LIMBIC TRH AFTER ELECTROCONVULSIVE SHOCK (ECS). A. Sattin* R.L. Lloyd, A.E. Pekary, M. Chilingar., Psychiatry, Medicine (Endocrinology) & Research Services, W. Los Angeles VA Medical Center & UCLA, Los Angeles, CA 90073.

The OBX model of depression was used in 2-mo. male Wistar rats (Simonsen, Gilroy, CA). The Porsolt pre-swim could not be used with OBX because of ceiling effects, so a modified FST was scored with a single 5 min immersion after ECS/sham ECS daily x 3. Sham ECS vs ECS ss's were subgrouped following bilateral OBX or sham OBX (4 groups). Only the OBX/sham ECS's showed high immobility compared to the other 3 groups. Thus, OBX prevents the burst of swimming seen after initial immersion of intact rats. This result cross-validates the forced-swim and OBX models of depression. Analysis of TRH and precursor TRH-Gly (1 day post-swim) showed the expected post-ECS increases (Sattin et al, Ann NY Acad Sci, 739: 135-153, 1994), but OBX/sham ECS showed significant increases of TRH in amygdala/entorhinal cortex and pyriform (olfactory) cortex. These post-OBX increases might be consequent to decreased release of TRH secondary to trans-synaptic degeneration, and consequent swim-immobility. Supported by VA Research Service.

95.2

EXPRESSION OF c-*fos* mRNA IN RAT BRAIN FOLLOWING KINDLING OF MYOCLONUS WITH CLOZAPINE: BEYOND THE DOPAMINE HYPOTHESIS J. Stevens*, D. Denney, P. Szot. Depts Neurology & Psychiatry Oregon Health Sciences University Portland, OR 97201 & GRECC VAMC Seattle, WA 98108

Seizures often improve symptoms of psychoses, including schizophrenia. Clozapine, which frequently ameliorates symptoms of schizophrenia in patients unresponsive to other antipsychotic agents, has CNS excitant properties that cause epileptiform EEG changes, myoclonus or seizures in 3-10% of patients who receive average clinical doses of the drug (*Neurology* 41:369, 1991). We previously reported a clear dose-response relationship for production of myoclonus by clozapine in partially restrained rats (*Biological Psychiatry* 37: 427 1995). Repeated administration of very low dose clozapine (1mg/kg) I-P, a dose that initially did not induce myoclonus or other behavior change, caused increasing myoclonus when repeated at weekly intervals. Myoclonus induced after repeated weekly 1mg/kg injection of clozapine is associated with expression of c-*fos* mRNA in ventral tegmental area and anterior thalamic nuclei. These results of kindling with clozapine are compared with c-*fos* expression in brains of similarly restrained rats given a single 1 mg/kg or 10 mg/kg dose of clozapine and to animals after kindling with clozapine without restraint. Expression of c-*fos* mRNA in these thalamic and midbrain nuclei indicates that myoclonus induced by kindling with clozapine is associated with activation of specific subcortical nuclei. This may relate to the unique therapeutic effect of this drug in schizophrenia.

95.3

PERSISTING EFFECTS OF ORAL PYRIDOSTIGMINE BROMIDE ON THE BEHAVIOR OF RATS. B.H. Natelson, J.E. Ottenweller, C. Goldstein, D. Beldowicz, W.T. O'Brien and W.N. Tapp*, R.J. Servatius. Neurobehavioral Unit (127A), VA Medical Center, East Orange NJ 07018 and Department of Neuroscience, New Jersey Medical School-UMDNJ, Newark NJ.

Pyridostigmine bromide (PB) is an anticholinesterase used for protection against nerve agents by troops during the Persian Gulf War. Questions have arisen as to whether its use could be contributing to some of the long-term, unexplained medical illnesses in these veterans. To understand whether PB could produce persisting effects, we gave rats about 2 mg/kg/day for 7 days in their drinking water. Young adult male Sprague-Dawley and Wistar-Kyoto rats received drinking water with PB, and controls had plain water. On the day after stopping PB, and 1 and 2 weeks later, startle responses to 73, 83 and 93 dB noises were assessed using whole body and eyeblink EMG responses. 48 hr after stopping PB, open field activity was measured, along with assessment of pain thresholds using hot-plate tests. The open field test was not affected by PB, but PB increased pain sensitivity 48 hrs after the last exposure. Startle responses were not affected 24 hr after the end of PB treatment, but one week later PB rats showed enhanced startle responding. Two weeks after discontinuing PB, treated rats still appeared to be sensitized to the startle stimuli compared to controls. It is unlikely that PB was directly active at the time of these behavioral tests. The persisting effects of PB on startle responding are not apparent just after treatment, but seem to emerge 1-2 weeks after the treatment had ended. Experiments are in progress to determine the duration of these effects in rats. Our results were unexpected and suggest that ingestion of PB may have long term consequences. The direct relevance of this animal work to the medical complaints of the Persian Gulf veteran is not known. Supported by VA Medical Research Funds for the New Jersey Center for Environmental Hazards Research.

95.4

DISRUPTION OF THE GENE ENCODING THE RIIβ SUBUNIT OF PKA ABOLISHES TRANSCRIPTIONAL AND BEHAVIORAL RESPONSES TO HALOPERIDOL IN THE DORSOLATERAL STRIATUM M.R. Adams* E.P. Brandon, E. Chartoff, R.L. Idzerda, D.M. Dorsa and G.S. McKnight Depts. of Pharmacology and Psychiatry, University of WA, Seattle WA 98195.

Administration of typical antipsychotic drugs (APD's), such as haloperidol, promotes a dramatic increase in the transcription of the neurotensin/neuromedin N (NT/N) gene in neurons of the dorsolateral striatum (DLST) and shell sector of the nucleus accumbens. Previous studies have shown that APD's bind to and antagonize D2 receptors and increase intracellular cAMP. In order to determine whether haloperidol-induced NT/N gene expression is mediated via the cAMP-PKA-CREB pathway, we examined the effects of haloperidol on NT/N gene expression in mice in which genes that encode the various subunits of the PKA enzyme have been disrupted. In mice, carrying a disruption of the gene encoding RIIβ, the predominant regulatory subunit of PKA in the striatum, induction of the NT/N and c-fos gene in the DLST is completely abolished. In addition, an acute behavioral response to haloperidol, catalepsy, is also significantly attenuated in these animals. Thus, these animals are unresponsive to effects of haloperidol that are thought to involve the motor striatum. The data suggest that PKA activation is an early and essential step in APD induced transcriptional effects. They also indicate a potential role for neurotensin in cataleptic effects of haloperidol. Supported by the GM32875 (GSM) NS20311 (DMD) and MCBTG, T32GM07270 (MRA)

95.5

INCREASED EXTRACELLULAR BRAIN GLUTAMATE CONCENTRATIONS AND CONCOMITANT LOSS OF NON-NMDA BINDING SITES IN RATS WITH ACUTE LIVER FAILURE

A. Michalak, C. Rose J. Butterworth, D.D. Mousseau* and R.F. Butterworth, Neuroscience Research Unit, Hôpital Saint-Luc (University of Montreal), Montreal, Québec, Canada H2X 3J4

It has been suggested that hepatic encephalopathy is the consequence of glutamatergic synaptic dysregulation (Butterworth, *Mol. Pharmacol.* **2**, 229, 1992). In order to evaluate glutamatergic function in acute liver failure, rats with ischemic liver failure following portacaval anastomosis and hepatic artery ligation were stereotactically implanted with microdialysis probes (CMA 12 1 mm x 0.5 mm with a 20,000 m.w. cut off) in frontal cortex and glutamate concentrations were measured as their o-phthalaldehyde derivatives by HPLC at various times during progression of encephalopathy. In a separate series of experiments, NMDA and non-NMDA binding sites were assessed by quantitative receptor autoradiography using ^{3}H-MK801 or ^{3}H-5-fluorowillardiine (respectively) in the brains of animals with acute liver failure at coma stages of encephalopathy. Ischemic liver failure resulted in a significant increase in extracellular concentrations of glutamate in frontal cortex, an increase which was positively correlated with CSF ammonia concentrations in these animals. Extracellular glutamate concentrations in frontal cortex of rats with acute liver failure were increased 3-4 fold at coma stages at which time, densities of binding sites for the non-NMDA receptor ligand ^{3}H-5-fluorowillardiine were significantly decreased in frontal cortex, olfactory tubercle, hippocampus, hypothalamus and cerebellum. ^{3}H-MK801 binding site densities were within normal limits. This, acute liver failure results in increased extracellular concentrations of glutamate and a concomitant loss of non-NMDA binding sites consistent with the hypothesis that alterations of glutamatergic synaptic function are responsible for the neuropsychiatric symptoms encountered in acute liver failure (funded by MRC Canada and The Canadian Liver Foundation)

95.6

EARLY BREAKDOWN OF THE BLOOD-BRAIN BARRIER AND CONCOMITANTLY INCREASED IMMUNOSTAINING FOR MACROPHAGE LYSOSOMAL ANTIGEN (ED1) IN THIAMINE DEFICIENCY. D.K. Leong*, L. Oliva, J. Kril and R.F. Butterworth, Neuroscience Research Unit, Hôpital Saint-Luc (University of Montreal), Montreal, Quebec, Canada H2X 3J4

Immunohistochemistry with antibodies to astrocytes (polyclonal glial fibrillary acidic protein; GFAP); and macrophages (monoclonal ED1), were used to examine the glial response in brains of rats at different stages of thiamine-deficiency. In addition, an antibody to serum albumin was used to study the integrity of the blood-brain barrier. The results of the study reveal early, region-selective, increases of immunostaining for ED-1 and serum albumin in brains of thiamine-deficient rats, prior to the appearance of neurological symptoms of thiamine deficiency or major histopathological lesions. Increased ED1-immunostaining, suggestive of an early macrophage response, and concomitantly increased albmin-immunoreactivity suggestive of an early breakdown of the blood-brain barrier, were confined to the inferior olive, inferior colliculus and medial thalamus, regions of the brain which ultimately manifest marked neuronal loss in thiamine deficiency. Histological studies of neurologically symptomatic thiamine-deficient rats revealed significant neuronal loss, pallor of the neuropil and marked gliosis in vulnerable brain regions, while immunohistochemical studies of the same regions showed marked increases of GFAP-immunostaining, indicative of reactive gliosis. This region-selective macrophage response resulting from increased permeability of the blood-brain barrier may underly the phenomenon of selective regional vulnerability in thiamine deficiency. (Funded by the Medical Research Council of Canada)

95.7

INCREASED GAD mRNA IN THE RAT DENTATE GYRUS FOLLOWING REPEATED ELECTROCONVULSIVE SHOCK. S. R. Wright*[1], C. A. Stewart[2], P. Naylor[1], I. C. Reid[2] and R. C. A. Pearson[1]. [1]Dept. of Biomedical Science, University of Sheffield, S10 2TN, UK. and [2]Dept. of Psychiatry, University of Dundee, DD1 9SY, UK.

Male hooded Lister rats were assigned to one of two groups. One group (n=5) received a course of electroconvulsive shock (ECS) treatments induced transcranially under inhalation anaesthesia using ear-clip electrodes (200 V, 50 mA for 2 s). A total of 10 ECS were given spaced at 48 hr intervals. The other group (SHAM, n=5) received identical handling and anaesthesia but no seizure was induced. Approximately 24 hr following the last ECS or sham treatment the rats were sacrificed and their brains suitably prepared for in-situ hybridization histochemistry.

A [^{35}S]dATP 3'end labeled 30'mer oligo probe for GAD mRNA was hybridized to 10μ cryostat prepared sections. The slides were exposed to photographic emulsion (Ilford K5) for 4 weeks. Computerized image analysis of mRNA levels for GAD, measured as mean grain count per unit area, showed an increase in the granular layer (ECS 3.59±0.9 SD, sham ECS 2.47±0.33 SD) and molecular layer (ECS 3.27±0.59, sham ECS 2.18±0.41) of the dentate gyrus (two-tailed student "t" test, granular layer $p<0.005$, molecular layer $p<0.001$).

We postulate the increased production of GAD 24hrs following ECS is to maintain local inhibition and modulate our reported increase in the CA3 AMPA receptor population in the same animals (Mol Brain Res 35 (1996) 349-353). A more global effect may well have important implications for the therapeutic and adverse effects of ECT in humans. *Supported* by The Wellcome Trust.

95.8

INCREASED PLASMA LUTEINIZING HORMONE (LH) AFTER ELECTRO-CONVULSIVE SHOCKS (ECS) IN RATS AND HUMANS. Th. Steimer, J.L. Moreau, P. Lemoine, M.L. Aubert, P. Schulz*. Department of psychiatry, Geneva, 1225 Switzerland; Preclinical CNS research, Hoffmann-La Roche, Basel; Biological psychiatry unit, Lyon, France; Department of pediatrics, Geneva, Switzerland.

Hormones of the hypothalamo-pituitary-gonadal (HPG), axis notably the gonadotropin-releasing hormone (GnRH or LHRH) and gonadal steroids such as testosterone (T) and/or oestradiol, might have anti-depressant properties, or potentiate other treatments in patients and animal models of depression. We studied the acute effects of ECS on hormones from the HPG axis in depressed patients and male Wistar rats. In rats, plasma LH was significantly elevated 20 and 60 min after ECS (30mA, 0.5 sec). Plasma T was also increased at 60 min. Plasma prolactin (PRL) was higher in ECS-treated as compared to non-shocked control rats 5 and 20 min after ECS, whereas corticosterone (B) was only augmented after 20 and 60 min. In human males (n=4), a substantial and reproducible increase in lasma LH and PRL was observed 15 min after ECS. The HPG axis could be involved in the antidepressant effects of ECS.

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95.9

ANXIOLYTIC ACTIONS ARE MEDIATED BY SEROTONIN (5-HT)$_{1A}$ AUTORECEPTORS: S 15535 AND 8-OH-DPAT BLOCK ULTRASONIC VOCALIZATIONS (USV) AND AGGRESSION IN A WAY 100,635-REVERSIBLE FASHION, M. Brocco*, K. Bervoets, B. De Ladonchamps, S. Veiga and M.J. Millan, I.D.R.S., 125 Chemin de Ronde, 78290 Croissy, France.

The relative involvement of pre and postsynaptic 5-HT$_{1A}$ receptors in decreasing anxiety is controversial. Here, we examined this question using selective ligands with differential efficacy at these sites: 8-OH-DPAT (8-hydroxy-2-(di-n-propylamino) tetralin) (8DP), an agonist at both pre and postsynaptic sites; WAY 100,635 (N-{2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl}-N-(2-pyridinyl) cyclo-hexanecarboxamide) (WAY), an antagonist at both pre and postsynaptic sites and S 15535 (4-(benzodioxan-5-yl)1-(indan-2-yl)piperazine)), an agonist at pre and an antagonist at postsynaptic sites. USVs were measured over 5 min by a bat-detector in rats pre-exposed (-24 h) to a mild foot-shock (0.8 mA, 8 sec). Aggression (resident vs intruder) was tested over 3 min in mice isolated for 6 weeks. S 15535 or 8DP were given -30 min, and WAY, -60 min. Doses (base) are means ± S.E.M. in mg/kg, s.c..

	VEH/ VEH	VEH/ S 15535	VEH/ 8DP	WAY/ VEH	WAY/ S 15535	WAY/ 8DP
USV (sec)	206±25	40.8±15.0#	0.4±0.2#	222±38	236±57*	196±48*
Attacks (number)	19±4	2.1±1.1#	0±0#	21.0±2.7	19.0±4.2*	31.0±3.1*

VEH = vehicle. Doses of S 15535/8DP were 0.63/0.16 for USVs, and 2.5/0.63 for aggression. # $P<0.01$ to VEH/VEH; *$P<0.01$ to VEH/S 15535 or VEH/8DP.

S 15535/8DP inhibited USVs and reduced aggression at Inhibitory Dose$_{50}$s of 0.3/0.01, and 1.4/0.1, respectively. WAY (0.16) blocked these actions and was inactive alone. Since S 15535 shares agonist properties of 8-OH-DPAT at presynaptic but *not* postsynaptic 5-HT$_{1A}$ receptors, it is suggested that 5-HT$_{1A}$ autoreceptors mediate their anxiolytic actions in these procedures.

This study was supported by Servier Pharmaceuticals.

95.10

COMPARISON OF THE EFFECTS OF CP-93,393 AND BUSPIRONE ON NE AND 5-HT RELEASE: MICRODIALYSIS STUDIES IN HIPPOCAMPUS OF FREELY MOVING RATS AND GUINEA PIGS.

Y. Lu*, T. Clarke, A.W. Schmidt, J. Sprouse and H. Rollema
Department of Neuroscience, Pfizer Central Research, Groton CT 06340

CP-93,393 is a novel anxiolytic/antidepressant, possessing both 5-HT$_{1A}$ agonist and α_2 antagonist properties. We measured the effects of 5-HT$_{1A}$ agonists (CP-93,393, buspirone, 8-OHDPAT) and α_2 antagonists (CP-93,393, idazoxan, 1-PP) on 5-HT and NE release in the hippocampus of freely moving rats (5-HT) and guinea pigs (NE).

CP-93,393, buspirone and 8-OHDPAT decreased 5-HT release to 25-40% of basal levels in rat hippocampus, with ED$_{50}$'s of 1.5, 0.7 and 0.02 mg/kg (sc) respectively, which correlated well with their K$_i$ values for 5-HT$_{1A}$ binding in rat cortex.

The compounds also produced an increase in NE release, but did so via two distinct mechanisms. All doses of buspirone tested (0.3 - 32 mg/kg) increased NE release 1.5 to 2-fold via 5-HT$_{1A}$ activation, since the NE increase was completely blocked by the 5-HT$_{1A}$ antagonist WAY 100635. Apparently, α_2 effects do not contribute to the buspirone-induced NE increase, despite the fact that 1-PP markedly increases NE release. The effects of buspirone were comparable to those of 8-OHDPAT. Low doses of CP-93,393 also increased NE release via 5-HT$_{1A}$ activation (blocked by WAY 100635), but doses of 10 mg/kg and above produced a robust NE increase, mediated by α_2 receptor blockade, which was only slightly attenuated by WAY 100635 pretreatment. The effects of 10 mg/kg CP-93,393 were very similar to those of 1 mg/kg of the α_2 antagonist idazoxan.

These results confirm that CP-93,393 acts *in vivo* as a 5-HT$_{1A}$ agonist, decreasing serotonergic transmission, and as an α_2 antagonist, increasing noradrenergic transmission.

95.11

EXPRESSION AND REGULATION OF MULTIPLE FORMS OF CYTOCHROME P450 IN RAT AND HUMAN BRAIN, P.S. Tirumalai[1], S. Bhamre.[1], M.R. Boyd[2], S.H. Koslow[3]* and V. Ravindranath[1], Dept. of Neurochemistry, National Institute of Mental Health and Neuroscience, Bangalore 560029, India[1], Lab. of Drug Discovery Research and Development, DTP, NCI, NIH, FCRDC, Frederick, MD 21702[2] and Division of Brain and Behavioral Sciences, NIMH, NIH, Rockville, MD 20857[3].

Cytochrome P450 (P450) are important xenobiotic metabolizing enzymes. P450 mediated bioactivation of xenobiotics in the brain could lead to irreversible damage, while *in situ* metabolism of psychoactive drugs can mediate pharmacological modulation at the site of action. Multiple forms of P450 are present in rat brain and are selectively induced by phenobarbital (PB), ethanol or dexamethasone. The isoforms of P450 are variably distributed in brain regions and their transcriptional regulation differs from that of the liver. PB administration resulted in increased transcription of P4502B in rat liver 12 hr after the dose, while similar elevation in brain mRNA was seen after 1 day. The induction of P450 by PB was observed in the brainstem (BS) and thalamus (TH), while it was unaffected in hippocampus(HP) and cerebellum (CE). Chronic ethanol administration resulted in increased P450 levels in HP and TH while it was unchanged in CE and BS. The expression of multiple forms of P450 was also observed in human brain regions obtained at autopsy from traffic accident victims. Localization of multiple forms of P450 in rat and human brain by immunocytochemistry and in situ hybridization revealed the predominant presence of P450 in the neuronal cells, the site of action of psychoactive drugs. The variable response to psychoactive drugs seen in population groups is often not accountable by considering dissimilarities in hepatic metabolism. These studies demonstrate the regional and cellular diversity exhibited by the brain in the distribution and regulation of multiple forms of P450 and point to their possible role in the local pharmacological modulation of psychoactive drugs.

Grant support: US-India fund for cultural, educational and scientific cooperation.

95.12

IDENTIFICATION OF LITHIUM REGULATED GENES BY DIFFERENTIAL DISPLAY PCR. J. F. Wang* and L. T. Young. Departments of Psychiatry and Biomedical Sciences, McMaster University, Hamilton, Ontario, Canada, L8N 3Z5.

Lithium is highly effective in the treatment of bipolar disorder. Although its exact mechanism of action is as yet unknown, much evidence suggests that lithium regulates gene expression in brain. To identify lithium regulated genes (LiRGs), we used mRNA differential display PCR to screen differentially expressed genes in the presence or absence of 1 mM LiCl treatment for one week in C6 glioma cells. Four candidate genes were isolated and verified by northern blot analysis. Sequencing analysis revealed three previously unidentified cDNA fragments in addition to a sequence with 99% homology with the cDNA for 2',3',-Cyclic Nucleotide 3'-Phosphodiesterase type II (CNPaseII). Using quantitative slot blot analysis, we found that 1mM LiCl increased CNPaseII gene expression by 62%, an effect which did not occur after administration of sodium chloride or anticonvulsants (carbamazepine or sodium valproate) commonly used to treat bipolar disorder. Since CNPaseII is important in myelinogenesis and possibly neuronal growth and repair, the present findings suggest that lithium may regulate these processes which is consistent with its long term stabilization of bipolar disorder and prevention of subsequent relapses of mania or depression. (Supported by a NARSAD Independent Investigator's Award (L.T.Y.) and a grant from Abbott Laboratories.)

95.13

EFFECT OF LITHIUM ON TRANSCRIPTION FACTOR BINDING IN CULTURED CEREBELLAR GRANULE CELLS AND THE BRAIN OF RATS. N. Ozaki* & D.-M. Chuang.Section on Molecular Neurobiology, Biological Psychiatry Branch, NIMH, NIH, Bethesda MD 20892-1272

Lithium is most commonly used in the treatment for manic depressive illness. However, its underlying therapeutic mechanisms remain obscure. Using primary cultures of rat cerebellar granule cells (CGC) as a model, we have shown that long-term exposure of CGC to LiCl increases the levels of mRNA for c-fos and m_3-muscarinic acetylcholine receptors (Gao et al., Neurochem. Internatl. 22:395,1993). These results suggest a potential regulatory role of lithium at the transcriptional level. In this study we investigated the effects on AP-1 binding in CGC exposed to LiCl and in rats chronically fed with Li_2CO_3 for 2 or 4 weeks. Extracts of CGC and dissected brain tissues were prepared for AP-1 gel-shift analysis using consensus AP-1 sequence. We found that exposure of CGC to LiCl (0.1 mM-10 mM) for 1 to 7 days induced a concentration-dependent increase in AP-1 binding. The maximal increase reached at 0.5 mM of LiCl which is within the plasma therapeutic range of this drug. Using extracts prepared from the frontal cortex, hippocampus and amygdala and cerebellum, two AP-1 binding bands were detected on the gel. The upper binding band was significantly increased in the hippocampus and amygdala after treatment with lithium-diet for 4, but not 2, weeks. Conversely, the lower binding band was markedly increased in the frontal cortex after 4 weeks of lithium treatment. These results strengthen the hypothesis that lithium at the therapeutic range is a transcriptional regulator. Mechanism underlying the lithium-induced AP-1 binding *in vivo* and in cultured neurons are being investigated. (NIMH)

95.14

CONCORDANT REGULATION OF GENE EXPRESSION BY LITHIUM AND VALPROIC ACID IN VITRO. P. X. Yuan, G. Chen*, and H. K. Manji. Schizophrenia and Mood Disorders Clinical Research Division, Dept. of Psychiatry & Behavioral Neurosciences, Wayne State Univ. School of Medicine, Detroit, MI 48201.

Both lithium and Valproic acid (VPA) are clinically effective antimanic and mood-stabilizing agents, but their molecular mechanism(s) of action have not been elucidated. Recent studies have shown that both these agents produce strikingly similar effects on PKC isozymes and substrates; in view of their delayed onset of therapeutic efficacy, it is also noteworthy that these agents also regulate the activity of transcription factors in the AP-1 family. To date, however, there has not been any clear evidence that these effects on AP-1 are translated into effects at the gene expression level. In the present study, rat C6 glioma cells and human SY5Y neuroblastoma cells were transfected with luciferase reporter gene vectors driven by SV40 or MMTV-LTR promoter/enhancers. At clinically therapeutic concentrations, both drugs increased reporter gene expression several fold. These effects are both time- and concentration-dependent. The possible involvement of each of the DNA binding elements as well as of PKC isozymes in mediating these effects of lithium and VPA are currently under investigation. This regulation of gene expression by lithium and VPA may play a role in their long term efficacy.

95.15

DEPRESSION IN SYSTEMIC LUPUS ERYTHEMATOSUS - PRIMARY OR COINCIDENTAL? S. Denburg*, J. Brasch, R. Carbotte and J. Denburg. Depts. of Psychiatry and Medicine, McMaster University, Hamilton, ON, Canada L8N 3Z5

Although depression is the most frequent psychiatric presentation in lupus (SLE), there is little consensus as to whether it represents a primary manifestation of nervous system involvement in that disease. We undertook a pilot study comparing psychiatric symptomatology and neurocognitive function in depressed SLE patients (N=11), idiopathically depressed patients (N=8) and non-depressed SLE controls (N=7). We found no differences between the two depressed groups in cognitive, affective or somatic symptom endorsements, suggesting that the clinical presentation of depression in the two groups may be indistinguishable. In contrast, while verbal productivity was reduced in both depressed groups, the depressed SLE patients tended to perform more poorly than both the depressed and non-depressed controls on tasks involving sustained mental effort, verbal and nonverbal learning and visuospatial planning, pointing to the potential importance of cognitive variables in studying the etiology of depression in SLE.

Funded by the Ontario Mental Health Foundation.

95.16

THE ROLE OF THE BETA ADRENERGIC SYSTEM IN CCK-4 INDUCED PANIC SYMPTOMS. J-M Le Mellédo*, J Bradwejn, D Koszycki, J-P Boulenger, A Cadieux, I Jerabek, F Jolicoeur, F Bellavance, D.G Bichet. Psychobiology and Clinical Trials in Anxiety Research Unit,Clarke Institute,Toronto,Canada, M5T 1R8.

The involvement of the noradrenergic (NE) system, particularly the beta NE system, in panic disorder has been suggested for some time. However, attempts at blocking pharmacologically induced panic attacks with propranolol (a central and peripheral beta-1 and beta-2 NE blocker) have yielded inconsistent results. These inconsistencies might be explained by a failure to verify the effectiveness of beta- blockade. Our study objectives were to assess the effects of controlled beta-blockade on CCK-4 induced panic symptoms and associated biological changes in healthy male volunteers. The study was double-blind and placebo-controlled. The beta-blocking effects of 0.2 mg/kg i.v. of propranolol were controlled for every subject, using the Cleaveland method. This method consists of verifying that the injection of a predetermined CD25 dose of isoproterenol has no effect on heart rate following the administration of a beta blocker (CD25 is the dose of isoproterenol which increases the heart rate of a subject by 25 beats per minute). Subjects exhibiting beta blockade were included in the study. Two days later, the same dose of propranolol or placebo was administered to these subjects followed by an i.v. injection of CCK-4 (50µg). Preliminary results based on 30 subjects showed that pretreatment with propranolol, as compared to placebo, decreased sum intensity of panic symptoms ($p< 0.01$), reduced CCK-4 induced anxiety ($p<0.05$) and CCK-4 induced increases in heart rate ($p< 0.01$). In conclusion, our results suggest that the beta NE system mediates at least part of the CCK-4-induced effects. The results of the effects of propranolol on CCK-4-induced arginine vasopressin, oxytocin, cathecolamines and NPY release will be presented.

This study was funded, in part, by the Medical Research Council of Canada.

95.17

DETERMINANTS OF ELECTROPHYSIOLOGIC RESPONSIVITY IN PTSD, J.H. Casada, R. Amdur, E.A. Young*, and I. Liberzon. Department of Pychiatry, VAMC and University of Michigan, Ann Arbor, MI, 48105.

Post-traumatic stress disorder (PTSD) is an anxiety disorder that is characterized by intrusive, avoidant, and hyperarousal symptoms. Electrophysiological changes in skin conductance, heart rate, and skin temperature in response to trauma related stimuli are consistantly reported in this disorder, however little is known about the contribution of individual variations and stimulus qualities in determining electrophysiological responsivity. We have studied electrophysiological responses of PTSD patients and two groups of controls (total of 36 subjects) in several different settings and in response to a variety of different stimuli: trauma related, threatening, non-specific auditory and arousing visual stimuli. The electro-physiological indices chosen for study include those that reflect autonomic tone, such as skin conductance (SC), heart rate (HR), and temperature (T), as well as one that is independent of autonomic tone, corregator electromyogram (EMG). The preliminary data analysis showed the expected increases in SC, HR, and EMG and decreases in T during exposure to each stressful stimulus; however, when individual responses were examined across all of the experiments, individuals with PTSD were found to have more consistent responses to arousing stimuli. Further analysis will explore the contribution of individual and stimulus variables to electrophysiological reactivity. The results of this study support the hypothesis that PTSD patients are more autonomically reactive to both combat and nonspecific stimuli than controls.

Funded by the University of Michigan Department of Psychiatry.

95.18

CONTROL OF AGGRESSION AND VIOLENCE WITH CARBAMAZEPINE. C.C. Huang* Medical College of Wisconsin, Milwaukee, WI 53226.

The purpose of the study is to learn whether carbamazepine is effective in controlling impulsive aggression. Eighteen patients were chosen for this study (5 female, 13 male, age 18 to 56). 5 of these patients were diagnosed with schizophrenia, 6 with mental retardation, 4 with OBS, 2 with organic personality disorder and 1 with intermittent explosive disorder. All of these patients were hostile, aggressive and had acting out behavior prior to the treatment. Each incident of acting out behavior which required restraint was used as a measurement of the severity of the patients' aggression. Every patient was allowed to continue his concomittant medications, i.e. antipsychotics, anticholinergics, sedatives or antiepleptics throughout the course of study; then carbamazepine 300 to 800 mg per day was added to each patient's regimen. The number of incidents of acting out behavior in one month prior to carbamazepine treatment was compared with number of incidents in one month after the carbamazepine treatment. The results show a statistically significant difference between 2 experimental groups ($p=0.01$). The means are 5.722 versus 1.667. Carbamazepine might have a direct or indirect effect on the limbic system.

95.19

BLOOD FLOW IN COCAINE DEPENDENCE, Ronald I. Herning*, Warren Better, and Jean L. Cadet, Molecular Neuropsychiatry Section, Neuroscience Branch, NIH/NIDA, IRP, Baltimore, MD 21224

Blood flow of the anterior and medial cerebral arteries was measured by transcranial doppler sonography in cocaine-dependent individuals (n=21) and control subjects (n=21) to determine whether the EEG differences observed in cocaine abusers might reflect specific cerebral blood flow differences. Blood flow was measured within the three days and again after about 25 days after being admitted to an inpatient research ward. Resting EEG was recorded after the blood flow measurement on all subjects. The mean, systolic, and diastolic velocities on both arteries did not differ between the control and cocaine dependent subjects. A correlation was observed been the frontal and central EEG and blood flow measured in the control, but not the cocaine dependent subjects at the first recording. After about a month of abstinence, correlation began to emerge between the EEG and blood flow for the cocaine-dependent subjects. These preliminary findings support the notion that the relationship between EEG and cerebral blood flow is altered in cocaine dependent patients.

NEUROPSYCHIATRIC DISORDERS II

96.1

DOPAMINERGIC CORRELATES IN NON-PSYCHOTIC RELATIVES OF SCHIZOPHRENIC PROBANDS. F. Amin*, L.J. Siever, J.M. Silverman, C.J. Smith, P.J. Knott, K.L. Davis. Houston VA/Baylor College Medicine, Houston, TX, 77030.

First-degree relatives of schizophrenic probands frequently manifest attenuated characteristics of schizophrenia. Analogous to the symptoms of schizophrenia, many of these characteristics may be viewed as social deficit-related (more prominent in this population) or psychotic-like that are hypothesized to be associated with decreased or increased dopamine (DA), respectively; in different brain regions. It raises the possibility that DA abnormalities may be present in the relatives of schizophrenic probands. Plasma homovanillic acid (HVA), the major DA metabolite and a peripheral indicator of presynaptic DA activity, was measured in a group of non-psychotic, physically healthy, first-degree relatives (n=55) of schizophrenic probands. Deficit-related and psychotic-like symptoms were assessed on study mornings using the Positive and Negative Syndrome Scale.

Stepwise multiple regression analyses suggested that plasma HVA predicted statistically significant proportions of variability in both the deficit-related and psychotic-like symptoms, with significant negative partial correlations with deficit-related and positive partial correlations with psychotic-like symptoms. Not only schizotypal relatives but also the relatives who did not meet criteria for any DSM-III-R personality disorder were found to have significantly lower plasma HVA compared to a normal group. None of these results appeared related to the renal HVA excretion or the noradrenergic component of HVA, consistent with the notion that plasma HVA findings were due to brain DA. Our results are compatible with the view that brain DA may negatively and positively modulate the milder continuums of deficit and psychotic symptoms of schizophrenia, respectively, in the relatives of schizophrenic probands. Our results raise the possibility that DA dysfunction of schizophrenia may have genetic antecedents involving the presynaptic DA function. (Supported by grants from Scottish Rite Foundation and VA Merit Review program to Dr. Amin, and by a grant from NIH to Dr. Silverman)

96.2

EFFECTS OF SCHIZOPHRENIA AND TEMPORAL LOBE EPILEPSY ON MEMORY. W.S. Stone*[1], L.J. Seidman[1], R. Jones[1], R.H. Harrison[2], and A. Mirsky. [1]Dept. of Psychiatry, Harvard Medical School, [2]Dept. of Psychology, Boston University, [3]National Institute of Mental Health, Bethesda, MD.

Although impaired recall is well-documented in schizophrenia, the extent to which deficits in memory can be dissociated from other cognitive and demographic variables remains unclear. This issue was addressed by comparing learning and memory performance in patients with schizophrenia to groups of temporal lobe epileptics (TLE) and normal controls. Schizophrenic and TLE patients were significantly impaired in both verbal and visual modalities on subtests of the Wechsler Memory Scale, with schizophrenics generally performing most poorly. Since the schizophrenics consisted of a relatively non-chronic sample with a mean Full Scale IQ of 99.7, their memory disorder cannot be attributed to "schizophrenic dementia." Nor was the deficit related to sex, education, IQ estimate or attention span. "Savings" score measures of memory decay indicated that the loss of information over time (long-term memory function) in schizophrenia was quantitatively mild compared to that found in most neurologic groups with memory disorders. The extreme difficulty that schizophrenic patients had on an incidental recall task suggests that absence of instructional set added to an already existing abnormal rate of forgetting. Relatively unimpaired performance on paired associate learning suggests that, in this sample at least, schizophrenics benefited from retrieval cues and multiple trials comprised of short (nonsupraspan) bits of information. These results provide additional evidence that memory recall is more impaired than recognition in schizophrenic populations. More generally, they show that memory deficits in schizophrenia are not epiphenomena occurring secondary to deficits in overall cognitive function, but are more likely persistent sequelae of the disorder itself. Supported by research grants from NIMH (MH-37156, MH-26183, and MH-14915) and the Commonwealth Research Center of the Massachusetts Mental Health Center.

96.3

TEMPORAL DISINTEGRATION OF VISUAL PERCEPTION IN SCHIZOPHRENIA. R.Izawa, S.Yamamoto, A.Iwawaki, K.Ohta, E.Matsushima, H.Shibuya* and M.Toru. Dept. of Neuropsychiatry, Tokyo Medical and Dental Univ. Sch. of Med., Tokyo, Japan.

A perceptual disturbance in schizophrenia has been described as "fragmentation". However, the way in which the perception is fragmented and the relation between the perceptual fragmentation and the other symptoms remain obscure.

We investigated 24 schizophrenics and 14 normal controls by a newly devised psychological test and assessed the schizophrenic symptoms on 3 syndrome scores (Liddle,P.F.). The test consisted of 12 different geometrical figures. On a CRT, a part of the figure was presented, just like peeping through a hole in a black screen. The subjects were asked to move "the hole" freely by a mouse device in order to get the whole image by integrating temporally parts of the figure. Afterwards they were required to draw each figure from memory (recall) and to select a correct figure from 6 figures (recognition). The error scores in recall and recognition of schizophrenics were both significantly higher than those of normal controls. In schizophrenia, there were positive correlations between these scores and Disorganization syndrome score, but not Reality Distortion nor Psychomotor Poverty syndrome score.

These results suggest that schizophrenics may have impairment of temporal integration in visual perception and this perceptual disturbance and positive thought disorders may have a common basis, which can be interpreted as temporal disintegration.

96.4

NORMAL P300 AMPLITUDES IN LATE-ONSET SCHIZOPHRENIA AND RELATED PSYCHOSES. John Olichney,MD*[1], Vicente Iragui-Madoz,MD,PhD[1], Marta Kutas,PhD[2], Ralph Nowacki,AS[1], Dilip Jeste,MD[3] Departments of Neurosciences[1], Cognitive Science[2], and Psychiatry[3]; University of California, San Diego and San Diego Veteran's Affairs Medical Center; La Jolla, CA 92093.

The P300, an event-related potential elicited by task-relevant stimuli, is sensitive to the depth and speed of cognitive processing. The P300 amplitude has been found to be reduced in many studies of younger adult schizophrenia patients. This reduction has been related to severity of psychiatric symptoms and to temporal lobe abnormalities. Very little is known, however, regarding the P300 in late-life schizophrenia. We have studied 26 middle-aged and elderly (mean age = 61 yrs.) patients with schizophrenia (n=22) and related psychoses (n=4), including 13 patients with "late-onset"-- i.e. onset of psychotic symptoms after 45 yrs., and 13 normal comparison subjects on an Auditory Oddball P300 paradigm. Preliminary analyses showed reduced P300 amplitudes in the early-onset psychosis patients at posterior electrode sites, compared with late-onset psychosis patients of comparable age, education and sex. There was no significant intergroup difference in P300 peak latencies. Our results suggest that reduced P300 amplitudes in schizophrenia are related to age of onset.

Supported by NIMH grant MH49671 and NIH grant AG00658.

96.5

AUDITORY P300 AND IMPULSIVE AGGRESSION. J. E. Gerstle and M. S. Stanford*. Department of Psychology, University of New Orleans, New Orleans, LA 70148.

Impulsive aggression involves a hair trigger response to provocation with loss of behavioral control. This loss of control is not secondary to any medical or psychiatric disorder and, by virtue of the spontaneity of the act, is not planned. Subjects were 12 college students classified as impulsive aggressive by self-report and 12 nonaggressive matched controls. All impulsive aggressive subjects reported a lifetime history of physical aggressive outbursts. P300 was recorded during an auditory oddball task. Data were analyzed from 19 scalp electrodes using t-tests and topographical analyses. It was hypothesized that impulsive aggressive subjects would demonstrate significantly lower P300 amplitude. Between groups analysis indicated that impulsive aggressive subjects had significantly lower average frontal P300 amplitude when compared to matched controls (t[22] = 1.85, $p>0.05$). These findings are consistent with psychophysiological findings in impulsive aggressive incarcerated criminals and support the notion of a specific behavioral syndrome associated with spontaneous aggressive outbursts.

96.6

RELATION BETWEEN VISUAL SCANNING OF FACES AND SCHIZOPHRENIC SYMPTOMS. **R.B. Rosse, B.L. Schwartz*, S. Johri, and S.I. Deutsch. Psychiatry, Dept. of VA Medical Center and Georgetown University Medical School, Washington D.C. 20422.**

Eye movement disorders are consistently found in patients with schizophrenia and may represent a biologic marker of the disease. However, abnormal eye movements such as those in tests of smooth pursuit often are not associated with the patients' severity of illness. Here we present findings from two studies showing a relation between the severity of schizophrenic symptomatology and eye movement measures of visual scanning while patients viewed faces. In these studies, patients performed two tests on which they show facial processing deficits: gaze discrimination and recognition of facial expression. We observed that first, schizophrenic patients were impaired on these tests, and secondly, they produced fewer short-duration (<50 ms) fixations and saccades while viewing faces compared with controls. Importantly, the number of short-duration (<50 ms) fixations and saccades correlated with the patients' scores on a standard instrument used to assess the severity of psychopathology in schizophrenia, i.e., the Brief Psychiatric Rating Scale (BPRS). Patients who made fewer short-duration fixations and saccades had more severe symptoms of schizophrenia on the BPRS. Visual fixations and perhaps saccades of less than 50 ms that are produced while viewing a face are thought to reflect parallel, preattentive processes involved in perceiving configural or gestalt-like properties. These results suggest that patients with more severe psychotic symptoms engage in less preattentive processing, which may contribute to their facial processing deficits.

96.7

REACTION TIME AND PUPIL RESPONSE MEASURES SHOW REDUCED LATENT INHIBITION IN CHRONIC SCHIZOPHRENIA . L.A. Dunn* and R.J. Scibilia. Department of Psychiatry, Duke University Medical Center, Durham, North Carolina 27710.

Latent inhibition (LI) is an indirect behavioral measure of selective attention that is reduced in humans with schizophrenia (Baruch et al. J Nerv Ment Dis 176:598-606, 1988). A continuous performance task that measures LI has been developed that uses within subject controls and allows repeat testing. Letter stimuli are presented on a video monitor with a SI of 1 sec. and an ISI of 0.25 sec. Pupil size is monitored by infrared video. Subjects respond by releasing a button for each target X. The task is divided into four contiguous phases. During phases 1 and 3 20% of the stimuli are targets, 60% are letters, and 20% are a non-letter preexposure stimulus. During phase 2 a novel symbol always cues the target resulting in decreased reaction times. Phase 2 ends when 3 reaction times are lower than those in phase 1 or the subject has responded 33 times. During phase 4 the preexposed stimulus cues the target. Reaction times decrease as in phase 2. Trials to criterion are calculated for novel and preexposed phases. A subject demonstrates LI when the trials to criterion are higher in phase 4 than in phase 2. Pupil diameter is measured 60 times per second throughout the task. Pupil diameter changes are assessed by peak height and latency following target presentation. Reaction times were studied in 14 subjects with schizophrenia and 28 normal controls. LI was present in normal controls ($p<.001$) but not in subjects with schizophrenia. Pupil diameter changes were studied in 9 subjects with schizophrenia and 25 normal controls. LI was evident pupil diameter response to cued targets. Pupil diameter increase was smaller and earlier for preexposed cues in normal controls ($p< 0.001$ for each). No significant change was observed in subjects with schizophrenia. (Supported by NIMH #47503)

96.8

SACCADIC INHIBITION AMONG TOURETTE SYNDROME PATIENTS, R.H. Farber, B.A. Clementz, N.R. Swerdlow and V.D. Lehmann-Masten*. Departments of Psychology and Psychiatry, University of California, San Diego, La Jolla, CA 92093-0109.

Pathologies affecting cortico-subcortical circuitry result in characteristic saccadic eye movement response patterns. Tourette syndrome (TS) is theoretically associated with prefrontal cortex-basal ganglia pathology. Studies of saccadic performance among TS patients, therefore, may complement our understanding of this disorder's putative neuropathology. Sixteen TS patients and 8 nonpsychiatric subjects were administered: (1) a visually-guided "gap" saccade task in which a 200 msec delay period separated the offset of a central fixation point and the illumination of a ±4° peripheral target; and (2) an antisaccade task in which subjects were instructed to make saccades to the equal and opposite locations of pseudorandomly presented cues at ±8 and 16°. During the gap task, TS patients generated a significantly increased proportion of anticipatory saccades (RT<90 msec) and a significantly decreased proportion of regular reaction time saccades (RT>150 msec) than nonpsychiatric subjects (anticipatory: TS M=0.21, SD=0.12; nonpsychiatric M=0.06, SD=0.04; regular: TS M=0.46, SD=0.17; nonpsychiatric M=0.65, SD=0.16). The groups did not statistically differ on proportion of "express" saccades (90 msec<RT<140 msec). TS patients had significantly shorter amplitude express and regular saccades than nonpsychiatric subjects. During the antisaccade task, TS patients had a significantly decreased proportion of correct antisaccade trials than nonpsychiatric subjects (TS M=0.74, SD=0.17; nonpsychiatric M=0.91, SD=0.10). The groups did not statistically differ on either correct antisaccade or incorrect error saccade amplitudes. TS patients, however, had significantly increased saccadic reaction times on correct antisaccade trials (TS M=364.59 msec, SD=66.43; nonpsychiatric M=306.36 msec, SD=22.39). Collectively, the results are consistent with deficits of saccadic inhibition among TS patients presumably involving prefrontal cortex and associated subcortical circuitry. This work was supported by grants from the USPHS and the Tourette Syndrome Association.

96.9

FUNCTIONAL NEUROANATOMY OF SMOOTH PURSUIT EYE MOVEMENTS. D.E. Ross*, H.H. Holcomb, G.K Thaker, M. Zhou, D.R. Medoff, C.A. Tamminga. Maryland Psychiatric Research Center, P.O. Box 21247, Baltimore, MD 21228.

Background: Although a great deal is known about the functional neuroanatomy of smooth pursuit eye movements in humans, most of this information is based on indirect methods of observation, such as studies of non-human primates and humans with brain lesions. Objective: To determine the functional neuroanatomy of smooth pursuit eye movements in normal human subjects using positron emission tomography (PET). Methods: A single normal human subject has been tested so far using [O15] PET. There were three tasks: (1) at rest, with eyes closed; (2) fixation of a stationary target; and (3) smooth pursuit of a sinusoidally moving target. Eye movements were measured with infrared oculography. The data were analyzed using Statistical Parametric Mapping (SPM95). The condition (at rest, fixation or smooth pursuit) and covariate (mean global blood flow) effects were estimated according to the general linear model at each and every voxel (Friston et al., 1995). Regionally specific condition effects were determined using linear contrasts. Results: Large Z-scores (all Z's > 3.1) indicated robust activation of regions hypothesized to be active during smooth pursuit, including the following regions: (1) primary visual area, (2) middle temporal/ medial superior temporal area, (3) parietal eye field, and (4) frontal eye field. The locations and Z scores of the oculomotor regions corresponded well with previous reports. The number of subjects will be increased to N=6, and these data will be presented.

SUPPORT: NIH: CRC MH40279, R01 MH43031 and MH49826.

96.10

AVOIDANCE LEARNING DEFICITS IN SCHIZOPHRENIA. C.D. Fisher[1], M.H. Kosmidis[2], and B.D. Fantie[1]*. [1]Human Neuropsychology Lab, The American University, Washington, DC 20016, [2]Lab of Psychology and Psychopathology, NIMH, Bethesda, MD 20892.

Past investigations of automatic cognitive processing in patients with frontal deficits (i.e., CHI survivors) have suggested that using an aversively loud auditory stimulus can help them compensate for these deficits. Such a cue can enhance associative learning by drawing attention to the stimulus relevant to solving the problem. We administered two conditioned avoidance tasks, in which we altered the salience of the auditory UCS, to a group of schizophrenic patients and normal controls.

Preliminary data suggest that both groups had difficulty making the required association to avoid the nonaversive stimulus. On the aversive task, the normal controls benefited from the UCS and needed fewer trials to reach criterion ($U(6,23)=21$, $p<.01$), and both avoided ($U(6,23)=29$, $p<.05$) and escaped ($U(6,23)=30$, $p<.05$) faster than the schizophrenic group; the schizophrenic patients did not benefit from this salient stimulus. Within subject analyses revealed that the control group reached criterion on the aversive stimulus task faster than on the nonaversive stimulus task ($Z=-2.2$, $p<.05$), whereas the performance of the patients did not differ between the two conditions.

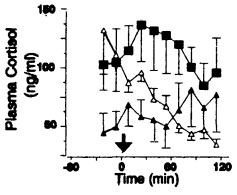
Schizophrenic patients did not differ from the control subjects in their performance on the more ambiguous, nonaversive task, but they did not benefit from the more salient, aversive stimulus as did the control group. This pattern of performance suggests that the schizophrenic group was not disorganized, but either that they had deficits in processing negative consequences or were less sensitive to the aversiveness of the stimulus.

Source of support: NIMH IRP

96.11

CORTISOL RESPONSE TO STRESS IN SCHIZOPHRENICS WITH WATER IMBALANCE M.B. Goldman*. Psychiatric Institute, University of Illinois in Affiliation with the University of Chicago, Chicago, IL, 60612

Hyponatremic schizophrenic patients are hypercortisolemic and have smaller hippocampi compared to matched schizophrenic controls or normals. This brain region has been linked to schizophrenia, water imbalance, and the restraint of neuroendocrine responses to psychological stress. In particular, glucocorticoid response to stress may be heightened and prolonged following hippocampal damage. We performed the cold pressor test in hyponatremic schizophrenics (HS) (n = 4: ■), normonatremic nonpolydipsic schizophrenics (NS) (n = 5: ▲), and normal controls (C) (n = 5: △) to assess if the HS demonstrate this pattern of glucocorticoid response to stress.



Psychiatric subjects were first stabilized on haloperidol for 2 wk on an inpatient psychiatric research unit. At 1430 h, an IV catheter was inserted and a blood pressure cuff attached. 90 min later, subjects immersed their dominant hand in ice water for 60 s (⬇), and measures were obtained at regular intervals for the next 2 h.

Basal cortisol (CORT) tended to be lower in NS than in the other groups ($P<0.06$) (data = mean ± SEM). The CORT response differed in HS vs. NS and C (fig.)($p<0.02$) as well as in NS vs. C ($p < .005$). Other responses (i.e. subjective pain, blood pressure, pulse, temperature, plasma glucose) were similar across groups.

These preliminary results are consistent with the concept that neuroendocrine response to psychological stress is enhanced in HS. **Sponsored by The Brain Research Foundation of the University of Chicago.**

96.12

EFFECT OF ANTIPSYCHOTIC DRUGS ON FORCE CONTROL IN SCHIZOPHRENICS. **P.E. Konicki*, D.W. Brescan, M.A. Fuller, G.E. Jaskiw, G. Jurjus, K.Y. Kwon, A. Popli and P.B. Vrtunski. Cleveland VAMC and Department of Psychiatry, CWRU School of Medicine, Cleveland, OH 44141.**

Although the motor effects of different antipsychotic drugs are well known, there are few objective techniques for measuring them. With the emergence of 'atypical' antipsychotic drugs, there are new opportunities to investigate the mechanisms of motor side effects. To this end, we studied 112 chronic schizophrenics and 20 normal control subjects using a force control (FC) task. All patients were on stable antipsychotic treatment with either clozapine (N=66), haloperidol (N=18), fluphenazine (N=24), or risperidone (N=7). The task consisted of subjects' index-finger pressure on a force-sensitive button and matching target and response lights. There were 42 trials of varying force. The steadiness error of force maintenance was the dependent variable. The largest steadiness error was in the clozapine group (2.769±0.478), followed by the haloperidol (2.666 ±0.624), fluphenazine (2.311 ±0.472), risperidone (2.154 ±0.325) and control groups (1.775 ±0.252), respectively. The overall difference was highly significant ($F(4(127) = 19.79$, $p<0.0001$). The significance was localized (Tukey test) to six of the 10 contrasts (the four which were not were between the neighboring pairs: clozapine-haloperidol, haloperidol-fluphenazine, etc.) We conclude that each antipsychotic drug may have its characteristic effects on the force control function. Two contrasts are particularly interesting: a) virtual absence of difference between clozapine and haloperidol, even though clinically the two are quite distinct, and b) wide separation between haloperidol and risperidone despite their clinical similarity.

(Supported by the Veterans Administration and USPHS grant MH-46630)

96.13

NMDA GLUTAMATE RECEPTOR HYPOFUNCTION: A MODEL FOR SCHIZOPHRENIA-LIKE COGNITIVE AND BEHAVIORAL SYMPTOMS J.W. Newcomer*, N.B. Farber, V. Jevtovic-Todorovic, G. Selke, A. Kelly, F. Williams, S. Craft, J.W. Olney. Washington University School of Medicine, St. Louis, MO 63110.

N-methyl-d-aspartate (NMDA) glutamate receptor hypofunction (NRH) is central to recent hypotheses concerning the neurobiology of schizophrenia. This experiment aimed to transiently induce mild schizophrenia-like behavioral and cognitive symptoms in young healthy adults using double-blind, "placebo"-controlled, randomized, within-subjects comparisons of 3 fixed subanesthetic, steady-state doses of intravenous ketamine, with detailed behavioral and cognitive assessments during each infusion condition.

Results (n=10) indicate dose-dependent increases in schizophrenia-like symptoms, including Brief Psychiatric Rating Scale (BPRS) positive ($F[3,27]=14.83$,$p<0.0001$) and negative symptom subscales ($F[3,27]=3.90$,$p=0.02$), Scale for Assessment of Negative Symptoms (SANS) total ($F[3,27]=4.49$,$p=0.01$) and affective flattening subscale ($F[3,27]=3.01$,$p=0.05$), and trend-level increased BPRS conceptual disorganization ($F[3,27]=2.65$,$p=0.07$). Ketamine also produced a dose-dependent decrease in immediate and delayed verbal recall ($F[1,9]=7.34$,$p=0.02$; placebo vs. high dose), consistent with a ketamine-induced decrease in retrieval and/or encoding. Ketamine-induced NRH produces mild schizophrenia-like psychiatric symptoms as well as impairment in verbal memory and learning, consistent with well-described impairments in memory and learning in schizophrenia. Ketamine-induced NRH offers a useful clinical model for studying schizophrenia-like cognitive and behavioral symptoms. Supported by a Scientist Development Award (SDA) from NIMH (MH01045; JWN), an SDA from NIDA (DA00290; NBF), and an Established Investigator Award from NARSAD (JWO).

96.14

APOE IN SCHIZOPHRENIA. D. Pickar, A.K. Malhotra, W. Rooney, J.N. Crawley*, A. Breier, D. Goldman. Experimental Therapeutics Branch, NIMH, Bethesda, MD 20892

APOE4, a naturally occurring allele of the gene encoding apolipoprotein E (ApoE), increases the risk of developing late-onset Alzheimer's disease. We have examined APOE allelic frequency in schizophrenia using the Haplotype Relative Risk technique (n= 56 families) and studied the association between APOE alleles with clinical aspects of schizophrenic inpatients (n=74). We find no increase in transmission of APOE4 in schizophrenia. We did observe, however, that patients with an APOE4 allele showed later age of onset ($p<.01$) and less positive ($p<.01$) symptoms when drug free and less treatment response to typical ($p<.05$) but not to atypical antipsychotics. These data suggest that APOE4 genotype may influence phenotypic expression in schizophrenia. Further investigation is underway in an independent group of schizophrenic outpatients.

NIMH/NIH (DIRP)

96.15

ASSOCIATION BETWEEN THE BDNF GENE AND SCHIZOPHRENIA. A.M. Vicente, F.M. Macciardi, M. Verga, H.B.Niznik, N. King, G. Bean, and J.L. Kennedy. Section of Neurogenetics, Clarke Institute of Psychiatry, University of Toronto, 250 College Street, Toronto, ON M5T 1R8 Canada.

Several lines of evidence suggest that a neurodevelopmental defect may play an important role in the etiology of schizophrenia. Alterations in the cerebral cortex and limbic system of schizophrenia patients have been reported, including changes in sizes of temporal lobe structures, altered sizes and numbers of specific types of neural cells, and disarray of neurons in the hippocampal region. These anomalies could be attributed to defective function of a number of interacting neurodevelopment molecules involved in neuronal differentiation, migration, and synaptic connectivity in the brain. The Brain Derived Neurotrophic Factor (BDNF) is a member of the neurotrophin family, and promotes the survival and differentiation of neurons during embryonic development. In the adult brain, BDNF is highly expressed in limbic regions, where it is involved in synaptic plasticity mechanisms, and has been shown to interact with dopaminergic and serotonergic pathways, repeatedly implicated in schizophrenia. We report the finding of association between a marker at the BDNF locus and schizophrenia ($\chi 2 = 10.401$, $p = 0.0155$, $df = 3$), using the Haplotype Relative Risk (HRR) method of analysis in a sample of 77 probands and their parents. In addition, we performed a Transmission Disequilibrium Test (TDT) and obtained a statistically significant result for two risk alleles in schizophrenia ($p = 0.0032$ and $p = 0.0110$). The results obtained represent moderate association, implying that BDNF is possibly a susceptibility locus, not necessary or sufficient for disease expression but significantly increasing the risk for schizophrenia.(Supported by MRC Canada & NARSAD)

96.16

ALLELIC VARIATION OF THE HUMAN HISTAMINE H_2 RECEPTOR GENE IS A MAJOR PREDISPOSING FACTOR FOR SCHIZOPHRENIA. P.R. Orange[1]*, P.R. Heath[1], S.R. Wright[1], C.N. Ramchand[1], L. Kolleiwicz[2], R.C.A. Pearson[1]. [1]Dept. Biomedical Science, University of Sheffield, Sheffield, S10 2TN, UK. [2]Claybury Hospital, Woodford Bridge, Woodford Green, Essex, IG8 8BY.

Evidence is rapidly emerging which suggests an involvement of brain histamine in the pathophysiology of schizophrenia. A potent histamine H_2 antagonist (Famotidine) has been shown to ameliorate symptoms of the disease. We have recently described a novel allelic variant of the human histamine H_2 receptor gene (NeuroReport 1996 7(7)). This study investigated the relationships between H_2 genotype and schizophrenia.

DNA was collected from schizophrenia sufferers (n=47) (DSMIII-R/ICD-9) and non-psychiatric controls (n=46). From these DNA samples a 266 base pair PCR fragment of the H_2 gene was produced. The PCR fragment was then analyzed by detecting single-stranded conformational polymorphism (SSCP), as well as investigating differences in *TaqI* restriction endonuclease digestion patterns.

An increase in the homozygous H_2R_{649Q} genotype in the schizophrenic population of $\times 2.57$ ($p<0.0001$) was shown. This data puts the attributable fraction of H_2R_{649Q} homozygosity for schizophrenia at 23.1%

These data show that another monoaminergic neurotransmitter is implicated in the pathophysiology of schizophrenia. Whilst the sample size is small, the high degree of significance suggests that the brain histaminergic system and in particular the H_2 receptor, are fundamental to the disease process.

Supported by The Schizophrenia Research Fund UK

96.17

LACK OF TRIPLET REPEAT EXPANSION IN β1 SUBUNIT OF NA^+,K^+-ATPase IN TEMPORAL CORTEX OF BIPOLAR PATIENTS. R.S. El-Mallakh, J. Kleinman and R. Li.* Department of Psychiatry, University of Louisville School of Medicine, Louisville, KY 40292. and Clinical Brain Disorders Branch, NIMH, Washington DC 20032.

The human genome posses many nucleotide repeat sequences. Recently, trinucleotide repeat expansion has been found to be strongly associate with several neuropsychiatric diseases. These diseases share common features such as anticipation (worsening disease expression across successive generations) variable penetrance, and isolated neuromuscular involvement with progressive symptomatic course. Bipolar illness possesses many of these characteristics. Early studies suggested that bipolar individuals may have an abnormality of sodium pump regulation. Since the β1 subunit of the sodium pump possesses a polymorphic triplet repeat sequence (CGG) in the untranslated 5' promoter region, we examined its sequence in post mortem brain tissue from bipolar and normal individuals. Total genomic DNA was isolated from temporal cerebral cortex in 5 bipolar and 5 normal individuals and further amplified by nested PCR. After total of 30 cycles, the PCR reaction mixture was analyzed by 3% agarose gel electrophoresis. PCR products were purified and used as templates for direct sequencing. Our results revealed no expansion of CGG repeats in β1 subunit isoform of sodium pump from bipolar patients (the number of CGG repeats were 7-8 in temporal cerebral cortex from both normal and bipolar individuals). We conclude that the abnormality of sodium pump in bipolar patients does not appear to be related to a triplet repeat expansion.

96.18

A FAMILY-BASED ASSOCIATION STUDY OF BIPOLAR AFFECTIVE DISORDER. M. Del Zompo*, M.P. Piccardi, G. Severino, R. Ardau, M. A. Palmas, A. Bocchetta. Department of Neurosciences "B.B. Brodie", University of Cagliari, I-09124 Cagliari, Italy.

Genetic factors play an important role in the pathogenesis of bipolar affective disorder, but traditional linkage studies have so far failed to identify major single genes. An alternative to linkage studies in common-complex diseases are association studies using markers of candidate chromosomal regions. In order to overcome the problem of population stratifications, family-based association methods have been proposed, using non-transmitted parental chromosomes as controls. We studied several different polymorphic markers in chromosomes from 44 triads, consisting of probands with bipolar disorder and their parents. No association was found between bipolar disorder and the dopamine D_3 receptor gene, which is located on chromosome 3q13.3. An association was found with the locus of glucose-6-phosphate dehydrogenase, but not with two additional markers of the same chromosomal region (Xq28). Other candidate genes are currently being investigated, including the dopamine D_4 receptor gene, located on chromosome 11p15.

96.19

DECREASED ACOUSTIC STARTLE PREPULSE INHIBITION IN TRANSGENIC MICE WITH AN IMPAIRED GLUCOCORTICOID RECEPTOR (GR) FUNCTION: IMPLICATIONS FOR AFFECTIVE AND ANXIETY DISORDERS. S.Beaulieu*, J.R. Glowa, N. Barden and R.M. Post. Biological Psychiatry Branch, NIMH, Bethesda, MD 20892-1272.

A transgenic (TG) mouse model of impaired GR function and secondary ACTH and corticosterone hypersecretion has previously been developed (Pepin et al., Nature, 355, 1992; 725-728). We have demonstrated that TG mice have a significantly higher startle response than control (CT) animals before treatment ($p< 0.0001$). It had been previously established that a 2 week treatment with desipramine was sufficient to normalize the level of GR mRNA and GR. This treatment (10-20 mg/kg/day s/c) reduced the startle response of TG mice to levels indistinguishable from those of saline treated CT. In an attempt to further characterize the startle response of these TG mice and the possible mechanisms for the increased startle response, we studied the effectiveness of prepulse inhibition (PPI). The CT and TG animals were placed in the startle chambers for a 5 min acclimatization period with a 70 dB background noise. A test session consisted of 24 trials: 13 startle trials of a 110 dB acoustic startle stimulus (100 msec), and 11 PPI trials of 85 dB (30 msec) that preceded the startle trial by 100 msec. All trials were separated by periods of background noise that varied in duration and averaged 15 sec. The TG mice showed a consistently decreased percentage of prepulse inhibition of the startle response (40%) compared to control animals (64%) ($p<0.002$). This suggests that an impaired GR function leads to a decreased effectiveness of the PPI circuit to negatively modulate the startle circuit. This may play a role in the increased response to anxiogenic stimuli observed in these animals and possibly, in patients with anxiety and affective disorders. (Supported by the Medical Research Council of Canada and The Stanley Foundation)

97.1

HISTORICAL PERSPECTIVES ON PHARMACOLOGICAL TREATMENT OF DEPRESSIVE DISORDERS. L.H. Schneider* and R.B. Murphy. Innova Biomed, Inc., Irvington NY 10533 and New York University Department of Chemistry and Center for Neural Sciences, New York, NY 10003

The pharmacological armamentarium of antidepressant drugs has expanded vastly since the original postulation of a role for biogenic monoamines in affective disorders. The present-day proliferation of new classes of rationally designed selective agents such as SSRIs, SNRIs, and the like somewhat obscures the fact that a variety of therapeutics have been in use in Western medicine for the treatment of depressive illness for several centuries. At the time they were prescribed these therapeutics were widely believed to be useful and effective, based principally upon empirical observation. In this poster we trace the historical evolution of antidepressant agents which have been commonly employed in practice. We examine the place of these therapeutics as an important component of the overall treatment strategies which were historically employed. The period from the early Nineteenth century up to the present day is examined. This analysis affords a social as well as a scientific perspective on the evolving roles of therapeutics in general through this period.

Supported in part by Innova Biomed, Inc.

97.2

DELIRIUM: A HISTORICAL REVIEW OF REPUTED CAUSES. K.A. Hesse, G.W. Hesse* Geriatric Medicine, Deaconess Hospital, Boston, MA 02215

Delirium is a common phenomenon in acutely ill patients. Studies suggest up to 30% of medical patients over age 65 may be delirious during an acute hospitalization. This poster reviews historical efforts to understand the causes of this familiar clinical experience often associated with dire prognosis. The early Greeks and Romans were aware of the syndrome complicating fever. Hippocrates described many cases associated with infection in his Book of Epidemics. Asclepiades suggested it was caused by a stoppage or obstruction of the corpuscles of the membranes of the brain while Soranus believed the stoppage occurred in the "sensory passages". Some thought it arose from disorders of the bowel or the diaphragm. Galen proposed a disease of yellow bile associated with dryness and heat and this view persisted through the middle ages. Toxins (henbane) and intoxications (wine) were recognized as causes by Jewish, Byzantine, and Arabic writers. By the 16th century, over heating or over cooling of the brain were thought to be etiologies competing with a proposed moral disorder of the soul. 17th century writers suggested heat or fever increased blood velocity resulting in abnormal secretion of material from the brain while 18th century theory proposed toxic particles produced from the body out of food caused excessive heat and the resultant changes in blood velocity. Delirium tremens was described by the 19th century and attempts were made to localize the various symptoms of delirium to anatomical areas of the brain. The modern era has classified the causes of delirium as intoxications, withdrawals, infections and metabolic disturbances. However, the underlying mechanism of brain dysfunction remains poorly understood and neuroscientists are still struggling to elucidate the final common pathway producing delirium.

97.3

MIND AND THE NEUROSCIENCES. J.G. Foy*. Dept. of Psychology, Loyola Marymount University, Los Angeles, CA 90045.

One of the most important questions facing neuroscience today is the nature of the relation between neural and mental events. Neuroscience research in the 1990s, the Decade of the Brain, has been a period of unparalleled data-gathering, a time where basic questions of the science have predominated. Many would argue, however, that despite this rapid expansion of our knowledge about the brain, we are no closer to answering the fundamental question about the relation between mind and the brain than Aristotle or Descartes. A paradigmatic reluctance in the neurosciences to address these broader questions has been tempered somewhat by Francis Crick and Christof Koch's encouragement of questions pertaining to consciousness, but a lack of communication between disciplines concerned with the mind, (e.g. philosophy, psychology and the neurosciences) about things of the mind, is still prevalent. Applied subfields of these disciplines (e.g. cognitive science and neuropsychology) have grown remarkably in number and in size in recent years, suggesting that there is considerable interest in addressing these questions. I propose that an historical look at the development of the disciplines of psychology, philosophy, and neuroscience in the last 100 years may shed some light on current debates about the relation between mind and brain, and whether, as we approach the 21st Century, we are asking the appropriate questions.

Supported by an LMU Faculty Research Grant

97.4

STEREOTACTIC CEREBRAL LOCALIZATION: ORIGIN, EVOLUTION AND FUTURE. R.L. Jensen* and J.L. Stone. Department of Neurological Surgery, Loyola University Medical Center, Maywood, IL 60153.

The precise localization of intracranial targets based on extracranial and more recently on intracranial landmarks has been an important technique for neuroscience experimentation as well as for diagnosis and treatment of a number of neurological diseases. We describe the origin of this work with the cadaver studies of Paul Broca, early animal studies with guided electrodes, and Zernov's "encephalometer" which was used on human patients. The first stereotactic instrument was the Horsley-Clarke frame and extensive animal studies were carried out using this device. This instrument was used on humans as well and influenced the work of Spiegel and Wycis who designed and used the first stereotactic frame made exclusively for humans. A number of stereotactic instruments and atlases evolved from this early human work. With the emergence of better radiographic, CT and MRI imaging techniques, frame-based and frameless stereotactic techniques are currently available. We describe the principles behind these methods, the origin and evolution of their use and future of stereotactic cerebral localization.

97.5

THE EVOLUTION OF THE BRAIN/MIND IN HORROR AND SCIENCE FICTION. S. R. Ginn. Department of Psychology, East Carolina University, Greenville, NC 27858-4353.

With apologies to Gene Roddenberry, I and most neuroscientists would agree that the brain/mind is the "final frontier." We spend our lives studying this complex landscape in order to more clearly elucidate the mechanisms by which this most complex of entities works. Nonetheless, we must also be cognizant of the fact that the majority of the public views scientists with suspicion, if not hostility. Such a view may be perpetuated by popular fictional accounts of the "mad scientist" in his laboratory (and in this case I am referring to men only because the majority of "mad scientists" are men). These scientists violate every ethical principle known to man and woman and the professional societies in an effort to test their (rather outlandish) hypotheses. Such efforts include altering the structure of the DNA, specific genes, proteins or hormones to change man into various subhuman creatures (alligators, flies, wolves, pre-humans, etc.) and have been recorded by authors from H. G. Wells to Dean Koontz. In addition, various organs, including human and nonhuman brains, have been transplanted from human to human, human to nonhuman and nonhuman to nonhuman (including "aliens"). This presentation is intended to provide a humorous look at the work of these "mad scientists" as they search for the answers to the meaning of the brain and/or mind. However, the fact that the general public frequently believes that such research is not only possible, but actually happening somewhere (presumably in a Department of Defense facility), is frightening. Suggestions for combatting the negative perceptions of scientists will be encouraged.

97.6

THE HISTORY OF ALZHEIMER'S DISEASE FROM THE PERSPECTIVE OF PATHOLOGICAL VS. NORMAL PROCESSES OF AGING. J. A. Martin* and C. W. Cotman. Dept. of Psychobiology, Univ. of Calif., Irvine, CA 92697-4540.

The incidence of dementia has reached epidemic proportions in the industrialized world because better health care has successfully prolonged people's lives. Though many factors can contribute to dementia, half of the cases are ascribed to Alzheimer's disease.

Many ancient civilizations, such as the Chinese with their Taoist philosophy, shared the belief that health and longevity required harmony, or balance of certain principles, such as Yin and Yang, whereas disease and death resulted from an imbalance. However, the Indians categorized aging as a specific form of illness called a "natural disease," and the Greeks viewed aging and death as normal processes of life, resulting from the inevitable loss of heat from the heart. Although there is only limited mention of dementia in medically related literature, there is evidence that these early societies were aware of its existence through literary allusions.

The neurophysiology associated with dementia was not described until the late 19th century. In 1892 Blocq and Marinesco described plaques in the brain of an elderly epileptic patient. In 1906 Alzheimer described neurofibrillary tangles in a demented woman's brain, suggesting that it was a distinct type of disease. His student Perusini made detailed descriptions of the neuropathology in his patients, but Alzheimer later described strong reservations about these cases being a distinct disease. Fischer clarified the significance of these findings through his own detailed descriptions and with the first clinico-pathological correlational studies. Despite Alzheimer's reservations, Kraepelin identified this newly distinguished form of dementia as Alzheimer's disease in his organization of psychiatric disorders.

Currently, Alzheimer's disease is well recognized and may affect nearly half of the population over age 85. Some studies show that 84% of normal aged brains have plaques and 97% have tangles. Other studies indicate that aged brains can have severe neuropathology without the expected level of dysfunction. Thus, the history continues as to whether Alzheimer's disease is a pathological process or an exaggerated normal consequence of aging.

97.7

A HISTORY OF ALZHEIMER'S DISEASE (AD): 1880 INDEX MEDICUS TO INTERNET. Henrik K. Kulmala* Grayslake, IL 60030

As research into AD has increased since the 1970's, this study sought to explore the nature of AD research and reporting starting in 1880 and the current state of the internet [world wide web (WWW)] as a source of information on AD. Two data sources were examined: Index Medicus starting in 1880 and the WWW using WebCrawler® in April 1996.

Dr. Alzheimer, a German physician, first described a unique disease to a convention of Southwestern German psychiatrists in 1906 and published the first account of this disease in 1907. The first reference to AD appeared in a book published in 1910 by Dr. Kraepelin, Alzheimer's superior. This account was so much more colorful and detailed than Alzheimer's paper, that it was usually cited. Kraepelin made a distinction between AD (early onset) and senile dementia in the book. Dr. Alzheimer conducted an active, self-funded research program during his career, dying after an extended illness in 1915.

From 1911-14, AD was indexed under Insanity, presenile and there were 20 papers. There was no reference to AD from 1915-17 and only 12 papers under this heading. One reference to Alzheimer appeared in 1922: an account by Kraepelin of the deaths of Alzheimer, Brodmann, and Nissl. The early 1940's saw a decrease in the number of papers on AD and the size of the Index Medicus, with a large increase after WWII. In 1970, there were 54 papers on dementia, presenile and 59 on psychoses, senile, both references to AD. In 1980 there were 97 and 77, respectively. AD became a separate heading in 1984, with >200 references and more every year since.

A search of the WWW revealed 496 documents, although many of these were resumes and alphabetical WWW subject listings. Several offered information on AD, including grants information and data on the disease.

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97.8

1896-1996: THE CENTENNIAL OF THE "AXON"

Marina Bentivoglio* Institute of Anatomy & Histology, University of Verona, Italy.

The term axon was introduced in 1896 by Rudolph Albert von Koelliker (1817-1905) to designate the *"axis cylinder prolongation"*. The modern nomenclature of the "neuron", term coined by Wilhelm Waldeyer (1836-1921) in 1891, already equipped with "dendrites", term coined by Wilhelm His (1831-1904) in 1889, was thus established one century ago. However, the earliest studies on the axon, christened in the middle of the debates on the neuron theory, date back to the beginning of the 18th century. The microscopist Antoni von Leeuwenhoek (1632-1723) provided in 1717 the first observation of nerve fibers (*"very minute vessels"*). In line with the current concept that animal spirit flowed in cavities along the nerves, von Leeuwenhoek believed that the axon was a tubular space. Abbot Felice Gaspare Fontana (1730-1805) is credited with the first description of nerve fibers and the envelope probably representing their myelin sheath. Fontana, director of the Museum of Natural Sciences in Florence, was interested in the biological effects of the viper venom. Using a magnifying glass, Fontana performed a careful dissection of the nerve *"with a very sharp needle"*, then immersed the nerve in water so that the threads floated in it. In an addendum on the structure of nerves to his work on the viper venom published in 1781, Fontana described that *"a nerve is formed of a large number of transparent, homogeneous, uniform, and simple cylinders... filled with transparent, gelatinous fluid... Each of these cylinders receives a cover in the form of an outer sheath... A very large number of transparent cylinders together can form a nerve."* The neurohistologist Robert Remak (1815-1865) described in 1838 that the axons (*"the primitive bands"*) originated from the nerve cell bodies (*"nucleated globules"*). In his posthumous work published in 1865, Otto Friedrich Deiters (1834-1863) provided an unequivocal illustration of the *"main axis cylinder"* (as opposed to the *"small axon cylinders"*, emanating from protoplasmic processes, that represented distal dendritic arborizations). With the revolutionary progress of the neuron doctrine and its official name, the axon was thus ready for the 20th century.

97.9

THE CNS MYELINATING CELL: OLIGODENDROCYTES - A BRIEF HISTORY OF TIME. S.D. D'Souza, A.F. Sadikot*, V.W Yong and W. Feindel. Montreal Neurological Institute, Montreal, QC H3A 2B4.

Oligodendrocytes were among the last cells of the CNS to be described, owing to their refractoriness to the metallic stains in use in the 1800s. Cajal's gold chloride-sublimate method which selectively labelled astrocytes, predicted the existence of a "third element" within the classic neuroglia. Robertson (1899), using his platinum stain, recognized a group of cells (oligodendrocytes) that he erroneously called "mesoglia", based on his belief that they were of mesodermal origin. Rio-Hortega (1919), using his silver carbonate stain that selectively impregnated the "third element" of Cajal, proved that it was made up of two distinct cell types, which he named "microglia" and "oligodendrocytes" (OLs). He made the distinction between "interfascicular" OLs that were situated in rows between myelinated axons of the white matter, and "perineuronal satellite" OLs of the grey matter. Having observed a great variability in the morphology of OLs, Rio-Hortega (1928) proceeded to subdivide OLs into four prototypes: types I, II, III and IV.

From their anatomical position and the fact that their appearance in development corresponded with the period of myelination, Rio-Hortega (1921) and Penfield (1924) proposed that OLs were involved in myelination in the CNS. Tissue culture studies (Lumsden and Pomerat, 1951) and electron microscopy studies (Farquhar and Hartman, 1957; Peters, 1960; Bunge et al., 1962; Mori and Leblond, 1970) provided definite proof that OLs were the myelinating cells of the CNS. In demyelinating diseases of the CNS such as multiple sclerosis (MS), OLs and their myelin membranes are the selective targets of immune-mediated destruction. Certain white matter areas, however, are spared in MS. Could it be that the various types of OLs have different susceptibilities to immune effector mechanisms?

97.10

THE CONCEPT OF HYPOTHALAMIC INTEGRATION IN THE *"ANOTHOMIA"* OF MONDINO DEI LIUZZI DA BOLOGNA.

^§R. Toni*, ^F. Ruggeri, §F. Briganti, °RM Lechan,

*^Human Anatomy, *§°Internal Medicine-Endocrinology. °NEMCH-Tufts Univ. Boston, MA USA and ^§Univ. Bologna, Italy.*

The *"Anothomia"* of Mondino dei Liuzzi was the leading text of human anatomy during the XIV century in Europe. Using a recent translation from Latin to Italian of this text (Giorgi PP, Pasini GF, 1992), we have analyzed the description given by Mondino of the region of the cerebral third ventricle, believed to be involved in the regulation of the "entire animal behavior" through the contribution of both "physical sensations" (*"sensata"* in Latin) and "abstract feelings" (*"non sensata"* in Latin). In addition, he reports that discrimination of these two types of information occurs in the third ventricle region through the action of the faculties of "reasoning" (*"virtus cogitativa"* and *"merito"* in Latin) and "memory" (*"memorata"* in Latin). Although this description condenses old principles coming from Aristotle, Galen and Avicenna, Mondino dei Liuzzi put forth the new idea that the region of the third ventricle is involved in the integration of sensory, emotional and cognitive information to regulate animal behavior. Therefore, his description represents one of the most ancient observations concerning the function of the diencephalon as a regulator of body homeostasis and implicates him as a forerunner of the concept of hypothalamic integration.

97.11

DAVID HARTLEY'S (1749) DOCTRINE OF NEURAL VIBRATIONS: ENLIGHTENMENT OR ACCIDENT? R. B. Glassman* and D. Spadafora. Depts. Psychol. and History, Lake Forest College, Lake Forest, IL 60045.

The doctrine of vibrations, and a notable pre-Hebbian, pre-Pavlovian formal examination of associations, are aspects of David Hartley's 1749 treatise, Observations on Man, His Frame, His Duties, and His Expectations. According to D. N. Robinson (Intellectual History of Psychology, Macmillan, 1976), Hartley's text, "is a treatise in *modern* psychology." The "famous theory of vibrations was the first detailed modern treatment of physiological psychology, and it won Hartley considerable renown ..." (Spadafora, The Idea of Progress in Eighteenth-Century Britain, Yale, 1990). How could Hartley have chosen such an apt metaphor for neural activity, with its (as we now know) frequency-modulated action potentials, before anything was known about its electrical basis? Was this a mere intellectual accident? One root of the idea may be sought in Hartley's several references to Newton's conception of light as particles that have vibratory effects and an octave property (e.g., Hartley, pp 18, 41; Newton's Opticks. Dover, 1740/1979, pp 126, 280, 344). Hartley also extensively considered the question of *continuity* from body to cortex of the "medullary substance," i.e. nerves and CNS white matter - whose fibers had to have a uniform "pellucid" texture (Hartley, p 17). We hypothesize that Hartley's vibration metaphor was a rational inference; his book suggests awareness that a neural system must include a medium in which communication of patterns finely differentiated in time and space supports the organization of behavior.

97.12

Historical Poster Exhibit: Temple S. Fay M.D. 1895 - 1963

Alfred R. Henderson[1] and Dong Jiang[2]* 1 U.S. Department of State (retired), 2 Molecular Neuropharmacology Section, ETB/NINDS/NIH, Bethesda, MD 20892

Neurosurgeon, neurologist, educator, provocative investigator and unconformatable harbinger of numerous clinical investigations. Professor of Neurosurgery and Neurology, Temple University. Devised new approaches to the management of trigeminal neuralgia, head trauma and epilepsy (by intense dehydration), CO_2 therapy for spastic disorders and seizures. First to reduce total body temperatures (breaking the thermal barrier) in the treatment of malignant tumors thus establishing clinical hypothermia. Founder of a method of treatment of brain-injured children, mainly cerebral palsy, utilizing phylogenetically functional pattern movements. Established the Neurophysical Rehabilitation Clinic in Chestnut Hill, Pa for this purpose. Co-founder of the Harvy Cushing Society and American Academy for Cerebral Palsy. The exhibit will contain appropriate photographs and reproductions of significant published materials.

97.13

The Complexity of Glio-architectonics: Golgi studies of Retzius (1894).

B. Quinn.* Div. Neuropathology, NYU Medical Center, New York NY 10016.

Until recently, astrocytes had received relatively little attention in most neuroscience research. Traditionally, forebrain astrocytes included fibrous and protoplasmic types, and ill-defined functions included ion buffering or other 'supportive' functions of astrocytes. However, in the early 1890's, Golgi studies by Retzius and others revealed a complex world of glial architectonics and a diversity of astrocytic morphologies. These morphologies included 'caudate' astrocytes, extending from the pial surface down to cortical laminae I/II; fibrous astrocytes, large and small protoplasmic astrocytes, small shrub-like forms, giant spider-like forms, perivascular forms, and additional specialized astrocytes such as the Bergmann glia of the cerebellum. Retzius described his studies in great detail in 1894 as a long article in the *Biologische Untersuchungen*. The work may mark an apogee of glial architectonic studies, because later versions of the Golgi stain were often titered to impregnate neurons and avoid astrocytic impregnations. The later studies of Schroeder (1935) will also be discussed. The difficulty of visualizing the architectonics of astrocyte systems may have contributed to viewing them as rather inconspicuous, uniform cells which become reactive or hypertrophic in disease. It is interesting to look back on Retzius' exhaustive morphologic studies today, now that many additional functions and regional specializations of astrocytes are being proposed, such as production of various neurotrophins (e.g., glial-derived neurotrophic factor, GDNF), neurotransmitter metabolism (particularly glutamate), and modulation of the perisynaptic environment. Sponsored by the CorText Institute and the Stanley Foundation.

(1) Retzius (1894) Die Neuroglia des Gehirns beim Menschen u. Saugethieren, Biologische Untersuchungen 6:1-28. (2) Schroeder (1935) Gliaarchitektonik des Zentralnervensystems, in: Bumke & Foerster, Handbuch der Neurologie, (I) Allgemeine Neurologie: Anatomie, pp. 791-810.

97.14

RECOVERY OF FUNCTION AFTER EXTENSIVE LESIONS IN THE FOREBRAIN: THE EXPERIMENTS OF F. GOLTZ IN THE LIGHT OF CURRENT NEUROSCIENCE. A. Aschoff and G. F. Jirikowski*, Dept. Anatomy II, FSU-Jena, Teichgraben 7, 07743 Jena, Germany

120 years ago the physiologist F. Goltz from Strassburg developed a new method for experimental forebrain lesions in dogs. He used the cutting force of a water beam to destroy large parts of the cortical gray matter. Thus, he was able to keep the animals alive, and to conduct observations on resulting deficits and their subsequent functional recovery. He noted a correlation between the degree of deficit and the size, but not the site of the lesion. By inflicting painful stimuli through an automated device he described the occurrence of somatosensory deficits and rapid recovery. He also found impaired of motor functions and visual deficits even after lesions restricted to the frontal cortex. Despite extensive cortical damage some sensory, visual, or motor functions were subsequently restored, albeit subtle somatosensory, visual and motor impairment persisted. Goltz' interpretation of his findings was concordant with current knowledge, and in disagreement with the views of Flourens and Hitzig (recovery based on structural redundancy), and the functional substitution theory of Carville and Duret. Goltz explained the deficit of function with the suppressive effect of the lesion on intact lower centers of the brain, anticipating the diaschisis concept of Monakow. He clearly stated that these transient functional deficits are caused by the loss of regulatory input and not by "vegetative" factors as blood flow or nutrition. Consequently, the resultant specific functional deficit should be determinable only after recovery from the lesion-induced inhibition has occurred. In this he opposes the view of strict localization of function within the CNS, and argues, that lesion-induced impairments reflect the function of intact, rather than damaged brain areas.

97.15

WILDER GRAVES PENFIELD AND THE SPANISH NEUROGLIAL TECHNIQUES, V. Balasingam, V.W. Yong*, W. Feindel. Montreal Neurological Inst., McGill Univ, Montreal, Quebec, Canada, H3A 2B4

Wilder Graves Penfield (1891-1976), the American-born Canadian Neurosurgeon, played a prominent role in bringing the Spanish metallic impregnation techniques for the identification of neuroglia into North American neuroscience and neuroclinical spheres. Penfield was initially introduced to the histological study of the central nervous system (CNS) under the guidance of Sherrington at Oxford, England. As a visionary neuroscientist, Penfield travelled to Madrid, Spain, to study with the eminent Spanish neuromicroscopists Ramón y Cajal and Pio del Rio-Hortega to expand his understanding of the structure and function of neurons and glia in the CNS. It was here that Penfield began his seminal work on astroglial and oligodendroglial reactivity and the changes in microglia after brain injury by using the Spanish methods (Rio-Hortega and Penfield, 1927). He modified the original Spanish techniques and utilized them for studies involving the cytological classification of gliomas, the mechanisms of brain healing and the evolution of vasoglial scars in association with focal epilepsy. From these neuroglial studies he also contributed to future generations of neuroscientists and pathologists by providing expert technical instruction on these methods. A careful observer, Penfield is remembered as a tireless neurosurgeon who contributed to laying the ground-work for many modern studies on neuroglia and its role in brain function.

97.16

THE PLATES OF PIETRO BERRETTINI DA CORTONA: A NEUROANATOMY AHEAD OF ITS TIME. R. Olry, M.-G. Martinoli*. Dept of chemistry-biology, Univ. of Trois-Rivières, Qc, Canada G9A 5H7.

The twenty-seven anatomical plates of Pietro Berrettini da Cortona (1596-1669) are rightly regarded as Baroque masterpieces. They were probably engraved in 1618 by Luca Ciamberlano (monogram on plates 1 and 4), but were published over a century later (first edition 1741; second and last edition 1788). On most of the plates, the anatomy of nervous structures (central, peripheral or autonomic) is emphasized, leading to the assumption that they were originally intended to illustrate a book of neuroanatomy or neurology. This putative book and its author are unfortunatelly unknown. However, the analysis of the minute details on these plates allows a better understanding of the neuroanatomical knowledge in the early seventeenth century, and get to reconsider some landmarks in history of morphological neurosciences. The course of trigeminal nerve is well depicted, over hundred and fifty years before the celebrated monograph of Giovanni Battista Palletta (1784), and the emergence of cranial nerves and their course through the base of the skull is comparable with Samuel Thomas Soemmering's description of 1778. The plates of Berrettini da Cortona were therefore much ahead of their time, and their undeniable artistic features unfortunately pushed their scientific content into the background.

97.17

WALTER HEILIGENBERG (1938-1994) AND HIS CONTRIBUTIONS TO BEHAVIORAL PHYSIOLOGY. G.K.H. Zupanc*. Department of Physical Biology, Max Planck Institute for Developmental Biology, D-72011 Tübingen, FRG.

Walter Heiligenberg, born on 31 January 1938 in Berlin and tragically killed in an air-plane crash at Pittsburgh (Pennsylvania) on 8 September 1994, was one of the most prominent pioneers in modern neuroethology. He completed his Ph.D. thesis in 1963 under the guidance of two famous biologists: Konrad Lorenz from the Max Planck Institute for Behavioral Physiology in Seewiesen and Hansjochem Autrum from the University of Munich. Following an invitation by Theodore H. Bullock, he joined, in 1972, the Neurobiology Unit of the Scripps Institution of Oceanography of the University of California, San Diego (UCSD), where he remained as a member of the faculty until his death.

In the course of his scientific career, Walter Heiligenberg made significant contributions to the analysis of behavior and especially to our understanding of how the brain controls behavioral patterns. At Seewiesen, he developed techniques to describe the temporal structure of behaviors by mathematical terms, and he succeeded in a quantitative demonstration of the phenomenon of "heterogeneous summation." Heiligenberg also showed that releasing and motivational effects are just the two end-points of a spectrum of possibilities that might occur after behavioral stimulation.

At UCSD, he analyzed neural mechanisms that control behavior by using the "jamming avoidance response" of weakly electric fish as a model system. He rigorously revealed the computational rules and the details of the neuronal network underlying this type of behavior. These achievements provided insights into general principles of neural algorithms and network organization, thus making them applicable to a wide array of neuronal systems.

The results of Walter Heiligenberg's work are likely to remain of major influence on ethologists and neuroethologists for many years to come.

Supported by grants from the Max Planck Society.

97.18

WENDELL JORDAN KRIEG: NEUROANATOMIST, ARTIST AND THE CONCEPT OF ELECTRONEUROPROSTHESIS. D.E. Haines and A. Subramony. Department of Anatomy, The Univ. of Mississippi Med. Ctr., Jackson, MS 39216 and Yale Univ., New Haven, CT 06520.

In 1949 Wendell Jordan Krieg (b. April 13, 1906) was Professor of Anatomy at Northwestern University. He had just completed 2 years ('46-'48) as Director of the Institute of Neurology at Northwestern and several years on the faculty as Associate Professor ('44-'46) and Professor ('46-'48) of Neurology. He had already established a reputation in neuroanatomy and, by this time, had published 3 books including the first edition of his textbook Functional Neuroanatomy ('42, '53, '66). Based on his broad-based research on the nervous system Krieg (in '49) originated what he called the concept of electroneuroprosthesis. He postulated that electrical appliances could be devised that would deliver patterned electrical stimulation to various areas of the nervous system (special emphasis on the cortex); these appliances would restore vision, hearing, and perhaps motor abilities. Krieg's ideas were novel, significantly ahead of his time, and met with considerable skepticism especially among his clinical colleagues. Although not pursued by Krieg, his ideas foretold the later development of a variety of neuroprosthetic devices. Krieg's publications, especially his 15 books, were illustrated by drawings of unparalleled detail, accuracy, and 3-dimensions. He executed these stunning illustrations by his own hand; they are unique among neuroanatomy textbooks published in the U.S. This poster reviews the career of W.J. Krieg with special emphasis on his scientific contributions, his unusual artistic works, and his theories on electroneuroprosthesis.

97.19

THE SESQUICENTENNIAL OF THE FIRST PUBLIC DEMONSTRATION OF SURGICAL ANESTHESIA. S. M. Dodek III *, N. R. Myslinski, J. Buxbaum, C. Truax. OCBS Department, Univ. of Maryland Dental School. Baltimore, MD 21201.

On October 16, 1846, William G.T. Morton, a Boston dentist and medical student, first publicly demonstrated the use of general anesthesia. The substance was ether, and the operating room at Massachusetts General Hospital remains in use today as a memorial called "the Ether Dome".

Having heard that a medical student had developed a method for eliminating surgical pain, skeptical doctors and nurses gathered impatiently in the gallery of the operating room on a Friday morning in 1846. The surgeon was Dr. Warren. There were also several strong men available to hold the patient down in case the procedure did not work. William Morton administered the anesthesia and the operation was completed with the patient showing no signs of pain. The strong men were not needed. An eminent surgeon in the audience, Dr. Bigelow, remarked, "I have seen something today that will go around the world."

Within months, the use of ether spread to other cities in the U.S. and Great Britain, eliminating the suffering of millions of people and allowing for the development of many surgical procedures that would have been impossible without it.

Dr. Morton did not fare as well. He initially withheld the chemical nature of the substance, calling it "letheon". In 1848 he was granted a patent, but did not experience great wealth from his discovery. A provision of the patent permitted certain charitable hospitals to use "letheon" free of charge. Congress suggested a gift of $100,000 for the discoverer of anesthesia, but the money never came forth. Over the decades following the first use of anesthesia, others claimed its discovery. One claimant was Charles Jackson, Mortan's chemistry teacher. Another claimant was Horace Wells, known for his use of nitrous oxide. Dr. Morton died in poverty on July 15, 1868 in New York's Central Park.

97.20

CENTENARY OF GAULE AND LEWIN; A LANDMARK IN THE HISTORY OF CELL COUNTING AND STEREOLOGY METHODS. John C. Hedreen* and Jean Paul Vonsattel. New England Medical Center and Massachusetts General Hospital, Boston, MA.

One hundred years ago, in 1896, Justus Gaule and his student Th. Lewin published in the Centralblatt für Physiologie 10:437-440 and 465-471 a paper entitled "On the number of nerve fibers and ganglion cells in the spinal ganglion of the rabbit." In this paper they pioneered an unbiased method for converting the number of profiles of cells in the sections (ie., the number of pieces that a cell is cut into by sectioning) to the total number of cells. In a sample of neurons that they could follow through serial sections, they found the total number of profiles for the sampled neurons, and thence the mean number of profiles per neuron. They then divided this number into the total number of profiles counted in the ganglion to obtain the total number of neurons in the ganglion. In the 32nd spinal ganglion of a rabbit they counted 71,264 neuron profiles in 10 µm sections through the ganglion, and calculated 3.5 profiles per neuron. Thus their final count of neurons in the ganglion was 20,361. For comparison, they counted cells with nucleoli in the same sections, finding a total of 21,678, which they felt was too high because of counting some cells twice. Their admirably direct method for converting profile number to cell number did not come into general use, although a few papers in the 1930's [Davenport and Bothe, 1934; Foley and DuBois, 1937] did use such an approach when counting nucleoli. Coggeshall and colleagues [1984, 1990; Pover and Coggeshall, 1991] reinvented and perfected this procedure, which they called the empirical method, only to abandon it finally, as being less efficient than the disector method.
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97.21

A HISTORICAL REVIEW OF FUNCTIONAL BRAIN MAPPING TECHNIQUES. N. Prakash*. Department of Psychobiology, University of California, Irvine, CA 92717.

Current functional brain mapping techniques include positron emission tomography (PET), intrinsic signal optical imaging (ISI), functional magnetic resonance imaging (fMRI), and voltage-sensitive dye imaging (VSD). Historically, these four techniques originated from three different principles: 1) active nerves become more opaque, 2) active neurons require more blood flow, and 3) active tissue shows oximetric changes.

Early work in 1892 by C.F. Hodge followed by D.K. Hill and R.D. Keynes in 1949 led to the concept that there are changes in nerve opacity directly correlated with electrical stimulation. These changes were found to be amplified by the addition of dyes and throughout the 1970's L.B. Cohen and his collaborators established the *in vivo* technique of VSD. In 1986, based on similar techniques as VSD, A. Grinvald and collaborators established ISI as a method for functional brain mapping.

C.S. Roy and C.S. Sherrington speculated in 1890 that, "The brain...vascular supply can be varied locally in correspondence with local variations of functional activity." It was only in 1967 that J. Risberg and D.H. Ingvar first confirmed this speculation in humans using radioactive gases and scintillation detectors. Improved multi-detector systems led the development of PET and in 1986 P.T. Fox and collaborators reported the first mapping of the human visual cortex based on blood flow changes using PET.

Work by D. Keilin in 1925 followed by G.A. Millikan in 1933 led to the development of oximetry, which is the measurement of spectral changes that occur as hemoglobin is reduced. Oximetric techniques were further developed by B. Chance and collaborators starting in the 1950's and are currently used in clinical techniques, such as near infrared spectroscopy. Similarly, the intrinsic paramagnetic properties of deoxyhemoglobin led to the first successful fMRI based on oximetric principles by K.K. Kwong and collaborators in 1992 (after initial work by J.W. Belliveau and collaborators in 1991). Finally, in 1990 R.D. Frostig and collaborators reported that opacity, oximetric, and blood flow changes are the three major signals in ISI.

97.22

A RETROSPECTIVE LOOK OF THE UNIVERSITY OF MEXICO'S DEPT. OF PHYSIOLOGY TO ITS PRESENT DAY STATUS. **E. Gijòn, X. García, L. Cartas and R. Drucker-Colín* Dept. de Fisiología, Facultad de Medicina, UNAM. México, D.F. 04510, México.**

In the past, physiology in Mexico followed the prevalent ideas of the scientific world, since the founding fathers were all trained abroad. The aim of this work is to revisit the Department of Physiology of the School of Medicine at the Universidad Nacional Autónoma de México (UNAM), because it was the first Department of Physiology in all of Mexico, founded in 1934 by José Joaquín Izquierdo and years later was consolidated by Rosenblueth and Del Pozo, who started the kernel of physiology in México with a strong emphasis in Neuroscience. The first group of physiologists were Alcocer, Alonso de Florida, García Ramos, Guevara-Rojas, Hernández-Peón and Puche. Previous reviews in 1968, 1979, 1986, 1990 and 1993 showed that physiology is one of the most productive scientific disciplines in the medical sciences. Recent changes in the department of physiology has resulted in increasing staff, research lines, grants and technical support, which is reflected in its publication level which is similar sometimes to larger University Centers, Institutes and Schools. For example, its publications in the last three years equals the production that some research institutes show during much longer periods. This suggests that physiology remains one of the most productive scientific disciplines in México.

97.23

THE ORGANIZATION OF AGGREGATES; NEUROSCIENCE INSTITUTES AND THEIR LEADERS. L.H. Marshall* and S. Bedi.[1] Neuroscience History Archives, Brain Research Institute, and [1]Dept. of Physiological Sciences, UCLA, Los Angeles, CA 90095-1761.

The philosopher/physicians of ancient times found that by congregating together for discourse, writing, and teaching they could more efficiently refine and spread their ideas and concepts than if they pursued a solitary life. Thus was formed in 4th c. Greece, Plato's academy teaching a cephalocentric view of the body, and Aristotle's lyceum with a cardiocentric opinion. During the Dark Ages, Avicenna in Persia spread Greek anatomic knowledge which returned to the West in the teaching texts of Mondino and Vesalius. In the late 19th c., European istitutes were organized around distinguished men, especially in Germany. An International Association of Scientific Academies recognized eight institutes for neurological research, one of which was the Wistar Institute of Philadelphia, where Henry Donaldson (1857-1938) worked. Other North American institutes were in Montreal, New York, and elsewhere; in Chicago, Northwestern University built around Stephen Walter Ranson (1880-1942), who had been a student of Donaldson's. When Ranson's student, Horace Winchell Magoun (1907-1991) planned the Brain Research Institute at the University of California, Los Angeles, it was a shining example of the new American style of research facility with a team approach to brain and behavior that crossed departmental and disciplinary boundaries.

97.24

NEUROSCIENCE IN ANTIQUARIAN CHILDREN'S BOOKS: FROM THE *ORBIS PICTUS* OF COMENIUS TO LEWIS CARROLL'S *SYLVIE AND BRUNO*. R.A. Johnson*. Neuroscience History Archives, Brain Research Institute, and Department of Special Collections, University Research Library, UCLA, Los Angeles, CA 90095 (e-mail: rjohnson@library.ucla.edu).

Several neuroscience-related topics were serendipitously identified in the course of a recent Department of Education Title IIC-sponsored project which created online catalog records for 10,000 pre-1900 titles in the Children's Book Collection at UCLA. Johann Amos Comenius's *Orbis sensualium pictus* (1658), considered the first educational picture book for children aside from illustrated alphabets, combined woodcuts with simple Latin and German sentences to produce "a nomenclature and pictures of all the chief things that are in the world, and of men's employments therein." Chapters on anatomy and sensation included two depictions of the brain: a cartoon-like horizontal section through the head and a cerebrum revealed in the manner of 16th-century anatomist Andreas Vesalius's scalp-draped dissections. The 18th and 19th century fascination with all things electrical was reflected in a treatment of Luigi Galvani's renowned and mythologized experiments, in Peter Parley's *A glance at the physical sciences* (1844). *A legacy of affection, advice, and instruction* (1827) described the cerebral and cerebellar ablation experiments of Pierre Flourens, in one of a series of letters from a retired governess to her charges. Experiments by Jules Jean César Le Gallois on the respiratory center of the medulla were filtered through the abolitionist gauze of the animal welfare movement's rhetoric at century's end in Susanna Watts' poem, *Adventures of Bigio* (1831). Science informed fiction in the latter half of the century; *e.g.*, among carefully-indexed topics such as "how to improve (the) character of fairies," Lewis Carroll's *Sylvie and Bruno* (1889) nestled the "curiously slow action of nerves," "inversion of images on (the) retina," and a comparison of "nerve-force and free-will." Although there are few neuroscientific allusions buried in surviving books, what is striking is the detail in which concepts were described and the depth of understanding which the authors conveyed in works that were explicitly intended for juvenile audiences. Citations for appropriate works which the presenter might not have encountered are sought and welcomed.

98.1

CREATING INEXPENSIVE INTERACTIVE MULTIMEDIA INSTRUCTIONAL PROGRAMS AND TEACHING AIDS FOR THE WORLD WIDE WEB. R.A. Wheeler, S.T. Neary, C.L. Jollie, A.E. Baldwin, & J.T. Cannon*. *Departments of Psychology, Computer Science, and Neuroscience Program, University of Scranton, Scranton, PA 18510-4596.*

Recently, we (Meyers *et al.*, 1993) described the integration of interactive multimedia programs into a behavioral neuroscience course. These programs were created using an array of costly hardware and software. Our most successful creation was a sheep brain neuroanatomy guide available at no cost through FTP on the Internet and currently used world-wide. Our original combination of hardware and software had two disadvantages: 1) its cost (NSF grant USE-9250437), and 2) the software did not run on other computer platforms. To address the latter, we translated our sheep brain guide into Hypertext Markup Language (HTML) for Netscape 2.0 compatible browsers (*http://academic.uofs.edu/department/ neuro).* Although this HTML conversion necessitated a reduction in image size and resolution to accommodate web bandwidth restrictions, the utility of the program has been increased: 1) the operating system problem is largely eliminated, and 2) the software can be used without requiring the downloading, extracting and housing the original 16 MB program file. A web format has provided some unexpected benefits, not the least of which is that multimedia applications can be generated for a fraction of our original cost. Quality video images can be obtained with a standard camcorder coupled to a Snappy™ framegrabbing device (< $200). These images can be edited and labeled using software bundled with Snappy™. Multimedia HTML programs can be generated with a standard text editor or any of a growing number of inexpensive/free HTML editors. The functionality of HTML is rapidly expanding and, unlike dedicated Multimedia authoring software, is not likely to suffer from rapid obsolescence. Finally, the Web generates both greater student interest and more direct contribution to the authoring process than does idiosyncratic dedicated software. Our students are required to maintain home pages with course-related materials such as: current research topics and related Web links; histological images; honors theses; and "virtual" poster presentations.

98.2

THE WORLD-WIDE WEB UNDERGRADUATE BEHAVIORAL NEUROSCIENCE RESOURCE SITE PROJECT: PROMOTING SCIENTIFIC LITERACY FOR THE NEXT GENERATION.

E.P. Wiertelak*, Department of Psychology, Macalester College, St. Paul, MN 55105.

The past 24 months has seen explosive growth in the use of the internet for pleasure and profit, as well as academic pursuits. While internet resources present unparalleled opportunities for curricular enhancement across academic disciplines, college students primarily use the internet for recreation and correspondence, utilizing e-mail, world wide web (WWW) browsers, newsgroups and gopher services.

The goal of the curricular program discussed here was to develop new instructional WWW mechanisms to augment our successful WWW diary and home page projects, and further enhance learning outcome in neuroscience via student involvement with the WWW. Initial exercises were designed to introduce WWW searching and provide real experience in programming HTTP through creation of home pages. This was followed by posting of sequential HTTP diary entries into each student's home page hierarchy. Following this training, students were divided into groups, which were charged with creating complete, one-stop resource sites for undergraduate-level student inquiries related to behavioral neuroscience. Each site covers a single topic, and features a hypertext-enhanced bibliography. Our experience has been that the inclusion of service-oriented, cooperative projects has provided students with increased insight into their own studies, and has given other neuroscience students at the undergraduate level sites containing easily understood yet comprehensive treatment of neuroscience-related topics.

98.3

NEUROSCIENCES ON THE INTERNET. N. A. Busis*. University of Pittsburgh School of Medicine, Pittsburgh, PA 15213.

A database of Internet neuroscience resources was first published on the World Wide Web in December, 1994 and has been regularly updated. The Uniform Resource Locator (URL) of the US site is <http://www.lm.com/~nab>. There are mirror sites in the UK and Germany. Data was collected from Internet search services, announcements in USENET newsgroups, links on relevant World Wide Web pages or published in World Wide Web directories, and suggestions from others. Over 2400 links to neurobiology, neurology, neurosurgery, psychiatry, psychology and cognitive science sites are included (as of April, 1996). The database can be searched interactively or can be browsed via a hierarchical Table of Contents. It lists other neuroscience Internet guides, academic home pages, professional organizations, meetings, imaging resources, software, journals, patient information, newsgroups, mailing lists and Web forums. Some biomedical and World Wide Web links and a feedback form are included.

Currently, the optimal technique to find specific Internet neuroscience resources or to browse through the available sites is by consulting specialized neuroscience indexes and general Internet indexes and search services. This database will co-evolve with the World Wide Web and will serve as a testbed for new methods of information retrieval concerning Internet neurosciences resources.

98.4

USING THE WORLD-WIDE-WEB AS A TOOL FOR AN INTER-DISCIPLINARY APPROACH TO THE SCIENTIFIC STUDY OF EMOTION.

J-M Fellous* and E. Hudlicka, Volen Center for Complex Systems, Brandeis University, Waltham MA 02254-9110 and GTE Research Lab, Waltham.

The scientific study of emotion has always suffered from a lack of inter-disciplinarity. Recent advances in the neuroscience of fear conditioning and emotional disorders have contributed to a better understanding of the neural substrate involved. However, such results are for their most part largely unavailable to other disciplines such as experimental and cognitive psychology or artificial intelligence. The aim of the present work is to design and implement a web site dedicated to the scientific study of emotion, focusing on integrating and inter-relating the major results in neuroscience, cognitive and experimental psychology and artificial intelligence. It is intended as both a teaching tool and a research tool. It offers both overviews, and links to in-depth analyses located locally or remotely in the laboratories involved, ensuring up to date information.

This page contains a summary of the major results and bibliography in the three disciplines mentioned above. The neuroscience section includes the main results and theories on fear conditioning, the limbic system, and the neuro-pharmacology of emotional disorders, addressing simultaneously several levels of neural complexity. Results in each of the three disciplines are linked to results of the two others, whenever possible, providing focused 'gateways' from one discipline to another. This page also includes an extensive historical survey of the theories of the biological basis of emotion, from Plato to the beginning of the 20th century, with links to original online texts and artworks. Finally, it includes a section on relevant online conference announcements, journal and book publications, and links to various laboratories, individuals and institutions currently working on this topic. For information, contribution and free access, please contact fellousj@volen.ccs.brandeis.edu.

98.5

DRUGS AND THE BRAIN: A WORLD WIDE WEB TUTORIAL IN NEUROPSYCHOPHARMACOLOGY. A.B. Chinn and K.A. Trujillo*, Psychology Program, California State University, San Marcos, CA 92096-0001.

Understanding the ways that specific drugs affect the brain and behavior is a critical component of a variety courses in the neurosciences, behavioral biology and psychology. However, many undergraduate students have difficulties grasping such material. This arises in part due to the new vocabulary students must acquire in order to understand the topic, but also because it is by nature an interdisciplinary topic, covering areas ranging from molecular biology to human behavior.

In order to supplement material for such courses and offer students an opportunity to better understand how drugs affect the brain and behavior we are designing a World Wide Web (WWW) tutorial devoted to neuropsychopharmacology. This hyperlinked tutorial is intended to give students exposure to the topic in a self-paced, interactive manner so that they can learn the material without being intimidated by it. In addition, students will be able to explore areas beyond the scope of their course material, gaining a deeper understanding of the field as a whole. To supplement the content we will include links to bibliographic information and a glossary of scientific terms. Each section will also include thought-provoking questions and quizzes that will allow students to utilize and process the information and gauge their understanding of the material. Finally, we will include links to other relevant WWW sites to provide an easy mechanism for students to further explore this topic.

It is hoped that this WWW site will serve as a valuable instructional tool for faculty teaching courses that include discussion of neuropsychopharmacology, a learning tool for students taking courses in this area, and an informational resource for anybody with an interest in the ways that drugs affect the brain and behavior.

Supported by the University Fund for Technological Innovation on Campus, CSU San Marcos.

98.6

TEACHING ON THE INTERNET: ENHANCEMENT OF THE UCLA SCHOOL OF MEDICINE NEUROANATOMY CURRICULUM. A.F. Cannestra*, C. R. Houser, and A.W. Toga, Lab. of Neuro Imaging, Division of Brain Mapping, Departments of Neurology and Neurobiology, UCLA School of Medicine, Los Angeles, CA 90095

We constructed an Internet page for the UCLA School of Medicine neuroanatomy course to aid the communication of complex anatomical relationships, dynamic processes, and clinical imaging not practically presentable within the constraints of lecture or text. Material presented was coordinated with the course dissection schedule, reinforcing the students learning and contextualizing anatomic information within exemplary clinical presentations. References of recent functional research and hyperlinks to other neuroscience Internet sites were included to increase the integration of neuroscience research and medical school education. Anatomical and functional neuroimages, technical descriptions of imaging techniques, integrative quiz questions, 3D neuroanatomical surface models, animations, and course administration information were incorporated into the site. Examples of CT, MRI, fMRI, PET, OIS, angiography, MR angiography, gross specimens, and cryosection specimens were provided within the following topics: the cerebral vasculature, ventricular system, cerebellum, brainstem, diencephalon, and telencephalon.

Internet access was a major advantage to this form of teaching. Student access to computers in libraries and student lounges in combination with remote modem access allowed flexible perusal of the information. Small group sessions were provided for students introducing the usage of the Internet and web site. Instructors were available to discuss and review on-line material during dissection hours. Electronic mail scripts were integrated into the Internet site to allow students to electronically ask questions remotely. Student evaluation of the Internet web site was positive indicating this will be a continuing part of the UCLA School of Medicine neuroanatomy course. AFC is supported, in part, by a Medical Scientist Training Program grant (GM08042)

98.7

TUTORIAL-BASED ACCESS TO A WEB ATLAS OF NEUROANATOMY
J.W. Sundsten*, J.F. Brinkley and K. Mulligan. Dept. of Biological Structure, University of Washington, Seattle, WA 98195-7420.
The Digital Anatomist Program's image-based interactive brain atlas has appeared in CD-ROM format, on the Internet in a custom client-server framework and most recently on the World Wide Web (http://www9.biostr.washington.edu/da.html). Most of the original material is organized by region, objects, serial slices and 3-D computer graphic images. We are now preparing additional means of arranging and accessing this image database in a systematic fashion for our course needs. We first developed a new set of atlas chapters, each of which contains a set of objectives, images (objects buttoned and with captions) and summary drawings. The current emphasis is neuroanatomical, but the material is ordered into functional units such as development and gross topography, general and special sensory systems, motor systems, brainstem and cranial nerves, cerebellum, basal ganglia, thalamus and cortex, and limbic system. The images are from departmental sources: the Digital Anatomist image database, a set of in-house teaching slides, and new material (processed with Photoshop software) from one of our course syllabi that is in digital format. Secondly, we are developing a Web-based tutorial, implemented in Netscape FRAMES, that contains links to appropriate frames from the Web version of the image-based atlas. The tutorial guide can be readily updated and tailored to individual instructor needs, making it possible, for example, to present class laboratory assignments, changes in schedules, and customized quizzes on a daily basis if necessary. These steps are moving us directly toward on-line interactive instruction for independent student use. A demonstration of the new chapters will be shown on CD-ROM, along with sample images from the Web-based tutorial. Supported in part by NLM Grant LM04925 and the Human Brain Project Grant DC/LM02310.

98.8

NEUROSCIENCE 4 AND THE WORLD WIDE WEB: WEBNOTES AND VIRTUAL LABORATORIES
Paul C. Knox, P.A. Spooner, G.W. Arbuthnott* Centre for Neuroscience, University of Edinburgh, Crichton St, Edinburgh, Scotland.
Neuroscience 4 is an undergraduate (Honours) course offered to final year Science students and intercalating Medical and Veterinary students in the University of Edinburgh. The course consists of five, five week modules. Two are taken by all students; the other three are chosen from ten optional modules. These cover the whole spectrum of contemporary neuroscience, from molecular aspects of the subject through to visual psychophysics. At any one time, teaching may be taking place in several different geographical locations across the city. Thus communication of both administrative information (timetables, deadlines) and learning materials (reading lists, seminar synopses, seminar material) is a major problem. In addition there is increasing pressure on laboratory space and staff time.

We have tackled this by placing information on the World Wide Web, and by beginning to develop "virtual laboratories" using Java. The University of Edinburgh provides open access microcomputing laboratories at all of the main University sites and machines in these labs have Netscape 2 installed. As we had established a Web server in March 1995 with pages containing information about research and postgraduate teaching in the Centre for Neuroscience (URL http://sirius.lns.ed.ac.uk/), it seemed a natural progression to develop an undergraduate information resource. The Web pages are maintained on a Sun Sparcstation 5, running the CERN HTTPD Server software. There are approximately 80 files taking up 250Kb of disc space. Student use is tracked by examination of the Server logs. We are developing a series of online lecture notes on various topics and links to other relevant material ("WebNotes"), which complement material given in seminars. In addition, using Java "applets" we are able to simulate experiments (freeing up laboratory space) and provide students with data to be analysed, interpreted and presented.

98.9

TEACHING OF NEUROSCIENCE IN SWEDEN: THE USE OF COMPUTER ASSISTED LEARNING (CAL).
T. Kling-Petersen* and M. Rydmark. MICE@mednet, University of Göteborg, Medicinaregatan 7, 314 90 Göteborg, SWEDEN.

At the medical faculty, University of Göteborg, the department of Medicinal Informatics and Computer assisted Education (MICE) is responsible for the development and implementation of computer based learning. The preclinical efforts have traditionally been divided into 3 blocks:

NeuroAnatomy - This block contains in-house developed software designed to teach the various aspect of the brains anatomy:
- 3D Brain - Virtual Reality modelling of structures of the human brain.
- BrainTeach - 2D sliced brain organised in frontal or horizontal series.
- BrainRadiology - software utilizing alternative imaging techniques like MRI, X-ray and others to visualise structures.

NeuroHistology - The software provides an interactive view of the cell- and tissue types of the brain organized after structures. The software also covers the organisation of structures as well as development.

Pharmacology - This block utilizes interactive simulations to visualize pharmacological issues.
- RabbitIntestine - Simulation of pharmacological agents effects on the peristaltic movements of rabbit jejunum.
- PharmacoKinetics - Simulation of the human body's effect of administered pharmacological agents.

MICE@mednet is an organization consisting of scientists devoted to using the computer as a tool for education. The organisation spans several areas of medicine, ranging from basic, preclinical education to clinical specialist training.

98.10

USING "SLICE OF BRAIN" VIDEODISC AS AN IMAGE AND VIDEO RESOURCE FOR PROBLEM-BASED NEUROSCIENCE TEACHING.
S. S. Stensaas*, Educ. Cent., Cornell Univ. Med. Coll., N.Y.10021

With the increased availability of computers and the popularity of Problem-Based Cases a pilot neurology course used "BookSlate" for organizing and launching resources for each case. BookSlate is an application created on top of HyperCard as an easy way for students, residents and faculty, who do not know scripting, to quickly produce case presentations. Cornell has used the application in a different way. Each case listed and linked the appropriate multimedia instructional resources for each case on a "slate".

A ready source of images for internal use was "Slice of Brain", a videodisc of 20,000 slides including 5000 gross and microscopic images. In addition, 7000 neuropathology and 8000 neuroradiology images can be used. There are over 150 video clips of patient signs and symptoms, reflexes, seizures, and rotating brain reconstruction's. AV equipped multimedia PCs or Macs permit direct video input from vidfeotape or videodisc. A videodisc index allows the user to quickly find and select an image for digitizing. Other linked resources were Stedman's dictionary, Netter's Atlas, sites on the WWW, scanned EEGs and EMGs, transcripts for first year and teaching software for neuroanatomy. The use of "BookSlate" provided: (1) An enhancement of the paper cases we obtained from Harvard University, and (2) an organizer and launch pad for material. The BookSlate, disc and index will be demonstrated.

98.11

ATTITUDES OF HEALTH SCIENCE STUDENTS TOWARD COMPUTER-BASED MULTIMEDIA LECTURING IN NEUROSCIENCE.
C.L. Cleland*. Dept. of Physiology and Biophysics, University of Iowa, Iowa City, IA 52242

Although computer-assisted teaching is becoming more commonplace in neuroscience education, its efficacy has not yet been clarified. The goal of this study was to survey student attitudes toward computer-based multimedia lecturing to identify common views and determine whether attitudes correlate with the type of student or computer experience and skill.

Computer-based neuroscience lectures were delivered to students in medicine, dentistry, pharmacy, nursing, and engineering. The lectures (6-10) were similar in different classes (3 different classes), contained extensive multimedia content (digital video, animation, sound, graphics, images and interactive simulations), and were given by a single lecturer. Attitude questionnaires were distributed immediately following the lectures. Questions (n=30), based on a modified 1 (positive) - 5 (negative) Likert scale, covered attitudes toward various aspects of the lecture, student background and computer experience and views. Questionnaires were distributed at the beginning of class and then collected 10 minutes later.

Responses were obtained from 520 students. Overall, students felt that the computer improved understanding (2.02 $\pm$0.8 SD), made the class more interesting/fun (1.99$\pm$0.9) and should be used more often (1.96$\pm$0.08). Animations were identified, by score (1.82$\pm$0.9) and comments, as being particularly valuable. Favorable attitudes toward computer-based lectures (e.g., understanding) were significantly correlated with favorable attitude toward computers (cc=0.69, $p<0.002$), but interestingly, not with subjective or objective computer skills (cc=0.17).

Supported by the Department of Physiology and Biophysics, The University of Iowa (OCRME, ITS, Council on Teaching) and Microsoft Corporation.

98.12

USE OF COMPUTER ASSISTED INSTRUCTION (CAI) FOR TEACHING BASIC NEUROSCIENCE TO PHYSICAL THERAPY (PT) STUDENTS.
S.D. Stoney, Jr.*, W.J. Jackson and T. M. Nosek. Dept. of Physiology and Endocrinology, Medical College of Georgia, Augusta, GA 30912.

PT students were asked to study portions of a CAI application covering basic neuronal physiology, the physiology of sensory and motor systems, higher brain functions, and an introduction to clinical cases. This application was originally designed for freshmen medical students. Groups of 4 to 6 students worked together using 7 portable computers in the department and 4 computers at the school library. Of 21 students who completed the course evaluation, 85% used the computer for more than 6 hours, either alone or in a group. Although 62% agreed that learning from the computer kept their attention better than learning from their textbooks, 29% disagreed. However, if they had been able to use their own computer, 85% thought that learning from the computer would be more effective than using a textbook. Clinical cases, animations, and questions with answers were judged to aid in understanding the material by 90%, 100%, and 95% of the students, respectively. Text and figures, animations, and questions with answers were rated as "moderate to very efficient" for learning by 95%, 95%, and 100%, respectively. 90% of students indicated that they would like to have their own computer for studying this kind of material. The major negative comments concerned the inefficiency of working in groups and the time it took to go through the material. The data suggest that integrated neuroscience information in a CAI format can be a useful method for teaching Physical Therapy students.

98.13

EVALUATION OF A PROBLEM-BASED APPROACH AND THE USE OF "SLICE OF LIFE" MATERIALS IN A MEDICAL NEUROSCIENCE CURRICULUM. K.G. Ruit*. Dept. of Anatomy and Cell Biology, University of North Dakota School of Medicine and Health Sciences, Grand Forks, ND 58202.

Our neuroscience curriculum has been a "traditional" one in that students were given enormous amounts of facts in a didactic setting with little emphasis on small group process or clinical problem solving. As a result, students rated the neuroscience course among the worst in their medical school experience. Since students in our curriculum do not have required 3rd or 4th year clerkships in neurology, we saw it as essential that their exposure to neuroscience in the 1st year be substantive yet productive. Therefore, we have sought to take a less all-encompassing approach with more of an emphasis on the delivery of essential facts, clinical application, and the use of multi-media technology.

Over the last three years we have: instituted the evaluation of "paper cases" within student small groups, reduced lecture hours by 40%, introduced the use of HyperBrain software and the Slice of Brain videodisk, established expert panel discussions, and given students actual patient exposure. Students have dramatically improved their overall performance averages (to midterm: 1992: 80.87%; 1996: 89.58%). Students are able to recognize clinical presentations of neurologic disorders and localize lesions with no difficulty and attribute it to the clinical problem-solving emphasis and the use of multi-media technology. Neuroscience is now considered one of the best experiences of their medical school experience.

Through our recent efforts in getting students thinking about neuroscience rather than simply doing neuroscience, we are convinced that even in the absence of a required 3rd year neurology rotation, they are now prepared for 4th year electives in neurology based on their comprehensive yet efficient exposure to neuroscience in their 1st year.

98.14

SENEX: OBJECT-ORIENTED PRESENTATIONAL TOOLS FOR MOLECULAR NEUROSCIENCE. S.S. Ball and V.H. Mah* UCSF/Fresno, Valley Medical Center, Dept. of Medicine, Fresno, CA 93702.

SENEX is an evolving set of computer tools for molecular neuropathology featuring computer-based presentations linked to a large knowledge base. Slide presentations use a standard projection system connected to a powerbook computer. Slides may provide in addition to the projected image: 1) explanatory text; 2) links to the underlying SENEX knowledge base; 3) a free form query into the SENEX knowledge base; 4) access to any slide in an entire slide collection. The underlying knowledge base has been built by customizing the behavior of an object-oriented computer language to fit the domain of molecular neuropathology. Domain-specific considerations include contextual information, properties common to specific classes of molecules, the concepts of generalization and uncertainty, multiple layers of structure within molecules, and different logic for comparing different molecular features. The SENEX knowledge base contains molecular data derived in part from four large databases: 1) ProSite database of peptide motifs & electronic textbook of biochemistry; 2) Swiss-Prot database of protein sequences; 3) the Enzyme database; 4) Mendelian Inheritance in Man. Molecular data from the SENEX knowledge base are displayed through graphical presentations of virtual molecular objects accompanied by explanatory or supplemental text and images of gross and histopathologic features. Molecular and disease-related data is presented in a hierarchical and overlaid fashion which allows specific retrieval or focused exploration in a mouse-oriented intuitive graphically oriented environment. This empowers a speaker with tools at his/her disposal previously unavailable.

98.15

INTEGRATION OF BRAINSTEM AND SPINAL CORD FUNCTIONAL ANATOMY IN A CHART FORMAT FOR TEACHING AND REFERENCE. K. H. Taber*, L. A. Hayman, S. A. Berman, C. Sonne. Baylor College of Medicine, Houston, TX 77030 and Harvard Medical School, Boston, MA 02115

The goal of this project was to create a set of "user friendly" schematics of the brainstem and spinal cord utilizing the rules derived from information design theory. [1,2] These charts are designed to enrich teaching of neuroscience by providing a link between the disciplines of neurology, anatomy and radiology. The format is oriented to match the imaging procedure (myelography and computed imaging), rather than the "upside-down" format of other sources. One poster color-codes structures needed for movement. It illustrates the nuclei and fibers which mediate coordination and individual muscle groups (e.g., eyes; body; face). The companion chart shows the structures needed for different types of sensation. This allows the reader to easily find the areas which must be scrutinized in patients with specific motor or sensory loss.

1. Tufte ER. Envisioning Information. Cheshire, CT: Graphics Press, 1990. 2. Tufte ER. The Visual Display of Quantitative Information. Cheshire, CT: Graphics Press, 1983.

98.16

THE ANIMATED BRAIN: A MULTIMEDIA BIOPSYCHOLOGY TEXT. T.J. Voneida*, Neurobiology Department, N.E. Ohio Universities College of Medicine, Rootstown, OH 44272, and R. Vardaris, Psychology Department, Kent State University, Kent, OH 44242.

This computerized courseware is designed for those interested in relating the basic functional anatomy of the human central nervous system to behavior. It is complementary to the "Graphic Brain - Neuroanatomy" (Voneida, '91), with greater emphasis on behavior, and less on neuroanatomical detail. It is particularly well suited for undergraduate psychology and related courses in paramedical, nursing, occupational and physical therapy programs. Fully animated, interactive graphics are correlated with text (audio and on screen visual).

Introductory chapters relate to historical landmarks in neurobiology, methods of study, neuroembryology, development of behavior, and basic neurophysiology , all of which are graphically animated. Each sensory system is introduced by a video sequence related to that particular modality. The video fades to animated graphics in which sensory impulses move from receptor into peripheral nerve, then throughout their central trajectory, with synapses illustrated by colored flashes. Motor pathways also begin with videos of behavior (concert pianist; runner), fading to motor cortex and pyramidal cells. Descending tracts are traced to terminations on ventral horn interneurons and motor neurons. Motor impulses are then followed graphically to effectors. Cerebellum and basal ganglia are presented in a similar fashion, with emphasis on their modulatroy role. Hypothalamus, limbic and autonomic systems are discussed in terms of their role in homeostasis and emotional behavior. Cortical function, including language and memory, are related to cortical localization, cytoarchitectonics and interhemispheric activity. Final chapters deal with consciousness, cognition and cortical malfunction.

Graphics and text related to functional neuroanatomy are accompanied by animated presentations of perceptual phenomena and other psychological correlates of CNS function.

98.17

VIDEO PRESENTATION AND CLINICAL CASE DISCUSSION IN NEURO-ANATOMY LABORATORY SESSIONS. S.S. Palmer* and E.O. Emenike. Dept. of Physiol./Neurosc., St. George's Univ. School of Medicine, Grenada, West Indies.

Lab sessions in Neuroscience at St. George's Univ. School of Medicine are composed of three parts. Topics in the course range from intracellular signalling to neurologic disease processes. There are 72 lectures including six near finals on clinical cases. There are 10 lab sessions.

Each lab begins with a 20 minute video showing the structures to be identified. One hour is spent viewing brains and models. The last 30 minutes of the 2-hour session is spent discussing a clinical case related to the structures identified. For example, the first lab covers brain arteries and the case is a stroke of the middle cerebral artery. Students immediately see the importance of learning the structures they are required to know by learning how physicians make a diagnosis based on signs and symptoms caused by dysfunctions of particular brain nuclei and tracts.

Students have indicated the value they place on both the video presentations and the clinical case discussions for learning neuroanatomical structures and neuroscience in general. Results of a survey of 200 students will be presented. A handout indicating the composition of each lab and the cases discussed will be given to interested neuroscientists.

98.18

CONNECTING NEUROSCIENCE AND THE ARTS: A CREATIVE PROJECT IN THE NEUROSCIENCE CLASSROOM. B.L. Mania-Farnell*. Department of Biological Sciences, Purdue University Calumet, Hammond, IN 46323.

Science students associate science with logical procedures and critical thinking. They do not necessarily associate science with creativity or an artistic aspect. Successful scientists however, develop both of these qualities. Creative thinking often leads to innovative discoveries. Artistic skills are used to enhance presentation of data, with critical points brought out in color slides, videotapes, and computer graphics. The artistic aspects of science becomes even more important as scientists try to communicate complex ideas to the public.

In order to encourage students in Neuroscience 525, an introductory course for upper level undergraduates and master's candidates, to appreciate the creative/artistic aspects of science, a creative project was incorporated into the course. Students were asked to write and present a paper on a neuroscience related topic of their choice. Additionally, they were asked to incorporate a creative form of expression into their presentation. The form of expression was left open, with the possibility of using poetry, painting, dramatic interpretation, or any other media. The inclusion of a creative project prompted the students to examine their topic from another perspective and provided them with the opportunity to integrate science and the arts. Overall, the addition of a creative aspect stimulated student interest and enthusiasm and appeared to increase student efforts in preparing their presentations.

98.19

TEACHING NEUROSCIENCE PRINCIPLES IN A COMBINED ON-SITE AND DISTANCE EDUCATION ENVIRONMENT. W. R. Klemm*, Dept. VAPH, Mail Stop 4458, Texas A&M University, College Station, TX 77843.

In a 3-credit-hour course for graduate students and undergraduate Honors students, lectures (1-2 hours) were presented every Monday in a two-way televideo studio. Throughout the week, the 15 on-site students and three off-site students worked as three teams in a hypertext workspace environment called FORUM™. The text was *Understanding Neuroscience* (Mosby, 1995), which seemed ideal for stimulating insights and debate, because it presents a "bare bones" summary of 80 core principles of neuroscience. By Wednesday noon of each week, each student submitted in FORUM two insightful questions about each week's reading, accompanied by answers (Q&As). Students used in-context hypertext links to critique each other's Q&As, with the aim of perfecting one Q&A that they would submit as the group's "Best Q&A of the Week." Students were graded individually on Q&As and as a group on their "Best Q&A." Students graded each other on the quality of Q&As and on group helping behavior. The final exam was drawn from open-ended study questions, which were first group answered in FORUM and certified by me when acceptable.

This report presents examples of student Q&As, participation statistics, and summary of student opinionnaires. I enjoyed teaching this way and found it more refreshing than my traditional lecturing approach. Students were much more engaged in learning, and they benefitted from the diverse perspectives and insights of fellow students.

TEACHING OF NEUROSCIENCE: CURRICULAR INNOVATIONS

99.1

MEDICAL NEUROSCIENCE; INTEGRATION OF BASIC AND CLINICAL SCIENCE, PHYSICAL EXAMINATION, AND PROBLEM-SOLVING IN A TOTAL IMMERSION FORMAT. J.C. Pearson† and J.B. Dean*. Depts. of Anatomy† and Physiology & Biophysics, Wright State University, School of Medicine, Dayton, OH 45435.

Our objectives were to develop a medical neuroscience course that: is oriented towards students entering primary care residencies (~ 65% of each class); integrates basic and clinical disciplines with introductory physical examination instruction; and, develops collaborative learning and problem-solving skills. The total immersion course meets every day (~ 5 hrs. /day) for four weeks at the end of Year I. Learning experiences include: integrated lectures that introduce basic material in a case-oriented format; laboratories in which clinical scenarios are discussed while examining traditional and computer interactive lab material (see Breyley et al., this session) in small groups; case problem-solving sessions that involve group discussion followed by presentation to peer groups of 16-20 students; clinical workshops that emphasize basic science mechanisms; and, neurologic examination sessions consisting of plenary presentations of examination technique followed by individual instruction and small group practice. Weekly multiple choice examinations (80% of final grade) test content from all sessions including lab. Short quizzes given in problem-solving sessions and clinical workshops reinforce creative thinking (10%). Physical exam skills are tested using an Objective Structured Clinical Examination (OSCE) administered at the end of the course (10%). Students overwhelmingly agreed that clinical workshops (96% agreement), case problem-solving sessions (94%), and neurologic exam sessions (86%) successfully integrated basic science principles and clinical material.

99.2

INTERACTIVE SOFTWARE DEVELOPMENT FOR INTEGRATED MEDICAL NEUROSCIENCE EDUCATION. G. Breyley†, S.I. Barnett, and J.C. Pearson*. Interdisciplinary Teaching Lab.† and Dept. of Anatomy, Wright State University., School of Medicine, Dayton, OH 45435.

In the integrated Medical Neuroscience course at WSU (see Pearson and Dean, this session), students have identified two areas in which they require particular help. One is learning functional neuroanatomical pathways, and the other is characterizing neurologic disorders. To help the students with these tasks, we have developed two interactive software applications: (1) "TractFinder", designed to introduce students to neuroanatomical pathway study; and (2) the "VideoClip Library of Neurologic Disorders", which is a compilation of quicktime movies of neurologic disorders most frequently encountered by the primary care physician. TractFinder incorporates myelin-stained central nervous system sections and two-dimensional pathway tracings to allow students to display individual somatosensory pathways. Raw media for this program was acquired from 35mm color slides and enhanced with Adobe Photoshop 3.5. TractFinder was created with Macromedia Director 4.0. The VideoClip Library was created with Apple Media Tool 2.0 to help students identify signs of motor and mentation disorders. Hypertext links to video clips of specific phenomena allow for easy identification. These clips were digitized from the University of Washington's Motor and Mentation videodiscs and the Slice of Brain I videodisc using Adobe Premiere. Evaluation of both programs will occur during the four-week neuroscience course.

99.3

PERFORMANCE-BASED ASSESSMENT OF NEUROLOGICAL PROBLEM SOLVING FOR SECOND YEAR MEDICAL STUDENTS. K.L. Lovell,*B. Mavis, K. Ogle. Depts. of Pathology and Family Practice and Office of Medical Education, Research and Development. Michigan State University, E. Lansing, MI 48823.

A performance-based assessment format was developed to evaluate the ability of second year medical students to apply clinical neuroanatomy concepts in localization of lesions and a focussed neurologic examination. The objective was to create a low-cost, participatory experience that would involve integration of clinical skills neurologic exam and basic science neuroscience concepts, especially localization of lesions and neuropathology. The format included three stations: a computer-based case with six questions, a neurologic exam on a simulated patient (a classmate simulated the patient presented in the computer case), and a write-up of assessment and plan. Two cases were created, with half of the class acting as "doctor" and half acting as "patient" for each case. One case was designed to be more difficult in order to test different levels of problem-solving. The evaluation was not part of a course grade, but students were provided with feedback on their performance. Conclusions from quantitative scoring and qualitative feedback from students include the following: (1) even though all students had just finished courses covering the basic science concepts and clinical skills, many students had a great deal of trouble with integration and application of neurologic concepts; (2) students gave high ratings to the experience as practice in application of concepts in a simulated patient encounter; (3) acting as the "patient" as well as the "doctor" provided a learning experience. This type of problem-based assessment will be modified for the future based on results and feedback, and will be conducted several times during the second year.

99.4

TEACHING NEUROSCIENCE AT THE UNIVERSITY OF NEW MEXICO SCHOOL OF MEDICINE: A COMBINED PROBLEM-BASED AND TRADITIONAL APPROACH. L.C. Saland*, D.D.Savage,L.D.Partridge, N.I.Perrone-Bizzozero, R.G.Franchini, and K.J.Fiedler, Depts. of Anatomy, Pharmacology, Physiology, Biochemistry, Psychiatry, and Neurology, Univ. of New Mexico Sch. Med., Albuquerque, NM.

The medical curriculum at the University of New Mexico School of Medicine has undergone significant change in teaching methods since 1993. Neuroscience in the first year of medical education now combines basic science topics and clinical skills with a patient case-based set of tutorial interactions, in a 9 week period. All medical students work in tutorial groups with two tutors, generally one basic scientist and one clinician, both neuroscience experts. Cases studied in tutorials reflect both neurologic and psychiatric problems which are designed to address specific important basic science concepts as well as clinical issues. Lectures, laboratories, and clinical skills sessions run concurrently with tutorials. A concerted effort has been made to minimize added time commitments of involved faculty. The new teaching format requires cooperation on an ongoing basis among basic science and clinical faculty at a level not generally attained prior to instituting this curriculum.

99.5

EARLY PARTICIPATION OF MEDICAL STUDENTS IN BASIC RESEARCH AND PROBLEM BASED LEARNING. X. García*, S. Morales, A. Hamabata, E. Piña, M. A. Lastíri, and E. Gijón. School of Medicine, Research Center and Educational Services CISE, UNAM, and Research Center of Advance Studies, CINVESTAV, IPN. México D. F. 04510, México.

PAEA is an institutional program of high academic demand at National Autonomous University of México. In the school of medicine it was started a change in teaching method for medical students. Students with higher scores were selected in each year promotion from a diagnostic examination, to form the kernels of educational quality formerly called NUCE (Núcleos de Calidad Educativa) as part of the high academic demand program PAEA (Programa de Alta Exigencia Académica). Problem based learning was introduced replacing traditional teaching in 25% of the available time. Early participation in basic research has been implanted with a model of two students tutored by a researcher. Partial results show that this student-centered active learning leaded the students to follow scientific methodologies for conducting supervised experimentation, capturing data, analyzing results and writing reports that were adequate for specialized meetings and publication, about interest topics for health sciences knowledge and it also prepared them for postgraduate studies.

99.6

CLINICAL CORRELATION POSTER PRESENTATIONS IN MEDICAL NEUROBIOLOGY. H. F. Martin III*
Dept. of Physiology, The Medical Univ. of South Carolina, Charleston, SC 29425

Near the end of the Medical Neurobiology course, students are asked to choose a neurological disease to investigate. This year, instead of a written paper, the presentation was in the form of a poster session. The assignment had two parts: 1) a disease or disorder was chosen, and a "patient scenario" was prepared which typified the disorder (to include symptoms, history, physical examination findings, lab tests, etc.); and 2) a poster presentation was prepared to discuss the disorder.

The aim of the discussion was to explain to the patient and/or patient's family what is happening to the normal structure and function of the brain as a result of the disease. Students were expected to explain why the particular symptoms resulted from the disorder, what was the prognosis for recovery or progression, and how treatment was intended torestore function.

Directing the explaination to the patient was intended to stress the role of patient education in medical practice. Presenting the results in the poster format allowed the students to learn from the other student projects as well as their own.

99.7

ANNUAL SYMPOSIUM ON CRANIAL NERVES: HAVING FUN WITH NEUROANATOMY. J.L. Culberson*. Department of Anatomy, West Virginia University Health Sciences Center, Morgantown, WV 26506.

As careers in health sciences expand, more students are studying structural biology, including Neuroanatomy. Physical therapists in particular have a substantial interest in basic neurology. Many undergraduates with modest scientific preparation now enroll in specialized biomedical curricula where even academically talented students may be intimidated (or overwhelmed) by the content and pace. While teaching a brief course in Functional Neuroanatomy for 22 years, I worried that some of these very bright folks (and I) might benefit from an occasional diversion from the usual instructional paradigm, and so introduced student presentations in the course.

Each year now we hold a cranial nerve symposium. Small groups of students are assigned one nerve (or a group of related nerves). They prepare a 15-20 minute presentation which is to be both instructive and entertaining. Student work generated in this small project exceeded my expectations from the outset and continues to be outstanding. It has sufficient notoriety that other faculty and older students return for an "annual review" of cranial nerves. Students do usually achieve the most important objective, the identification of those features of a nerve that physical therapists need to know. This often differs from what a neurology-biased anatomist would have emphasized. Beyond content 'though, the entertainment aspect of the symposium has exposed a degree of talent and creativity that we seldom recognize in our students. We now enjoy original poetry, songs (lyrics and music), original artwork, videos (both seriously documentary and outrageously funny) and most recently, a wide range of game shows. This poster will share some of this student material in the interest of stimulating other course planners to look for ways to increase student participation in and enthusiasm for their own learning.

99.8

AN EASY WAY TO LEARN THE NAMES OF GYRI (AND SULCI) OF THE HUMAN BRAIN. Glenn H. Kageyama*, Department of Biological Sciences, California State Polytechnic University, Pomona, CA 91768; Department of Anatomy & Neurobiology, College of Medicine, Univ. Calif., Irvine, CA 92717.

Learning the names of all the major gyri and sulci of the human brain can be a tedious undertaking by medical students. An easy system for learning these names has been used successfully at Cal Poly and at the UCI College of Medicine. After first identifying the landmarks that delineate the 5 lobes of the brain (including limbic), all other structures are learned by presenting them as structurally related sets of three. For example three vertical lines can be used to indicate the (1) precentral, (2) central and (3) postcentral sulci; these sulci delineate the vertical (1) precentral and (2) postcentral gyri, which are linked together medially by the (3) paracentral lobule. The temporal and anterior part of the frontal lobes are presented together, because each can be divided into (1) superior, (2) middle and (3) inferior (temporal or frontal) gyri. The parietal lobe is divided into the (1) superior parietal lobule and the (2) supramarginal and (3) angular gyri. Lastly the occipital lobe, when viewed from a posterior position can be divided into the (1) lateral occipital gyri, (2) the cuneus and (3) lingual gyrus. Cerebral structures can also be presented in sets of three in basal; and medial views of the brain. One advantage that this simple method provides is that within a very short period of about 30+ minutes, undergraduates and Medical students can easily master the names of all of the major gyri and sulci of the human brain. Further reinforcement is provided by superimposing the (1) functional organization of the brain (cortical areas), and (2) the blood supply to the brain, and by reviewing the (3) functional deficits that would be produced by lesions in different parts of the brain. Color-coded illustrations will be presented. Support: Cal Poly Pomona RSCA Grant #1-11496 (Kageyama), & NIH Grants: DC 00450 & NS 30109 (RT Robertson & GH Kageyama).

99.9

HEMISPATIAL NEGLECT: CASE STUDIES EMPHASIZING MECHANISMS OF ATTENTION AND SENSORIMOTOR INTEGRATION.
Dr. Joseph J. Warner* Dept. of Neuroscience, University of Florida College of Medicine. Hemispatial Neglect has been defined as the inability to recognize, report, or respond to stimuli from one side of space. (Heilman). Early studies associated unilateral neglect with lesions of the right parietal lobe. Hemispatial neglect has since been reported with other right hemispheric cortical and subcortical lesions. Analysis of hemispatial neglect has focused on neural mechanisms of attention and sensorimotor activity, and on the association of hemispatial neglect with right hemispheric lesions. Animal and human studies have demonstrated unilateral neglect with lesions of the mesencephalic reticular formation, superior colliculus, thalamus, basal ganglia, internal capsule, and multiple cortical regions, and have led to the concept of the "corticalization" of attentional mechanisms with phylogenetic advancement. Neuropsychological and physiologic studies suggest right hemispheric dominance for attentional direction. Studies of cortical and subcortical systems in primates have provided a foundation for models of cerebral mechanisms for attention and hemispatial exploration. The analysis of hemispatial neglect thus extends from nonspecific arousal and specific afferent systems through assessment of "stimulus significance," modulation of afferent transmission, sensorimotor integration, and response generation. Clinical cases, including neuro-behavioural testing, C.T. and/or M.R.I. studies, and neuropathologic findings, are presented emphasizing systems organization and structure-function correlations. This format is an effective approach to the neuroanatomic and physiologic aspects of systems organization, including perspectives of basic and clinical neuroscience, for students in medical neuroscience as well as for residents in neurology, neurosurgery, and psychiatry.

99.10

TEACHING ETHICS TO RESEARCHERS: PROVIDING INSTRUCTION AND ASSESSING COMPETENCY. B.A. Fischer and M.J. Zigmond*. Survival Skills and Ethics Program, University of Pittsburgh, Pittsburgh, PA 15260.

We believe that responsible conduct is an integral part of "doing science" and have developed a model that reflects this philosophy. Graduate students in the Dept. of Neuroscience receive instruction in *ethical sensitivity* (being able to identify ethical issues and the norms of their field) and *moral reasoning* (a problem-solving approach). Instruction in ethics is integrated throughout both the required science courses and workshops on "survival skills" (professional development). For example, in the neurochemistry course students discuss ethical issues related to conflict of interest in drug testing, and in the workshop on writing, topics such as authorship and integrity in graphics are addressed. Thus, students are sensitized to many of the ethical dimensions of their work. Based on the method of Bebeau, et al (1995), students also receive formal instruction in moral reasoning. This provides individuals with a tool for evaluating and resolving ethical dilemmas, including novel situations as might arise following the development of new technology. Furthermore, using Bebeau's method we systematically measure a student's ability to develop a well-reasoned response to an ethics case. *Competency* in this skill is now measured as part of the comprehensive exam. We believe that our model has several advantages over more conventional approaches: (1) Training in ethics occurs over 2-3 years rather than a few weeks or months. (2) Senior scientists who can act as role models provide most of the instruction. (3) Material is included that typically is not covered in more traditional courses. (4) Students learn tools for tackling yet undiscovered ethical issues. And, perhaps most importantly, we feel such a program (5) conveys to students that responsible conduct is an essential part of being a researcher. (Support: NSF, IBN 94-13145; NIMH, MH18273; Univ. Pittsburgh.)

99.11

A SUPPORT PROGRAM OF INSTITUTIONAL IMPROVEMENT OF TEACHING. E. Gijón*, M. A. Lastiri, X. García, and L. Cartas. Sch. of Medicine, Sch. of Psychology, and Research Center and Educational Services CISE, UNAM. México D. F. 04510, México.

PAPIME is an institutional program that induces changes toward a teaching improvement. The main goal of this program is to improve teaching quality in National Autonomous University of México. The aim of this work is to present the progress of this program at the School of Psychology, within the scope of our project teaching strategies of neuroscience and basic processes and implantation of new technologies.Our project is devoted to solve some of the problems that we found in the field of the education in Psychology and Medicine, especially in neuroscience, working along with teachers of the subjects that are imparted in the first three years of the licentiate in Psychology. The project was organized in two stages. In the first one we organized a workshop of teaching methodologies centered in contents for teachers in order to structure didactically their teaching units and also a workshop for using authorship tools, such as HyperCard, Digital Chisel for Macintosh platform and ToolBook for PC platform. In a second stage teachers are elaborating specific didactic resources to topics of their programs in a first prototype level in paper and they are developing didactic resource prototypes by computer.

99.12

MEDICAL NEUROANATOMY: RELATING STRUCTURE AND FUNCTION. Edwin S. Purcell*[1] and Ann S. Stano[2]. [1] Department of Anatomy, University of Osteopathic Medicine and Health Sciences, Des Moines Iowa 50312; [2] Lake Erie College of Osteopathic Medicine, Erie, PA 16509.

At Lake Erie College of Osteopathic Medicine (LECOM) neuroscience is presented in a holistic system approach combining anatomy, biochemistry, physiology, pharmacology, pathology and neurology. While this format intrinsically provides clinical correlation for basic science material, in 1996, the neuroanatomical component was specifically designed to emphasize the relationship of structure and function in a clinical context. The rationale was that information is more readily assimilated and retained if its relevance to medical practice is clear. Further, this approach provides a way to reinforce overlap between disciplines. Specific aspects of the course design included: sequential presentation of material in a hierarchical manner, assignments in the atlas to correlate with each lecture, full scale "mock practicals" with each laboratory and small group analysis of clinical scenarios which were presented to the entire class. Examinations mirrored the course content with 50% of lecture based questions relating structure and function and a full 50% of the laboratory questions being second order. We are currently evaluating the efficacy of this approach to the teaching of neuroanatomy. The initial response from the students was very positive. Among the factors we will examine is the performance of this class on part 1 of the medical licensing examinations in 1997.

99.13

NEUROSCIENCE FOR NURSE ANESTHETISTS. D.D. Rigamonti*, J.W. Tsao and H.J. Bryant. Uniformed Services University of the Health Sciences, Bethesda, MD 20814-4799.

Neuroscience courses have become commonplace in medical and graduate school programs but are not usually part of nurse anesthetist's programs. The Council on Accreditation for Nurse Anesthetists outlines a variety of requirements related to the nervous system that students must master for successful administration of anesthesia. To meet these requirements, we have designed a unique course taught by a diversified clinical and basic science faculty drawn from the School of Nursing, the School of Medicine, affiliated hospitals and federal agencies.

The course is taught in the summer and fall semesters. The summer semester provides a review of basic neurophysiological concepts and an examination of gross central nervous system structures. This portion runs concurrently with a separate human anatomy dissection course. The fall semester provides an in depth review of neurophysiological concepts related to peripheral nerves, spinal cord segments and brain, such as, compound action potentials, myotatic delay and averaged evoked potentials. These principles are demonstrated in laboratories utilizing human subjects and computer assisted data acquisition. In addition, lectures, clinical correlations and computer aided instruction are routinely used to teach central nervous system organization. Great emphasis is placed on the understanding of peripheral and central mechanisms of local and general anesthetics and their affects on the nociceptive system

Thoroughout the course of instruction, individual students are assigned to each session to lead discussion with visiting faculty and student study groups.

This course has been offered for the last two years and based on student surveys has been successful in effectively communicating principles of neuroscience necessary for clinical practice.

99.14

TEACHING NEUROSCIENCE WHEN THERE'S NO FORMAL NEUROSCIENCE AT A COMPREHENSIVE I INSTITUTION. M.C. Zrull*, R.E. Bowman, and A.J. Trivette. Department of Psychology, Appalachian State University, Boone, NC 28608.

Appalachian State University (ASU) is a Comprehensive I university offering bachelors and masters degrees in a variety of traditional disciplines. Neither an undergraduate nor a graduate degree in neuroscience is offered. Unlike many of the programs described in previous Teaching of Neuroscience posters, ASU does not offer a minor, a concentration, or even a course in neuroscience. Yet, both undergraduate and graduate students in the Department of Psychology can gain a respectable background in neuroscience. This poster is presented because we don't believe our situation is unique, we believe existing flexibility in degree programs can be used to teach foundations of neuroscience, and we are interested in suggestions to improve our approach.

At ASU the B.S. in psychology does not require a minor. Rather, an interdisciplinary concentration is required. Through careful advising a set of courses from psychology, biology, exercise physiology, etc. can be combined to provide a classroom foundation for future study in neuroscience. The M.A. in general psychology offers similar flexibility after a core courses. The program follows a mentorship model usually found in doctoral programs and requires "selected topics" courses. Courses covering topics like behavioral or cognitive neuroscience, endocrinology, neuroethology, etc. are possible. Our approach to laboratory experience is important. Students are integrated into ongoing research projects and the mentorship model is extended to the graduate-undergraduate relationship. While experiments may combine to reach the faculty member's goal, each student in our lab is given enough of a role in one (or more) experiment(s) that he/she can present the work in an appropriate setting. Our hope is to present our current model and to learn about ways to enhance our programs. Some of the presented activities were partially supported by NIH grant DC02476 and Dept. of Ed. grant P217A50512.

99.15

USING WRITING TO TEACH NEUROSCIENCE TO NON-SCIENCE MAJORS. Peter A. Pawson*. Dep't of Biology, Utica College of Syracuse University, Utica, NY 13502.

I teach Introductory Neuroscience to sophomore non-science majors (OT and PT majors) at a small liberal arts college. As part of a college-wide effort to increase writing across the curriculum, I have developed a "Writing-Intensive" course that incorporates writing-to-communicate (a formal term paper and essay exams) and writing-to-learn (in-class and out-of-class informal writing assignments) into its curriculum. The term paper is designed to familiarize students with the primary Neuroscience literature and the process of writing a scientific paper, as well as to give them an opportunity for an in-depth study of some aspect of the Neurosciences that interests them. Several elements contribute to the success of the paper writing project: students are given a detailed guide to the mechanics of writing a scientific paper; the paper involves a multiple revision process, including peer reviews. The ungraded, informal writing assignments are designed to allow students to use writing to introduce themselves to topics and to formulate their own connection with the material. These writing elements have produced more sophisticated term papers, demonstrating an increase in both student comprehension and overall science literacy. (Supported by a grant from Utica College).

99.16

Mad Cow Disease: A Topic for Undergraduate Neuroscience

C. A. Paul and B. S. Beltz*; Wellesley College, Wellesley, MA 02181

The recent crisis in England over Mad Cow Disease (MCD) has caused a panic among the English meat eating population and British farmers. MCD results in bovine spongiform encephalopathy (BSE) and is possibly linked to the human neurodegenerative disease, Creutzfeldt-Jakob disease (CJD). The connections between these diseases have been suspected for the last 5 years, however no action was taken until a recent report indicated a rapid rise of CJD in young people. There are many questions arising from how this affair was handled by the government.

This multi-faceted problem provides an opportunity for teaching undergraduates the inter-relationships between health issues, basic science, economics and politics. Most of our students will not become neuroscientists. However, our expectation is that all students will be educated, scientifically literate citizens. Whether they become scientists, politicians or journalists all students need to acquire skills to address complex issues such as MCD.

This poster will focus on:

History and Epidemiology - the possible origins of MCD from the sheep disease scrapie; the human disease kuru; spontaneous mutations leading to low levels of the disease; and the transmission of CJD between humans due to tissues extracted from CJD infected cadavers.

Biology - explanation of the infectious nature of a prion, an infectious protein; spontaneous mutations of a gene which lead to production of this protein.

Pathology - how does the dementia arise, and what are symptoms of CJD?

Politics - how should public policy be determined?

Economics - how can the need for profit be balanced against scientific and medical concerns?

A series of questions will be included to facilitate discussion on this topic.

99.17

INCORPORATING SELF-PACED LABORATORY, GRANT WRITING AND SERVICE LEARNING COMPONENTS INTO A BEHAVIORAL ENDOCRINOLOGY SEMINAR. C. A. Frye*. Neuroscience Laboratories, Depts. Psychology & Zoology, Connecticut College, New London, CT 06320.

Independent reports indicate that self-paced laboratories (Jacobs, 1980), grant writing (McCormick, 1995) and service learning (Kelland, 1995) are effective tools which bring students closer to the material. These pedagogical techniques were utilized in conjunction with a seminar in Behavioral Endocrinology. Briefly, 8 seniors chose topics within the field of Behavioral Endocrinology. In the first part of the semester, each student led one weekly seminar meeting. Later in the semester, students wrote mock grant proposal, complete with methods, budget, etc., on their selected topic. One or two grants were then presented weekly and critiqued in a model grant review session in which the class served as the panel. Each student acted as primary and secondary reviewers on another grant proposal. Students reported that they found these reviews extremely valuable for improving the final quality of their papers. Throughout the semester, at each seminar meeting, students were informed of the weekly schedule of events that were occurring in the behavioral endocrinology research laboratory. Students were encouraged to participate in the events that were of interest to them. Students reported that this informal laboratory component facilitated their learning of "hand-on" aspects of how to answer their research questions. Students reported that this experience was invaluable and necessary to gain a coherent understanding of the research papers they had read and to facilitate the methodological component of their grants. Students' participation necessitated substantial commitment of those currently working in the Behavioral Endocrinology Research Laboratory. Thus, seminar students were required to perform a 1-2 hour service learning task. Students found the service learning component so valuable that they exceeded course requirements by providing a 3 hour seminar at a nearby high-school during *Brain Awareness Week*. Students' feedback indicate the informal laboratory, grant writing and service learning were excellent and complimentary additions to the seminar. *Supported by NSF CAREER grant 9514463*

99.18

EXPLORING NEUROSCIENCE THROUGH SENSORY SYSTEMS, A SHORT COURSE AT THE ADVANCED HIGH SCHOOL OR UNDERGRADUATE LEVEL. C.L. Miller* Brandies University, Norman S. And Eleanor E. Rabb School of Summer, Special and Continuing Studies, P.O. Box 9110, Waltham, MA 02254-9110.

This course was designed to teach basic neuroscience concepts using sensory systems. Interactive teaching methods such as hand-on demonstrations, and in-class experiments help the students learn about the basics of neuroscience by studying their own senses. The course is taught in a semi-flexible format that allows students to direct the lecture by expressing what topics are most interesting to them, and providing questions for the class to address.

By introducing a neuroscience course at the high school or introductory college level common misconceptions about the field of neuroscience, such as "all Neuroscientists are brain surgeons" can be dispelled. By introducing the topic of neuroscience in the context of sensory systems, students feel comfortable approaching this difficult discipline, because they can relate what they have learned to their own sensory experiences.

The course content includes: An introduction to nerve cells and how they communicate, specialized human sensory receptors and their structures, sensory information processing, sensory perception, and minimal neuroanatomy.

99.19

BrainLink®: TEACHING NEUROSCIENCE TO ELEMENTARY AND MIDDLE SCHOOL STUDENTS. J.H. Dresden*, N.P. Moreno and B.Z. Tharp. Baylor College of Medicine, Houston, TX 77030.

Scientists, clinicians and educators at Baylor College of Medicine have worked together to create instructional materials for teaching neuroscience concepts to elementary and middle school children. (Some activities can be used at the high school level.) After three years of development, review and field testing, the *BrainLink* project is now in its third year of national dissemination of the materials and workshops in their use. Both development and dissemination activities have been supported in part by the National Institutes of Health, Science Education Partnership Awards program.

The *BrainLink* materials are ready-to-use and are designed to promote the fun and excitement of "doing" science in schools, museums or community settings, or at home. Topical units include Brain Comparisons, Motor Highways, Sensory Systems and Memory & Learning. Each unit contains three interrelated components: a guide to classroom activities, an adventure storybook and a mini-magazine that can be used with groups or at home for parent and community involvement.

BrainLink materials provide a unique vehicle for use by scientists in outreach programs with students, as well as for enrichment of the science curriculum in schools. Neuroscience content is presented in ways that make it accessible to laypersons and to children. Regional dissemination centers providing information, materials and workshops for assistance in using *BrainLink* are located at Baylor College of Medicine, Boston University School of Medicine, the University of Kentucky College of Medicine and the University of California, San Francisco. Evaluation research findings will be presented, along with a display of the materials and their use.

99.20

INTEGRATION OF SCIENCE DISCIPLINES IN A HIGH SCHOOL SCIENCE PROGRAM THROUGH NEUROSCIENCE RESEARCH. R. E. Landsman*, L. I. Perrotti, D. M. Niedosik and D. DeWitt. Science Dept., Academy for the Advancement of Science and Technology, Hackensack, NJ 07601.

Over the past four years, our new high school has focused on the development of an integrated interdisciplinary science curriculum in which, annually, students simultaneously learn biology, chemistry, physics and mathematics, and many are engaged in research in our in-house scientific research program. The research projects focus on state-of-the-art neuroscience studies in behavioral toxicology (Prabhakar and Landsman, *Soc. Neurosci. Abst.* 20, #159.16, 1994), neural control of behavior (Lin, Gupta, Perrotti, and Landsman, *Soc. Neurosci. Abst.* 22, in press, 1996), neuroendocrinology and neuroimmunology.

The goal of our research program is to use neuroscience research as a method for demonstrating to the student the need for interdisciplinary understanding of biological phenomena as well as the need to use the scientific method. This program 1) prepares students for the recent upsurge in undergraduate neuroscience programs, 2) addresses the need for change in the present secondary educational system regarding writing ability and critical thinking skills, 3) infuses technology into education, 4) addresses the need to instill in students ethical values and practices in the use of the scientific method, and 5) stimulates interest across all sciences through the use of interdisciplinary techniques.

Funded by the Bergen County Technical Schools' Board of Education.

99.21

ELEMENTARY SCHOOL CHILDREN USE THE INTERNET TO SHARE THOUGHTS ABOUT A DAY IN THE LIFE OF A NEUROSCIENTIST. D. L. Colbern* and L. M. Bargabus. Brain-Exchange Electronic Mentorship Network, Venice, CA 90291, and UCLA Brain Research Institute, Los Angeles, CA 90095

An important and current trend in education is to treat science as synonymous with "critical thinking" and to engage students in curriculum where career awareness is emphasized and science and technology are portrayed as forces likely to impact the future. BEEMNET, the Brain-Exchange Electronic Mentorship Network, is a research-oriented, educational organization that uses the World Wide Web to link elementary school children and their teachers with practicing neuroscientists. An important goal of the network is to provide and support mentorship activities between children, teachers, and neuroscientists; the use of information technology enables access to resources for all children.

We asked 965 children (grades 3-6) in nine urban and rural elementary schools across the United States (California, Colorado, Illinois, Tennessee, and New York) to "write a story about a day in the life of a neuroscientist" from the time that person wakes up until the time they go to sleep. Children were told only that "a neuroscientist is a person who studies the brain and nervous system." The resulting essays provided clear evidence of the baseline level of knowledge children have regarding the enterprise of neuroscience. Selected stories were published on BEEMNET where neuroscientists could use various aspects of the children's stories to help communicate real life as a scientist. Neuroscientists learned what perceptions children, and perhaps the public, hold regarding their profession. Stories and drawings can be found on the World Wide Web at http://www.beemnet.com/. *Supported by NIDA grant DA 10059-01.*

99.22

FROM BALLOONS TO BRAINS: SCIENCE FOR THE ELEMENTARY GRADES. J.M. Kerns*, J. Harrington, M. Blenz and K. Botticelli. Dept. Anatomy, Rush Medical College, Chicago; Lincoln School, District 97, Oak Park, IL. 60612.

The Global Village Project of Oak Park School District 97 has for five years formed an educational partnership between working scientists and students in grades 3 - 8, designed to spark interest and excitement in science . Thirty-two volunteer scientists from 14 different area institutions establish interactions with the teachers and students from ten grade schools. These hands-on learning experiences consist of 5-6 classroom visits, two field experiences in a working environment, and a Young Scientists Conference, which also includes parents and community members in an interactive workshop setting. Our particular theme involved the use of balloons filled with air, helium and gelatin to demonstrate the following: 1) the use of the scientific method; 2) how neuron shapes influence neuron function; 3) how the brain is protected from injury; 4) what happens to neurons when they are injured; 5) influences affecting the ability of neurons to regenerate and heal themselves. Several experiments were conducted where small student groups pose a scientific problem, apply an experimental method, collect data, form conclusions, and make practical applications. In addition, students used a novel drama setting to act out nerve regeneration. During a 1½ hr tour experience, students worked with microscopes, computers, a photographic darkroom, models and gross brain specimens to see the benefits of viewing the brain in various slices. Lecture material was kept at a minimum, favoring the small group direct learning experiences.

The overall impression of these scientist-teacher-student interactions is that students develop a more accurate view of scientists and a more keen interest in their work by such exploratory and facilitated experiences, independent of the choice of specific content and curricula. Balloons and brains just happen to be a convenient bridge to enhance the love of knowledge, teamwork activity and problem solving skills. *Funding in part due to a grant from the Oak Park Education Foundation.*

100.1

COMPUTER SIMULATION FOR TEACHING THE PHYSIOLOGICAL BASIS OF RESTING MEMBRANE POTENTIAL. J.E. Salceda-Ruanova and E. Soto*. Instituto de Fisiología, Universidad Autónoma de Puebla, México.

An interactive computer simulation for teaching the physiological basis of resting membrane potential is presented. The program was developed using Visual Basic™ and runs under Windows™. It allows the user to record with intracellular electrodes and determine, under controlled conditions, the membrane potential and the equilibrium potential for each ion. The variables controlled by the user are: intra and extracellular concentrations of sodium, potassium and chloride ions, the permeability coefficients for the ionic species considered and the temperature. The program includes a voltage clamp option, which allows the user to impose a membrane potential value and study the resulting driving force for each ion. Concentration-membrane potential and concentration-equilibrium potential curves may be obtained at any time. The simulation has been tested in general physiology courses and has shown to be a useful tool as a complement to the experimental and theoretical study of the ionic basis of membrane potential.

100.2

A MULTIMEDIA COURSE IN BEHAVIOURAL NEUROSCIENCE. J.Cranney*, J. Kehoe, and G. Weidemann, Psychology, University of New South Wales, Sydney, 2052, Australia.

For both ethical and practical reasons, there has been increasing pressure to convert "wet lab" experimental teaching, utilising live animals in the demonstration of principles of behavioural neuroscience, to a "dry lab" format with simulation of animal experiments. In this presentation, three computer simulations will be demonstrated: RabbitLab, DrugLab, and NeuralNetLab. RabbitLab allows a 2nd or 3rd year Neuroscience or Psychology Student to simulate the lesioning of one of a number of different areas in the rabbit brain, and to look at the effects of that lesion on one of a number of different classical conditioning paradigms. The student can then take that data, analyse it, and write up the simulated experiment as an experimental lab report. In DrugLab, students can choose one of many drugs, and test the effects in rats on one of two startle paradigms, startle potentiation and prepulse inhibition. In NeuralNetLab, students interact with a simulated neural network for a simple conditioned reflex, during which some of the basic principles of learning, such as the Hebbian adaptive unit rule. [Supported by a UNSW Discretionary Development Grant]

100.3

DIFFERENTIATION IN PC12 CELLS: A STUDENT LABORATORY IN DEVELOPMENTAL NEUROBIOLOGY. E.M. Adler*. Department of Biology, Williams College, Williamstown, MA 01267

The PC12 rat pheochromocytoma cell line, which resembles immature adrenal chromaffin cells when grown under standard culture conditions and differentiates towards a neuron-like phenotype when grown in the presence of nerve growth factor (NGF) (Greene and Tischler, 1982), provides an ideal model system for introducing students to cell culture and to questions pertaining to cellular and developmental neurobiology. This upper level lab starts with a demonstration of cell culture, following which the students practice sterile technique. They grow cells under various experimental conditions, make morphological observations, harvest and count the cells, and assay them spectrophotometrically for acetylcholinesterase (AChE) activity.

I have used well established properties of this easily grown cell line to illustrate various developmental questions. Students have compared the effects of NGF and dexamethasone on cell morphology to illustrate the influence of environmental factors on differentiation of cells of sympatho-adrenal lineage; have compared the effects of NGF and the adenylate cyclase activator forskolin on neurite development and AChE activity to examine regulation of different traits in response to activation of distinct second messenger pathways; and have compared the effects of different substrata -- collagen and polyethylenimine -- on neurite outgrowth. The laboratory can easily be developed in various directions. As one example: RNA can be extracted and subjected to Northern analysis to determine whether observed changes in AChE activity occur at a pre or post-translational level. Student generated data will be presented.

100.4

CHANGES IN UNDERGRADUATE PHYSIOLOGY LABORATORY INSTRUCTION TO INCORPORATE INVESTIGATIVE LABORATORIES AND TO IMPROVE SCIENTIFIC COMMUNICATION SKILLS. J. K. Ono*, R. A,. Koch, and K. Dickson, Dept. of Biological Science, California State University, Fullerton, CA 92634.

In most undergraduate institutions, a student's first encounter with the neurosciences is through a physiology or psychology course. At California State University, Fullerton (CSUF), the primary physiology course for the Biology major is Mammalian Physiology. The laboratory portion of the course consists of three 3-hour laboratory sections that meet once a week, with a maximum enrollment of 20 students per section. Prior to the Fall, 1995 semester, the laboratory consisted of typical, didactic laboratory exercises with expected outcomes. Implementation of a National Science Foundation Improvement of Laboratory Instrumentation Program award has led to the incorporation of investigative experiments and group presentation of analyzed data. Much of this change has focused on the intensive use of computers for data acquisition, analysis, and group presentations. Data acquisition is carried out through transducers connected to MacLab interfaces and software. Widely used commercial software (Microsoft Office) is used for data analysis, graphing, and presentation graphics. Students work in groups of 3-5 and are assigned a completed experiment to present to their section. The group is responsible for analyzing the data from all 3 sections and for creating a professional presentation in Powerpoint. The presentation is displayed through an LCD projection system in the format used at professional scientific meetings. For most students, this is their first encounter with the intensive use of computers for these functions and we would like to share our experiences to those faced with introducing similar components to their teaching laboratories.

Supported by NSF Improvement of Laboratory Instrumentation Program Award.

100.5

THE SHERLOCK HOLMES LAB: AN INVESTIGATION OF NEUROMUSCULAR PHYSIOLOGY. P.J. Schwartz* and E.M. Adler. Depts. of Biology and Psychology, Williams College, Williamstown, MA 01267.

It is not uncommon for students to perform laboratory exercises mechanically with little real appreciation or understanding of the basic underlying principles. One way around this problem is to engage their interest in some problem, and then actively involve them in designing experiments to solve it. We have developed a laboratory for first year college students that uses this approach as an introduction to the physiology of neuromuscular transmission. The laboratory is presented as an unsolved case investigated by Sherlock Holmes. The basic scenario is that three persons have been found dead from respiratory paralysis. Scotland Yard assumes that all three have been killed with the same toxin and, hence, by the same individual. Sherlock Holmes questions this assumption and bemoans the lack of knowlege of neuromuscular physiology in the late 19th century. Enter your class. Their mission is to determine whether the "toxins" are in fact identical or whether the murders are the work of separate individuals.

Students are provided with the frog sartorius nerve-muscle preparation for experimentation. They are then presented with one of three different solutions that blocks muscular contraction in response to nerve stimulation. We used 0 calcium + EGTA, d-tubocurarine, and lidocaine. Students design experiments to identify the toxin. Each experiment can be used as the basis for an extended discussion on physiology or more extensive experimentation. For example: extracellular recording from the frog sciatic nerve to determine if a toxin blocked action potential propagation led into a classic experiment on nerve properties like conduction velocity and the refractory period.

100.6

LABORATORY EXERCISES IN NEUROBIOLOGY: CUSTOMIZED LAB MANUALS. A.C. Mills*[1], B.R. Johnson[2], and D.U. Silverthorn[3], [1]Middle Tennessee St. U., Murfreesboro, TN 37132, [2]Cornell Univ., Ithaca, NY 14853, [3]Univ. Texas, Austin, TX 78712.

Traditional laboratory manuals are often criticized for being too "cookbook" in nature: not stimulating original thought and experimental design, lacking an emphasis on development of critical thinking skills, and also for not closely fitting the instructor's preferences for the range of exercises offered. We are trying to improve the choice of laboratory exercises for physiology instructors by gathering investigative lab exercises from faculty at colleges and universities around the world, putting the exercises into a uniform format and editing the content. Adopters of the manual can choose from the total selection offered, for a custom-published manual for their laboratory courses, available in 1997.

A variety of exercises is offered, from low to high tech, from computer-based to those using more traditional physiology equipment, from labs appropriate for non-science majors and pre-college students to others more geared toward upper-division biology majors with a strong biology background. Protocols that require the use of live animals as experimental subjects vary in species requirement, from invertebrates to humans, depending on the exercise. The individual lab exercises for neurobiology range from neuronal simulations, to updated traditional exercises such as recording extracellular potentials from the earthworm nerve cord, to integrating whole brain function such as observing the changing accuracy of a motor task by a student wearing prism goggles. Each manual will also include informative chapters on animal use and care, lab safety, experimental design, and science writing.

General physiology exercises are also available. The project is sponsored by Prentice Hall Life Sciences Publishing.

100.7

INTRODUCTION TO ELECTROPHYSIOLOGICAL TECHNIQUES AND PRINCIPLES USING WEAKLY ELECTRIC FISH. M. C. Fields, 21st Century Biology Class, R.D. Fields*. Sidwell Friends School, Washington, D.C. 20016.

The electric organ discharge (EOD) of the weakly electric fish *Eigenmannia* and *Gnathonemus* has been recorded and mapped in three-dimensional space using electrophysiological recording methods. This exercise familiarizes students with the equipment and techniques used by electrophysiologists: preamplifiers, oscilloscopes, electrodes, grounding and shielding, and computer acquisition and analysis of data. Students learn basic neurophysiological concepts including muscle physiology, anatomy and biochemistry, membrane potential, sensory physiology, behavior, and evolution. The fish are available from aquarium stores and are not harmed by the investigation, making this exercise ideal for the high school classroom. The electric dipole created by the fish and the changes that occur when the fish encounter conductive and nonconductive substances are measured and modeled by computer. Students researched the literature and talked with scientists about the ecology, evolution, and behavior of these fishes from South America and Africa. Students developed and refined their own experimental protocols for observing the behavior of the fish, monitored the changes in the EOD in response to social interactions and the presence of predators, and utilized computer software to model and analyze their data. As a final exercise, students wrote a lab book with complete protocols and background information. This poster represents the work performed by these students.

100.8

PROJECT CRAWDAD: A PROGRAM TO PROMOTE USE OF INVERTEBRATES IN UNDERGRADUATE NEUROSCIENCE COURSES. R.A. Wyttenbach, B.R. Johnson, and R.R. Hoy*. Neurobiology and Behavior, Cornell University, Ithaca, NY 14853-2702.

The "Crawdad Project" is a three-year program funded by the National Science Foundation with the aim of promoting the use of invertebrates in undergraduate physiology and neuroscience courses. The program will culminate in the production of a laboratory manual and instructional videotape at the end of the three years. During each year of the program, a workshop for college teachers will be conducted at Cornell University. The purpose of the workshop is twofold: first, to give teachers hands-on experience with invertebrate preparations so they can incorporate them into classes and second, to allow ongoing testing and evaluation of instructional materials in the making. The first workshop took place this June.

The exercises to be presented are inexpensive, easy to prepare, and straightforward for students. They use simple invertebrate preparations to illustrate fundamental processes of all nervous systems. The use of commercially cultured invertebrates reduces cost and administrative overhead as well as potential ethical and environmental objections on the part of students. We have have successfully used and refined all of these exercises at Cornell and elsewhere over several years.

The laboratory exercises are based primarily on crayfish superficial flexor muscle and innervation, crayfish stretch receptor, and snail brain. Topics to be covered include motor innervation, neuroanatomy, sensory systems, ionic bases of resting and action potentials, synaptic transmission, synaptic plasticity, and central rhythms.

For more information, send email to crawdad@cornell.edu or visit the web site at http://www.bio.cornell.edu/neurobio/hoy/crawdad/home.html.

Supported by NSF grant 9555095.

100.9

NEURONET: A COMPUTER TOOL FOR TEACHING ANALYSIS OF MULTIPLE SIMULTANEOUS RECORDINGS BY CROSS-CORRELATION. I. Espinosa*. Lab. of Cybernetics, Physics Dept., Sch. of Sciences, Mexico National University, Mexico, DF 04510.

Cross-correlation analysis as developed by Gerstein and colleagues has become a useful tool in neuroscience for discerning functional connectivity and temporal synchronization in neuron assemblies. Our lab has produced a simple collection of computer programs that allows to analyze and display spike trains generated by one of MacGregor's neural simulators (Neural and Brain Modeling, Ac. Press 1987). A network can be designed on the screen and the corresponding datafile is written and if redirected to the simulator an output file is produced which contains the spiking times for each neuron and fiber in the simulation. The spike trains can be analyzed by a cross-correlation program and displayed as a raster plot. In such a manner, networks can be formed where different types of neuron interactions are induced. The student learns what connections and parameters to change to produce independent spike trains, direct connections (excitatory and/or inhibitory) and shared inputs. Similarly, different types of neural oscillators and other nets can be studied. With this training tool the task of analyzing real data from multiple simultaneous recordings becomes easier and the production of wiring diagrams is also facilitated.

100.10

ELECTROPHYSIOLOGY OF THE UNDERGRADUATE NEUROSCIENCE STUDENT: A LABORATORY EXERCISE IN HUMAN ELECTROMYOGRAPHY. R.C. Lennartz*, Department of Psychology and Program in Neuroscience, Williams College, Williamstown, MA 01267.

Undergraduate neuroscience laboratory courses usually include at least one electrophysiology exercise, such as the frog sciatic nerve or cockroach leg preparation. Often, however, it is not evident how well students appreciate that the electrical activity that they observe in these reduced, nonmammalian preparations is also taking place in humans. Thus, an ideal supplement to such exercises is one in which students record potentials from themselves. Although *neural* activity can be recorded as the electroencephalogram and evoked potentials, the averaging and/or analysis required makes it difficult for the student to immediately observe a relation between behavior and electrical activity. This poster demonstrates how the recording of muscle activity (electromyography, or EMG) can be done as an undergraduate laboratory exercise in electrophysiology. EMG is easily recorded from the skin surface; this is routinely done in the field of psychophysiology. Students get immediate visual (and auditory, with the addition of an audio monitor) feedback of how their behavior relates to the electrical activity of muscles. This laboratory exercise can serve as a framework in which to discuss terms such as motor unit, and be used to demonstrate concepts such as recruitment, flexion and extension, the stretch reflex, etc. This is a suitable supplement to preexisting electrophysiology exercises, as the equipment -- preamplifier and oscilloscope -- is the same, requiring only the change of filter settings and perhaps amplifier gain. In addition, there is no additional expense or inconvenience involved in the purchase, housing, and feeding of the animals.

Supported by a grant from the Essel Foundation.

100.11

A LABORATORY EXERCISE THAT INTRODUCES LANGUAGE MINORITY GENERAL EDUCATION STUDENTS TO THE NEUROSCIENCES. B.L. Krilowicz,* H.Henter and L.Kamhi-Stein. Dept. of Bio/Micro. and Sch. of Ed., Cal. State University, Los Angeles, CA 90032.

CSULA is an urban university with a diverse student body. Many students at CSULA are high risk, language minority students (i.e. immigrant or native-speaking English bilinguals) and are the first in their families to attend university. A quarter long neuroscience exercise was developed for a general education biology course with the primary goals of introducing non-majors to: 1) scientific investigation, 2) scientific writing, 3) on-line scientific bibliographic databases, and 4) the scientific literature. Throughout development of this exercise, emphasis was placed both on academic content and methods to improve the academic language skills of the students. Students begin by collecting data on their own circadian rhythms in autonomic (heart rate), motor (eye-hand coordination) and cognitive (adding speed) function. Students write a journal-style scientific paper using pooled class data. Students are prepared to write the paper by several methods that are designed to improve academic language skills. First, they spend one laboratory period in the library where they receive hands-on training in the available bibliographic resources and are asked to complete an exercise demonstrating basic proficiency in utilizing these resources. Secondly, overheads of a "model" student paper are used to show what should appear in each section of their paper. The overheads indicate how the "model" paper fulfills criteria given in a section of the laboratory manual entitled "How to write a scientific paper". Only one section of the paper is covered each week. Finally, the students write drafts of each section of the paper before the final version is submitted. All drafts are critiqued and returned to the students at least two weeks prior to the due date for the final paper.

100.12

HANDS-ON ILLUSTRATION OF SYNAPSES. P.B. HERNANDEZ*. Biology Department, Abilene Christian University, Abilene, TX 79699.

The objectives of this demonstration or group activity are to convey the concepts that: (1) a neurotransmitter selectively binds to a receptor, and (2) neurotransmitter actions may be inhibitory or excitatory.

Conveying the concept of the receptor-substrate specificity for neurotransmitters may be easily role played by students with the use of balloons (which represents receptors) and "pop-ice" (which represents synaptic vesicles with neurotransmitters). Select 4 students for a demonstration or work in groups of 4. Their body is the soma, one arm is a dendrite, and the other arm is an axom. One person's dendritic arm will have balloons of one color on its fingertips, which represents a specific type of receptor. The other 3 students each with a "pop-ice" synaptic vesicle of a different color in their axonal hand, stand next to the dendritic arm with balloons. These students attempt to "synapse" with the postsynaptic neuron. But the dendritic arm accepts only 1 type of neurotransmitter, the neurotransmitter that is the same color as the balloon receptor. The type of neurotransmitter accepted determines the action on the postsynaptic terminal.

After establishing the neurotransmitter-receptor specificity, you may designate different colors to represent different types of neurotransmitter and their receptors. Also, tell the students if the neurotransmitters are excitatory or inhibitory. Using the previous scenario, 3 presynaptic neurons (each with a different neurotransmitter) will attempt to synapse with a postsynaptic neuron. The postsynaptic neuron now has several types of receptors (different color balloons) on its dendritic hand. The students can now deduce

100.13

UNDERGRADUATE NEUROBIOLOGY LAB INDEPENDENT RESEARCH PROJECTS: DOWN REGULATION OF SIGNAL TRANSDUCTION PROTEINS. M.E. Morton*, M.P. Leslie and M.A.Marinello. Department of Biology, College of the Holy Cross, Worcester, MA 01610.

The Cellular and Molecular Neurobiology course at Holy Cross is an undergraduate laboratory-intensive course which places emphasis on student involvement in investigative, hands-on learning. Students engage in multi-week experiments developed to mimic a research experience rather than a laboratory exercise. The final project of the semester is a four-week, independent investigation in which small groups of students execute self-designed experiments. During the fall of 1995, groups of students investigated three related aspects of carbachol-induced down regulation of the mAChR signal transduction pathway in B104 and IMR-32 neuroblastoma cell lines. One group investigated the comparative concentration-dependent down regulation of mAChR protein expression by carbachol in the two cell lines; another investigated the down regulation of G-protein mRNA expression by carbachol; a third investigated whether or not down-regulation could be prevented by down-stream activation of the pathway by forskolin. A combination of [^{3}H]QNB binding and solution hybridization analysis was used. These projects demonstrate directly the sophistication with which undergraduate students can engage in cellular and molecular neurobiology research as well as the feasibility of including independent research projects within the context of an undergraduate laboratory course. Supported by grants from NSF # USE-9250833 and the Howard Hughes Medical Institutes.

100.14

AN UNDERGRADUATE LABORATORY COURSE IN CELLULAR NEUROBIOLOGY. A. K. Snyder, E. Eisenberg, E. Kotlar, V. C. Kotak and S. J. Fluharty*. Biological Basis of Behavior Program, University of Pennsylvania, Philadelphia, Pa, 19104.

The Laboratory in Cellular Neurobiology is one of the few courses in the country that offers advanced undergraduates an opportunity to gain hands-on experience in the art of intracellular electrophysiology and synaptic physiology with pharmacological manipulations. Every week, students employ single-electrode, current-clamp technique in isolated nerve-ring preparations from the snail, *Helisoma trivolvis* (generously donated by Dr. S.B. Kater and Dr. C. Cohan). This course first introduces students to neuronal firing patterns in the CNS including pace-making, delayed firing, and bursting. Single spike dynamics are also examined. Students then learn to appreciate the role of underlying ionic currents by employing techniques such as voltage stepping and pharmacological manipulation. This single semester course is designed to accommodate 10-12 students and is taught with the help of 3 previously trained undergraduate teaching assistants. The class meets twice a week. Practical skills are taught during an eight hour lab session, and data and papers are discussed during an additional hour-long recitation. On a weekly basis, students collect, present, and discuss data from their own intracellular recordings. In addition, they read and discuss relevant research papers and learn to apply their new knowledge to the interpretation of their own data. Finally, students develop and execute their own research projects which they present in a formal paper and poster session. In the past, students have examined a broad range of topics including the effects of nitric oxide on bursting neurons, physiological characterization of glutamate receptor subtypes, and synaptically-evoked prolonged hyperpolarization. Student have consistentlty rated this lab experience as exceptional and demand for participation in this course always exceeds the number of available positions. (Supported by the BBB program at the University of Pennsylvania).

100.15

ULTRASONIC VOCALIZATIONS: A VERSATILE BEHAVIORAL ASSAY FOR USE IN UNDERGRADUATE BEHAVIORAL NEUROSCIENCE INSTRUCTION. M. Kerchner*, Department of Psychology, Washington College, Chestertown, MD 21620-1197.

A variety of rodents and other laboratory mammals produce ultrasonic (> 20kHz) vocalizations. This poster describes how these calls are employed in our undergraduate neuroscience laboratory curriculum. Sample laboratory exercises will be presented from the following courses:

Biopsychology. We employ adult male mouse USVs as a behavioral assay of sexual motivation following castration. Using various metabolites of testosterone, e.g., estradiol, dihydrotestosterone, in a replacement paradigm illustrates the roles that these steroids serve in activating components of male sexual behavior.

Psychopharmacology. Here infant mouse USVs are employed as an assay for the anxiolytic properties of various drugs, e.g., diazepam (which decreases USV) and the opiate antagonist naloxone (which increases USV). Variations of the exercise demonstrate the phenomena of tolerance and cross-tolerance.

Comparative Psychology / Neuroethology. Nagel's classic article "What is it like to be a bat?" describes the basic dilemma facing comparative psychologists - "How do we know what it's like to be another creature?" This issue is confronted by students as they employ USV to locate, identify, and track local bat populations as they feed on insects. This exercise leads to a discussion of the neural mechanisms that are employed by echolating bats to locate prey and by the prey as they avoid the bats.

In addition to being extremely versatile, the cost of equipping an undergraduate laboratory for quantifying USVs is relatively low.

100.16

USE OF NADPH-DIAPHORASE HISTOCHEMISTRY IN AN UNDERGRADUATE NEUROBIOLOGY LABORATORY. H. Itagaki*. Dept. of Biology, Kenyon College, Gambier, OH 43022.

Nitric oxide (NO) has been heavily implicated in a variety of cellular signalling roles across a wide range of organisms. Its synthesizing enzyme, nitric oxide synthase (NOS), produces NO from L-arginine. NOS has also been shown to have NADPH-diaphorase activity (Hope *et al.*, 1991). By using a relatively simple histochemical technique to detect the NADPH diaphorase activity, it possible to localize the NOS (Hope and Vincent, 1989).

We have begun using NAPDH-diaphorase histochemistry in our upper-level undergraduate neurobiology laboratory to stain nervous systems from various invertebrate preparations (*e.g.* fly, moth, caterpillar, cricket, crayfish), looking for NOS activity. The advantages of this lab include: 1). ease of protocol - as the preparations can be processed in whole-mount; 2). ease of generating student excitement - as students are aware of the surge in interest in NO research over the past decade, and they can try preparations that have not been assayed before; and 3). ease of teaching students the idea of comparative studies - as related organisms can be assayed to look for homologies in the CNS.

For more detailed study of stained tissues, we have also modified standard protocol (Elphick *et al.*, 1995) to allow sectioning of tissue embedded in paraffin after the NADPH-diaphorase histochemistry. This avoids the use of a cryostat, which is often difficult to use for many students, and may not be widely available for use in undergraduate labs.

Supported by NIH DC01939, NSF USE-9152088, and Kenyon College.

100.17

PAIN MANAGEMENT THE RAT. **F.V. Abbott* and M. Bonder**, Dept of Psychiatry, McGill Univ, Montreal, PQ, Canada, H3A 1A1.

Clinical recommendations for analgesics in laboratory rodents are usually extrapolated from basic research. The animal models of pain often involve withdrawal reflexes evoked by threshold-level stimuli, while pain associated with surgery or disease involves injury and inflammation. Agents tend to be chosen as exemplars of a drug class, without regard for whether the route of administration is practical, the drug has useful kinetics, and side effects are tolerable. Here we discuss clinical assessment of pain in small animals and present data on the efficacy of drugs from four classes using the formalin test as a model of injury-induced pain.

Formalin (50 µl, 2.5%) was injected sc into a rat's paw and the behavioural response (elevation or licking of the paw) recorded. Buprenorphine and dipyrone completely suppressed pain responses at 0.1 and 200 mg/kg, respectively. When formalin was injected 6 h after buprenorphine or dipyrone, pain scores were 30% of control scores. In the absence of pain and handling, 0.6 mg/kg buprenorphine was lethal to 25% of rats. Locomotor activity was slightly depressed by 300 mg/kg dipyrone. Xylazine suppressed pain responses at 2 mg/kg, but analgesia had decreased to less than 50% by 2h, and effects were highly variable thereafter. At 8 mg/kg rats were unresponsive to strong pinch. Acepromazine, marketed primarily as a tranquillizer, reduced pain to 20% of control scores at 2.5 mg/kg, and this level of analgesia was still present at 6 h. Neuroleptic effects were prominent at 5 mg/kg. These data identify three very good analgesics that would be suitable for moderate to severe post-surgical pain when administered Q8-12h. Practical routines for use of analgesics in research are discussed. *Supported by MRC Canada*

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SYMPOSIUM: PAIN AND THE CEREBRAL CORTEX. K.L. Casey, Univ.of Michigan (Chairperson); A.D. Craig, Jr., Barrow Neurol. Inst.; G. Duncan, Univ. Montreal; M. Gabriel, Univ. Ill.; B.L. Whitsel, Univ. N.Carolina.

Both early and contemporary clinical and experimental observations have shown that several regions of the cerebral cortex affect pain, each in unique, but as yet incompletely defined, ways. The results of these studies have encouraged the continuing reconsideration of cortical functions in pain. The "internal phrenology" of strict, psychologically defined functional localization continues to give way to the development of hypotheses that consider the plasticity of the forebrain and the importance of interactions among physiologically specialized cortical regions. Ken Casey will review this information to provide a background for the several specific questions to be addressed by recent anatomical, physiological, behavioral and functional imaging studies. A.D.(Bud) Craig will discuss new evidence about the functional anatomy of ascending spinothalamic and thalamo-cortical pathways that project specific information about pain and temperature to the primate insular, anterior cingulate, and somatosensory cortices. Gary Duncan will review the results of recent studies that combine psychophysical measurements of pain with the functional imaging of synaptically induced cortical and subcortical regional cerebral blood flow responses in humans. Barry Whitsel will present new information, obtained from direct near-infrared optical imaging and neuronal recording, about anterior parietal cortical responses to noxious and innocuous stimuli delivered to the same skin site in monkeys. Mike Gabriel will discuss combined behavioral and multi-site neurophysiological recording studies. These investigations reveal the successive engagement of distinct brain circuits as an animal learns to avoid pain-signaling acoustic stimuli. The involvement of nociceptive processing in the development of the mnemonic functions of these circuits will be considered. Time will be provided for questions from the audience after each presentation and for audience participation in a discussion period following the formal lectures.

102

SYMPOSIUM. SINGLE NERVE CELLS AS COMPLEX COMPUTING DEVICES: INTEGRATING EXPERIMENTAL AND COMPUTATIONAL APPROACHES. A.Borst, Max-Planck-Institute (Chairperson); J.P.Miller, UC Berkeley; I.Segev, Hebrew Univ.; A.Borst, Max-Planck-Institute; J.M.Bower, CalTech.

Recent advances in electrophysiological and optical recording techniques reveal an astonishing physiological complexity of individual neurons. The symposium illustrates how information theoretic analysis and detailed computer models help to understand the functional consequences of this complexity for the computational capabilities of nerve cells. John Miller shows how an advanced statistical analysis of neural signals has yielded model-independent assessments of the quantity and quality of information encoded by neurons in the cricket cercal sensory system. These studies have also provided insight into the nature of neural coding by ensembles of neurons in this system. Idan Segev's talk focuses on the effect of the morphology of neurons on the site of action potential initiation. He shows that the geometry per se critically determines this site and that, in central neurons (e.g., neocortical pyramidal cells) the neighborhood of the soma is the favorable region for spike initiation. This result is explained utilizing a new parameter, the input delay, which characterizes how fast the excitable current escapes from a given input site. Using fly visual interneurons as experimental system, Alexander Borst talks about the spatial and temporal filter properties of dendrites. A particular focus is given on the relationship between these input-output characteristics and the distinct passive and active membrane properties of individual neurons. James Bower demonstrates how the interaction between modeling and experimental efforts have lead to a new understanding of the cellular mechanisms underlying the response of the cerebellar Purkinje cell to synaptic input. The results require a major reexamination of the function of cerbellar cortex. Taken together, the talks emphasize the need of a combined effort of experimentalists and theoreticians to achieve an understanding of the computational properties of the brain's basic building blocks.

ALZHEIMER'S DISEASE: MECHANISMS OF CELLULAR INJURY

105.1

POTENTIAL LINKS BETWEEN ALZHEIMER'S DISEASE AND BRAIN INJURY FROM MICROEMBOLI RELEASED DURING CARDIOPULMONARY BYPASS. W.R. Brown*, D.M. Moody, M. Tytell, V.R. Challa, and H.S. Ghazi-Birry. Departments of Radiology, Neurobiology & Anatomy, and Pathology; Bowman Gray School of Med., Winston-Salem, NC 27157.

Traumatic brain injury can lead to amyloid deposition and Alzheimer's disease. Also, ischemic brain injury can lead to amyloid precursor protein deposition, but it is unknown if this can progress to amyloid deposition and Alzheimer's disease. Permanent neurobehavioral deficits are found in 19-36% of patients undergoing cardiopulmonary bypass (CPB) and at 5 years after CPB there is an unexpectedly steep cognitive decline. In this study we investigated the brain pathology resulting from CPB-induced brain microemboli (Moody et al. Ann Neurol 1990;28:477) and looked for links with the pathogenesis of Alzheimer's disease. Brain tissues from CPB patients were obtained at autopsy, fixed in 70% ethanol, embedded in celloidin, and stained with histochemical and immunocytochemical markers. We found thousands of microemboli with evidence of very small areas of ischemic damage in the nearby tissues, including vascular degeneration, swollen astrocyte endfeet, upregulation of constitutive heat shock protein 70 around the vessels, and one microinfarct with hemorrhage. In the case with the most microemboli, we also found huge numbers of heat shock protein-positive granules that appeared similar to dystrophic neurites which were also found in the brains of Alzheimer's patients. We conclude that CPB can cause multiple microscopic ischemic brain injuries that may lead to neuropsychological deficits and perhaps eventually to Alzheimer's disease. Supported by grants NIH NS20618 and NIH NS27500

105.2

NON-AMYLOID COMPONENT PROTEIN (NACP) REACTIVITY AND mRNA EXPRESSION IN CEREBRAL VESSELS IN ALZHEIMER'S DISEASE.

D.R.D. Premkumar*, T. Saitoh and R.N. Kalaria.

Departments of Neurology and Pathology, Case Western Reserve University, Cleveland, Ohio and Department of Neurosciences, University of California at San Diego, San Diego, Caljfornia, USA

NACP is a recently identified protein that is highly expressed in brain. The cleaved product, NAC binds tightly to amyloid ß protein of Alzheimer's disease (AD). NAC is also associated with cerebral amyloid angiopathy. Here, we used immunochemical and RT-PCR methods to study the expression of NACP in cerebral vessels and other brain tissues from AD subjects compared to normal aging controls. Immuoblotting revealed that the 19 kDa NACP reactivities were consistently increased in soluble fractions of buffer extracted cerebral vessel preparations from AD subjects compared to controls. This was not apparent in other brain tissues. We also noted NACP mRNA expression in neuronal regions, the cerebral vessels and choroid plexus. The mRNA expression was highest in the hippocampus and relatively low in the cerebellum. Contrary to the increased protein levels, we observed decreased NACP mRNA expression in cerebral vessels from AD subjects compared to controls but this was not readily apparent in any other region examined. Our results suggest AD specific changes in NACP of non-neural tissues as well. Supported by grants from NIH.

105.3

VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) LEVELS ARE UNCHANGED IN ALZHEIMER'S DISEASE. A. Baird[1]*, V. Kuo-LeBlanc, P. Song, A.M. Gonzalez[1], and E.G. Stopa. Dept. of Pathol., Brown Univ. Sch. of Med./RIH, Providence, RI and [1]PRIZM Pharmaceuticals, San Diego, CA

Vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (FGF-2) have both been shown to have an affinity for heparin. Because of this affinity, FGF-2 normally binds to the structurally related heparan-sulfate proteoglycans (HSPGs) present in the extracellular matrix. In a previous study we demonstrated FGF-2 levels are increased in Alzheimer's disease, and FGF-2 immunoreactivity was clearly present within neuritic plaques and neurofibrillary tangles. We have also previously shown that despite its heparin-binding properties, VEGF immunoreactivity showed no apparent association with the B/A4 amyloid continuing structures found in Alzheimer's disease.

In this study, we utilized an ELISA assay (R&D Systems) to quantitate VEGF in 3 areas severely affected by AD: amygdala, A20, and A28; and 2 areas which are less severely involved by the disease: caudate nucleus and A4. Human brains were obtained within 18 hours of death. The areas of interest were carefully dissected from the fresh brain and snap frozen in liquid nitrogen.

The concentration of VEGF was generally found to be highest in the amygdala. In all 5 areas, VEGF levels ranged from 0.06-0.45 ng/mg protein and were virtually unchanged in Alzheimer's disease (0.05-0.42 ng/mg protein). FGF-2 levels by contrast ranged from 15.78-20.61 ng/mg protein and were increased 2-3 fold in Alzheimer's disease, 31.89-65.30 ng/mg protein ($p<.0001$).

These data confirm the previous observation that despite its heparin-binding properties, VEGF does not appear to be influenced by B/A4 amyloid deposition. They also suggest that certain heparin-binding growth factors may be more critical than others in the pathogenesis of Alzheimer's disease. *(Supported by AG10682)*

105.4

ASSOCIATION BETWEEN THE MITOCHONDRIAL tRNA^{Gln} A4336G MUTATION AND NEURODEGENERATIVE DISEASE

R. Egensperger, S. Kösel, N.M. Schnopp, P. Mehraein and M.B.Graeber*

Molecular Neuropathology Laboratory, Institute of Neuropathology, Ludwig Maximilians-University, 80337 Munich, Germany

Mutations of mitochondrial DNA (mtDNA) are being increasingly implicated in the pathogenesis of neurodegenerative disease. Specifically, a mitochondrial tRNA^{Gln} sequence variant (A4336G transition) has been suggested to occur more frequently in Alzheimer's (AD) and Parkinson's diseases (PD) than in controls [Wallace D.C. (1994) PNAS 91:8739]. The finding is of great interest as this mutation may represent the first known genetic susceptibility factor shared by two common neurological disorders. We have analyzed 27 neuropathologically confirmed cases of AD, 39 cases with Lewy-body PD and 100 controls without clinical and histological evidence of neurodegeneration. Presence of the mtDNA^{A4336G} transition was tested by *Ava*II restriction enzyme analysis of PCR products amplified from formalin-fixed and paraffin-embedded brain tissue. In addition, all diseased brains were genotyped for alleles of the apolipoprotein E (ApoE) gene. One out of 27 AD cases and two out of 39 PD cases carried the mtDNA^{A4336G} mutation whereas no mutation was found in 100 age-matched controls ($p<0,05$). Interestingly, the patient carrying the mtDNA^{A4336G} mutation was homozygous for ApoE ϵ3 and showed a high number of neurofibrillary tangles. Our data support the hypothesis that the mitochondrial A4336G mutation represents a genetic susceptibility factor for PD and AD. They are further in line with the view that point mutations in mitochondrial genes could play a causative role in the oxidative phosphorylation deficit known to occur in both AD and PD. Finally, it is tempting to speculate that defects in oxidative phosphorylation may predispose to the formation of neurofibrillary tangles.

105.5

ALZHEIMER DISEASE CELLS HAVE DEFECTIVE DNA REPAIR OF FREE RADICAL-INDUCED DNA DAMAGE: IMPLICATIONS FOR NEURODEGENERATION AND ANTIOXIDANT THERAPY. J.H. Robbins*[1], R. Parshad[2], K.K. Sanford[1], F.M. Price[1], L.K. Melnick[1], L.E. Nee[3], M.B. Schapiro[4] and R.E. Tarone[1]. [1]NCI, [3]NINDS, [4]NIA, NIH, Bethesda, MD 20892 and [2]Howard Univ., Washington, DC 20059.

We previously showed that xeroderma pigmentosum (XP) and familial and sporadic Alzheimer disease (AD) cells appear to have a similar defect in repairing DNA damage induced in cultured skin fibroblasts or blood lymphocytes by fluorescent light (FL). FL damages DNA in cells in tissue culture indirectly by generating free radicals, and XP cells [which are defective in nucleotide excision repair (NER) of damaged DNA] are unable to repair a type of free radical-induced DNA damage (Satoh et al., PNAS 90, 6335-9, 1993). We report in detail [Parshad et al., PNAS 93, (May 14), 4738-42, 1996] all these FL test results on AD and XP cells, together with studies using the free-radical scavenger theaflavin mixture (TFM), a highly purified extract of polyphenol antioxidants extracted from black tea. Our tests use chromatid breaks to measure ability of skin fibroblasts or PHA-stimulated lymphocytes to conduct NER or respond to unrepaired DNA lesions. All 16 familial AD patients (representing 4 families) and all 11 sporadic AD patients were abnormal in our tests, whereas at least 28 of 31 normal donors had normal responses. Lymphocytes from a sporadic AD patient and a normal donor exhibited normal NER of 254-nm UV-induced DNA damage when TFM (lot LN-0046-01, Lipton Tea Co., Englewood, NJ) was present during irradiation, indicating that the NER pathway was not affected by TFM and that 254-nm UV-induced DNA damage was not mediated via free radicals. However, when normal cells were irradiated with FL in the presence of TFM, no NER was apparent, indicating that TFM had prevented the free radical-induced DNA damage which AD cells cannot repair. When the AD cells were irradiated with FL in the presence of TFM, the damage which AD cells cannot repair was also prevented. If death of neurons occurs in AD patients because of their inability to repair normally-occurring free radical-induced DNA damage, as is believed to be the case in XP, antioxidant preventive therapy may delay or prevent neurodegeneration in AD.

105.6

ACTIVATION OF MAP KINASE BY ATP-DEPLETION IN PC12 CELLS IS MEDIATED BY CALCIUM: RELATION TO ALZHEIMER'S DISEASE. Y. Luo*, P. Kumar, P. Russell and V. M. Ingram. Dept. of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139.

Brain aging and Alzheimer's disease (AD) are accompanied by a decreased ATP metabolism and by substantial changes in calcium homeostasis. We have previously demonstrated that partial depletion of ATP in NGF-differentiated PC12 cells with an uncoupler of OXPHOS, FCCP, produced phosphoepitopes on cytoskeletal proteins similar to those seen in Alzheimer brains. We hypothesize that ATP depletion can elevate the intracellular calcium concentration , thereby activating MAP kinases.

Using a specific immunoprecipitate kinase assay we observe an increased activation of MAP kinases in PC12 cells treated with FCCP or oligomycin. FCCP produces a transient two-fold increase in ERK1 and ERK2 activity towards TAU after 10 min. Oligomycin, in contrast, significantly increases the activity of ERK2 at 1 to 2 hours after treatment, but not at 10 min. MonoQ FPLC confirms this conclusions. Western blotting of MonoQ fractions shows increased phosphorylation of ERK1 and ERK2 as gel mobility shifts. The activation of ERK1/ERK2 by FCCP or oligomycin correlates with tyrosine phosphorylation of the kinases detected by an antibody specific to phosphorylated ERK1/ERK2. A calcium ionophore, ionomycin, which raises internal calcium, also increases ERK1/ERK2 activity 2.5-fold after treatment for 10 min. Fluorometric measurements demonstrate that FCCP and ionomycin, but not oligomycin, cause a rapid and lasting rise of $[Ca^{2+}]_i$ in the PC12 cells. This suggests that FCCP-stimulated ERK1/ERK2 activation is mediated by calcium.

FCCP treatment of PC12 cells for 2 hr induced the translocation of ERK2 to the nucleus, and decreased several tyrosine phosphoepitopes. It appears that increases in $[Ca^{2+}]_i$, as a consequence of ATP depletion, activate the MAP kinase cascade. The resultant hyperphosphorylation of TAU and phosphorylation of transcription factors, which lead to expression of new genes, may well be relevant to the neurotoxicity seen in Alzheimer's disease. (Y.L. is a Fellow of the Lucille P. Markey Foundation.)

105.7

ALTERED CALCIUM RESPONSES IN FIBROBLASTS FROM ASYMPTOMATIC MEMBERS OF ALZHEIMER'S DISEASE FAMILIES. R. Etcheberrigaray*†, N. Hirashima‡, J. Prince‡ and D.L. Alkon‡. †Lab. of Applied Neuroscience-GICCS, Georgetown University Medical Center, Washington, DC 20007 & ‡Lab. of Adaptive Systems-NINDS, National Institutes of Health, Bethesda, MD 20892.

We have shown that fibroblasts from Alzheimer's disease (AD) patients exhibit distinct cellular and molecular alterations. These alterations include functional absence of a 113 pS K^+ channel, absent or greatly reduced tetraethylammonium(TEA)-induced Ca^{2+} responses, enhanced IP_3-induced Ca^{2+} release, and reduced levels of Cp20 (a memory related GTP-binding protein). The combination of these alterations can be use as an index for AD. Recent results also suggested (Kim et al, PNAS, 92:3060-3064, 1995;Hirashima et al, Neurobiol. Aging, in press) that some of these alterations might be present in asymptomatic members of AD families. To further explore these observations, using fluorescent Ca^{2+} imaging we studied cell lines from asymptomatic (A) individuals including "escapees" (with ages above the families' age of onset, N=3) and subjects at 50% "risk" (with one direct relative affected and ages below the families' age of onset , N=7). This combined group was compared to AD (N=22) and controls (N=23). All but one (Italian family 1097) of the asymptomatic individual are from the Canadian family 964. Over 50 cell were measured in each cell line. The overall comparison between the three groups was highly significant for the TEA induced Ca^{2+} response, $p < 0.0001$ (Kruskal-Wallis). Post test analyses (Dunn's) revealed that both A and AD have significantly lower TEA responses than controls ($p < 0.01$ and $p < 0.001$ respectively). The same analyses revealed remarkably similar TEA responses between A and AD. The bradykinin and bombesin (IP_3-mediated) responses of A had values higher (although not statistically significant) than controls and lower (not significant) than AD. However, the combined "index" (Hirashima et al, Neurobiol. Aging, in press) identified 8 out of 10 of the asymptomatic individuals members of AD families as having an AD profile. These results strongly suggest that these molecular alterations are present in varying degrees before clinical symptoms arise and when combined as an index they could provide an early diagnostic tool in familial cases.

105.8

IMPAIRMENT OF Ca-HOMEOSTASIS IN CENTRAL NEURONS AND ASTROCYTES BY APOLIPOPROTEIN E AND ßA4.

W. Müller*[1], K. Berlin[1], F. Wüstenberg[2], H. Scharnagl[3], W. März[3] and T.G. Ohm[2] [1]Physiologisches Institut, [2]Anatomisches Institut, Charité, 10117 Berlin, [3]Klinische Chemie, Universität, 79106 Freiburg i. Br., Germany.

Neurodegeneration appears to be a primary mechanism in the expression of Alzheimer's disease and ßA4-Amyloid (ßA4) as well as apolipoproteinE (ApoE) might have a role in this process. We used Fura-2/AM loaded rat hippocampal cultures and digital ratio-imaging to study acute effects on Ca-homeostasis after coincubation of ApoE and ßA4-Amyloid (ßA4) for at least 12 hours at 37° C. In order to study direct postsynaptic effects, all experiments were performed in the presence of CNQX (10 µM). Transient application of recombinant apolipoproteinE3 (ApoE3, 20nM for 3 min) increased free Ca in neurons as well as astrocytes slightly by 60±37 nM. ßA4 (aa1-43, 5 µM) raised free Ca in neurons and astrocytes by 165±50 nM (mean±std). Application of ßA4-ApoE3 resulted in a strong and overadditive effect, i.e. Ca-levels were increased by 313±60 nM. In control experiments with a reversed ßA4 (aa 40-1) Ca-increases were small (ßA4(40-1): 65±5nM, ßA4(40-1)-ApoE3: 116±12nM). We conclude that apolipoproteinE plays an important role in cellular Ca-homeostasis, most likely via a specific binding of several ßA4s, thereby damaging neurons directly and possibly indirectly through astrocytes.

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105.9

Differentially elevated intracellular Calcium levels by Apo E-isoforms in the presence of ßA4-Amyloid.

F.Wüstenberg; K. Berlin[1]; H. Scharnagl[2]; W. März[2]; T.G. Ohm*; W. Müller[1]. Institut für Anatomie, [1]Institut für Physiologie, Charité, 10117 Berlin, Germany; [2]Abteilung klinische Chemie, Universität Freiburg , Freiburg, Germany

Apolipoprotein E allele 4 (Apo E4) is an important risk factor in late-onset familial and sporadic Alzheimer's disease (AD). It has been shown that AD-associated amyloid-ß peptides (ßA4) bind to Apo E protein and trigger elevated intracellular free-Calcium levels ($[Ca]_i$), which lead to the expression of AD-related epitopes and cell death. Our working hypothesis in this study was that $[Ca]_i$ are still higher in the presence of Apo E protein and the extend of the increase in $[Ca]_i$ is dependent on the Apo E isoform present in the complex. We monitored $[Ca]_i$ via slow-scan ratio-imaging of cultured hippocampal neurons preloaded with Fura-2, immediately after extracellular application of either 20 nmol/L recombinant Apo E 3 or recombinant Apo E 4, resp., either with or without 1µmol/L ßA4 peptide, preincubated for at least 12 hours at 37°C. We found that Apo E3 protein moderately elevates $[Ca]_i$ to 63 nmol/L over resting level, whereas after application of Apo E4 the mean increase of $[Ca]_i$ was 77 nmol/L. After application of Apo E / ßA4 complexes we recorded significantly higher $[Ca]_i$. With Apo E3/ßA4 complexes $[Ca]_i$ was increased 5fold to 313 nmol/L over resting level, and after Apo E4/ßA4 complexes $[Ca]_i$ was highest with a 434 nmol/L increase, which is 6fold as compared to Apo E4 alone. These findings correspond to the notion that Apo E4 protein itself does not destroy neurons itself, but it can exert its influence via the complex of Apo E4 and ßA4, as soon as ßA4 is present extracellularly. Our data, especially the extremely high $[Ca]_i$ after application of Apo E4/ßA4 complexes correspond to the epidemiological data associating the Apo E4 allele with an early onset of AD. Apo E3/ßA4 complexes still trigger marked elevations of $[Ca]_i$, which can be explained by the susceptibility

105.10

A NOVEL HUMAN FE65-LIKE PROTEIN INTERACTS WITH THE CYTOPLASMIC DOMAIN OF THE ß-AMYLOID PRECURSOR PROTEIN

S. Guénette*, J. Chen, P.D. Jondro and R.E. Tanzi. Genetics and Aging Unit, Department of Neurology, Mass. General Hospital East.

To identify cellular components necessary for the intracellular trafficking of and/or function of cell surface ßPP we screened for proteins that interact with the cytoplasmic domain of ßPP with the "interaction trap". Using this approach we identified a novel human gene which we have named hFE65L. The predicted open reading frame of the hFE65L gene is 57% identical to the 499 overlapping amino acids of the rat FE65 gene and represents one of three members of a novel human gene family. In contrast to the rat FE65 gene the hFE65L gene is expressed in all tissues tested. Amino acid sequence alignment of members of the human FE65 gene family reveals the presence of two tandem phosphotyrosine interaction (PI) domains. The cytoplasmic domains of the ßPP homologues, APLP1 and APLP2, were also tested for interaction with hFE65L. Only APLP2 was found to interact with hFE65L in the interaction trap assay. We confirmed these interactions *in vivo* by successfully co-immunoprecipitating endogenous ßPP and APLP2 from mammalian cells overexpressing a hemaglutinin-tagged hFE65L fusion protein. Our results also show that the C-terminal PI domain of hFE65L is sufficient for interaction with APP *in vivo*.

The PI domain of the protein Shc, is known to interact with the NPXYp motif present in the cytoplasmic domain of a number of different growth factor receptors. Furthermore, the NPXY motif of ßPP has previously been shown to modulate the internalization and trafficking of ßPP. We hypothesize that the PI domain present in the C-terminal moiety of the hFE65L protein binds the NPXY motif of ßPP and APLP2 and plays a role in the internalization and/or function of these molecules.

Supported by the American Health Assistance Foundation.

105.11

CLEAVAGE SITE-SPECIFIC CELL DEATH SWITCH ANTIBODY LABELS ALZHEIMER'S LESIONS. B. Teter*[1], F. Yang[1], C. Wasterlain[1], M. Smulson[2] S.A. Frautschy[1] and G. M. Cole.[1] [1]Sepulveda VAMC, GRECC 11E, Sepulveda, CA 91343 and Depts. Med. & Neurol., UCLA., [2]Dept. Biochemistry, Georgetown Univ. School of Med., Washington, D.C. 20007

Neurofibrillary tangle (NFT) bearing neurons in Alzheimer's Disease (AD) brain are associated with slow or delayed cell death and dementia, but the causal mechanism involved is obscure. The Tunel method for in situ detection of apoptosis DNA fragments labels rare cells occasionally including NFT-bearing neurons in AD brain. An apoptotic cascade can be mediated by cysteine proteases in the ICE or CED-3 family which cleave targets including poly(ADP-ribose) polymerase (PARP) to generate a 24kD DNA binding domain fragment (PARP-24) separated from the activated catalytic domain that depletes NAD and ATP reserves. A PARP-24 specific antibody was developed which immunoprecipitated 35S-labeled PARP-24 derived from in vitro translated 35S-PARP treated with apopain extract from human neuronal cells. This antibody was shown to label PARP-24 in apoptotic human neuronal cells in culture and Tunel positive neurons in rat brain. The antibody clearly labeled rare apoptotic neurons with nuclear fragmentation in AD hippocampus. These morphologically apoptosing neurons were sometimes associated with either ß-amyloid or NFT identified by the PHF-1 tau antibody. Surprisingly, anti-PARP-24 also labeled the majority of NFT and many dystrophic neurites and curly fibers. Isolated NFT directly labeled strongly with the antibody, similar to that found with PHF-1. A PARP-24 band at ~24kD was identified on Western blots from NFT preparations and from AD hippocampus. These data suggest that long before final apoptotic degeneration, subthreshold apoptotic cleavage of PARP occurs producing PARP-24 which binds NFT. We interpret this data to indicate widespread subthreshold activation of a cell death pathway in AD brain which can be detected with this novel antibody. Supported by AG9009, AG11125 (GMC), CA25344(MS).

105.12

ENDOCYTOSIS AND CATHEPSIN CONTENT OF EARLY ENDOSOMES ARE INCREASED IN NEURONS IN ALZHEIMER'S DISEASE. A.M. Cataldo*, J.L. Barnett, and R.A. Nixon. Labs. Molec. Neurosci., McLean Hosp., Dept. Psychiatry, Harv. Med. Sch., Belmont, MA 02178

Alterations of APP, PS1, PS2 and Apo E-ε4 are risk factors for Alzheimer's disease (AD). Trafficking of these proteins via endosomal-lysosomal (E-L) interactions represents a common pathway through which their etiologic influences may be exerted. Using specific markers, we identified different functional populations of neuronal endosomal and lysosomal compartments and abnormalities of these organelles in affected AD neurons. In this study, we report striking alterations in the early endocytic pathway of cortical AD neurons using the early endosomal marker rab 5 and the more general acidic vacuolar system markers, Cat D and B. Rab 5 antiserum labelled a population of intracellular vacuoles in close proximity to the plasmalemma and distinct from the perinuclear location of late endosomes. The total number of early endosomes in AD brains was equal to or less than controls, but total endosomal volume averaged 3± 1.5-fold larger than normal, implying marked upregulation of endocytosis. These large rab 5-positive early endosomes were not seen in a survey of neurons from other neurodegenerative diseases. We found that Cats D and B colocalized with rab 5 in vacuolar compartments of AD neurons to a greater extent than in controls. These data suggest that increased endocytosis and the more frequent occurrence of Cat D in abnormal early endosomes of affected AD neurons may be a response to accelerated membrane turnover. Colocalization of hydrolases, including Cat D, a potential Aß-producing secretase, in the same E-L compartments as APP, suggests a potential mechanism to account for the close relationship between E-L system activation and ß-amyloidogenesis. We recently found that early endosome size was larger in some forms of FAD vs sporadic AD suggesting a link between alterations in endocytosis and genetic risk factors for AD. (NIH)

105.13

ALZHEIMER-SPECIFIC ANTIBODIES DEMARCATE PROGRESSION INTO ANAPHASE. Alex Dranovsky[1,2], Sergey Lyubsky[2], Inez Vincent[3], Peter Davies[3], and Dmitry Goldgaber[4]*. [1]M.S.T. Program, Depts. of [2]Pathology and [4]Psychiatry, S.U.N.Y., Stony Brook, NY 11794; [3]Dept. of Pathology, Albert Einstein College of Medicine, Bronx, NY 10461.

Recently, monoclonal antibodies TG-3 and TG-4 specific for brains of patients with Alzheimer's disease (AD) were described. Surprisingly, both antibodies stained exclusively mitotic cells in culture (Vincent *et al.*, 1996, J. Cell Biol.132:413-425). Using laser confocal microscopy the temporal and spatial distribution of epitopes recognized by TG-3 and TG-4 was studied throughout the cell cycle in human epithelial HEp-2 cells. Granular staining was first observed in prometaphase. As cells progressed into early metaphase, the pattern changed, the intensity of fluorescence increased, and the staining appeared to envelop the chromosomes. The bright chromosome enveloping staining reached its highest intensity in metaphase and then disappeared abruptly demarcating the entry into anaphase. Cells in anaphase and telophase were not stained. In interphase, a weak dot-like fluorescence was observed in the nuclei of some cells. The unique phase-specific pattern was clearly distinct from the well characterized pattern revealed by the mitosis-specific MPM-2 antibody. MPM-2 has been shown to recognize several phosphothreonine-containing proteins in all phases of mitosis including anaphase and telophase. MPM-2, TG-3 and TG-4 each recognized a distinct pattern of proteins in cell lysates analyzed by immunoblotting. The role of proteins recognized by TG-3 and TG-4 in the cell cycle and chromosome segregation is under investigation.

Supported by Metropolitan prize to DG.

CHEMICAL SENSES

106.1

CHARACTERIZATION OF *SMELL IMPAIRED* GENES OF *DROSOPHILA MELANOGASTER*. R. R. H. ANHOLT*, N. H. KULKARNI and T. F. C. MACKAY. Depts. of Zoology and Genetics, North Carolina State University, Raleigh, NC 27695.

Drosophila melanogaster allows a combination of genetic, molecular and behavioral approaches to investigate how the concerted expression of ensembles of genes shapes odor-guided behavior.

We measured avoidance responses to benzaldehyde of 379 inbred lines of *Drosophila melanogaster*, containing single *P*-element (*P[lArB]*) insertions, and identified 14 lines with aberrant olfactory behavior. The *P[lArB]* insertions mapped to different locations on the second and third chromosomes and these olfactory loci were named *"smell impaired (smi)"* followed by their chromosomal band designations. Enhancer trap analysis revealed expression of 10 *smi* genes in olfactory organs (the third antennal segment and maxillary palps). In addition, 4 of the *smi* mutants show a sexually dimorphic phenotype with larger impairment in the female. Line *smi97B* shows the largest olfactory impairment with slight sexual dimorphism and highly localized expression in the third antenna segment.

P[lArB]-tagged DNA sequences can be rescued as inserts in pBluescript and used to identify genomic clones. Four genomic clones have, thus far, been isolated and their *P[lArB]* insertion sites mapped. Fragments of these genomic clones currently are used to screen a *Drosophila* head cDNA library to identify the *smi* gene products.

The phenotype of *smell-impaired* mutants can be restored through excision of the *P*-element. For line *smi97B* 20 revertants have been obtained with phenotypes ranging from homozygous lethal and semi-lethal to partial or complete reversion. Correlations of behavioral phenotypes with gene deletions due to imprecise excisions of the *P*-element together with a reduction in mRNA for the gene product will confirm that aberrant olfactory phenotypes indeed result from the *P*-element insertions.

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106.2

CHARACTERIZATION OF A SUBFAMILY OF ZEBRAFISH ODORANT RECEPTOR GENES: SEQUENCE, DEVELOPMENTAL ONSET OF EXPRESSION, AND LINKAGE. A.L. Barth*, J.C. Dugas, N.J. Justice, and J.Ngai. Department of Molecular and Cell Biology, Univ. of Calif. Berkeley, CA 94720.

In order to examine models of odorant receptor gene regulation, we undertook an analysis of a group of highly related odorant receptor genes in the zebrafish. A complete subfamily of genes, defined by the number of cross-hybridizing bands on a conventional high stringency Southern, was selected for comprehensive study. cDNAs for all five members of this subfamily of receptors were cloned from an olfactory cDNA library and sequenced. They exhibit between 72 and 79 percent identity at the amino acid level, versus about 30 percent identity to less-related zebrafish odorant receptors. None of these receptors cross-hybridize to each other on high stringency in situ hybridizations of adult olfactory tissue. We examined the onset of expression of the different subfamily members on embryos at developmental stages varying from 24 hours to 3 weeks post-fertilization. Results showed that each subfamily member exhibited a different time of expression onset and that four out of the five receptors were expressed before 48 hours post-fertilization.

We next investigated genetic linkage of receptors from this subfamily. Analysis by pulse-field gel electrophoresis shows that receptors expressed early in the development of the olfactory epithelium may be genomically linked to receptors expressed much later. These results may have implications for models of receptor gene regulation that suggest linked genes are regulated similarly. (Supported by NIH R01 DC-02253, the Pew and McKnight Foundations, and the NSF.)

106.3

INCREASED APOPTOSIS IN THE EMBRYONIC ZEBRAFISH TELENCEPHALON FOLLOWING REMOVAL OF THE OLFACTORY PLACODE. L.K.Cole* and L.S. Ross. Neurobiology Program, Dept. of Biological Sciences, Ohio University, Athens, OH 45701.

Apoptosis is a common type of cell death characterized by endonuclease fragmentation of the DNA. One proposed cause of apoptosis in neural development is a lack of trophic support during a critical period. In a previous study, apoptosis was observed in the telencephalon during the period when olfactory receptor axons are innervating the anlage of the olfactory bulb (Cole, Murray, and Ross, submitted). Afferent olfactory axons may support their target within the CNS, and cells within the target that fail to receive trophic support may be eliminated via apoptosis. To test this hypothesis, we unilaterally ablated the olfactory placode. The unoperated side served as a control. Apoptotic cells within the telencephalon were labeled using the TUNEL method. The number of apoptotic cells in the telencephalon of the operated side was significantly increased by 30 hours postfertilization (PF), as compared to the control side. By 36 hours PF, the difference in the two sides was substantially reduced, and by 50 hours PF, no significant difference was seen in the number of apoptotic cells on the control and experimental sides. These results suggest that during a critical period following axon arrival, trophic factors released by the ingrowing olfactory nerve prevent apoptosis of their target cells within the telencephalon. Supported by NSF IBN-9222896.

106.4

ODOR-STIMULATED CALCIUM SIGNALING IN CILIA AND DENDRITES OF OLFACTORY RECEPTOR NEURONS. F. Zufall*, T. Leinders-Zufall, M. N. Rand, G. M. Shepherd and C.A. Greer. Sections of Neurobiology and Neurosurgery, Yale Univ. Sch. of Med., New Haven, CT, 06510.

Odor detection takes place in the cilia of olfactory receptor neurons (ORNs), resulting in a rapid rise in cAMP which directly activates cyclic nucleotide-gated cation channels (CNG channels) allowing Na^+ and Ca^{2+} to enter the cell. Secondary to this response is a persistent increase in cGMP also leading to activation of CNG channels. Several recent observations have suggested that both cAMP and cGMP-induced Ca^{2+} signals act in concert to mediate sensory adaptation and regulate the sensitivity of olfactory transduction (Leinders-Zufall and Zufall, this meeting). It is therefore critical to gain detailed information about the spatial and temporal properties of ciliary Ca^{2+} gradients in ORNs. Using confocal imaging of salamander ORNs that were loaded with the Ca^{2+} indicator dye fluo-3AM (20 μM), local Ca^{2+} transients were resolved in individual cilia after a brief pulse of odor stimulation. Interestingly, an odor pulse led to the generation of Ca^{2+} transients with very similar temporal characteristics simultaneously in all cilia. This result supports the view that the cilia of a given cell possess similar properties with regard to their odor sensitivity, and that a given ORN integrates signals from different cilia. With repetetive stimulation, the primary Ca^{2+} transients declined over time showing signs of long-lasting adaptation while basal Ca^{2+} levels increased. This time-depedendent decline in responsiveness as well as portions of the basal Ca^{2+} signals could be abolished by inhibitors of the CO/cGMP second messenger system giving further support to our proposal that persistent activity of the cGMP pathway contributes significantly to Ca^{2+} levels and olfactory adaptation. Another series of experiments tested the effects of thapsigargin (100 nM), which irreversibly depletes Ca^{2+} from IP_3-sensitive stores, indicating that this treatment had relatively little effect on the generation of odor-dependent Ca^{2+} signals.

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106.5

ROLES OF CHORDA TYMPANI AND GLOSSOPHARYNGEAL NERVES IN NACL AND SWEET TASTE M.A. Barry*. Neuroscience Program, Univ. of Connecticut Health Center., School of Dental Medicine, Farmington, CT 06030-3705

Our studies examine the roles of specific peripheral nerves in the mediation of different taste qualities in golden Syrian hamsters. Behavioral techniques were utilized to examine the role of the chorda tympani (CT) nerve in NaCl taste. The roles of the CT and lingual branch of the glossopharyngeal (IX) nerves in NaCl and sucrose taste were assessed by changes in the central expression of c-*fos* protein following taste stimulation. In behavioral experiments, the CT nerve was sectioned bilaterally, and the CT or IX were sectioned unilaterally for c-*fos* studies. An intact CT nerve is required for normal preference and conditioned aversion behavior to 0.1-0.15 M NaCl. Also, the NaCl-taste induced expression of c-*fos* in parabrachial nucleus neurons is dramatically reduced by CT but not IX section. Our results in combination with those from other laboratories suggest that a unique sodium sensitivity is mediated by the CT, even though fibers in other taste nerves are responsive to NaCl. A new finding is that 0.1-0.25 M sucrose-taste induced c-*fos* expression in parabrachial nucleus neurons was reduced by IX but not CT section. Based on tract tracing experiments, these activated cells are in a location which is the target of afferent inputs from regions of the solitary nucleus with CT afferent input. Thus the lack of CT mediation of sucrose activation of these cells is not related to a regional lack of neuronal inputs. Behavioral and electrophysiological studies in other laboratories suggest that sweet taste is mediated primarily by the greater superficial petrosal (GSP) and CT nerves. However, some neurons in IX respond best to sweet compounds and not all behavioral responses to sucrose are eliminated by bilateral section of both the CT and GSP. Our results show that sweet taste, mediated by IX, but not the CT, activated and induced the formation of c-*fos* in central neurons, and suggest a specialized role for nerve IX in sweet taste.

Supported 2P50-DC00168-14A1 from NIDCD, NIH.

106.6

URINARY ODOR PROFILES DURING EQUINE ESTRUS AND DIESTRUS. W. R. Klemm* and Weidong Ma. Dept. VAPH, College of Veterinary Medicine, Texas A&M University, College Station, TX 77843-4458.

Horses use olfactory signals to coordinate reproductive behavior. The odor of an estrous mare is said to be detectable by stallions as far away as a half mile. We tested the hypothesis that profiles of volatile components in mare urine may differ in estrus and diestrus. Mare urine was analyzed by capillary gas chromatography (GC) to establish any qualitative and quantitative differences that may have potential value for chemical communication. Forty-five different volatile compounds were detected. Of these, 17 major GC peaks were common to all chromatograms. The profile of estrous urine was distinguished by the presence of a unique peak that was not present in diestrous samples. Numerous constituents exhibited endocrine dependence: 7 peaks had greater magnitude at estrus, whereas 4 peaks decreased their sizes at the same time. Statistical analyses also indicated that the normalized areas of many peaks changed significantly across the estrous cycle: the ratios were increased in 9 peaks, decreased in 6 peaks, and remained unchange in 2 peaks at estrus.

Because estrous urine, but not diestrous urine, elicits sexual behavior in the stallion, the unique peak, together with the peaks increased concentrations at estrus, may represent important chemical signals that stallions use to detect urinary estrous odors.

106.7

FUNCTIONAL ORGANIZATION AND PHARMACOLOGY OF RAT OLFACTORY BULB REVEALED BY OPTICAL IMAGING. S. Yagodin*, V. Aroniadou-Anderjaska, L.A. Zimmer, M. Ennis, M.T. Shipley and A. Keller. Dept. Anatomy, University of Maryland School of Medicine, Baltimore, MD 21201.

Synaptic interactions in an *in vitro* preparation of the rat main olfactory bulb were studied with high-speed, multiple site imaging of voltage-sensitive dye signals. Four hundred μm thick horizontal slices of olfactory bulbs from 3 to 4 week old rats were stained with RH-155 (100μM), placed in an interface chamber mounted on an upright microscope and perfused with oxygenated physiological solutions. Optical signals were collected through a 30X (0.9 N.A.) water-immersion objective and projected on a 100 element photodiode array. Stimulation in the olfactory nerve layer (0.2 to 0.5 ms, 0.1 to 0.5 mA) evoked a TTX-sensitive compound action potential (5 to 10 ms duration) that propagated from the stimulation site (0.3±0.04 m/s) into individual glomeruli. Smaller, longer-lasting depolarizing potentials (80 to 120 ms) were recorded from the glomerular, external plexiform and mitral cell layers. The amplitude of these potentials was largest in the glomerular layer and they were reversibly blocked by removing Ca^{2+} from the bath solution, indicating that they represent postsynaptic potentials evoked in neuronal elements within the glomeruli. The amplitude of these potentials was significantly reduced (-85%) by the non-NMDA receptor antagonist CNQX (10μM) and almost completely abolished by further addition of the NMDA receptor antagonist APV (50 μM). This suggests that olfactory nerve inputs to olfactory bulb neurons are mediated by glutamatergic receptors of the NMDA and non-NMDA types. The amplitude of the synaptic potentials was dramatically attenuated (80 to 90%) by application of the $GABA_B$ receptor agonist baclofen (10μM) but were not affected by the $GABA_A$ antagonist bicuculline (100μM). These, and other electrophysiological data from our laboratory, suggest a $GABA_B$-mediated *presynaptic* inhibition of olfactory nerve inputs to the olfactory bulb. Supported by PHS:NIH grants NS31078, DC02588 & DC00347.

106.8

A METHOD FOR GENERATION OF OLFACTORY STIMULI FOR fMRI. N. Sobel*[1], V. Prabhakaran[1], J.E. Desmond[2], G.H. Glover[3], E.V. Sullivan[4], J.D. Gabrieli[2]. Prog. in Neuroscience[1], Depts. of Psychology[2], Radiology[3], and Psychiatry & Behavioral Sciences[4], Stanford University, Stanford, CA 94305.

fMRI offers the means to functionally categorize brain regions involved in olfactory processing. To this end, we constructed a two channel air dilution olfactometer which operates within the fMRI environment. The olfactometer generates 30 L/min of activated-charcoal-cleaned air, passed through glass, temperature-controlled odor-saturators, up to a nasal mask equipped with 5 one-way valves permitting air to freely leave the mask. Switching vacuum lines achieved a stimulus rise-time below 500 msec. Subjects practiced pharyngeal closure such that the air was flowing out of the one-way valves except when the subjects sniffed. Each experiment consisted of 8 alternating 40s blocks of odor/no-odor; the odor in Exp. 1 was vanillin; Exp. 2, decanoic acid; Exp. 3, random alternations of the two odors. Odor concentration was set to levels above threshold following a full-length pretest. Every 8s, subjects were prompted by an on-screen instruction to sniff and to respond by pressing a button (balanced over conditions) indicating odor identification. The olfactometer, on-screen messages, and response recording were computer controlled. Functional data were acquired from eight 4mm slices, 2mm skip, taken at an oblique plain from frontal to temporal pole (31° to AC-PC) at 1.5 T using a T2* sensitive gradient echo spiral sequence (TR=720, TE=40, flip angle=65°). Data were analyzed using the cross-correlation method of Friston et al (1994). We observed significant activation ($p<.025$) in 3 healthy, right handed men in piriform cortex, entorhinal area, temporal pole, insula, and inferior medial-frontal gyrus, findings that are consistent with human lesion studies. Two subjects had asymmetric activation of orbitofrontal gyri, greater on the left, a pattern opposite to that observed in the only PET study of olfaction. This difference may be due to our use of different odors (pure olfactants as opposed to trigeminals) or to our currently small sample size.

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106.9

SEGREGATED PATHWAYS WITHIN THE ACCESSORY OLFACTORY BULB?
C. Jia* and M. Halpern. Program in Neural & Behavioral Sci., Box 5, SUNY. Hlth. Sci. Ctr. at Brooklyn, 450 Clarkson Ave., Brooklyn, NY 11203.

Previous studies have shown that there are two populations of receptor neurons in the vomeronasal organ (VNO). The Giα2-expressing population is located in the middle layer of the vomeronasal epithelium and projects to the anterior part of glomerular layer of the accessory olfactory bulb (AOB). The Goα-expressing population is located in the deep layer of the vomeronasal epithelium and projects to the posterior part of the glomerular layer of the AOB. Since the glomerular layer is the place where terminals of the vomeronasal receptor neuron form synapses with dendrites of AOB neurons, it is possible that the terminals in the anterior and the posterior compartments synapse with dendrites of different AOB neurons. In order to test this, AOB neurons were labeled with Lucifer Yellow and by Golgi impregnation methods and the distribution of dendrites of the labeled neurons was analyzed in opossums (*Monodelphis domestica*). Twenty-four mitral/tufted cells were injected intracellularly with Lucifer Yellow in lightly fixed, parasagittal (300 μm) sections of the AOB. Result showed that the apical dendrites of many mitral/tufted cells in the AOB terminated in more than one glomerulus. Some of the mitral/tufted cells sent apical dendrites to the anterior compartment of the glomerular layer, while other mitral/tufted cells sent apical dendrites to the posterior compartment. No mitral/tufted cell with apical dendrites entering both anterior *and* posterior glomerular layer was found. On the other hand, the secondary dendrites of the mitral/tufted cells spread extensively in the mitral/tufted cell layer but they did not enter the glomerular layer. Similar results have been obtained by Golgi impregnation method. These observations suggest that the anterior and posterior compartments of the glomerular layer are innervated by different populations of mitral/tufted cells in the AOB and therefore the pathways from the different populations of vomeronasal receptor neurons continue to be segregated in the AOB.

Supported by NIDCD Grant DC02745.

SECOND MESSENGERS AND PHOSPHORYLATION I

107.1

EFFECTS OF D2 DOPAMINE RECEPTOR STIMULATION ON MITOGEN-ACTIVATED PROTEIN KINASES (ERKs and JNKs).
Seema Basu, Donald H. VanLeeuwen and Robert G. MacKenzie*. Parke-Davis Pharmaceutical Research, Division of Warner-Lambert Company, Ann Arbor, MI 48105.

CHOpro-5 cells expressing human D2 dopamine receptors were used to study D2 receptor mediated stimulation of [3H]thymidine uptake. Cells were challenged with the D2 agonist quinpirole or the growth factor insulin. Both ligands stimulated [3H]thymidine uptake by approx. 20-fold. Pertussis-toxin treatment blocked the response to quinpirole but not to insulin. The effects of quinpirole and insulin on ERKs were studied because activation of these kinases are typically associated with mitogenic indices such as stimulation of [3H]thymidine uptake. Quinpirole and insulin both tyrosine phosphorylated ERK2 to the same extent. Quinpirole and insulin also activated ERK2 to the same extent (20-fold above basal). Pertussis toxin blocked the activation of ERK2 by quinpirole but not by insulin. Insulin stimulated the tyrosine phosphorylation of the insulin receptor substrate-1 (IRS-1) and the activity of the p85-associated PI3-kinase enzyme but quinpirole did not. Both insulin and quinpirole increased GTP-loading of ras. Both insulin and quinpirole tyrosine phosphorylated the adapter protein, Shc, suggesting that this might be a crossover point of G-protein coupled signalling onto the growth factor pathway. Interestingly, quinpirole but not insulin, strongly stimulated JNK1 activity and the quinpirole effect was completely blocked by pertussis toxin. Results from these experiments suggest that quinpirole and insulin stimulate [3H]thymidine uptake via activation of the ERK2 growth factor pathway but initiation of the signal differs between G-protein coupled and growth factor pathways. (Supported by Warner-Lambert.)

107.2

PROSAPOSIN AND PROSAPTIDE STIMULATE TYROSINE PHOSPHORYLATION AND ACTIVATION OF THE MAPK CASCADE IN PC12 CELLS.
W.M.Campana, M. Hiraiwa, K.C. Addison and J.S O'Brien * Department of Neurosciences, University of California, San Diego, Center for Molecular Genetics, La Jolla, CA 92093-0634

Prosaposin is a 66 kDa glycoprotein which has neurotrophic activity *in vitro* and *in vivo*. The trophic sequence has been pinpointed to a sequence of 22 amino acids (^{8}CEFLVKEVTKLIDNNKTEKEI^{29}L) in the domain for saposin C. In this study, we demonstrate that prosaposin and the 22-mer peptide, prosaptide, activate the MAPK cascade in PC12 cells. Binding studies using ^{125}I-prosaposin and ^{125}I-prosaptide revealed a single class of binding with a Kd of 10 nM and a Kd of 19.6 nM, respectively. Both prosaposin and prosaptide rapidly stimulated tyrosine phosphorylation. Western blotting experiments using a polyclonal antibody prepared against phosphorylated MAPK demonstrated a 15-20 fold stimulation of MAPK phosphorylation by prosaptide as well as prosaposin in the pM ranges. In contrast to NGF, phosphorylation of MAPK after treatment with prosaposin was transient since signaling was reduced after 30 minutes. Activation of MAPK was decreased when ^{21}Asn within prosaptide was changed to ^{21}Asp. Prosaposin and prosaptide also increased phosphorylation of the upstream signaling protein, Shc, and promoted prosaposin induced association of Grb2 with phosphorylated Shc. These findings demonstrate a role for src homology (Sh2) proteins and MAPK pathways in prosaposin signaling.

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107.3

VILIP - A MEMBER OF A FAMILY OF NEURONAL CALCIUM-BINDING PROTEINS: FUNCTIONAL STUDIES IN TRANSFECTED CELLS.
K.-H. Braunewell, W. Wetzel* and E. D. Gundelfinger, Federal Institute for Neurobiology (IfN), P.O. Box 1860, D-39008 Magdeburg, F.R.G.

VILIP (visinin-like-protein) is a member of a new sub-family of EF-hand Ca^{2+}-binding proteins, which also includes visinin, recoverin/S-modulin and frequenin. VILIP is expressed by a subset of neurons in the chicken brain, in the olfactory epithelium of rat and in the retina of different species at the time of their terminal differentiation. The molecule is localized in cell bodies as well as in axonal and pre- or post-synaptic structures. Two of the four EF-hand structures of VILIP are able to bind Ca^{2+} or Mg^{2+} with affinity constants in the μmolar and mmolar range, respectively. In order to determine the possible cellular function of the protein we transfected several non-neural and neural cell lines.

In transfected C6 glioma cells VILIP was shown to be myristoylated at its N-terminus. The myristoylation is most likely a prerequisite for the calcium-dependent membrane association of the otherwise cytosolic protein. This is in agreement with co-localization studies in PC 12 cells, where VILIP expression is strongly associated with membrane structures, such as the actin-rich cortical cytoskeleton. A strong immunoreactivity is also observed at cell-cell contact sites. We looked for the possible involvement of VILIP in calcium-regulated cellular signalling. In transfected glioma cells VILIP strongly enhances cAMP production following ß-adrenergic stimulation of the adenylate cyclase. Our results suggest that VILIP can act as a Ca^{2+}-sensor molecule. It may transmit Ca^{2+}-signals from or to the membrane-associated cortical cytoskeleton, thereby affecting processes, such as neurotransmitter release, synaptic plasticity or second messenger cascades. *This work is supported by DFG*

107.4

IN-SITU STUDIES OF CALCIUM DIFFUSION AND ENDOGENOUS CALCIUM BUFFERING CAPACITY IN CULTURED *APLYSIA* NEURON.
M. Gabso E. Neher* and M.E. Micha. The Hebrew University of Jerusalem, Israel, and the Max-Planck-Institut Fur Biophysilalische Chemie, Gottingen, Germany.

Two processes determine the range of intracellular calcium (Ca) operation as a second messenger: Ca-diffusion and Ca-removal. To date no *in-situ* experimental data are available to estimate the parameters underlying these processes separately. Attempts to characterize Ca-diffusion by imaging techniques suffer from the fact that presence of indicator dyes may strongly influence the processes under study.

In certain limiting cases Ca-diffusion can be described by an apparent Ca-diffusion which depends on D_{Ca}, the diffusion coefficient of the free Ca, on D_e, the diffusion coefficient of endogenous Ca-buffers, and on D_f, the diffusion coefficient of the indicator dye according to $D_{app}=(D_{Ca}+D_e\kappa_e+D_f\kappa_f)/(1+\kappa_e+\kappa_f)$ where κ_e and κ_f denote the so-called Ca-binding ratios (dCa_{bound}/dCa_{free}) of the endogenous buffer and the indicator dye, respectively.

In the present study we evaluated D_e and κ_e by measuring D_{app} in the large cylindrical axons of cultured metacerebral neurons isolated from *Aplysia* ganglions. As a prerequisite we had to measure D_f from images of the spatiotemporal distribution pattern of fura-2 pentapotassium pressure injected into the axon. The value obtained was 102μm^2/s. κ_f was calculated from the known intracellular fura-2 concentration and its K_D of 760nM. D_{app} was measured by fura-2 ratio imaging, analyzing the spread of pressure micro injected Ca^{2+} into axons.

From the plot of D_{app} as a function of κ_f (for fura-2 concentrations in the range 25-175μM) we estimated D_e to be as low as 1-20μm^2/s and κ_e to be 20-40, assuming a value for D_{Ca} of 220μm^2/s. D_e and κ_e have to be considered as lumped values for mobile and fixed endogenous Ca-buffers. We noted that fura-2, even at very low concentrations, strongly influences Ca-redistribution.

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107.5

NITRIC OXIDE INDUCES THE CONFORMATIONAL CHANGE OF NEURON-SPECIFIC PROTEIN KINASE C SUBSTRATE, NEUROGRANIN. F.-S. Sheu*, H.-M. Chen, H.H. Miao, S.Y. Au and K.Y. Chui. Dept. of Biochemistry, Hong Kong University of Science & Technology, Clear Water Bay, Kowloon, Hong Kong.

In the brain, the possible role of nitric oxide (NO) as signaling messenger has been linked to its involvement in synaptic plasticity of long-term potentiation (LTP), an electrophysiological model of learning and memory, which can be triggered in several brain regions including the hippocampus and the neocortex. The neural target(s) of NO is an important subject related to the mechanisms underlying the biological functions of NO in the brain. Since protein kinase C (PKC) activation leading to its substrates phosphorylation is observed during LTP expression, we seek to explore whether a soluble PKC substrate, neurogranin (NG) localized at the postsynaptic site, could be served as a molecular target of NO. NG binds to calmodulin (CaM) in the low [Ca^{2+}] and releases CaM in response to the increased intracellular [Ca^{2+}] upon NMDA receptor activation after LTP induction. The resultant released of CaM plus increased [Ca^{2+}] provides signals to activate NO synthase to produce NO from arginine. We approached the effect of NO on NG by testing whether NO generating agents could alter the biophysical and biochemical properties of purified NG in vitro. We found that several NO generating agents including diethylamine nitric oxide and some NO-amine complexes which can release NO spontaneously in the aqueous solution, oxidized NG leading to its conformational change to form intramolecular disulfide bond(s) as evidenced by the increased electrophoretic mobility of NG in SDS-PAGE. The oxidation effect of NO on NG was reversible by reducing agents such as DTT added back to the oxidized NG. The conformational change of NG from the reduced to the oxidized form was further examined by circular dichroism. The reduced NG showed a predicted secondary structure of α-helix, turn and random coil whereas the oxidized form became unfolded to near random coil. Work is in progress to determine the potential biological significance of NO effect on NG. [Supported by Hong Kong RGC Direct Allocation Grant (95/96. SC02)].

107.6

Mn^{2+}-DEPENDENT PHOSPHORYLATION INHIBITS BRAIN NITRIC OXIDE SYNTHASE ACTIVITY IN THE ADULT POSTSYNAPTIC DENSITY. J.L. Xu*, P.C. Suen, H.T.J. Mount, S.Y. Lin and K. Wu. Department of Neuroscience and Cell Biology, Robert Wood Johnson Medical School, UMDNJ, Piscataway, NJ 08854

Nitric oxide (NO) plays key roles in diverse processes, including neurotransmission in the peripheral and central nervous systems. Synthesis of NO from L-arginine is a rate-limiting step in NO action and is catalyzed by nitric oxide synthase (NOS). Activity of NOS is regulated, in turn, by phosphorylation. Electron microscopy and Western blots reveal that NOS is an intrinsic component of the postsynaptic density (PSD), a disc-shaped structure apposed to the inner surface of the postsynaptic membrane. In this study, we examined the effects of local protein tyrosine kinase (PTK) and cAMP-dependent kinase (PKA) on NOS activity in the isolated PSD faction of adult rat cerebral cortex. Phosphorylation in the presence of Mn^{2+}, an activator of PTK, resulted in a dramatic decrease in PSD NOS activity, as measured by induction of NO-stimulated NAD incorporation into glyceraldehyhyde-3-phosphate dehydrogenase. In contrast, cAMP-dependent phosphorylation of PSD proteins had no effect on NAD incorporation, consistent with the observation that PKA-dependent phosphorylation of purified NOS does not alter activity of the enzyme. Our findings suggest that activation of PTK reduces NO formation by inhibiting NOS activity.

(Supported by NIH grant HD 23315)

107.7

SECOND MESSENGER MECHANISMS UNDERLYING THE ENHANCEMENT OF Ih BY SEROTONIN IN THE LOBSTER STOMATOGASTRIC GANGLION. C.M. Hempel[1], P. Vincent[2], R.Y. Tsien[3] and A.I. Selverston[4]* Depts. of Biology[1,4] and Pharmacology[2,3] and HHMI[3], UCSD, La Jolla, CA 92093. Department of Biology, UCSD, La Jolla CA 92093.

The neuronal circuitry of the crustacean stomatogastric ganglion can be reconfigured by a number of neuromodulators. These modulators act in part by altering the voltage dependent currents in the constituent neurons, presumably via second messenger pathways. For example, serotonin enhances the hyperpolarization-activated, inwardly rectifying current, Ih. We examined the signal transduction mechanisms underlying this effect in cultured stomatogastric ganglion neurons. Serotonin enhanced Ih, with an EC_{50} of approximately 20 nM. Three pharmacological agents (forskolin, IBMX and 8-bromo-cAMP), which are known to elevate intracellular cAMP levels, all enhanced Ih. Fluorescence imaging with the cAMP indicator, FlCRhR, revealed that serotonin increased cAMP concentration. These results suggest that the action of 5HT is at least partially mediated by the cAMP signal transduction pathway. Further pharmacology will determine whether 5HT is acting via the cAMP-dependent protein kinase (PKA), or by a PKA-independent mechanism. Supported by NIH grant #25916 to A.I.S., NS27177 to R.Y.T and a Lavoisier fellowship to P.V.

107.8

FUNCTIONAL DIVERSITY OF *DROSOPHILA* CALCIUM/CALMODULIN DEPENDENT KINASE II ISOFORMS. B. GuptaRoy, K. Beckingham† and L.C. Griffith* Department of Biology and Center for Complex Systems, Brandeis University, Waltham MA 02254 and †Department of Biochemistry and Cell Biology, Rice University, Houston, TX 77251.

Expression of a gene for a specific peptide inhibitor of calcium-calmodulin dependent protein kinase II (CaM kinase II) in *Drosophila* produces defects in both associative and non associative learning (Griffith, et. al. Neuron **10**: 501-9). To understand the molecular basis of this phenomenon we have cloned and characterized several isoforms of CaM kinase II from *Drosophila*. These isoforms are produced by alternative splicing from a single gene. Seven of these isoforms were expressed in a COS cell expression system and purified for functional characterization. All these show nearly identical biochemical properties , except for a slightly elevated Km for CaM of one isoform (GuptaRoy and Griffith, J. Neurochem. **66**: 1282-88). However, these isoforms showed differences in activation by two series of CaM mutants. They also appear to have differential ability to phosphorylate known substrates of CaM kinase II, such as Eag, synapsin 1 and tyramine β hydroxylase.

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107.9

MECHANISM OF ANGIOTENSIN II REGULATION OF NEURONAL AT_1 RECEPTOR PHOSPHORYLATION. H. Yang, D. Lu, W. G. Luttge* and M. K. Raizada. Department of Physiology, University of Florida, College of Medicine, Gainesville, FL 32610.

Phosphorylation is a key regulatory event in agonist-induced desensitization and internalization of G-protein coupled receptors. Our objectives in this study were to determine if Angiotensin II (Ang II) regulates phosphorylation of AT_1 receptor (R) and if so, to determine the involvement of various kinases on this phosphorylation. Ang II caused a time- and dose-dependent stimulation of AT_1R phosphorylation. A maximum phosphorylation of ~6-fold was observed with 100 nM Ang II, was blocked by AT_1R antagonist, losartan, and was associated with an internalization of the AT_1R. Phospho-amino acid analysis of Ang II-treated neurons revealed a predominance of phosphoserine residues. Immunoblotting indicated that AT_1R co-precipitates with MAP kinase, suggesting the role of this enzyme in phosphorylation of AT_1R. This conclusion was further supported by the following observations. (a) AT_1R was phosphorylated by MAP kinase at serine residues. (b) Exogenous MAP kinase stimulated phosphorylation of AT_1R at serine residues that is comparable with Ang II. (c) U98059, a MAPKK inhibitor, blocked Ang II stimulation of AT_1R phosphorylation, and (d) MAP kinase phosphorylation of AT_1R leads to a loss in the ability of AT_1R to bind Ang II. These observations suggest that Ang II stimulates phosphorylation of AT_1R at serine residues, an effect mediated by MAP kinase. This phosphorylation is associated with internalization of these receptors. (Supported by NIH grant 33610).

107.10

G PROTEIN ALPHA SUBUNITS ASSEMBLE TO FORM LARGE FILAMENTOUS STRUCTURES IN VITRO. S. Roychowdhury [1], J. R. Hepler[2], M. E. Knight,[1] and M. M Rasenick[1]*, [1]Dept. of Physiology & Biophysics, U. Illinois College of Medicine, Chicago, IL 60612 and [2]Dept. of Pharmacology, U. Texas Southwestern Medical Center., Dallas, TX 75235

Results from several laboratories suggest that G proteins exist as heterotrimers in vivo, consisting of α and βγ subunits, α being the site of GTP binding. Recently, we have observed that α subunits of Gi1 or Gs are capable of forming larger polymeric structures in vitro, as judged by electron microscopy. Purified, recombinant Gsα (0.5-1 mg/ml) assembled to form regular arrays of filaments, 5-15 nm wide and hundreds of micrometers long, when incubated at 37°C for 30 minutes in presence of GTP or GDP. The filaments were found largely in bundles, occasionally twisted at irregular intervals. Filaments were not evenly distributed throughout the grid, suggesting an unfavorable nucleation step. Many isolated short filaments of variable lengths were also observed. Purified Gi1α assembled to form long filaments as well as isolated short filaments. Gi1α filaments were relatively straight and appeared to be wider (15-30 nm) than Gsα filaments. In some filaments protofilaments were easily detectable. Myristoylated-Gi1α was the better candidate for polymer formation than non-myristoylated Gi1α. The formation of regular polymers is dependent upon incubation of Gα with GTP or GDP, suggesting that, due to intrinsic GTPase activity, it is the GDP bound form of Gα which polymerizes. This is consistent with the recently reported crystallographic structure of Gi1α, which suggests that GTP hydrolysis triggers changes in the Gα molecule allowing the GDP form of Gi1α to attain a conformation capable of forming a head to tail polymer. We are currently trying to optimize the conditions for Gα assembly and to determine the role of nucleotides in that process by using nucleotide analogs and a GTPase deficient mutant of Gi1α. While the biological relevance of G protein polymers has yet to be determined, two possible roles are suggested. 1) G protein polymers might channel information to specialized regions, such as dendritic spines and 2) through interaction with cytoskeletal elements, G protein polymers might participate in synaptic remodeling. Supported by NIMH

107.11

INDUCTION OF NFAT-MEDIATED TRANSCRIPTION BY G_q-COUPLED RECEPTOR ACTIVATION. V. Boss*, D. J. Talpade and T. J. Murphy, Dept. of Pharmacology, Emory Univ., Atlanta, GA 30322

The nuclear factor of activated T cells (NFAT) is well known as an inducible transcription factor activated by antigen stimulation of the T-cell receptor in lymphocytes. Stimulation of NFAT-mediated transcription is now reported in PC12 cells, as well as in Jurkat T cells, following activation of a neurotransmitter receptor. The muscarinic receptor agonist carbachol induces robust luciferase responses in Jurkat and PC12 cells expressing an NFAT-luciferase reporter construct and an m3 muscarinic receptor that couples to phospholipase C-catalyzed phosphoinositide hydrolysis through G_q. Cyclosporin blocks this response. In PC12 cells expressing a G_i-coupled m2 muscarinic receptor, carbachol induces NFAT-mediated luciferase activity that is strictly dependent upon co-expression of a chimeric $G\alpha_q/\alpha_i$ subunit, which confers G_q-effector coupling on G_i-linked receptors. Taken together with reports identifying NFAT isoforms in neural tissue, these findings suggest that NFAT may play a role in neuronal signal transduction, and that neurotransmitters acting on G_q-protein coupled receptors may serve as physiological stimulators of NFAT.

Supported by NIH grants HL48252 and HL52810.

107.12

PROSTAGLANDIN $F_{2\alpha}$ IS REQUIRED FOR NMDA RECEPTOR-INDUCED TRANSCRIPTION OF c-*fos* mRNA IN DENTATE GYRUS NEURONS. L. S. Lerea*[1], N. G. Carlson[1], M. Simonato[1], J. D. Morrow[2], L. J. Roberts[2] and J. O. McNamara[1]. [1]Department of Medicine, Division of Neurology, Duke University Medical Center, Durham, NC, 27710, [2]Department of Pharmacology, Vanderbilt University, Nashville, TN., 37232

Activation of NMDA receptors has been linked to lasting physiologic and pathologic changes in the mammalian nervous system. The cellular and molecular mechanisms underlying permanent modifications of neuronal structure and function following brief episodes of neuronal activity are not known. Immediate early genes (IEGs) have been implicated in the conversion of short-term stimuli to long-term changes in cellular phenotype via regulation of target gene expression. The intracellular signaling pathways coupling activation of cell-surface NMDA receptors to nuclear IEG transcription are poorly understood. Here we show, for the first time, that NMDA receptor activation *in vitro* triggers the synthesis and release of two diffusible prostanoids, $PGF_{2\alpha}$ and PGE_2. We further demonstrate that $PGF_{2\alpha}$, but not PGE_2, is required for NMDA-dependent transcription of the IEG c-*fos* in neurons derived from the dentate gyrus. These findings identify the diffusible messenger $PGF_{2\alpha}$ as obligatory for NMDA receptor-mediated transcription of a nuclear IEG and reveal a novel mechanism by which activation of a cell surface receptor leads to a diffusible signal that can influence gene transcription.

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107.13

LIGAND INDEPENDENT ACTIVITY OF HUMAN ESTROGEN RECEPTORS IS TYROSINE KINASE DEPENDENT. J.J. Mulchahey[1]*, P.M. Plotsky[1], C.B. Nemeroff[1], E. v. Angerer[2], G.K. Stalla[3], and C.J. Newton[3]. [1]Department of Psychiatry & Behavioral Sciences, Emory Univ. School of Medicine, Altanta, GA 30322, [2]Institute for Pharmacy, Univ. Regensburg and [3]Max Planck Institute for Clinical Psychiatry, Munich.

Rodent estrogen receptors (ER) display a ligand-independent ability to drive estrogen-responsive reporters. Our study sought to extend our previous studies of this phenomenon to include human ER (hER) and to investigate the molecular mechanism underlying the intrinsic transcription factor activity of hER. When hER-containing breast cancer cells (MCF-7) stably transfected with a tk-ERE-luciferase reporter plasmid were cultured in steroid free conditions, basal expression of luciferase was detected. Expression was positively coupled to exogenous estradiol (421±63% of control; mean±SEM; n=6; $p<0.05$ at 1 nM). Basal luciferase expression could be reduced (52±17% of control; n=3; $p<0.05$) by treatment with the pure antiestrogen ZM182780 (10 nM), demonstrating a ligand-independent activity of hER. Treatment of unstimulated cells with the protein kinase C (PKC)-activator phorbol myristate acetate (0.1 μM) increased luciferase expression and this increase could be prevented with the PKC inhibitor chelerythine chloride (6.6 μM). Chelerythine alone had no effect on luciferase expression, suggesting that the PKC pathway is not involved in ligand-independent expression. In contrast, the tyrosine kinase inhibitor herbimycin (2.0 μM) was able to reduce basal expression of luciferase in this system (53±12% of control; n=5; $p<0.05$), suggesting that the ligand-independent activity of hER is at least partially dependent on tyrosine kinase phosphorylation of the ER. These findings offer new information of the mechanism of ligand-independent action of the hER. Additionally, our findings offer insight into the possible modulation of estrogen feedback to the CNS as well the estrogen-dependent and -independent proliferation of human breast cancers. Supported by the Emory Univ. Psychiatry - MPI Psychiatry Exchange Program and RISP grant MH 51761.

REGENERATION I

108.1

LOCALIZATION OF TRANSMITTER ENZYMES, CYTOSKELETAL ELEMENTS AND GROWTH FACTORS IN WHOLE MOUNT PREPARATIONS OF IMMATURE OPOSSUM CNS. J.M. Luque[1] E. Reinhard[1], B.M. Riederer[2], D.F. Kusewitt[3] and J.G. Nicholls[1]*. [1]Dept. Pharmacol. ,Biozentrum, Univ. Basel, 4056, Switzerland, [2]Inst. Anat. Univ. Lausanne, 1005, Switzerland, [3] Lovelace Inst., Albuquerque, NM 87108,

The CNS of the neonatal opossum (*Monodelphis domestica*) can be isolated in its entirety and maintained in culture where it not only survives but shows remarkable regeneration. Procedures have been devised for visualising the distribution and sites of synthesis for key signaling molecules in transparent whole mounts of the CNS. We have surveyed the major groups of catecholaminergic cell bodies and processes by applying antibodies against tyrosine hydroxylase. The developmental sequence is comparable to that in eutherian mammals with the exception of the olfactory bulb: in new born opossum the dopaminergic neurons are detected at a far earlier stage of development. Similarly, antibodies against neurofilaments, microtubule associated proteins and GAP-43 have been used to evaluate neurite outgrowth during development and regeneration. To examine cells expressing mRNAs encoding basic fibroblast growth factor, we used RNA probes generated from opossum FGF-2 cDNA. Labeled cells were already detected at the day of birth. They were distributed in proliferation regions, in discrete areas of septum, colliculus and cerebellum as well as the choroid plexus. By contrast message for low affinity nerve growth factor receptor was most abundant in spinal motoneurons. These experiments set the stage for imaging specific neuronal populations and assessing changes that occur after pharmacological manipulations or after lesioning the CNS.

Supported by grants from the Swiss Nationalfonds and from the International Research Institute for Paraplegia.

108.2

DEVELOPMENT AND REGENERATION OF MONOSYNAPTIC CONNECTIONS IN THE NEONATAL OPOSSUM (MONODELPHIS DOMESTICA) M. Lepre, W.B. Adams* and J.G. Nicholls, Department of Pharmacology, Biozentrum, University of Basel

A first aim of these experiments was to determine at what stage of development dorsal root fibres make monosynaptic contacts with cervical motoneurons in the same and in adjacent segments. At birth, stimulation of a dorsal root generates a short latency response in ventral roots followed by polysynaptic reflexes. When dorsal and ventral roots were stained with HRP, DiI or DiO afferent fibres were seen to end in the dorsal horn and on dendrites of motoneurons with enlargements that resembled synaptic boutons. Such boutons were seen in close apposition to motoneurons of the same segment and of neighboring rostral and caudal segments in pups aged 0 -9 days.

In experiments to study regeneration lesions were made by crushing the spinal cord between two segments. After culture for 6-7 days, electrical stimulation of the dorsal roots once again induced discharges in ventral roots. Labeled fibres were observed that had grown beyond the crush toward the motoneurons.

We are now making intracellular recordings and using electron microscopy to establish unequivocally whether connections observed in developing and regenerated spinal cord are monosynaptic as well as polysynaptic.

Supported by grants from the Swiss Nationalfonds and from the International Research Institute for Paraplegia.

108.3

NERVE GRAFT PREDEGENERATION ENHANCES PREFERENTIAL MOTOR REINNERVATION (PMR)

T.M. BRUSHART*. Depts. of Orthopaedics and Neurology, Johns Hopkins Hospital, Baltimore, Maryland 21287

Motor axons regenerating after transection of mixed nerve preferentially reinnervate distal motor branches. Collaterals of a single motor axon often enter both sensory and motor Schwann cell tubes of the distal stump; specificity may be generated by pruning collaterals from sensory pathways while maintaining those in motor pathways (J. NSci. 13:2730). These experiments evaluate PMR in fresh and predegenerated nerve grafts. Surgery was performed on the proximal femoral nerves of 250 gm female SD rats. In twenty animals, an inverted "Y" graft of femoral trunk and distal cutaneous and muscle branches was transposed to the opposite leg and sewn in place with 10-0 sutures. In 20 others, the graft was predegenerated by proximal crushes 4 and 2 weeks before transposition. The specificity of motor axon regeneration was evaluated at 3 mos. by applying HRP to one terminal branch and Fluoro Gold to the other. The mean number of motoneurons projecting axons correctly to the muscle branch and incorrectly to the cutaneous branch was significantly different in both groups (Graft:M=186,S=124, DL(double-labeled)=10; Predegenerated Graft:M=245, S=94, DL=16: for both sensory/motor differences $p<.01$). However, significantly more motoneurons projected correctly after predegeneration ($p=.01$). The sensory/motor difference, an index of specificity, was also greater (151 vs. 62, $p=.001$). Predegeneration of nerve grafts thus greatly enhances the specificity of motor axon regeneration (PMR). This could reflect clearance of inhibitory molecules from the graft, increased production of a contact or trophic factor within the muscle branch, or both. (supported by the Curtis Research Fund and JHU Orthopaedics)

108.4

EVIDENCE FOR PREFERENTIAL MOTOR REINNERVATION (PMR) IN NONHUMAN PRIMATES,

R. D. Madison*, S.J. Archibald, and C. Krarup. Div. of Neurosurgery, Duke Univ. Med. Ctr., Durham, NC 27710, and Dept. of Clinical Neurophysiol. Univ. Hosp., Copenhagen, DK

PMR refers to the proven demonstration that motor neurons which project to the rat femoral nerve preferentially reinnervate a terminal motor vs. cutaneous nerve branch (J. Neurosci. 8, 1026-1031; 1988). Motor unit counting of median nerve innervated muscles in the hand was used to assess the possibility of PMR occurring in nonhuman primates. Binomial probability statistics were used to determine the "expected" number of returning motor units if regeneration occurred randomly. Median nerves transected at the wrist were repaired by either direct suture (N=4), or by collagen nerve guides to bridge an unstructured 2 cm (N=6), or 5 cm (N=7) nerve gap. There were significantly more regenerated motor units found in all groups 2-3 years post-repair (range of $\bar{x}\pm$SEM of 13 ± 1 to 18 ± 1; from baseline of 147 ± 14) compared to that expected by chance (6 ± 2; $p<.01$). This is strong evidence that PMR influences regeneration accuracy in nonhuman primates. Supported by NS22404-11 (RDM), VA Merit Review (RDM), and Integra LifeSciences (RDM), Danish Med. Res. Council (CK).

108.5

DEFICIENT ACTIVATION OF MICROGLIA DURING OPTIC NERVE DEGENERATION.

S. Rotshenker* and F. Reichert, Dept. of Anatomy & Cell Biology, Hebrew University-Hadassah Medical School, Jerusalem 91120, ISRAEL.

Optic nerve (ON) injury is followed by the slow removal of myelin: considerable amounts of myelin are still present months after ON transection. Microglia, the potential scavenger cells, are present in-vivo throughout intact and degenerating ON. The question thus arises as to why is myelin removal by phagocytosis so inefficient. One possibility is that microglia are not capable of phagocytosing myelin. Alternatively, microglia may not become activated in-vivo to the extent that they will phagocytose myelin. We studied ON degeneration in relation to the expression of cell surface molecules that are relevant to phagocytosis, and are characteristically displayed by activated macrophages capable of myelin phagocytosis: MAC-1 (CD 11b, a complement receptor), FcγII/III receptor (FcR), MAC-2 (a galactose specific lectin, and a macrophage/microglia activation marker), and F4/80 (a murine specific macrophage/microglia antigen). In-vitro, the vast majority of microglia, but no other nonneuronal cell type, expressed all four molecules and phagocytosed myelin. In-vivo, intact ON displayed high levels of MAC-1, little FcR and F4/80, and no MAC-2. The expression of these molecules was upregulated differentially in in-vivo degenerating ON: MAC-1 uniformly, FcR and F4/80 variably, and MAC-2 sporadically. Thus in-vivo, only a small proportion of microglia become activated after ON injury to the extent of co-expressing all four molecules. The sporadic pattern of distribution of MAC-2 expression correlated best with a similar sporadic pattern of structural degeneration. Thus in-vivo, ON injury is followed by deficient microglia activation, which we suggest contributes to the slow clearance of myelin.

108.6

CONNECTIONS BETWEEN REGENERATING RETINA AND BRAIN IN ADULT GOLDFISH.

M.K. Powers*, P. Melzer, A.E. Lindsey and R.M.Wall. Vanderbilt Vision Research Center, Vanderbilt University, Nashville, TN 37240.

The CNS of adult goldfish regrows after surgical or pharmacological lesions. We have previously shown that a new neural retina, regenerated in vivo in adult fish, is capable of providing visual information to the animal. The electroretinogram returns, as do simple reflexive visual behaviors such as the dorsal light reflex (DLR) and optokinetic nystagmus (OKN). Connections must therefore be made from the new retina to the brain, a finding supported by Stuermer's observation that ganglion cell axons from regenerated retina do reach the tectum. However, we have also found that complex, learned visual behaviors, which are likely to be mediated through tectal connections, may not return. Here we report results of 2-deoxyglucose (2DG) studies designed to determine whether tectal neurons can be activated through new retinal projections. Fish with retinas destroyed either surgically or by intraocular ouabain injection were injected IP with 5 uCi/30ul 2DG at times up to 130 days following retinal destruction, then stimulated visually and sacrificed. Enucleated animals showed clear differences in brain activity, with the intact eye strongly stimulating the tectum and the enucleated not at all. Up to 81 days following ouabain injection, the regenerating retina had significantly less input to the tectum than the normal eye, producing very little activity. At longer times more activity was present, but only in some cases. We conclude that tectal input is not necessary for DLR and OKN, which return fully by 80 days regeneration, and we speculate that deficits in vision observed with other behaviors may be related to abnormal connectivities between regenerating ganglion cell axons and tectal neurons.

Supported by The National Institutes of Health (NEI) and Vanderbilt University.

108.7

REGULATION OF NEUROPEPTIDES IN AXOTOMIZED SYMPATHETIC NEURONS *IN VIVO* AND *IN VITRO*

L. Klimaschewski*, S. Kroesen, R. Fischer-Colbrie and C. Heym. Institute of Anatomy and Cell Biology, University of Heidelberg, 69120 Heidelberg, FRG.

The peptides galanin and neuropeptide Y (NPY) are elevated in the rat superior cervical ganglion after axotomy of the postganglionic branches. These changes have been attributed to increased synthesis of cytokines. 'Knock-out'-studies indicated that leukemia inhibitory factor (LIF) which is synthesized in non-neuronal cells after lesion may be responsible for the up-regulation of various peptides. On the other hand, LIF *decreases* NPY and its mRNA in sympathetic neuron cultures. This apparent contradiction was investigated by determination of NPY levels and NPY mRNA in lesioned ganglia. In addition, the regulation of galanin by LIF and by non-neuronal cells was examined in neonatal sympathetic neuron cultures.

Compared to control ganglia, the number of NPY immunoreactive neurons decreased whereas the density of NPY positive neuronal processes increased significantly one week after transection of the major postganglionic nerves. The nerve fibers extended into both carotid branches and ramified at the lesion site. Although the number of NPY mRNA positive neurons was not different from controls, the average grain density per neuron decreased by 40 % consistent with an effect of LIF on this neuron population. When axotomized ganglia were decentralized simultaneously, a threefold elevation of NPY immunoreactivity was detectable by radioimmunoassay and an additional increase in numerical densitiy of NPY immunoreactive nerve fibers was observed. Further reduced levels of NPY mRNA were found within postganglionic neurons. This synergistic effect of combined axotomy and decentralization on peptide content was detected for galanin also. Moreover, the up-regulation of galanin was observed in cultures of sympathetic neurons too. Addition of LIF to the medium significantly enhanced this effect. Supported by the DFG (Zi 110/22-3).

108.8

DUAL FLUORESCENCE STAINING DEMONSTRATES THE MORPHOLOGICAL RELATIONSHIP BETWEEN REGENERATING AXONS AND BLOOD VESSELS.

M.I.Hobson, G.L.Ferri*[1], C.Green, R.Brown, G.Terenghi. Blond McIndoe Centre, East Grinstead Sussex, U.K. and Dept. of Citomorfologia, University of Cagliari, Italy[1].

Nerve regeneration is dependant on many cellular and humoral factors and recently the role of neurotrophic factors has been widely investigated. Although it has been observed that an increase in capillary and permeability accompany axonal regeneration, the role and importance of angiogenesis is still poorly understood. It was the aim of this study to clarify the morphological relationship between regenerating axons and blood vessels.

Orientated mats of fibronectin, which support axonal growth and are initially avascular, were grafted into 1 cm defects in rat sciatic nerves. To follow the growth of neural and vascular elements, grafts were harvested between 3 and 30 days, sectioned longitudinally and stained with antibodies to endothelial cells (RECA-1), Schwann cells (S-100) and axons (Pan Neurofilament Marker and PGP). Dual fluorescence staining enabled the simultaneous visualisation of these elements within the same section and by superimposing their images vie double exposure photography, it was possible to show their true relative positions. Graft vascularisation came initially from the muscle bed. Vessel and axonal orientation were closely related. Schwann cells and axons extended together from the proximal nerve trunk, never exceeding the area of vascularisation and were more numerous in well vascularised regions with longitudinally orientated vessels. The results are suggestive of an interaction between blood vessels and regenerating axons, indicating regeneration may be enhanced by manipulating vascularisation.

Funding sources: grants; Ava Gardner Trust, Smith Charities

108.9

NEUROTROPHIN-3 ENHANCES PERIPHERAL NERVE REGENERATION. G.D.Sterne, C.Green, R.Brown, G.Terenghi*. Blond McIndoe Centre, East Grinstead and University College Hospital, London, UK.

Neurotrophin-3 (NT3) belongs to the Neurotrophin family of growth factors and acts on a distinct subpopulation of primary sensory neurons. As little is known about its action following nerve injury, we have investigated the effect of NT3 delivered directly to the injured nerve using fibronectin mats. These mats have previously been shown to support enhanced nerve regeneration when used in conjunction with nerve growth factor.

An established embryonic chick dorsal root ganglia bioassay was used to confirm that fibronectin can bind and release bioactive NT3. NT3-impregnated mats (500ng/ml) were grafted into 1cm defects of rat sciatic nerve. Plain mats were used as controls. Penetration distance and volume of axonal regeneration into the graft were quantified by computerised image analysis of immunostaining for axonal markers (GAP43, CGRP, SubP, VIP and NPY) up to 8 months postoperatively. The same markers were also used to analyse neuropeptide expression within dorsal root ganglia supplying the injured nerve.

Maximal NT3 effect occurred at day 15. For GAP43 axons both distance (NT3: 6.57±1.1mm; control: 3.99±1.2mm, $p<0.01$) and staining area (NT3: 0.137±0.041mm^2; control: 0.077±0.018mm^2, $p<0.01$) were significantly enhanced. Interestingly, increased axonal regeneration was noted in each neuronal subpopulation investigated. These results demonstrate that NT3 can contribute to an enhanced rate and volume of nerve regeneration.

Funding Sources: Grants :- The Geoffrey Burton Charitable Trust and Glaxo Holdings.

108.10

IN VIVO STUDY OF SUBSTRATE PREFERENCES OF REGENERATING PERIPHERAL AXONS

L.J. Chamberlain, I.V. Yannas*, H-P. Hsu, G. Strichartz, and M. Spector.
Massachusetts Institute of Technology, Cambridge, MA, 02139;
Brigham & Women's Hospital; and Brockton/West Roxbury VA Medical Center

The long term goal of our work is to identify the chemical composition and pore characteristics of a family of substrates which facilitates regeneration of a peripheral nerve trunk. Previous studies, in a transected rat sciatic nerve model, had shown that a highly porous analog of the extracellular matrix (ECM) was required to induce peripheral axon regeneration across a gap greater than 10 mm ensheathed by a silicone tube. Electrophysiological studies led to the finding that maximal functional recovery was observed when the average pore diameter of the ECM analog was 5-10 μm.

In the present study, the silicone tube containing the ECM analog was replaced with a collagen tube. Six groups of rats (n=9) received the tubular implants, which were either empty or filled with a copolymer of type I collagen and chondroitin-6-sulfate with axially oriented pores, 5-10 μm in diameter. The sciatic nerve of adult female Lewis rats was transected at mid-thigh and a 10 mm gap produced. Histomorphometric procedures were used to study axon diameter distributions (30 weeks) and electrophysiological parameters of excised specimens were evaluated (40 weeks).

The total number of myelinated axons per nerve increased threefold in the presence of the matrix in both the porous collagen and silicone tubes. For each group, the mode of the diameter distribution was 3 μm. In the matrix-filled tubes and in the autograft controls, diameters were as large as 9-11 μm. In the empty tubes, the maximum diameters were 6-8 μm. We conclude that axons which exceed 8 μm in diameter are formed only in the presence of the ECM analog. In addition, collagen can be used to replace silicone as a tube material. Electrophysiological testing is in progress to assess the degree to which conduction and function have recovered.
(This work was supported by the VA Rehabilitation R & D Service.)

108.11

AXON ELONGATION INTO COLLAGEN-GLYCOSAMINOGLYCAN IMPLANTS FOLLOWING SPINAL CORD INJURY. Spilker, M.H., Yannas, I.V., Hsu, H.-P.†, Norregaard, T.V.††, Kostyk, S.K.*, Spector, M.† Massachusetts Institute of Technology, Cambridge, MA 02139; †Brigham & Women's Hospital; ††New England Deaconess Hospital; *Brockton/West Roxbury VA Medical Center and Harvard Medical School.

Axons of the adult mammalian central nervous system (CNS) demonstrate limited spontaneous ability to regenerate following injury. Recent evidence suggests that the cellular and extracellular environment of the adult CNS, including the glial and connective tissue scar, impede regeneration by creating an environment that blocks the elongation of regenerating axons. At present, the substrate preferences of elongating axons *in vivo* are not well understood. The goal of this research was to study the healing process following spinal cord injury in the presence and absence of a family of well characterized analogs of extracellular matrix (AECM). Extensive data have shown that one such highly porous AECM composed of type I collagen and chondroitin-6-sulfate induces regeneration of peripheral nerves across a 15 mm gap. These results suggest the possibility of synthesizing an appropriate AECM which promotes regeneration of axons in the CNS.

In the current study, a 5 mm gap was created in the spinal cord of adult Sprague-Dawley rats by performing complete transections at T7 and T9 and removing the intervening spinal cord tissue. Either an empty collagen tube or one containing a specific AECM, of known chemical composition and pore structure, was placed in the gap. The rostral and caudal spinal cord stumps were inserted into the ends of the tube. After 30 days, a continuous cable of tissue had formed within the tube connecting the rostral and caudal stumps. This tissue contained numerous myelinated axons (mean of 313 for six animals). Immunostaining for 68 kD neurofilament confirmed the elongation of axons into the implant. Antibodies for GFAP, S-100, and OX-42 (macrophages and microglia) were also used to determine the cellular composition of the bridging tissue. The results show that implants containing AECM provide a controlled environment at the site of spinal cord injury which supports axon growth. (Supported by NSF grant 9315572-BES)

108.12

FK506 AND CYCLOSPORIN A: COMPARATIVE DOSE-DEPENDENCY STUDIES ON THE RATE OF AXONAL REGENERATION IN THE RAT SCIATIC NERVE. B.G. Gold*†^, M.S. Wang† and M. Zeleny-Pooley†. †Center for Research on Occupational & Environmental Toxicology and ^Department of Cell & Developmental Biology, Oregon Health Sciences University, Portland, OR 97201.

FK506 and cyclosporin A are immunosuppressant drugs presently used for organ tranplantions. The immunosuppressant action of these drugs is due to calcineurin (protein phosphatase 2B) inhibition, mediated via binding to distinct binding proteins (immunophilins): FK-binding-protein (FKBP) and cyclophilin for FK506 and cyclosporin A, respectively. We previously reported (Gold et al. *Rest Neurol Neurosci* 6:287, 1994; *J Neurosci* 15:1705, 1995) that FK506 also speeds functional recovery by increasing the rate of axonal regeneration for sensory fibers in the sciatic nerve following nerve crush; a (subcutaneous) 1 mg/kg dose increased regeneration rate (by 16%) from 3.8 mm/day (in controls) to 4.4 mm/day. In the present study, we have extended these initial studies by examining the dose-dependency for FK506's regenerative effects. In addition, we asked whether cyclosporin A shares FK506's regenerative properties. Rats (2-5/group) given a bilateral sciatic nerve crush (at the junction of the L4 and L5 spinal nerves), received daily subcutaneous injections of FK506 (5 or 10 mg/kg) or cyclosporin A (10 mg/kg); axotomized control animals received plecebo (Fujisawa). At 11 or 14 days, the L4 or L5 dorsal root ganglia (DRG) were exposed and radiolabeled with ^{3}H-leucine. Animals were killed 24 hours later (12 and 15 days, respectively), the nerves removed, cut into 3-mm segments and the radioactivity determined by liquid scintillation spectrophotometry. The maximal extent of outgrowth from the crush site was determined from the front of the resultant radioactivity profiles. Regeneration rates were calculated by regression analysis between the 12 and 15 day time-points. FK506 (5 mg/kg) increased regeneration rate (by 34%) to 5.1 mm/day; 10 mg/kg produced a smaller increase in rate (by 24%) to 4.7 mm/day. Cyclosporin A (10 mg/kg) only increased the rate (by 10%) to 4.2 mm/day; dose-dependency studies are being conducted. Thus, FK506 may increase regeneration via a FKBP-pathway distinct from immunophilin-calcineurin inhibition. *We thank Fujisawa for its generous gift of FK506. Supported by NS19611.*

108.13

ACTIVATION OF RECEPTORS FOR INTERFERON GAMMA (IFN-γ) IN ENDOTHELIAL CELLS AND REACTIVE ASTROCYTES AFTER SPINAL CORD INJURY. M. Risling*, T. Lindholm, T. Carlstedt, M. Meier, M. Mahlman, S. Cullheim, S. Lake and A.-M. Sjögren. Dept. Neurosci., Karolinska institute, Doktorsringen 17, S-171 77 Stockholm, and Pharmacia Upjohn, Stockholm, Sweden.

Spinal motoneurons in the adult cat and rat regenerate central nervous system (CNS)-type axons after lesions in the ventral funiculus of the spinal cord. Regrowing motor axons penetrate the CNS-type of scar tissue and enter adjacent ventral root fascicles. The traumatic scar tissue comprises a loose trabecular web of processes from reactive astrocytes and invading leptomeningeal cells. These two cell types have receptors for neurotrophins. The reactive astrocytes express neurotrophins, VEGF and receptors for prostaglandins. Matrix molecules such as laminin, tenascin and collagens are upregulated and there is a persistent defect in the blood-brain barrier. Cytokines have been suggested to play a key role in the early response to CNS trauma, by regulating the synthesis of neurotrophins and matrix molecules. In this study we have assessed the possibility that the cytokine IFN-γ may play a role in CNS scar formation.

Adult rats were subjected to an incision in the ventral funiculus of the spinal cord or to ventral root replantation after avulsion injury. 1 - 7 days later the animals were killed and the lesion area was sectioned with a cryostat and incubated with antibodies against the α- or β- subunit of the receptor for IFN-γ. A subpopulation of the endothelial cells of the spinal cord were found to carry IFN-γ receptors. An upregulation of class II MHC antigen was detected in the vasculature 1-3 days after the lesion. CD4+ T helper cells were present in the lesion area adjacent to the blood vessels. The inducable form of NOS (iNOS or macNOS) was present in association to some blood vessels and in scar tissue macrophages. JAK 1 immunoreactivity and positive staining for several members of the STAT family, including STAT 2 was observed in astrocytes in the lesion area. A redistribution of the interferon-stimulated gene factor 3 (ISGF3) was observed in scar tissue cells. These changes are consistent with a role for IFN-γ in the early events of CNS scar formation.
Supported by: Pharmacia Upjohn and the Swedish MRC (8657)

108.14

FK-506 IMPROVES BEHAVIORAL RECOVERY AND UPREGULATES NEURONAL EXPRESSION OF GAP-43 AFTER SPINAL CORD INJURY IN RATS J.R. Madsen*, P. MacDonald, N. Irwin, and L.I. Benowitz. Dept. Neurosurgery, Children's Hospital and Harvard Medical School, Boston, MA 02115.

FK-506, a drug widely used clinically as an immunosuppressant, has been shown to stimulate neurite outgrowth *in vitro* and to diminish the size of cerebral infarct after stroke in rats. These effects may be mediated through FKBP-12, an FK-506-binding protein which is abundant in neurons. The FK-506/FKBP complex inhibits the activity of the phosphatase, calcineurin, one of whose principal substrates is the neuronal growth-associated phosphoprotein GAP-43. We administered FK-506 (0.5 mg/kg) intravenously 10 min, 24 and 48 hr after inducing spinal cord injury at the T10 level by photothrombosis. Behavioral recovery was evaluated 1 through 28 days later using the Tarlov scale and the inclined plane, two measures of hindlimb function. Neuronal sprouting was assessed using immunohistochemistry to detect GAP-43. Behavioral and anatomical studies were carried out completely blinded to treatment regimen. Animals treated with FK-506 showed a significant improvement in neurological function 28 days postinjury relative to controls, both on the Tarlov scale and the inclined plane ($p=0.024$, $p=0.046$, respectively, two-tailed *t* tests, $df = 44$). In addition, FK-506 treatment caused a dramatic, two-fold increase in the number of GAP-43 immunoreactive neurons per mm^2 in the vicinity of the lesion ($p = 0.026$). Lesion volumes did not differ significantly between groups. Thus, FK-506 improves functional recovery after spinal cord injury and this may be mediated by an increase in neuronal sprouting. *Support: NIH NS01471(JRM), EY05690 (LIB).*

109.1

MAGNETIC RESONANCE IMAGING IN LESCH-NYHAN DISEASE: CORRELATION OF CAUDATE NUCLEUS VOLUME AND COGNITIVE FUNCTIONING. J. C. Harris,* Div. of Child & Adolescent Psychiatry, Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21287. D. Schretelen, N. Bryan, D.F. Wong.

Lesch-Nyhan disease, the most severe form of HPRT deficiency, is a rare sex-linked metabolic disorder with an unusual neurological and behavioral phenotype. Although first described in 1964 in two brothers who presented with hyperuricemia, mental retardation, choreoathetosis and self-destructive biting, little progress has been made since the early description in determining the pathophysiology of the neurological and behavioral symptoms and the extent of cognitive impairment. Despite the extent of the movement disorder, CT and MRI scans are generally reported to be normal. However, in a recent MRI study, we found consistent reduction in volumes of the caudate nuclei of six young men with this disease compared to 9 normal controls. We now report correlations between these volumetric MRI findings and the cognitive test performance of six patients. In this sample, the mean (± SD) volumes of the left and right caudate nuclei were 2349±257 and 2502±366 cu. cm., respectively. Analyses revealed a significant correlation (r =.78; p =.037, one tailed) between the left caudate volume and performance on one neuropsychological test of global cognitive functioning. Moderately strong correlations also were found between the left caudate volumes and tests of reasoning (r=.69; p=0.067, one tailed), orientation (r=.66; p=.077, one tailed) and receptive vocabulary (r=.47). Correlations between the right caudate volume and all four cognitive measures were much weaker. This pattern of results is similar to a recent study of left vs. right caudate volumes in relation to the cognitive test performance of patients with Huntington's disease. Supported in part by HD 24061.

109.2

LONGITUDINAL STUDY OF BRAIN MORPHOLOGY WITH MRI IN FIRST EPISODE PSYCHOTIC DISORDERS Jeffrey Lieberman, M.D.,* Houwei Wu, M.D., Rael Strous, M.D., Miranda Chakos, M.D., Jose Alvir, Ph.D., Bernhard Bogerts, M.D., Gus DeGreefe, M.D., Robert Bilder, Ph.D. Mental Health and Neuroscience Clinical Research Center University of North Carolina School of Medicine Chapel Hill North Carolina 27599-7160

Despite substantial evidence that the neuropathology of schizophrenia arises early in neurodevelopment, the clinical course of the illness suggests the presence of a neurodegenerative process. Previous studies examined this issue by longitudinal study of brain morphology to determine if the neuropathology of schizophrenia was progressive. Findings of these studies are limited by studying small or chronic patient samples and/or the use of unreliable image acquisition or analysis methods .

We examined volumes of regions of interest (cerebral cortex, ventricular system, mesiotemporal structures and the caudate nucleus) The sample included 39 patients ascertained in their first episode of schizophrenia (21 male, 18 females) mean (SD) age 26.6 (6.3) yrs., and 18 control subjects 29.2 (8.8) yrs. . Subjects underwent MRIs on at least two occasions 24 months apart. MR scans were morphometrically assessed using a computer based semiautomated program that includes segmentation and stereologic algorithms for volume determination. Neither patients nor controls showed mean group changes in cortical, mesiotemporal and ventricular volumes. However, longer durations of psychosis were associated with greater increases in ventricular volume (r=.047, p<.02). Patients with persisting negative symptoms defined by the deficit state had greater increases in ventricular volume (F=214, p<.0001.There was a 5.7 % increase in caudate nuclei volumes in patients which was correlated with duration of treatment.

These results suggest that there are subtle changes in brain morphology in patients with schizophrenia. This appears to be an effect of drug treatment for basal ganglia structures but for ventricular volume is associated with disease course and possible pathophysiological progression. (source of support: grant)

109.3

FOCAL NEUROANATOMIC SUBSTRATES IN LATE-LIFE MAJOR DEPRESSION, A. Kumar*, D. Miller, P. Cowell, W. Bilker, J. Zhisong, and G. Gottlieb. Univ. of Pennsylvania Sch. of Med., Philadelphia, PA 19104.

The purpose of our study was to examine MRI determined volumetric measures of the frontal and temporal lobes in subjects with Late Life Major Depression (LLD) and to compare them to similar indices obtained from non-depressed controls. Our study groups comprised of 41 subjects who met DSM-IV criteria for Major Depressive Disorder (14 M, 27 W, Mean age=74.4 SD=6.6) and 31 non-depressed healthy controls (7 M, 24 W, Mean age=70.5 SD=6.4). The depressed subjects had Hamilton Depression Scale Scores of 15 or greater without any clinical evidence of dementia. They had several stable comorbid medical disorders and were free of other central nervous system disease. Axial spin echo images were acquired on all subjects using a GE signal scanner with head coil. The 5mm thick, contiguous slices were obtained using a repetition time (TR) of 3000 msec and echo time (TE) of 30 and 80 msec in planes parallel to the canthomeatal line. The neuroanatomical boundaries used in the image analysis have been previously described (Cowell et al. J. Neurosci 1994). Frontal and temporal lobe volumes normalized using total brain and intracranial volumes were used for comparison between groups. The two groups differed significantly on most normalized measures of frontal and temporal lobe volumes after correcting for age using linear regression (P< 0.05). These data demonstrate that focal neuroanatomical abnormalities occur in LLD and provide further evidence for a structural basis to depression occurring in late life.

FUNDING: NIH Grant

109.4

POTENTIAL INVOLVEMENT OF ANTERIOR CINGULATE DOPAMINE-D_2-RECEPTORS IN MAJOR DEPRESSION

R. Larisch[1], A. Klimke[2], H. Vosberg[1], S. Löffler[2], W. Gaebel[2], H.-W. Müller-Gärtner[1,3*] Clinic of Nuclear Medicine[1], Clinic of Psychiatry[2], Univ. of Düsseldorf; Institute of Medicine[3], Research Center Jülich.

Striatum and parts of frontal cortex have previously been shown to be involved in mood disorders based on studies of glucose metabolism and blood flow. The current study investigates the *dopaminergic* system in-vivo.

Eleven patients (eight females, three males) fulfilling DSM-IV criteria for major depression were prospectively investigated with the specific dopamine-D_2-receptor antagonist ^{123}I-iodobenzamide (IBZM), using single photon emission computed tomography before and under treatment with a selective serotonin re-uptake inhibitor (SSRI). The depression was rated using the Hamilton Depression Scale (HAMD).

In treatment responders, dopamine-D_2-receptor binding (ratio over cerebellum) increased from 1.95 ± 0.24 to 2.06 ± 0.35 in striatum, from 1.12 ± 0.32 to 1.27 ± 0.41 in anterior cingulate (mean ± SD). In non-responders, dopamine-D_2-receptor binding decreased from 2.14 ± 0.14 to 2.06 ± 0.34 in striatum, from 1.32 ± 0.04 to 1.10 ± 0.16 in anterior cingulate. Improvement of HAMD and changes of dopamine-D_2-receptor-binding were positively correlated in striatum (r = 0.63, p = 0.04) and anterior cingulate gyrus (r = 0.82, p = 0.02 after Bonferroni correction).

The study indicates an interaction between the serotonergic and dopaminergic system in anterior cingulate gyrus and striatum in-vivo. The results further strengthen the hypothesis that the dopaminergic system is involved in major depression. The data specifically suggest a role of the anterior cingulate gyrus and striatum in this mood disorder.

109.5

ANTIDEPRESSANT EFFECTS OF WITH RAPID-RATE TRANSCRANIAL MAGNETIC STIMULATION (rTMS) ARE ASSOCIATED WITH NORMALIZATION OF PREFRONTAL HYPOMETABOLISM. J.M. Tormos, F. Tarazona, C. Cañete, F. Pallardó, M.D. Catalá and A. Pascual-Leone* Unidad de Neurobiología, Dept. Fisiología, Univ. Valencia and Inst. Ramón y Cajal, Consejo Superior de Investigaciones Científicas, SPAIN

Stimulation of left prefrontal cortex using rTMS has been shown to improve clinical symptoms in medication-resistant, primary depression [Pascual-Leone et al. 1996]. However, the mechanisms of action of rTMS are unclear. Functional neuroimaging studies have demonstrated prefrontal hypometabolism in depression that correlates with the severity of depressive symptomatology [George et al. 1994]. We have studied the effects of rTMS on prefrontal hypometabolism in depression and their correlation with clinical improvement. We have studied 10 patients with medication-resistant primary depression (DSM IV). All were studied off all medications. We obtained HMPAO SPECT scans before and after 10 days of daily rTMS applied focally to the left prefrontal cortex in 20 trains of 10 s, 10 Hz, 90% of motor threshold intensity, and 50 s intertrain intervals. Hamilton depression rating scale and Beck inventory were used for clinical rating by a blinded investigator. In all patients we documented clinical improvement which was associated with a decrease in frontal hypometabolism. In addition, we have measured the effects of rTMS trains on cortical excitability probing it before and after rTMS with single and paired-pulse transcranial stimuli. Motor cortical excitability was shown to be enhanced by the rTMS trains applied to the depressed patients. All patients tolerated rTMS without complications, no seizures were induced by the applied rTMS parameters. These results illustrate the mode of action of rTMS in depression and support the notion of a pathophysiological link between prefrontal hypometabolism and depressive symptomatology. Non-invasive, non-convulsive rTMS can enhance cortical excitability and may develop into a therapeutic tool in primary depression.

Supported by grants from the Generalitat Valenciana and the DGICYT.

109.6

D2 RECEPTOR OCCUPANCY DURING RISPERIDONE AND CLOZAPINE TREATMENT IN CHRONIC SCHIZOPHRENIA: RELATIONSHIP TO BLOOD LEVEL, EFFICACY AND EPS, T-P Su (1), A Breier (1), R Coppola (2), K Hadd (1), I Elman (1), C Alder (1), AK Malhotra (1), E Watsky (2), J Gorey, CJ Hough (3)*, D Weinberger (2), D Pickar (1). (1) Experimental Therapeutics Branch, (3) Biological Psychiatry Branch, NIMH, Bethesda, MD (2) Clinical Brain Disorders Branch, NIMH, St. Elizabeths Hospital, Washington DC. Funded by NIMH/DIRP

We have previously reported that wide ranges of D2 occupancies (18 - ≥ 80%) are associated with favorable response to clozapine without the occurrence of extrapyramidal symptom (EPS) (Su et al. 1993). Risperidone has been found to produce high levels of D2 blockade without EPS (Busatto et al. 1995). The specific aim of this study is to compare D2 occupancy between risperidone and clozapine in chronic schizophrenics and to determine the relationship of blood level, clinical efficacy and EPS to D2 occupancy.

We have examined this issue with two separate studies. In study 1, five schizophrenics underwent ^{123}I-IBZM-single photon emission computed tomography (SPECT) scanning during treatment with clozapine (400 mg/day) and with risperidone (6 mg/day) in a double-blind, crossover fashion. In study 2 (double-blind), 7 patients on risperidone (4 - 8 mg/day) and 7 patients on clozapine (400 mg - 600 mg/day) matched for age, sex, and illness severity were scanned. Risperidone resulted in significantly higher D2 occupancy (study 1: 77 ± 6%, study 2: 70 ± 6 %) than clozapine (study 1: 52 ± 8 %, study 2: 45 ± 12 %) (t_4 = 5.5 and t_{12} = 4.9 respectively, p < 0.006). Both drugs did not demonstrate an association of symptom reduction to the changes of D2 occupancy (p = NS). While clozapine - treated patients did not display EPS, 9 of 12 patients on risperidone did have modest EPS (Simpson Neurological Rating:13.0 ±1.4). The other 3 patients were free of EPS even with high level of D2 occupancy (65 - 76 %). In the dose-response (D2 occupancy vs. blood level) studies, a curvilinear model fits for risperidone, whereas a linear regression for clozapine. These data confirm that risperidone is more potent D2 blocker than clozapine and suggest that clinical efficacy and EPS are not simply related to D2 occupancy. The character of risperidone to gradually saturate D2 receptors provide a basis for monitoring treatment response and EPS.

109.7

IN VIVO EVALUATION OF DOPAMINE SYNAPTIC FUNCTION IN UNTREATED SCHIZOPHRENIC PATIENTS. A. Abi-Dargham*, R. Gill, R. M. Baldwin, J. P. Seibyl, J. Krystal, D. S. Charney, R.B. Innis and M. Laruelle. Yale University and VA Medical Center, West Haven, CT 06516.

The following dopamine parameters were measured with SPECT in 23 patients with schizophrenia and 23 matched healthy controls: density of dopamine transporters (with [^{123}I]β-CIT), density of D_2 receptors (with [^{123}I]IBZM), and amphetamine-induced dopamine release (as measured by the displacement of [^{123}I]IBZM binding following amphetamine 0.3 mg/kg i.v.). Patients had no history of substance or alcohol abuse, and were neuroleptic-free since 160 ± 90 days. We observed no difference in [^{123}I]β-CIT specific to nonspecific ratio between schizophrenics (7.8 ± 0.4, SEM) and controls (8.4 ± 0.4, ns). Similarly, the D2 receptor binding potential was unchanged between patients (220 ± 18 ml/g) and controls (207 ± 14 ml/g, ns). In contrast, schizophrenics exhibited greater displacement of [^{123}I]IBZM following amphetamine (-17 ± 3%) than controls (-8 ± 2%, p = 0.02). Increased dopamine release following amphetamine was associated with transient emergence or worsening of psychotic symptoms (p = 0.001). Thus, the dopaminergic system shows exaggerated functional responsiveness in schizophrenia, but, in our hands, no changes in static markers such as D_2 receptors or dopamine transporters densities. Since similar alterations have been described in rats sensitized to amphetamine, these data suggest that schizophrenia is associated with an endogenous sensitization process.
Supported by NARSAD, NIMH and Veterans Administration.

109.8

SPECT MEASUREMENT OF HUMAN STRIATAL SYNAPTIC DOPAMINE CONCENTRATION IN THE RESTING STATE M. Laruelle*, C. De Souza, R. M. Baldwin, A. Abi-Dargham, M. Bowers, J. P. Seibyl, D. S. Charney, and R.B. Innis. Yale University and VA Medical Center, West Haven, CT 06516.

Endogenous dopamine concentration in the human striatum was estimated by comparing the binding potential (BP) of the selective D_2 radiotracer [^{123}I]IBZM before and after acute dopamine depletion. Dopamine depletion was achieved by administration of the tyrosine hydroxylase inhibitor alpha-methyl-para-tyrosine (AMPT, 1 g every six hours for two days). AMPT increased [^{123}I]IBZM BP by 28 ± 16% (± SD, n = 9, age 25 ± 4 y). Experiments in rodents showed that this effect was due to removal of endogenous dopamine rather than D_2 receptors upregulation. Dopamine synaptic concentration was estimated as 45 ± 25 nM. Thus, D_2 receptors are significantly stimulated by dopamine in the resting state in humans. These results demonstrate the feasibility of measuring dopamine synaptic concentration in the living human brain, by coupling D_2 receptors imaging with acute dopamine depletion. As opposed to the previously described measure of dopamine release following amphetamine administration (Laruelle et al., J. Nuc. Medicine, 1995, 36: 182-1190), this technique allows measuring dopamine synaptic concentration in the absence of pharmacological challenge and will be used to correlate dopamine concentration and clinical symptomatology in patients with schizophrenia.
Supported by Scottish-Rite Schizophrenia Research Program and Veterans Administration.

109.9

PATTERNS OF rCBF ACTIVATION FOLLOWING TRAUMA RELATED STIMULUS IN POST TRAUMATIC STRESS DISORDER Israel Liberzon*, Lorraine M. Fig , Stephan F. Taylor, Satoshi Minoshima, Robert A. Koeppe. Psychiatry and Nuclear Medicine, VAMC and University of Michigan. Michigan 48109-0720

Post Traumatic Stress Disorder (PTSD) patients exhibit memory abnormalities (intrusive memories, flashbacks and dreams), avoidance behavior and exaggerated responses to traumatic stimuli. PET and SPECT studies of PTSD patients suggest the presence of specific rCBF patterns of activation in this disorder. We have reported rCBF changes in PTSD patients, as compared to normal controls, in the medial temporal lobe, anterior cingulate and orbital frontal regions, hypothesizing that these findings reflect abnormal memory formations and alteration in the arousal mechanisms of PTSD patients.

Two SPECT studies with Tc-99m HMPAO were performed in 30 subjects after exposure to 1) combat sounds and 2) white noise, (via headphones at 75 dB) 48 hours apart. Electrophysiological indices of arousal - skin conductance, heart rate, and EMG were collected during the baseline and "activation" phase of the SPECT imaging and the results reveal enhanced skin conductance response in PTSD patients as compared to the control groups. In the current set of data we compare rCBF changes in PTSD veterans, combat controls and age and sex matched controls, examining both activation patterns differences and the correlations between the rCBF changes and subject's arousal levels as reflected by their electrophysiological indices.

This project was supported by RAG grant to L.M.F from VACO

109.10

SCHIZOPHRENIA IS ASSOCIATED WITH ENHANCED AMPHETAMINE-INDUCED DOPAMINE RELEASE, A. Breier*, T-P. Su, I. Elman , C. Adler , R. Saunders (2), A. de Bartolomeis, R. Carson (1), W. Eckelman (1), D. Pickar. ETB, NIMH, (1) PET Dept., CC, NIH, Bethesda, MD (2)CBDB, NIMH, St Elizabeths Hospital, Washington, D.C.

Dopamine overactivity has been the predominate pathophysiologic hypothesis of schizophrenia for the past two decades. A major line of evidence used to support dopamine's involvement in schizophrenia is enhanced sensitivity to agents that stimulate dopamine release, such as the psyhcostimulant amphetamine. We employed a novel PET method that quantifies dopamine release by estimating the amount of dopamine competes with the radiotracer 11-C-raclopride for dopamine D-2 binding, +and used this method to test the hypothesis that schizophrenic patients, in comparison to healthy controls, have greater amphetamine-induced striatal dopamine release. Eleven patients with DSM IV schizophrenia disorder (6 neuroleptic-naive; 5 neuroleptic withdrawn) and 12 age, gender, weight matched controls participated in the study. Amphetamine produced highly significant decreases in 11-C-raclopride striatal tissue concentrations in both healthy controls (2.52±0.13 vs. 2.1±0.09, t=6.7, p=0.00004) and schizophrenic patients (2.53±0.23 vs. 2.00±0.18, t=4.8, p=0.0007). Schizophrenic patients had greater amphetamine-induced changes in 11-C raclopride binding than controls. The patients had a +22.3 % (±8.9) change in striatal 11-C-raclopride tissue concentrations compared to +15.5% (±6.1) change in healthy controls (t=2.1, p=.04). 11-C-raclopride striatal binding changes were correlated with global symptom changes (r=0.66, p=0.02) in schizophrenic patients. These data provide direct evidence for enhanced dopamine release in schizophrenia.

Funded by NIMH/DIRP

109.11

KETAMINE EFFECTS ON HUMAN BEHAVIOR AND BRAIN METABOLISM: IMPLICATIONS FOR SCHIZOPHRENIA. A. K. Malhotra, J. H. Callicott,* D. A. Pinals, C. M. Adler, N. Weisenfeld, D. Pickar, A. Breier. Experimental Therapeutics Branch, National Institutes of Mental Health, Bethesda, MD 20892.

Several lines of evidence have suggested that dysfunction in the N-methyl-d-aspartate (NMDA) receptor is involved in the pathophysiology of schizophrenia. We have utilized an placebo-controlled infusion paradigm with subanesthetic doses (0.12 mg/kg bolus followed by 0.65 mg/kg/hr infusion) of the NMDA antagonist, ketamine, to probe human NMDA receptor function. In normal controls, ketamine produces significant thought disorder, perceptual abnormalities and cognitive impairments. A Positron Emission Tomography (PET) study with Fluorodeoxyglucose (FDG) has demonstrated that ketamine produces specific activation of prefrontal areas and that these metabolic effects correlate with ketamine-induced thought disorder in healthy volunteers. In a study of drug-free schizophrenic patients, ketamine exacerbated core psychotic and cognitive symptoms. The effects of the antipsychotic agents, fluphenazine and clozapine, on ketamine-induced psychotic symptoms were also examined. These data support the hypothesis that NMDA dysfunction is involved in schizophrenia, offer evidence for prefrontal involvement in NMDA-mediated psychoses and suggest that antipsychotic drugs can alter ketamine-induced psychosis.

NIMH/NIH (DIRP)

109.12

STATE- AND TRAIT-LIKE NEUROIMAGING ABNORMALITIES IN DEPRESSION: EFFECTS OF ANTIDEPRESSANT TREATMENT. WC Drevets*, JL Price, JS Simpson, RD Todd, T Reich, ME Raichle. Wash. U. Sch Med.; St. Louis, MO

In major depressive disorders, we previously distinguished abnormalities present only in the depressed state from those present in both depressed and remitted states. Blood flow was elevated in the left orbital cortex in depressed but not unmedicated-remitted subjects, while BF was elevated in the left amygdala in both states. In depressed bipolar subjects, we identified an area of decreased metabolism in the subgenual prefrontal cortex (SGPFC), which appeared to reflect reduced grey matter volume in the prelimbic anterior cingulate. To characterize the effects of antidepressant treatment (ADT) on these abnormalities, PET images of glucose metabolism (Siemens 953B/31 ^{18}FDG; FWHM=5mm FWHM) and MRI images (Siemens Vision 1.5T; MPRAGE sequence; 1x1x1.25mm voxels) were acquired in unipolar depressives (UPD) before and after AD drug treatment (n=20,age=36±10), in separate groups of bipolar subjects, one depressed (n=8, age 36±8.4) and the other medicated-remitted (n=8; age=34±6.8), and in controls (n=17; age=34±8). In UPD, left amygdala and posterior orbital metabolism were abnormally elevated [(10.1%;p<.01) and 16%(p<.01), respectively] before ADT and declined after ADT [5.8%(p<.05) and 10.1%(p<0.01) respectively]. The decrement in orbital metabolism correlated with the reduction in depression ratings (r=0.47; p0.05). Amygdala metabolism decreased in the subgroup who remained well on continued treatment (-6.6%; p<.01) but not in the subgroup who relapsed on continued treatment (+0.2%). In UPD, SGPFC volume was abnormally reduced (48%; t=4.7; p<0.001) and did not change following treatment (-1.1% ±18%). In bipolar subjects SGPFC volume was reduced in both depressed (42%, p<.005) and remitted groups (38%; p<.05) relative to controls. These data support hypotheses that in UPD elevated orbital activity is specific to the depressed state and abnormal amygdala activity may predispose to illness recurrence, and that in both unipolar and bipolar disorders, reduced SGPFC grey matter volume is stable across clinical states. Supported by MH51137, MH00928.

109.13

THE ROLE OF THE CINGULATE IN MOOD HOMEOSTASIS.
HS Mayberg*, SK Brannan, M Liotti, RK Mahurin, JS Brickman, JA Silva, JL Tekell, PA Jerabek, CC Martin, PT Fox. Research Imaging Center, UTHSCSA, San Antonio, TX 78284.

Using FDG PET in patients with depression, we previously described metabolic abnormalities in the anterior cingulate (BA 24b/a) that predict and differentiate drug Rx responders (R) from non-responders (NR): NR << controls (C) < R (Neurol 1996; 46:S327). The present study examined the added effects of 6 weeks fluoxetine Rx (double blind, placebo controlled) on mood, cognition and Cg metabolism as a function of treatment outcome in 13 patients with unipolar depression. Eight pts received active drug; R=3, NR=5 (defined by a >40% decrease in Ham-D). Baseline depression severity and cognitive performance were comparable for the two groups. Pre- and post-Rx FDG scans were compared using pixel-by-pixel contrasts, CDA (J Nuc Med 1988; 30:141). In the NR, Cg 24b/a were still both significantly hypometabolic (NR<C), showing further decreases in 24b, but a small increase in 24a. In contrast, R had a robust increase in both Cg24b and 24a, resulting in an even greater difference relative to controls (R>C). The Cg increases seen prior to Rx and potentiated by fluoxetine suggest an ongoing compensatory response to the depressed mood. Additional changes in Fr46/47, ant Ins and BA25 further implicate involvement of neocortical and limbic/paralimbic regions with reciprocal connections with the Cg24 in mood recovery. In NR, the Cg compensatory response was not just absent but paradoxical, and no changes in Cg targets areas were seen. These findings further support a critical role for Cg24a/b in the limbic-neocortical networks involved in normal and abnormal emotional states. (Supported by: NARSAD and Eli Lilly and Co.)

109.14

MULTISLICE PROTON MAGNETIC RESONANCE SPECTROSCOPIC IMAGING IN MONKEYS WITH MESIAL TEMPORO-LIMBIC LESIONS
A. Bertolino*, R.C. Saunders, V.S. Mattay, J.H. Duyn, J. Bachevalier, J.A. Frank, D.R.Weinberger. Clinical Brain Disorders Branch, NIMH, NIH Bethesda, MD 20892.

To establish the relationship between the time of occurrence of mesial temporo-limbic lesions (perinatal vs. adult) and chemical alterations in prefrontal cortical neurons (PFC), we studied 18 adult rhesus monkeys with long echo time (TE 272 msec) multislice proton magnetic resonance spectroscopic imaging (^{1}H-MRSI). ^{1}H-MRSI permits imaging of signals from metabolites such as N-Acetyl-aspartate (NAA, neuronal marker), choline-containing compounds (CHO) and creatine/phosphocreatine (CRE) from multiple 15 mm thick slices with a nominal voxel volume of 0.84 mL. 6 monkeys had undergone surgical ablation of mesial temporo-limbic structures including the hippocampus, amygdala and entorhinal cortex within 3 weeks of birth; 6 monkeys received the same lesion at approximately five years and 6 monkeys were normal controls. Regions of interest (ROI) were drawn blindly on coplanar MR images for prefrontal cortex (PFC), sensory-motor cortex and centrum semiovale (CSO). Statistical analysis (3x2 ANOVA with significance level at $p<0.05$) showed significant bilateral reductions only of NAA/CRE, NAA/CHO and NAA PFC/NAA CSO ratios exclusively in the PFC of monkeys with neonatal lesion with respect to both other groups. No significant difference across groups in the frontal lobe volume, nor any significant correlation between the latter and metabolite ratios in PFC was found. Our results indicate that neonatal perihippocampal damage affects prefrontal neuronal chemistry in adult monkeys. The basis of this effect does not appear to be a result of reduced frontal lobe volume nor a loss of connections *per se*, since analogous damage during adulthood does not have the same effect. This finding may have implications in understanding developmental aspects of prefrontal-temporolimbic connectivity, and the reduction of NAA ratios observed in patients with schizophrenia.

ACETYLCHOLINE RECEPTORS: NICOTINIC

110.1

ACETYLCHOLINE RECEPTOR CHANNEL-LINING RESIDUES IN THE M2 SEGMENT OF THE β SUBUNIT.
H. Zhang and A. Karlin*. Center for Molecular Recognition, Columbia University, New York, NY 10032.

We have applied the substituted-cysteine-accessibility method (SCAM) to the M2 segment and the M1-M2 loop of the β subunit. Each residue from βP248 to βD273 was mutated to Cys, and the mutant β subunits were expressed together with wild-type α, γ, and δ in Xenopus oocytes. All of the mutants gave robust ACh-induced currents. The accessibility of the Cys to the methanethiosulfonate (MTS) derivatives, MTSethylammonium(MTSEA), MTSethyltrimethylammonium (MTSET), and MTSethylsulfonate (MTSES), added extracellularly, was inferred from their irreversible effects on the current induced by ACh. The positions accessible to MTSEA added in the absence and in the presence of ACh are indicated below by asterisks:

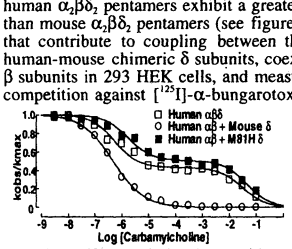
```
              250        260        270
               |    |     |    |     |
intra  PDAGEKMGLSIFALLTLTVFLLLLAD extra
  -ACh      ** *   *          * * *
  +ACh   *  ** *   *  *  **** * *
```

A subset was also accessible to MTSET, and the anionic MTSES penetrated as far as βV266C. These results imply that βM2 contributes to the channel wall and that its structure changes on the binding of ACh. From the pattern of accessibility, we infer that residues 255 to 265 may be α-helical and that residues 268 to 273 may form a β strand. The exposure in βM2 is significantly different from that in αM2 (Akabas et al., Neuron 13, 919, 1994). The accessibility of βG255C, βK253C, and βE252C to MTSEA in the closed state implies that the channel gate is close to the intracellular end of the channel. Supported by NS07065, MDA, and the McKnight Foundation.

110.2

IDENTIFICATION OF RESIDUES IN THE δ SUBUNIT THAT AFFECT COOPERATIVE INTERACTIONS IN THE NICOTINIC ACETYLCHOLINE RECEPTOR (nACHR)
P.A. Quiram*and S.M. Sine, Dept. of Physiology and Biophysics, Mayo Foundation, Rochester, MN 55905

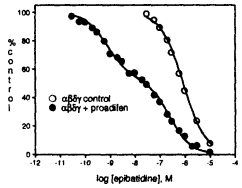
The nAChR is an $\alpha_2\beta\gamma\delta$ pentamer containing two binding sites formed by αγ and αδ subunit pairs. The γ and δ subunits are required for mediating cooperative interactions between the two binding sites and for maintaining the sites in the low affinity activatable state. Using mouse nAChR subunits, we previously demonstrated that omitting the γ subunit led to $\alpha_2\beta\delta_2$ pentamers that failed to maintain both cooperative interactions and low affinity of the two binding sites. Here we show that human $\alpha_2\beta\delta_2$ pentamers exhibit a greater loss of cooperative interactions than mouse $\alpha_2\beta\delta_2$ pentamers (see figure). To gain insight into residues that contribute to coupling between the binding sites, we constructed human-mouse chimeric δ subunits, coexpressed them with human α and β subunits in 293 HEK cells, and measured carbamylcholine binding by competition against [^{125}I]-α-bungarotoxin binding. Introducing mouse sequence into the N-terminal 81 positions followed by human sequence results in biphasic binding characteristic of the human δ subunit. Extending mouse sequence to position 174 restores monophasic binding characteristic of the mouse δ subunit. We conclude that residues between positions 81 and 174 mediate cooperative interactions between the two binding sites of the nAChR. Supported by NIH Grant NS31744



110.3

EPIBATIDINE SELECTS BETWEEN THE AGONIST BINDING SITES OF MUSCLE NICOTINIC ACETYLCHOLINE RECEPTORS.
R. J. Prince* and S. M. Sine. Dept. Physiology and Biophysics, Mayo Foundation, Rochester MN 55905

Previous workers have found epibatidine to have exceptionally high affinity for CNS nicotinic receptors (nAChRs) but lower affinity for muscle nAChRs. This study examines the interaction of epibatidine with the binding sites of muscle nAChR. We measured the competition of (±)epibatidine against the initial rate of [^{125}I]-α-bungarotoxin binding to mouse muscle nAChRs transiently expressed in 293 HEK cells. Fetal type ($\alpha_2\beta\delta\gamma$) nAChRs bound epibatidine with a K_{app} of 700 nM and a Hill coefficient of 0.9. However, in the presence of proadifen, a local anesthetic which desensitizes the receptor, the competition curve was biphasic with K_ds of 800 pM and 240 nM (see figure). The desensitized adult ($\alpha_2\beta\delta\varepsilon$) nAChR also showed distinct affinities for epibatidine with K_ds of 1.5 nM and 400 nM. In contrast, carbamylcholine does not select between the two sites in either fetal or adult desensitized nAChRs. We also expressed α,β and γ or α, β and δ to form $\alpha_2\beta\gamma_2$ or $\alpha_2\beta\delta_2$ pentamers. When converted to the desensitized state, $\alpha_2\beta\gamma_2$ bound epibatidine with an affinity of 3 nM whereas $\alpha_2\beta\delta_2$ bound epibatidine with an affinity of 500 nM. From these results we infer that the α-γ and α-ε subunit interfaces bind epibatidine with high affinity whereas the α-δ interface binds with low affinity. Supported by NIH grant NS 31744.



110.4

α–CONOTOXIN ImI (α-CTx-ImI) REVEALS THE PRESENCE OF TWO DISTINCT ELEMENTS IN THE Cl-DEPENDENT RESPONSE OF *APLYSIA* NEURONS TO ACETYLCHOLINE.
JacSue Kehoe, Micha Spira, & J. Michael McIntosh*. Laboratoire de Neurobiologie, Ecole Normale Supérieure, Paris, France; Deptartment of Neurobiology, Hebrew University of Jerusalem, Israel; Departments of Psychiatry and Biology, Univ. of Utah, Salt Lake City, Utah.

α-Conotoxins have proven to be very useful tools for discriminating among the wide variety of nicotinic receptors present in neuronal and muscle membranes. We have studied the effects of α-CTx-ImI (from a worm-eating conus) on the two nicotinic receptors previously shown to be present in molluscan neurons. One receptor mediates an increase in cationic conductance, and bears a pharmacological resemblance to the receptor mediating the fast EPSP in mammalian ganglionic neurons; the other mediates an inhibitory response by increasing Cl conductance, and resembles, pharmacologically speaking, the receptor of the frog neuromuscular junction.

In the present study, it was found that α-CTx-ImI, unlike α-bungarotoxin, blocks both of these responses, and in both cases the block was independent of holding potential. The IC_{50} for the inhibitory response is lower (under 100 nM) than that for the cationic response (between 150 and 200 nM). Furthermore, the toxin has revealed the presence in certain cells of two distinct elements in the Cl-dependent response: one rapidly desensitizing and blocked almost completely by 100 nM α-CTx-ImI; the other, non-desensitizing, and unaffected by a 10 μM concentration. These two elements are present in very different proportions on different identifiable cells. Finally, α-CTx-ImI has no effect on the Cl-dependent responses to GABA and glutamate, and hence it is the first antagonist found that discriminates completely between the cholinergic and non-cholinergic Cl-dependent responses.

Support: for JSK, CNRS; for MS, l'Université Pierre et Marie Curie, for JMM, NIH K20 MH00929.

110.5

α-CONOTOXIN MII (α-CTx-MII) INTERACTION WITH NEURONAL NICOTINIC ACETYLCHOLINE RECEPTORS. G. E. Cartier[1], D. Yoshikami[1]*, S. Luo[1], B. M. Olivera[1] and J. M. McIntosh.[1,2] Depts. of Biology[1] and Psychiatry[1], Univ. of Utah, Salt Lake City, UT, 84112.

The recently isolated peptide α-conotoxin MII selectively blocks α3β2 nicotinic acetylcholine receptors (nAChRs) expressed in *Xenopus* oocytes. Substitution of either the α3 or β2 subunit by one of its homologues leads to a 2-4 order of magnitude decrease in toxin sensitivity. Pre-exposure of α3β2 nAChRs to the competitive antagonist dihydro-β-erythroidine followed by exposure to MII leads to a rapid time course of recovery following drug wash out that is the same as that observed during recovery from exposure to dihydro-β-erythroidine alone, rather than the slower recovery seen following exposure to, and washout of, MII alone. This indicates that the two ligands share a common site of action on the receptor. The competitive action of MII is also suggested by results showing that MII-block is insensitive to membrane potential. Block of both α3β2 and α3β4 receptor activity upon exposure to MII follows a simple exponential time course. In contrast, the recovery of activity following exposure to saturating concentrations of MII and subsequent washout has a time course which is inconsistent with a simple bimolecular reaction. Instead, it appears that the toxin has two binding sites on the receptor, and occupation of either site by MII is sufficient to block receptor function.

Supported by NIH K20 MH00929 and P01 GM48677.

110.6

A RECOMBINANT ADENOVIRUS ENCODING ACTIVE NEURONAL BUNGAROTOXIN S. Gorman[1], N. Viseshakul[2], B. Cohen[2], S. Hardy[3], G. Grant[4], &J. Forsaych[1]*. [1]Dept. Anesthesia, UC San Francisco, CA 94143; [2]Division of Biomedical Sciences, UC Riverside, CA 92521; [3]Somatix Therapy, Alameda, CA 94501; [4]Dept. Molecular Biology, Washington University, St. Louis, MO 63110.

The availability of native neuronal bungarotoxin (NBT) is now very limited; hence, we and others have attempted to synthesize active recombinant NBT (rNBT). We used a novel Cre-loxP system to make an adenovirus that encoded NBT. This encoded NBT sequence was modified by attachment of bovine prolactin signal to aid secretion and a 6-histidine tail to aid purification of the protein. Western blot of medium from infected HEK-293 cells demonstrated that rNBT was indeed secreted. rNBT's ability to inhibit nicotinic acetylcholine receptor (nAChR) response to ACh was investigated in *Xenopus* oocytes that expressed neuronal nAChR subtypes $\alpha_2\beta_2$ and $\alpha_3\beta_2$. The recombinant toxin inhibited the response of $\alpha_3\beta_2$ type AChRs to ACh, and yet failed to inhibit the response of $\alpha_2\beta_2$ type AChRs. Further, the response of oocytes expressing muscle type nAChRs ($\alpha\beta\gamma\delta$), was not affected by rNBT. This supports the hypothesis that weak inhibition of muscle type nAChRs by native NBT preparations is due to contamination by α-bungarotoxin. This rNBT has two advantages over the yeast expression system: 1) the six histidine tag allows for purification and quatitation of the toxin, 2) the adenovirus vector may be used to infect neurons, thereby creating functional knock-outs of their nAChRs. Supported by a grant to JF by the NIH.

110.7

TWO PRIMARY GTS-21 METABOLITES ARE POTENT PARTIAL AGONISTS AT ALPHA7 NICOTINIC RECEPTORS EXPRESSED IN THE *XENOPUS* OOCYTE. W. R. Kem*, V. M. Mahnir, B. Lin and K. Prokai-Tatrai. Department of Pharmacology and Therapeutics, University of Florida College of Medicine, Gainesville, FL 32610-0267.

GTS-21, an Alzheimer's drug candidate undergoing clinical tests, is a partial agonist at nicotinic receptors containing alpha7-subunits (DeFiebre et al., 1995). This anabaseine derivative enhances cognitive behavior in rats, rabbits, and monkeys as measured by a variety of behavioral tasks. It rapidly enters the brain after oral as well as i.v. administration. Plasma and urine determinations of unchanged GTS-21 indicate that it is eliminated by a non-renal route, presumably biotransformation. Using liver homogenates, we have found two primary metabolites with HPLC retention times that match those of synthetic monohydroxy analogs of GTS-21 which would be generated by O-demethylation. These two compounds were examined pharmacologically by radioligand binding and electrophysiologically by measuring ionic currents induced by agonist interaction with *Xenopus* oocyte expressed nicotinic receptors. Both monohydroxy compounds were partial agonists when tested on expressed alpha7 homo-oligomers, with potencies greater than GTS-21. They also displaced iodinated alpha-bungarotoxin from rat brain membrane nicotinic receptors with higher affinity. It is possible that the relatively long duration of action resulting from a single sc injection of GTS-21 noted in some behavioral studies may in part be due to the actions of these GTS-21 metabolites.

Partially supported by Taiho Pharmaceutical Company.

110.8

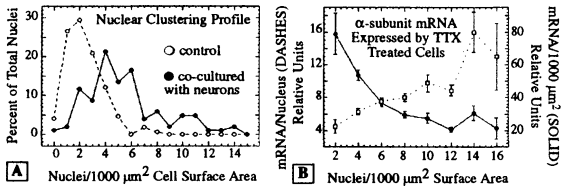
INDUCTION OF ACETYLCHOLINE RECEPTOR GENE EXPRESSION BY ARIA REQUIRES ACTIVATION OF MAP KINASE. J. Si[1], Z. Luo[2], S. Won[1], and L. Mei.*[1] [1]Dep. of Pharmacol., Univ. of Virginia Sch. of Med., Charlottesville, VA22908; [2]Diabetes Unit, MGH, Charlestown, MA 02129.

Transcription of genes encoding nicotinic acetylcholine receptor (AChR) subunits (α, β, γ or ε, and δ) is highest in nuclei localized to synaptic region of the muscle which contributes to maintain a high density of AChRs at the postjunctional membrane. ARIA (*ACh*R *i*nducing *a*ctivity) is believed to be the trophic factor utilized by motor neurons to stimulate AChR synthesis in the subsynaptic area. Treatment of muscle cells with ARIA causes tyrosine phosphorylation of the 185-kDa erbB proteins and activation of AChR subunit gene expression. To elucidate the signaling mechanism initiated by ARIA, we established stable C2C12 cell lines carrying nuclear lacZ gene under control of the mouse ε subunit promoter or chicken α subunit promoter. ARIA stimulated tyrosine phosphorylation of erbB proteins in these C2C12 cells within 15 sec with a peak at 5 min. Immediately following tyrosine phosphorylation of erbB proteins, mitogen-activated protein (MAP) kinase was activated which occurred within 30 sec and peaked at 8 min after ARIA stimulation, as assessed by retarded mobility in SDS-polyacrylamide gel electrophoresis and increased enzyme activity. Concomitantly, expression of AChR genes was induced by ARIA. ARIA-induced AChR gene expression was only observed in differentiated myotubes, but not in myoblasts. Treatment of C2C12 myotubes with PD98059, a specific inhibitor of MAP kinase kinase (MEK), abolished ARIA-induced activation of MAP kinase and stimulation of the AChR α and ε subunit gene expression. Furthermore, expression in C2C12 cells of dominant negative mutants of MEK1 or Raf1 diminished the induction by ARIA. By contrast, ARIA-induced activation of AChR gene expression was unaffected by wortmannin or rapamycin, inhibitors of phosphatidylinositol (PI) 3-kinase or S6 protein kinase, respectively. These results indicate that regulation of AChR gene expression by ARIA in C2C12 cells requires activation of the MAP kinase signaling pathway. (Supported by a NIH grant NS34062)

110.9

NUCLEAR CLUSTERING IN MYOTUBES IS PART OF AN ACTIVITY INDEPENDENT, COOPERATIVE MECHANISM OF ACHR mRNA UPREGULATION. K.A. Duca, K.-P. Chiu, T. Sullivan, S.A. Berman, S.Bursztain*. Laboratories for Molecular Neuroscience, McLean Hosp./Harvard Med. Sch., Belmont, MA 02178.

We investigated the relationship between nuclear cooperativity (as expressed by degree and type of nuclear aggregation) and upregulation of AChR subunit transcripts. We treated embryonic chick muscle cell cultures with muscle activity blocking agents, *viz.* decamethonium (DCM), d-tubocurarine (TBC), and tetrodotoxin (TTX) or co-cultured them with cholinergic neurons. We then examined the influence of muscle activity on the expression of AChR α-, γ-, and δ- subunit mRNAs and the degree of nuclear aggregation. mRNA was measured by *in situ* hybridization and nuclei visualized by *bis*-benzimide staining. All chemical treatments, as well as neuronal co-culture, resulted in an upregulation of mRNA expression, which also correlated with increased nuclear density. The pattern of nuclear aggregation was observed to be treatment dependent, with more and larger aggregates found when myotubes were co-cultured with neurons (A). Moreover, as aggregates became larger: 1) nearly all nuclei expressed mRNA and 2) the per nucleus mRNA output actually *decreased* while local accumulation remained high (B). We are testing the hypothesis that nuclear clusters stabilized via microfilaments and/or microtubules play a significant role in remodeling of the neuromuscular junction. This work was supported by NIH grants.



110.10

DOWNREGULATION BY CILIARY NEUROTROPHIC FACTOR OF NICOTINIC ACETYLCHOLINE RECEPTORS IN POSTNATAL RAT SUPERIOR CERVICAL GANGLION NEURONS. S. Huck and S. Boehm*. Department of Neuropharmacology, University of Vienna, A-1090 Vienna, Austria.

The cytokines ciliary neurotrophic factor (CNTF) and leukemia inhibitory factor have dramatic effects on the neurotransmitter phenotype and on neuropeptide expression in cultures from the postnatal rat superior cervical ganglion (SCG). We now report that CNTF also downregulates nicotinic acetylcholine receptors in these cultures.

SCG were dissected from early postnatal (P3-5) pups, dissociated and plated at high density on collagen-coated tissue culture disks (40.000 per disk, disk diameter: 5mm). Cultures were loaded with [3H]norepinephrine after 5-7 days *in vitro* and transferred to superfusion chambers. Superfusates were collected, and the spontaneous release of radioactivity, the release induced by electrical field stimulation, and the release elicited by nicotine in the absence or presence of tetrodotoxin (TTX) were determined by liquid scintillation counting.

Stimulated release was strictly dependent on the presence of calcium in the superfusion buffer. The presence of TTX allowed the distinction of 2 types of nicotine-induced [3H] release: one which depends on, and the other which is independent of action potentials. The two components have EC50 values for nicotine of 8.9 and 43.5μM, respectively. At 100μM nicotine, each component contributes about 50% to the total [3H] overflow. Electrically-induced release in cultures maintained in the presence of 20ng/ml CNTF did not differ from controls. However, the release induced by 10μM nicotine in the absence, or by 100μM nicotine in the presence of TTX was reduced by CNTF to 46% and 35% of controls, respectively. The sum of these observations implies that CNTF downregulates nicotinic acetylcholine receptors in rat superior cervical ganglion neurons.

Supported by the Austrian Science Foundation, project P11607-MED.

110.11

UBIQUITINIZATION OF THE ACETYLCHOLINE RECEPTOR COMPLEX FROM SKELETAL MUSCLE. J. P.O'MALLEY* & M. M. SALPETER, Section of Neurobiology & Behavior, Cornell University, Ithaca, NY 14853.

Acetylcholine receptors (AChRs) exist as two distinct physiological entities with different metabolic degradation rates. The two forms have been called Rr and Rs to designate rapidly degrading (Rr; t1/2 ≈ 1 d) and slowly degrading Rs AChRs (t1/2 ≈ 10 d in innervated muscle and ≈ 3d in denervated or cultured muscle). To date the molecular steps involved in AChR degradation are unknown. Since the two AChR populations can be spatially interspersed at the neuromuscular junction while exhibiting different half-lives, it is possible that AChR turnover may be achieved by individually marking each receptor for degradation. Indeed such a marking with ubiquitin, followed by the formation of a proteosome complex and protein degradation, has been observed for cytoplasmic, and more recently membrane associated proteins. To test if ubiquitin may be involved in AChR degradation we assayed normal and denervated adult as well as fetal and cultured rat muscle for ubiquitin. The tissue was homogenized and washed extensively to remove soluble ubiquitin, and the AChRs extracted in 1% Triton X-100. The AChRs were then affinity purified with α-bungarotoxin-Sepharose after which Western analyses using an anti-ubiquitin antibody was performed. We report that ubiquitin immunoreactivity co-purifies with the α-bungarotoxin (α-BTX) binding activity. We also report that the level of ubiquitinization of the α-BTX binding activity in each of the extracts differed, and was highest in tissue with the greatest Rs content.

These results suggest that ubiquitinization of either the AChR, or a receptor associated protein, may play a role in AChR degradation. The difference between Rr and Rs may, in part, reflect a regulation of ubiquitin binding or the formation of the proteosome complex after ubiquitinization.

We thank A. Haas for the anti-ubiquitin antibody.
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110.12

MAPPING NICOTINIC ACETYLCHOLINE RECEPTORS (nAChR) WITH PET. Y.-S. Ding, S.J. Gatley, J.S. Fowler*, N.D. Volkow, D. Aggarwal, J. Logan, S.L. Dewey, F. Liang, F.I Carroll, M.J. Kuhar, Chem and Medical Depts, BNL, Upton, NY 11973; Chemistry and Life Sciences, RTI, NC 27709; and Emory University, Atlanta, GA 30322.

This study provided the first evidence for using a positron emitter labeled epibatidine derivative to visualize nAChR in vivo with PET. The one-pot, two-step radiosynthesis afforded [^{18}F]norchloro-2-fluoro-epibatidine (NFEP) with a overall radiochemical yield of 55-65% in a synthesis time of 65 min with a specific activity of 4 Ci/μmol (EOB). The distribution of fluorine-18 in the baboon brain was heterogeneous with a strikingly high uptake and slow clearance in the thalamus as compared to the rest of the brain. The half-times for clearance from peak uptake for NFEP were 170, 50, 25 min for thalamus (TH), striatum (ST), and cerebellum (CB), respectively. The TH/CB ratio increased steadily, reaching a value of 4 at the end of study (3 h). In mice, a value of greater than 20 for both the SC/CB (SC:superior colliculus) and TH/CB ratios was reached at 90 min after the tracer injection, a distribution which strikingly resembles that of [^{3}H]norchloroepibatine in brain (Scheffel,et al, 1995). The pharmacological specificity of the in vivo binding was clearly nicotinic, as it was displaced by nicotine (TH/CB decreased to 1.1-1.5 after pretreatment with 3 mg/kg of nicotine), but not by benztropine, a muscarinic acetylcholine receptor ligand, nor by mecamylamine, an ion channel blocker of the nicotinic receptor which does not compete with the nicotinic recognition site. The extraordinary high thalamus to cerebellar ratio suggests a new approach to investigate the role of thalamus in the CNS system and its relationship to addiction. Supported by DOE/OHER; NINDS; NIDA.

110.13

DIFFERENTIAL EFFECTS OF CHRONIC NICOTINE TREATMENT ON NICOTINE-STIMULATED RUBIDIUM EFFLUX IN VARIOUS MOUSE BRAIN AREAS. A.C. Collins*, S.R. Grady, T.K. Booker, S.F. Robinson, A.E. Bullock, J.A. Stitzel, A.L. Clark and M.J. Marks. University of Colorado, Boulder, CO 80309

Chronic treatment of mice with nicotine results in the development of tolerance, as well as an increase in the number of nicotinic binding sites. In order to determine the effects of chronic treatment on nicotinic receptor function, mice were treated by continuous intravenous infusion of 0.0 or 4.0 mg/kg/hr nicotine for 10 days. After 12 hr withdrawal, crude P2 synaptosomes were prepared from eleven brain areas. $^{86}Rb^{+}$ efflux stimulated by 10 μM nicotine for 3 min was measured and the maximal response and rate of desensitization was calculated. The binding of [^{3}H]nicotine was also measured. Consistent with previous observations, a high correlation between $^{86}Rb^{+}$ efflux and nicotine binding was observed in control samples ($r=0.93$). Chronic nicotine treatment significantly increased [^{3}H]nicotine binding in 8 of 11 brain regions. However, the effects of chronic treatment on nicotine-stimulated $^{86}Rb^{+}$ efflux varied among brain areas: efflux from olfactory bulbs increased, efflux from thalamus decreased, and no significant change was observed in the remaining areas. The subsequent correlation of nicotine-stimulated $^{86}Rb^{+}$ efflux with [^{3}H]nicotine binding after chronic nicotine treatment was high ($r=0.91$), suggesting a continuing relationship between these two measures. The relative amount of efflux per binding site, as indicated by the slopes of the lines, was approximately 30% lower following chronic treatment. Whether this decrease resulted from a general reduction in function for the receptors or an increase in the number of nonfunctional receptors is unknown. The differential effect of chonic nicotine treatment on receptor number and function suggests a regional heterogeneity in receptor regulation that may be an important variable in the evaluation of the biological bases of tolerance and dependence.
Supported by DA03194 and DA000197.

VISUAL PSYCHOPHYSICS AND BEHAVIOR I

111.1

THE INTERACTION OF VISUAL ATTENTION, LEARNING AND CONTEXT ON LUMINANCE DISCRIMINATION IN HUMANS AND MONKEYS. M. Ito*, C.D. Gilbert and G. Westheimer. The Rockefeller University, NY, NY 10021.

To explore the role of expectation on early visual processing, we measured psychophysically the influence of spatial certainty on brightness discrimination. The subject had to indicate whether one of 4 lines, each presented in a different visual field location, was brighter or dimmer than a reference line. On some trials the observer was uncertain as to the position in which the odd stimulus would appear (uncertain condition), and on others the observer was cued to its position (certain condition). Initially, the discrimination was 2.2 fold better in the certain than in the uncertain condition. After 3 months of training, however, the discrimination in the uncertain condition improved so that it became equivalent to that in the certain condition. The number of possible positions was increased from 4 to 8 by adding stimuli in 4 new positions. In these new positions, the discrimination in the uncertain condition was 2.5 fold worse than in the certain condition, whereas both old and new positions had the same threshold in the certain condition. The new stimulus positions then showed a similar improvement with further training. We then explored the interaction between a contextual effect -- brightness induction of a target line by a second colinear line -- and attention. Under spatial uncertainty, there was a much greater brightness induction effect than when the subject anticipated the stimulus position. The fact that attentional influences show considerable positional specificity and affect simple attributes suggests the involvement of early visual processes. This non-retinal influence is in turn gated by context and by learning. Supported by NIH grant EY07968.

111.2

PERCEPTUAL LEARNING OF SPATIAL LOCALIZATION: SPECIFICITY FOR POSITION, ORIENTATION AND CONTEXT. *R.E. Crist, M.K. Kapadia, G. Westheimer and C. D. Gilbert**. The Rockefeller University, New York, NY 10021.

The specificity of perceptual learning for the particular stimulus parameters used during training can help determine the locus of both the discrimination and the learning effect itself. Training for several weeks with a bisection task, during which an observer was presented with three parallel lines and asked to determine the relative position of the central line, produced up to a three-fold threshold reduction. The improvement showed considerable retinotopic specificity with transfer limited to within a few degrees of the trained position. In addition, there was little transfer of improvement to a bisection task oriented orthogonally to the trained configuration. We examined the extent to which this learning would generalize to other stimulus configurations. In one experiment, the flanking lines of the bisection task were replaced with a single collinear reference to produce a vernier discrimination task—no transfer of improvement was observed. Furthermore, no evidence of transfer was seen when we changed the psychophysically relevant attribute of the stimulus from its position to its orientation, indicating that the learning effect was not general to all stimulus attributes at a particular visual locus. While the orientation and retinotopic specificity of the learning effect suggest cortical modifications early in the visual pathway, the specificity for particular stimulus configurations suggest that these changes are gated by contextual cues. Supported by NIH grants EY07968 and EY07138.

111.3

A TRAINABLE MODEL OF SALIENCY-BASED VISUAL ATTENTION. L. Itti, E. Niebur[1], J. Braun* and C. Koch, California Institute of Technology, 139-74, Pasadena, CA 91125 and [1]Johns Hopkins University, Baltimore, MD 21218.

We present a model of bottom-up selective visual attention, developed in accordance with the known physiology of the visual system of the macaque monkey. It comprises two interacting stages, the first being a fast and parallel pre-attentive extraction of visual features across 50 spatial maps (for orientation, intensity and color, at six spatial scales), and the second a slow and sequential focal attention shifting mechanism (using a Winner-Take-All neural network to select the most conspicuous image location, and an inhibition-of-return mechanism to generate attentional shifts). The link between the two stages is a "saliency map", which topographically encodes for the local conspicuity in the visual scene, and controls where the focus of attention is currently deployed [Koch and Ullman, *Human Neurobiol.* 1985;4:219-227]. Supervised learning can be introduced to bias the relative weights of the features in the construction of the saliency map and achieve some degree of specialization towards target detection tasks.

Despite its conceptual simplicity, this model reproduces psychophysical performance in stimulus-driven, task independent attention. Results with the model are comparable to humans' on simple psychophysical tasks (e.g. A. Treisman's pop-out and conjunctive search). Strong performance was also obtained in the detection of salient targets in natural color images, despite high noise, large variations in color and illumination, shadows, reflections and strong textures, which are reputed problematic for artificial vision systems (an interactive demonstration may be found at http://www.klab.caltech.edu/~itti).

(Supported by ONR, and by the NSF Center for Neuromorphic Systems Engineering at Caltech).

111.4

EFFECTS OF COVERT ORIENTING OF ATTENTION IN A MOTION DISCRIMINATION TASK. E. Zohary* and G. Kozminsky Dept. of Neurobiology, Hebrew University, Jerusalem, 91904, Israel.

A peripheral visual cue can shorten reaction times to detect a visual target in the cued area. Can covert attention also improve human psychophysical performance in a motion discrimination task? We used a random dot pattern whose motion coherence varied from trial to trial. Signal dots moving either up or down, were displayed inside an invisible rectangle subtending 5*5 deg centered either 5 degrees left or right of the fixation point, or at the center of gaze. Noise dots, moving at random directions, were displayed anywhere inside the central 20*20 deg of the visual field, to mask the location of the signal dots in the visual field. A spatial cue (an open rectangular frame), or a symbolic cue (an arrow) were used to inform the subjects the future location of the signal dots. The cue was displayed for 65 msec, and was followed by the test stimulus which was presented for 130 msec. Both types of cues lead to higher gains in psychophysical performance. The improvement was more prominent in the periphery than in the center of gaze. A second experiment revealed that the cue was ineffective if the noise dots were limited to the region of the signal dots. These results suggest that in cases of spatial uncertainty, orienting of attention can lead to gains in discrimination performance. In addition, a visual field left/right asymmetry in perceptual performance was often observed in the non-cued trials (Mean Left/Right threshold ratio: 1.97, t-test $P<0.001$, $N=13$), but not in the cued trials. Therefore, perceptual asymmetry is probably not due to left hemisphere superiority in motion discrimination, or a fixation shift. Rather, this asymmetry may result from an inherent bias in the allocation of attention. Supported by Israel Ministry of Science and Arts

111.5

VISUAL-SPATIAL ATTENTION AND EVENT-RELATED POTENTIALS: BRIDGING THE GAP BETWEEN MONKEYS AND HUMANS.
M. Girelli, M.T. McDermott, M.A. Ford, & S.J. Luck*, Department of Neurological and Visual Sciences, University of Verona, ITALY, and Department of Psychology, University of Iowa, Iowa City, IA, USA.

Single-unit recordings in area V4 and IT cortex of the macaque monkey have provided evidence that target detection in visual search tasks is accompanied by a suppression of information from the nontarget "distractor" items, beginning about 175 ms after search array onset. In previous event-related potential recordings from human subjects, we have characterized a component called "N2pc" that shares many properties with the single-unit suppression effect: 1) both start around 175 ms poststimulus; 2) both are observed primarily in the hemisphere contralateral to the target; and 3) both are eliminated when the distractors are removed. In the present study, we provided several tests of the hypothesis that the human N2pc component reflects the same suppression observable in the single-unit studies in the monkey. Specifically, we manipulated several variables that have been studied in previous single-unit recording experiments, including the density of the distractors, the difficulty of the discrimination, and the type of behavioral response. We obtained the following results: 1) the N2pc was larger when the target and a distractor were both presented in the same quadrant than when they were in opposite quadrants; 2) the N2pc component was considerably reduced in a simple feature detection task compared to a conjunction discrimination task; and 3) the N2pc for the feature detection task was larger when subjects were required to foveate the target than when they made a simple manual detection response. These results mirror the effects of similar manipulations in single-unit recording experiments, and this similarity supports the hypothesis that the human N2pc component reflects the same suppression mechanism that has been observed in the monkey single-unit recordings.
Supported by grant 95-38 from the McDonnell-Pew Program in Cognitive Neuroscience.

111.6

MOTION CAPTURE AND INDUCED MOTION CAN GENERATE CHANGES IN PERCEIVED SIZE AND ORIENTATION. V. S. Ramachandran* and K. McLain, Brain and Perception Lab UCSD, La Jolla, CA 92093-0109.

A yellow square is superimposed on an equiluminous grey background. If a sparse cluster of black dots is superimposed optically on the square and moved, the square appears to move as well even though it is physically stationary (Motion Capture, Ramachandran, 1985, *Nature*). We now find that: 1) if an expanding (or shrinking) array of spots is superimposed on the square, it can appear up to 20% larger (or smaller) than it really is -- an effect that occurs mainly at equiluminance. 2) If a shearing array of dots is superimposed on the square, it looks distorted into a trapezoid, i.e., orientation of edges changes. 3) If a yellow cross is used and if dots move horizontally on the vertical limb of the cross and vertically on the horizontal limb, the cross becomes "segmented" clearly into two separate bars sliding past each other. 4) If the yellow square in experiment 1 is made non-equiluminous, induced motion is seen instead of capture and, interestingly, the square actually looks smaller when the dot matrix expands.

We conclude that motion capture (and induced movement) can generate not only an illusory motion sensation, but changes in perceived size and orientation and the effect can also generate scene segmentation. The effect illustrates a surprising degree of interaction between cortical modules concerned with color, motion, shape and size. One wonders whether shape and size selective cells in DL, V4, and other extrastriate areas will respond to these illusions.

111.7

VISUAL PERCEPTION OF MOTION AND STRUCTURE-FROM-MOTION: A FUNCTIONAL MRI STUDY. V. Cornilleau-Pérès#, A.L. Paradis#, J. Droulez#, A. Berthoz# and D. Le Bihan^. #CNRS-Collège de France, 75270 Paris Cedex 06. ^Service Hospitalier Frédéric Joliot, CEA, Centre Hospitalier d'Orsay, 91000 Orsay, France. (SPON: European Neuroscience Association).

Motion parallax is a powerful cue for visual perception of three-dimensional shapes. Studies in primates have as yet failed to show visual cortical structures processing local spatial variations of retinal velocity. In this study we searched for cortical areas that are specifically activated by viewing a moving sphere, as compared to other stationnary or moving stimuli. Functional MRI images were acquired in 4 adult human volunteers using a 3T Bruker MR imager, while viewing random dots spread over 16 deg of visual angle. Dots could be stationnary (S), moving stochastichally (MS), in a coherent fashion of expansion-contraction (EX), or as if they belonged to an opaque sphere oscillating about one of its own frontoparallel tangents (SP). Three experiments were performed under alternating conditions: (1) S versus MS dots, (2) MS versus EX dots, (3) MS versus SP dots. In the first case (S vs. MS) strong bilateral activities in V5 and in the occipital lobe (OL) were observed in all subjects. In the MS vs. EX case, only a few voxels showed a correlation with the alternating stimulus. Most of them were located in the vicinity of V5 and OL, with an increase of the MRI signal for stochastic motion, as compared to coherent motion. In the MS vs. SP case, three subjects showed increased activity at the occipito-temporal junction bilaterally (anterior and ventral with respect to V5). The fourth subject exhibited activity in the posterior parietal and in frontal lobe. We conclude that part of the visual areas processing motion are in fact sensitive to strong local variations of the velocity field, rather than to expansion-contraction. Furthermore structure-from-motion seems to involve the occipito-temporal junction, although in a subject-dependent fashion.

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111.8

A RELATIVISTIC DESCRIPTION OF PERCEPTION, D. N. Levin*, 1720 N. Lasalle Dr., #25, Chicago, IL 60614.

We show how to use differential geometry to measure and characterize the perceptions of an observer of a continuum of stimuli. Because the method is not based on a model of perceptual mechanisms, it can be applied to a wide variety of observers and to many types of visual and auditory stimuli. The observer is asked to identify which small transformation of one stimulus is perceived to be equivalent to a small transformation of a second stimulus, differing from the first stimulus by a third small transformation. The observer's perceptions of a number of such transformations can be used to calculate an affine connection on the stimulus manifold. This quantity encodes how the observer perceives evolving stimuli as sequences of "reference" transformations. The internal consistency of the observer's perceptions can be characterized by the manifold's curvature and certain other tensors derived from the connection. Differences between the affine connections of two observers characterize differences between their perceptions of the same evolving stimuli. Therefore, the affine connections of two observers can be used to map a stimulus perceived by one observer onto another stimulus, perceived in the same way by the other observer. The technique was used to measure the affine connection of an observer of a dot moving on several different background graphics: a blank screen, a grid, or two converging lines, similar to those used to create the Ponzo illusion. Preliminary experimental results show to what extent the observer's sense of parallelism depended on the visual information contained in the background graphic. We will also demonstrate how the technique can be used to characterize an observer's perceptions of mixtures of two colors or two sounds.

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111.9

AFTER-IMAGES IN CORTICALLY BLIND VISUAL FIELDS
P.Stoerig*. Institute of Medical Psychology, Ludwig-Maximilians-University, Goethestr. 31, D-80336 Munich, Germany

Lesions that destroy or disconnect the striate cortex cause cortically blind visual fields. Forced-choice methods can reveal localization, detection, and discrimination of visual stimuli presented within absolute blind fields where the stimuli are not consciously seen by patients or monkeys with striate cortical ablation ('Blindsight'). To see whether an after-image can be induced in the blind visual field, a patient with an incomplete hemianopia was tested with high-contrast colour patterns. Following a 20s period of central fixation on the inducing pattern, the patient reported an after-image which reflected the negative of the 'seen' part of the pattern; the half that fell into the blind field did not produce an after-effect. Presentation restricted to the normal hemifield caused a normal after-image, while presentation restricted to the blind field in several instances caused an after-image which appeared as an unstructured lighter field without contour or colour. It follows that a simple phenomenal image can be elicited in a restricted cortically blind field. Topographically imprecise fibre systems projecting to striate cortex can exhibit unsuspectedly extensive plasticity (Sugita, Nature 380:523-526, 1996). If these mediate the phenomenal effect it should be absent in patients with complete hemianopia who retain no striate cortex.

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111.10

FRACTAL PROPERTIES OF DYNAMIC IMAGES AND HUMAN VISION.
Vincent A. Billock*, Gonzalo C. de Guzman, and J. A. Scott Kelso, Center for Complex Systems, Florida Atlantic University, Boca Raton, FL 33431.

Most natural images have $1/f^{\beta}$ amplitude spectra, where β is typically about 1.10. Recently it has been shown that human spatial contrast perception has a power law correlational structure which implies a $1/f^{\beta}$ spectrum for spatial vision, with a β closely matched to the exponent found for image processing (Billock, ARVO, 1996). This result is compatible with spatial frequency mechanisms at multiple scales and indicates that the visual system is adapted to the second order statistics of static natural images. Here, we extend this analysis to dynamic images and temporal vision. We analyzed scenes from several motion pictures. Each pixel in a scene was treated as a gray scale time series and Fourier transformed. The spectra of all the pixels were averaged. Each scene was well described by the power law $1/f^{\beta}$ ($.61 \leq \beta \leq 1.01$). Temporal perception was studied by analyzing the correlational structure of temporal contrast sensitivity using the mathematics of fractional Brownian (fractal) processes. Correlation of detection thresholds as a function of temporal frequency separation obeyed a power law, implying a $1/f^{\beta}$ spectra. β averages 1.35 for data taken at low to moderate spatial frequencies and 1.47 for data taken at high spatial frequencies (8-23 c/deg). This result may indicate a parvo/magno difference in temporal processing. Moreover, the difference between the exponents for motion pictures and temporal vision suggest a role for pursuit eye movements in optimizing the transduction of temporal information. Other psychophysical power laws may be specific instantiations of the general spatiotemporal power law relationship between the physical and perceptual worlds.

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111.11

WHEN THE BRAIN CHANGES ITS MIND: INTER-OCULAR GROUPING DURING BINOCULAR RIVALRY
I. Kovács*[1,2], T. V. Papathomas[1] & A. Fehér[1]. [1]Lab. of Vision Res. Rutgers Univ., Piscataway, NJ; [2]Eötvös Univ., Budapest, Hungary.

THE prevalent view of binocular rivalry holds that it results from reciprocal inhibition among monocular neurons. However, there is recent evidence that binocularly driven cells in V4 and MT reflect perceptual alternations in their firing pattern (Leopold and Logothetis, Nature 1996 379:549-552), suggesting that there is more to binocular rivalry than mere eye-competition. We have developed a method to study interocular interactions during rivalry. Conventional rivalry-inducing stimuli are dissimilar image pairs that are each coherent in their global structure (such as gratings of orthogonal orientations, or blobs of opposite colors). We replace these by complementary patchworks of intermingled rivalrous images. Can the brain unscramble the pieces of the patchwork arriving from different eyes to obtain a global percept? Percepts with conventional, globally coherent image-pairs (e.g., a monkey face vs. a jungle scene) are compared to those obtained with the patchwork image pairs (spatially complementing pieces of the monkey and the jungle in each image). We found that interocular grouping of image components occurs across extended images (15°x15°) in all tested conditions (color defined, orientation defined, natural, and moving natural images), implying that binocular rivalry goes beyond interocular suppression and follows more complex rules of perceptual organization. We suggest that interocular grouping is mediated by extremely interactive feedforward-feedback connections involving a large part of the cortical architecture.
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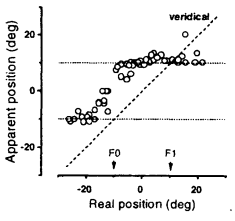
111.12

RESTRUCTURING OF PERCEPTUAL SPACE BEFORE SACCADES.
M.C. Morrone*, J. Ross and D.C. Burr Ist. di Neurofisiologia del CNR, Pisa, Italy and Dep. of Psychology, University of Western Australia, Australia.



We studied how the human visual system recalibrates visual co-ordinates to compensate for saccadic eye movements. Observers made horizontal saccades from a fixation point F0 at -10° to a target F1 at +10° on an otherwise featureless red screen; they reported the apparent position of a vertical green bar that was briefly displayed before, during or after the saccade. Bars presented 50 ms before the beginning of the saccade, or after its completion, were perceived accurately and veridically. However, bars presented immediately prior to the saccade were systematically mislocated, either in the direction of the saccade or in the opposite direction, depending on the spatial position of the bar (see figure). This result has been verified by various techniques including vernier offset estimation, and a forced choice annulling task. When four bars (straddling the saccade target) were displayed in the interval -25 to 0 ms, they were seen to be merged into 1 bar (forced choice). The results suggest that each saccade is accompanied by a shift in the retinal co-ordinate system, and a momentary compression of visual space centred around F0 and F1. The perceptual compression may be instrumental in ensuring a smooth transition from fixation to fixation.

Supported by Australian ARC and Italian CNR and MURST.

111.13

THE NEURAL SITE OF THE REDUNDANT-TARGETS EFFECT: EVIDENCE FROM EVENT-RELATED POTENTIALS
C.A. Marzi*, M. Girelli, A.E. Ipata & C. Miniussi.
Dept. Neurol. & Vis. Sci., U. of Verona, 37134 Verona, Italy.

The redundant-targets effect (RTE) consists in a decrease in reaction time (RT) following presentation of two or more stimuli versus one stimulus alone. It has been interpreted either as a probability summation phenomenon or as a neural summation effect. With the aim of casting light on its neural site we studied the event-related potential (ERP) correlates of the RTE in 12 normal human subjects performing in a simple visual reaction time (RT) task with stimuli (2.5°x4° rectangular checkerboards of 2.1 cycles/degree) lateralized to the upper or lower portions of the left or right hemifields. The electrodes (32) were placed over the scalp and referred to the mastoids. The behavioral data showed a robust RTE that was reliably present in the lower but not in the upper visual hemifield.

The main thrust of this study was that reliable evidence of a RTE was found for three main early components of the visual ERP namely C1, P1 and N1. All these components showed an overall shorter latency and a higher amplitude for bilateral than unilateral stimuli especially for lower hemifield presentations. It is particularly important to point out that the presence of a RTE in the C1 component (latency range: 40-80 msec) suggests that this effect is present already at the level of Area 17. In keeping with this we found a polarity reversal of C1 for stimuli presented to the upper and lower hemifields. Such reversal is considered a consequence of the peculiar retinotopic organization of the calcarine sulcus. The presence of a neural correlate at the level of Area 17 brings weight to the neural summation hypothesis of the RTE.

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111.14

SELECTIVE IMPAIRMENT OF MAGNOCELLULAR FUNCTION IN COMPRESSION OF THE ANTERIOR VISUAL PATHWAYS.
G. Tassinari*[1], C. A. Marzi[1], B. B. Lee[2], V. Di Lollo[3], D. Campara[1] (SPON: Eur. Brain and Behav. Soc.). [1]Dept. of Neurol. and Vis. Sci., Univ. of Verona, Italy, [2]M.P.I. for Biophys. Chem., Göttingen, Germany, and [3]Dept. of Psychol., Univ. of Alberta, Canada.

Two main parallel systems, magnocellular (M) and parvocellular (P), originating from different types of retinal ganglion cells, are known to be segregated in different portions of the pre-geniculate visual pathways. Their relative contribution to two main cortical streams, dorsal and ventral, is still under discussion, but it is reasonable to suppose that selective damage to the M or P subcortical system might interfere with specific aspects of processing within one or the other cortical system. Using two different apparent-motion tasks, we compared the performance of patients affected by compression of the ventral part of the anterior visual pathways with that of normal controls. In the first task, observers detected small displacements of a low-contrast stationary edge (Lee et al., *J. Neurosci.* 13: 1001, 1993), while in the second task they estimated the visible persistence of moving dots (Hogben and Di Lollo, *Percept. & Psychophys.* 38: 450, 1985). In the first task, patients were impaired following parafoveal presentation, particularly in the temporal portion of the visual field, while no group difference was present in the second task. However, in the second task, patients showed reduced suppression of visible persistence at long exposure durations. Three considerations support the hypothesis that these results represent a selective impairment of the M system in humans. First, it is known that afferents from retinal ganglion cells are segregated by size in the human pre-geniculate visual pathways, with the largest axons (presumably belonging to the M system) located ventrally. Second, patients with a ventral compression behave like P ganglion cells of the macaque monkey, which have previously been shown to exhibit elevated and unmodulated thresholds for apparent motion. Third, patients are less sensitive to the inhibitory signals that suppress visible persistence, which probably originate in the M system. *Supported by MURST and HFSP.*

112.1

INCREASED SUPEROXIDE DISMUTASE ACTIVITY PROMOTES NEURONAL SURVIVAL AND PROTECTS AGAINST L-DOPA-INDUCED TOXICITY. D. Sulzer, M.A. Mena, U. Khan, C.J. Epstein, S. Fahn*, and S. Przedborski. Dept. Neurology and Psychiatry, Columbia Univ., New York, NY 10032; and Dept. Pediatrics, Univ. California, San Francisco, CA 94143.

Postnatal midbrain neurons (PMN) progressively die in culture. We hypothesize that this occurs because of a diminished ability to detoxify free radicals. To test this possibility, we examined the fate of PMN from transgenic mice expressing human copper/zinc superoxide dismutase (SOD1) and from their non-transgenic littermates. Midbrain from transgenic pups had ~3-fold higher SOD activity than midbrain from non-transgenic pups. We found that transgenic neurons died significantly less than non-transgenic neurons and that increased SOD1 activity seemed to prevent the loss of tyrosine hydroxylase (TH)-positive neurons better than the loss of GABAergic neurons. We also found that neuronal processes from transgenic cultures exhibited greater density than those from non-transgenic cultures. To subject PMN to an oxidative stress, cultures were incubated with different concentrations of L-DOPA. Non-transgenic cultures incubated with 200 μM L-DOPA showed ~50% reduction in TH-positive neuron number and a dramatic increase in the number of dying neurons and apoptotic profiles. Transgenic cultures incubated with the same dose of L-DOPA showed no changes in the number of TH-positive neurons, of dying cells, or of apoptotic profile compared to vehicle incubated controls. These results indicate that (1) the progressive death of cultured PMN is mediated in part by free radicals and (2) chronic increased SOD1 activity promotes cell survival, neuronal process development, and protects against drug-related oxidative stress.

This work is supported by the APDA, NINDS, MDA, PDF, and Lowenstein Foundation.

112.2

COMPARATIVE STUDIES ON THE EFFECTS OF 7-NITROINDAZOLE ON MPTP AND METHAMPHETAMINE NEUROTOXICITY. J. E. Royland, M.W. Jakowec, J.W. Langston and D.A. Di Monte*. The Parkinson's Institute, Sunnyvale, CA 94089.

Previous studies have reported that 7-nitroindazole (7-NI), a selective inhibitor of neuronal nitric oxide synthase (NOS), is capable of preventing the neurotoxic effects of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). After exposure to MPTP, NOS could be stimulated *via* the rise of intraneuronal Ca2+ due to the activation of NMDA receptors. Excessive production of nitric oxide may ultimately lead to neuronal damage. Activation of NMDA receptors is thought to play a role in methamphetamine (METH) neurotoxicity. Therefore, it is possible that NOS stimulation and increased nitric oxide production could also occur after METH exposure. We tested this hypothesis by determining the effects of 7-NI on METH-induced striatal dopamine depletion in mice. METH alone (7.5 and 10 mg/kg x 4 injection at 2-hour intervals) caused a significant loss of striatal dopamine at 90 min, 1 and 5 days. Pretreatment with 7-NI (50 mg/kg, 20 min before each METH injection) potentiated METH-induced dopamine depletion at 90 min, while it protected at 1 and 5 days. 7-NI also protected against the loss of tyrosine hydroxylase-immunoreactivity caused by METH at 5 days. In comparison to the effects observed with METH, 7-NI prevented MPTP-induced dopamine depletion at 90 min as well as 5 days. Results of this study support a role of NOS in dopaminergic neurotoxicity while pointing to important differences in the mechanisms of dopamine depletion in the METH and MPTP models.

This work was supported by the Parkinson's Institute, the National Parkinson Foundation (JER) and The Mather and Lookout Foundations (MWJ).

112.3

OXIDATIVE STRESS-RESISTANT CELLS ARE PROTECTED AGAINST HALOPERIDOL TOXICITY. F. LEZOUALC'H, F. HOLSBOER and C. BEHL*. Max Planck Institute of Psychiatry, Clinical Institute, 80804 Munich, Germany.

The dopamine D2 receptor antagonist and neuroleptic drug haloperidol is cytotoxic for different neuronal and non-neuronal cells. The mechanism of haloperidol toxicity may involve oxidative stress resulting in cell death as cells are protected against haloperidol toxicity by the lipophilic free radical scavenger vitamin E. To further support this hypothesis, first we used clones of the rat pheochromocytoma PC12 cells which were selected for their resistance to the oxidative stressor amyloid ß-protein (Aß). Employing MTT assay as a first indicator of mitochondrial damage and propidium iodide stainings as cell lysis assay, we showed that Aß resistant cells were also resistant against haloperidol-induced cell death, even after a 24-h challenge with 100 μM haloperidol. The PC12 parental cells were not protected from this haloperidol toxicity. Second, the cells of the clonal hippocampal cell line HT22 transfected with the potential antioxidant, Bcl-2, were also protected against haloperidol toxicity. Taken together, these data strongly support the causative involvement of free radicals in haloperidol-induced cell death. Supported by the Max Planck Society.

112.4

TRANSGENIC MICE WITH HIGH LEVELS OF SUPEROXIDE DISMUTASE ACTIVITY ARE PROTECTED FROM THE NEUROTOXICITY OF 2'-NH_2-MPTP ON SEROTONIN AND NOREPINEPHRINE NERVE TERMINALS. A. M. Andrews*, B. Ladenheim, J. L. Cadet and D. L. Murphy. Laboratory of Clinical Science, NIMH, Bethesda, MD 20892 and Molecular Neuropsychiatry Section, NIDA, Baltimore, MD 21224.

Intraperitoneal administration of the MPTP analog 1-methyl-4-(2'-aminophenyl)-1,2,3,6-tetrahydropyridine (2'-NH_2-MPTP; 4 x 15 mg/kg) to CD-1 mice caused substantial decreases in cortical and hippocampal 5-hydroxytryptamine (5-HT) and norepinephrine (NE) levels to 20-30% of control 3 weeks post-treatment. The magnitude of the depletions was similar to those previously reported in Swiss Webster and C57BL/6 mice given 4 x 20 mg/kg 2'-NH_2-MPTP and in keeping with these prior studies, striatal dopamine levels were unchanged by 2'-NH_2-MPTP treatment in CD-1 mice.

Transgenic CD-1 mice producing high levels of human cytosolic Cu-Zn superoxide dismutase (SOD) were subsequently studied to assess the role of oxygen radicals in the mechanism of action of 2'-NH_2-MPTP. In homozygous SOD mice, 5-HT and NE levels were almost completely unaffected by 2'-NH_2-MPTP treatment while in 2'-NH_2-MPTP-treated heterozygous SOD mice, moderate depletions in cortical and hippocampal 5-HT (50-60% of control) and NE (30-40% of control) were observed. In addition, the density of [^{125}I]RTI-55-labeled 5-HT uptake sites was used to assess 5-HT terminal loss. In various cortical and hippocampal subregions of nontransgenic mice treated with 2'-NH_2-MPTP, the number of 5-HT uptake sites was reduced to 20-35% of control; however, regional 5-HT uptake site density in the 2'-NH_2-MPTP-treated homozygous SOD mice was only minimally affected while in the heterozygous SOD group, intermediate reductions in 5-HT uptake site density on the order of 55-80% of control were seen.

These results demonstrate that 2'-NH_2-MPTP is capable of causing selective 5-HT and NE neurotoxicity in CD-1 mice, and that mice genetically endowed with increased SOD activity are protected from these effects, thereby implicating superoxide radicals in the mechanism of action of 2'-NH_2-MPTP. (This work was supported by the Intramural Research Programs of NIMH and NIDA. A. M. A. was supported by an Intramural Research Training Award from NIMH.)

112.5

MDMA ACUTELY INCREASES BRAIN SEROTONIN SYNTHESIS WHILE FEW HOURS LATTER SYNTHESIS FALLS BELOW PRE-MDMA INJECTION: DOG PET STUDY. Mirko Diksic*, Sadahiko Nishizawa and Shadreck Mzengeza. Department of Neurology and Neurosurgery, McGill University, Montreal, Canada

3,4-Methylenedioxymethamphetamine (MDMA, "Exstasy") is an analog of amphetamine used as recreational drug of abuse, shown to be toxic in laboratory animals and in non human primates. The mode of the MDMA neurotoxicity is not well understood and there is no method to assess it in living human brain. We hypothesize that there is an acute activation of the brain serotonergic system with an increase in the serotonin synthesis, which produces a metabolic activation of the brain serotonergic system beyond the compensatory capacity, eventually resulting in neuronal death. This hypothesis was tested in the dog brain by assessing the influence of the MDMA on the brain 5HT synthesis. We measured brain serotonin synthesis rate using α-[^{11}C]methyl-L-tryptophan and positron emission tomography (J Neurochem 56:153-162, 1991) in dog under halothane anesthesia. The synthesis rate was measured before i.v. injection of 2 mg/kg MDMA, and 2 and 5 h after the MDMA injection. The rate of 5HT synthesis at 2 h after MDMA injection was about 9 times greater than that at the baseline. In contrast 5 h after MDMA the 5HT synthesis rate was about 3 times lower than that at the base line or about 27 times lower than that at 2 h after MDMA. The baseline 5HT synthesis rate was about 40 pmol/g/min. There was no influence of the MDMA injection on the plasma Trp concentration or the fraction of free Trp in plasma. Research was supported by the NS-29629.

112.6

ACTIVATION OF NEURONAL NITRIC OXIDE SYNTHASE: A LIKELY MECHANISM OF PARAQUAT-INDUCED LUNG INJURY. Alok Bandyopadhyay, Satish Rattan, and Sami I. Said*. Thomas Jefferson Univ., Philadelphia, PA 19107; Northport VA Med. Center & University Med. Center, Stony Brook, NY 11794-8172.

Excessive nitric oxide (NO) generation, usually resulting from induction of NO synthase in macrophages (iNOS), has been associated with acute inflammatory responses and tissue injury. Constitutive NO production, by endothelial cells and neuronal cells, is commonly viewed as physiological, although overactivation of neuronal NOS has been implicated in excitotoxicity of central neurons. We recently reported that the pro-oxidant herbicide paraquat induces acute lung injury via mechanisms involving excessive production of NO (*PNAS* 91: 7445, 1994). Because this injury occurred within 1 h, it seemed unlikely that iNOS was responsible. To ascertain the source of NO provoked by paraquat, we isolated RNA from rat tracheal tissue, including tracheal ganglion cells, after direct application of paraquat (0.5-5.0 mM) for 1-10 min. RNA was amplified by PCR, with neuronal NOS- and iNOS-specific primers, and NOS protein was measured by Western Blot. Neuronal NOS expression (NOS RNA/G3PDH RNA) was markedly enhanced following paraquat treatment, but iNOS was unaffected. The stimulated expression of neuronal NOS was dose-dependent: 5 mg paraquat produced maximal elevation, and the effect peaked at 10 min but was sustained for > 30 min. We conclude that : 1) Upregulation of neuronal NOS probably accounts for the stimulated NO production (and lung injury) induced by paraquat; and 2) activation of neuronal NOS, like that of iNOS, may contribute to tissue injury.

Supported by NIH Grants HL 30450 & DK35385, and by VA research funds.

112.7

OXIDATIVE STRESS IN THE BRAIN FOLLOWING INTRAVENTRICULAR ADMINISTRATION OF ETHYLCHOLINE AZIRIDINIUM (AF64A). N.V. Gulyaeva*, N.A. Lazareva, M.L. Libe, O.S. Mitrokhina, M.V. Onufriev, M. Yu. Stepanichev, and T.J. Walsh[1]. Dept. of Functional Biochemistry of the Nervous System, Inst. of Higher Nervous Activity and Neurophysiology, Russian Acad. of Sci., Moscow, Russia, 117865, and [1] Dept. of Psycho-logy, Rutgers Univ., New Brunswick, NJ, USA, 08903.

AF64A is a toxic analog of choline that disrupts high affinity choline transport and produces a persistent presynaptic cholinergic hypofunction. The observed neuroprotectant effects of Vitamin E in the AF64A model suggested that oxidative stress contributed to the cholinotoxicity of AF64A. The studies presented here examined whether intraventricular injection of AF64A produces oxidative stress in the brain of male Wistar rats. Indices of oxidative stress including thiobarbituric acid reactive species (TBARS), free radical generation using hydrogen peroxide-induced, luminol-dependent chemiluminescence (CL), and superoxide scavenging/generating activity were measured in cerebral cortex, hippocampus and the rest of the brain, without cerebellum, 1, 3, or 5 days after bilateral intraventricular injection of 3 nmoles of AF64A or artificial CSF (sham surgery). The sham operation itself induced oxidative stress throughout the brain (increased TBARS, CL, and superoxide generation). In addition to the oxidative stress of the sham surgery AF64A increased basal TBARS on day 1 and Fe/ascorbate-induced TBARS on days 3 and 5 throughout the brain. AF64A produced compensatory "antioxidative" changes as well with increased superoxide scavenging activity observed on day 3 and decreased basal TBARS on day 5. AF64A-induced changes specific to the hippocampus included a decrease of CL and an increase of superoxide scavenging activity on day 5. The increased superoxide scavenging activity persisted up to 126 days. The results of the present study provide the first direct evidence that AF64A induces oxidative stress following intraventricular injection.

This work was supported by a McDonnell Foundation grant.

112.8

EFFECT OF LONG-TERM ANTICHOLINESTERASE EXPOSURE ON NEUROMUSCULAR FUNCTION IN VITRO.
R. Drake-Baumann*[1,2] and F.J. Seil[1,2,3]. Neurology Research, VA Medical Center [1] and Depts. of Neurology [2] and Cell Biology & Anatomy [3], Oregon Health Sciences University, Portland, OR 97201.

We have used organotypic dorsal root ganglion-spinal cord explants co-cultured with skeletal muscle to evaluate the effects of long-term exposure to pyridostigmine bromide (PB), an acetylcholinesterase inhibitor, on dorsal root ganglia neurons, cholinergic ventral horn motoneurons and neuromuscular function. No alterations in morphological or electrophysiological properties were observed in dorsal root ganglia neurons or ventral horn motoneurons with exposure to 10^{-6}M PB for periods of up to three weeks in vitro. Neuromuscular function was selectively affected by PB treatment. Short-term treatment (minutes) with PB increased spontaneous muscle contraction. This potentiation is consistent with the inhibition of acetylcholinesterase and the consequent greater availability of acetylcholine at the neuromuscular junction. This effect could be blocked by succinylcholine. Long-term treatment (weeks to days) with PB decreased the contractile activity of muscle fibers and their sensitivity to externally applied acetylcholine. Morphological studies by light microscopy showed an enlargement of the acetylcholinesterase-positive regions of the muscle fibers. Our findings suggest that long-term exposure to PB causes acetylcholine receptor desensitization and morphological alterations of the muscle end-plate. (Supported by the Portland Environmental Hazards Research Center and the Department of Veterans Affairs.)

112.9

EFFECT OF LIDOCAINE ON CYTOPLASMIC CALCIUM IN THE ND7 NEURONAL CELL LINE. M.E. Johnson*, C.B. Uhl. Anesthesiology Dept., Mayo Foundation and Mayo Clinic, Rochester, MN 55905.

Recent clinical reports and animal studies suggest that lidocaine has a neurotoxic effect at high concentrations. Toxicity is unlikely to involve Na^+ channel block, which would preserve mitochondrial energy stores and minimize elevation of cytoplasmic calcium (Ca^{2+}_{cyt}). Rather, earlier studies suggested that local anesthetics harm the ability of intracellular organelles to sequester Ca^{2+}. We therefore tested the effect of lidocaine on Ca^{2+}_{cyt} in the neuronal cell line, ND7, using ratiometric, digitized, video fluorescence microscopy with fura-2.

During the 60 min experimental exposure, controls containing tris(hydroxy-methyl)-aminomethane equimolar to the lidocaine concentrations tested caused no change in Ca^{2+}_{cyt}. Increasing concentrations of lidocaine caused an increasing height and duration of Ca^{2+}_{cyt} change (see Table). Lidocaine 5.0% killed 2 of 10 cells. No cell death was observed at lower lidocaine concentrations.

	Ca^{2+}_{cyt} as % initial baseline, ±SE, averaged over indicated time period. Lidocaine added at 0 min.				
Lidocaine	<0 min	0-5 min	5-10 min	10-30 min	30-60 min
0.1%(N=11)	100±1	95±2	92±2*	90±1*	107±1*
0.5%(N=10)	100±4	123±6*	101±7	92±4	115±3*
1.0%(N=5)	100±4	141±4*	104±4	96±2	111±2
2.5%(N=20)	100±4	153±7*	130±7*	199±4*	339±3*
5.0%(N=10)	100±4	126±6*	262±27*	412±21*	320±7*

*P<0.05 compared to pre-lidocaine baseline, by two way ANOVA followed by Tukey's test for differences between time periods.

Supported by Mayo Foundation and American Society of Regional Anesthesia Carl Koller Grant.

112.10

BEHAVIORAL AND BIOCHEMICAL EFFECTS OF DISULFIRAM IN MALE AND FEMALE RATS. M.R. Rahman[1]*, M.M. Faraday[2], N.E., Grunberg[1,2], and G.P. Mueller[1]. [1]Neuroscience Program, [2]Medical and Clinical Psychology, Uniformed Services University of the Health Sciences, Bethesda, MD 20814.

Disulfiram (Antabuse™) is an inhibitor of alcohol dehydrogenase, and is widely used as an adjunctive therapy in the treatment of human alcoholism. Behavioral and biochemical neurotoxic effects have been reported in humans and rats. Recently, we reported that in male rats disulfiram significantly impairs muscle strength, balance, and coordination; decreases body weight; and increases latencies to respond to a nociceptive stimulus. In addition, peptidylglycine ∝-hydroxylating monooxygenase (PHM), the enzyme that catalyzes ∝-amidation and bioactivation of peptides used in intercellular communication, and levels of the ∝-amidated peptide ∝-melanocyte stimulating hormone (∝-MSH) were affected. The present experiment included male (n=32) and female (n=32) Sprague-Dawley rats, additional behavioral measures, and longer-term administration of disulfiram. The behavioral and biochemical findings replicate our recent report. Further, the behavioral and biochemical effects of disulfiram were differentially expressed in males and females. Implications for humans treated with Antabuse™ will be discussed.

Funding source: USUHS, DoD

112.11

AXONAL NEUROTOXICITY INDUCED BY ANTIVIRAL DIDEOXY-NUCLEOSIDES IN SENSORY GANGLION CULTURES, E. B. George*, Department of Neurology, Johns Hopkins University; Baltimore, MD 21287-6953

A cell culture model for the neurotoxicity of antiviral dideoxynucleosides was established and used to determine if pharmacological interventions which interrupt calcium-mediated axonal degeneration are protective in this system. The dideoxy-nucleosides 2',3'-dideoxycytidine (ddC) and 2',3'-dideoxyinosine (ddI) are antiviral agents which display dose-limiting neurotoxicity when used to treat HIV infection. Pharmacologic agents which block entry of extra-axonal calcium or the subsequent activation of calpains can prevent or delay axonal degeneration produced in tissue culture by either axotomy or acrylamide toxicity. The current study investigates whether the antiviral dideoxynucleosides can induce axonal degeneration in this tissue culture system and whether manipulation of calcium is protective.

Murine dorsal root ganglion explants were allowed to extend neurites in culture for 10-14 days. Healthy cultures were then exposed to dideoxynucleoside either in the presence or absence of calcium antagonists. The effects on the established neurites were monitored and dose-toxicity curves generated for the dideoxy-nucleosides. The calcium-antagonist induced shift in these dose-toxicity curves was used to detect protection from axonal toxicity. The antiviral dideoxynucleosides produce a dose-related distal axonopathy in established cultures of sensory neurons with features similar to acrylamide neuropathy. Simultaneous treatment with calcium antagonists or calpain inhibitors can be used to delay the progression of the toxic axonopathy in this culture system. Calcium-mediated axonal degeneration appears to be a final common pathway for many types of axonopathies, and dideoxy-nucleoside effects on mitochondria may interfere with calcium handling in axons.

Supported by NIH 5 K08 NS01504

112.12

BIOSENSORS UTILIZING NEURAL CELLS AS SENSING ELEMENTS [1]J.J. Hickman*, [2]D.A. Stenger, [3]G.T.A. Kovacs. [1]SAIC, Biotechnology Research and Applications, McLean, Virginia 22102, [2]NRL, Washington, D.C., 20375, [3]Stanford University, Stanford, CA 94305

We are building a self-contained system to allow automatous operation of a biosensor utilizing a living neural cell as the sensing element. The neural cells act as transducers as well as sensing elements for toxin detection. The receptors on the cell act as the sensor which responds to toxins by stopping the electronic signal (action potentials) generated by the cell. The electrical signal (or lack thereof) is sensed by an Au microelectrode placed close to the neuronal-derived cell and then is amplified by the accompanying microelectronics. The cell line needs to be treated as an electronics component and conditions manipulated to achieve optional properties for various applications. The longevity, sensitivity, electrical activity and placement of the neural cells are critical parameters. We have manipulated the parameters by using different growth conditions, surface modifications and subclass isolation and then studied the resultant cultures by electrophysiology, morphology, and surface analysis. The conclusion is that we can use a neural cell as a sensor element for toxin detection. This system will be described in detail as well as the results generated with biological and chemical toxins.

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113.1

NEUROTRANSMITTERS OF THE VESTIBULAR COMMISSURAL PATHWAY IN THE CAT. F.A. Chen* and R.F. Spencer. Department of Anatomy, Medical College of Virginia, Richmond, Virginia 23298-0709.

The vestibular commissural pathway interconnects the bilateral vestibular nuclei and is thought to play a role in the vestibulo-ocular reflex, the enhancement of vestibular responses, and recovery of function from peripheral lesions of the VIIIth cranial nerve through excitatory and/or inhibitory projections. Previous pharmacological studies have suggested that the inhibitory neurotransmitters involved in vestibular commissural inhibition may be GABA and/or glycine. In the present study, injections of biocytin were made into the medial vestibular nucleus of adult cats to label anterogradely the commissural synaptic endings in the contralateral medial vestibular nucleus. A combined preembedding immunoperoxidase and postembedding immunogold technique was used to localize the neurotransmitters (glutamate, aspartate, glycine or GABA) associated with biocytin labelled vestibular commissural endings by electron microscopy. Terminals immunoreactive for the inhibitory neurotransmitters glycine and GABA established synaptic connections with postsynaptic profiles that were non- immunoreactive for these neurotransmitters. Consequently, the direct inhibitory commissural pathway mediated by these neurones is presumed to synapse on contralateral excitatory (Type I) neurones. Biocytin labelled glutamate immunoreactive endings establish synaptic contact with postsynaptic profiles that were either immunoreactive or non-immunoreactive for glutamate, both of which were contacted by non-glutamate immunoreactive synaptic endings. Very few biocytin labelled synaptic endings were immunoreactive for aspartate, even though postsynaptic processes and non-biocytin labelled terminals that were aspartate immunoreactive were present in the MVN. The results of this study are consistent with the notion that the vestibular commissural pathway consists of both excitatory and inhibitory components, and that GABA and glycine are inhibitory neurotransmitters involved in vestibular commissural inhibition.

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113.2

EXCITATORY AND INHIBITORY INPUTS FROM SACCULAR PRIMARY AFFERENTS TO CAT SINGLE VESTIBULAR NEURONS. Y. Uchino, H. Sato* and H. Suwa. Dept. of Physiol., Tokyo Med.College, Tokyo 160.

Hair cells of the saccular macula display different morphological polarities, but little is known about their projection pattern on to single vestibular neurons. We studied the neural organization between vestibular neurons and saccular afferents which originated from hair cells with different morphological polarity. All cats were anesthetized with halothane. At the last stage of surgery, the cats were decerebrated. Excitatory (E) and inhibitory (I) postsynaptic potentials (PSPs) were recorded in vestibular neurons after focal stimulation of saccula macula. Focal stimulation was performed by changing polarity of the stimulus current via a pair of electrodes, one of which was placed in the rostro-ventral and the other in the caudo-dorsal part of the saccular macula. We recorded either monosynaptic EPSPs ($\leq$1.2 ms) or disynaptic IPSPs ($\geq$1.5ms) in 22 out of 36 tested neurons by changing the polarity of the stimulus current. In the other neurons, the response pattern was always the same regardless of the polarity; EPSPs (12/36) or IPSPs (2/36). Afferent information from one population of hair cells activated vestibular neurons monosynaptically, while that from hair cells located on the opposite side of the striola projected to the vestibular neurons disynaptically via inhibitory interneurons. Half of the saccular-activated neurons received excitatory and/or inhibitory inputs from other vestibular nerve branches. The neural circuits from the saccular afferents to the vestibular neurons may be responsible for the increase in the sensitivity to vertical linear acceleration. The described circuit also seemed to have input noise-resistant characteristics.

113.3

Firing Behavior of Central Vestibular Neurons during Voluntary Head Movements. R.A. McCrea*, G.T. Gdowski, T. Belton, and R. Boyle[2] The Committee on Neurobiology, University of Chicago, Chicago, IL; [2]Dept .Otolaryn., Oregon HS Univ, Portland, OR

Single unit recordings were obtained from 60 secondary vestibular neurons in alert, squirrel monkeys whose head was free to move ±45° in the yaw plane. Secondary vestibular neurons were identified by the short latency (<1.3 ms) orthodromic activation following single shock electrical stimulation of the ipsilateral vestibular nerve. The monkeys were seated on a vestibular turntable that allowed whole body rotation of the monkey in the yaw plane. The vestibular sensitivity of a cell was assessed by passive whole body rotation with the turntable during temporary head restraint. Only units that responded to passive rotation in the plane of the horizontal semicircular canal were recorded. The firing behavior was then recorded during active, self generated saccadic and smooth pursuit head movements.

Different secondary vestibular neurons responded in different ways during active head movements. The responses of only a few "pure" vestibular neurons could be predicted from their responses during passive whole body rotation. They were insensitive to active head movements, but they continued to respond to passive whole body rotation during saccades. Neurons that exhibited pauses during ocular saccades, including position vestibular pause neurons, usually were inhibited during head saccades, regardless of the direction of the saccade. In most cases, the duration of the inhibition was best correlated with the gaze velocity of the saccade, and cells resumed firing before the head movement component of the saccade was completed. Vestibular neurons that burst during ocular saccades showed a variety of responses during gaze saccades; a common response was a burst discharge related to the ocular saccade combined with weak inhibition or excitation related to the head component of the saccade that was opposite in direction to the preferred vestibular on direction. Passive perturbations of the head during saccades were usually capable of evoking responses in the vestibular on direction.

Although most secondary vestibular neurons are capable of responding to passive head perturbations during active head movements, the vast majority do not code the head velocity related to self generated head movements. The implication is that vestibular reafference may not play an important role in coordinating eye and head movements during voluntary gaze shifts.

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113.4

Influence of Neck Rotation on the Firing Behavior of Secondary Vestibular Neurons in the Alert Squirrel Monkey. G.T. Gdowski*, R.A. McCrea, T. Belton, and R. Boyle[2] The Committee on Neurobiology, University of Chicago, Chicago, IL 60637, [2]Dept. Otolaryngol. & Physiol., Oregon Health Sci. Univ., Portland OR 97201.

The influence of neck rotation on secondary vestibular neurons is behaviorally dependent on whether the head movement is in pursuit of a target or reflexive to stabilize the head during passive whole body rotations (vestibulocollic reflex, VCR). The neck sensitivity (H_N) of secondary vestibular neurons was measured as the ratio of sinusoidal fits applied to desaccaded records of unit rate and neck velocity during passive body rotations while the head was held stable in space (2Hz, 20°/s). Spontaneous records of gaze and head position were also obtained to determine the unit sensitivity to static eye and head position. Most units, including position vestibular pause units, had *weak* neck sensitivities (H_N<0.3) which were difficult to assess during spontaneous movements. In most cases, the unit response was in phase with neck velocity and in the opposite direction of the units vestibular sensitivity.

Target pursuit produces neck and vestibular inputs having *identical* directions. A similar head and neck movement was obtained by passively rotating the head while the body was held stable in space. Unit responses to this stimulus could be predicted using an antagonistic combination of neck and vestibular sensitivities.

The VCR evokes head movements in the *opposite* direction of the body rotation. VCR-related head movements were evaluated by comparing responses to passive whole body rotations (2Hz, 20°/s) while the head was fixed and while the head was free to move (±45° in yaw). Most secondary vestibular neurons exhibited little change in their response in the presence and absense of VCR compensatory head movements. This lack of change appears to be due to an a synergistic combination of neck and vestibular sensitivities.

These results suggest that while unit responses during reflexive or passive head and neck rotations can be predicted with vestibular and neck sensitivities, they are insufficient to predict the unit responses during voluntary head movements.

Work supported by NIH (DC02072 and EY08-041).

113.5

ACTIVITY OF MEDIAL VESTIBULOSPINAL (MVST) NEURONS DURING APPLIED AND ACTIVE HEAD MOVEMENTS IN THE SQUIRREL MONKEY. R. Boyle[1]*, R.A. McCrea[2], T. Belton[2], and G. Gdowski[2]. [1]Depts. Otolaryngol. & Physiol., Oregon Health Sci. Univ., Portland, OR 97201; [2]Dept. Pharmacol. & Physiol. Sci., Univ. Chicago, Chicago, IL 60637.

Secondary MVST neurons were identified using electrical stimulation techniques in the alert squirrel monkey prepared for chronic single-unit, eye and head movement recordings. Animals were free to make active head on shoulder movements of ±45° in yaw and ±10° in pitch; the head could also be coupled to the chair to deliver passive head movements in space. Cell discharge was examined during combined eye and head saccades up to 400°/s, applied whole body rotation with or without head restraint, applied rotation of head on shoulder, and applied neck rotation with the head held stationary in space. The latter tests were done to assess a cell's neck afferent input. Our main findings are that 1) MVST cells carry signals related to the passive, but not active, component of a head movement during gaze saccades; and suggests that they continue to receive vestibular inputs during head saccades, but that the inputs related to the head saccades are cancelled. Consequently, MVST cells do not encode head velocity in space during self-generated head movements. 2) MVST cells do not code neck position and receive weak neck afferent inputs during passive neck rotation; and suggests that either an efference copy mechanism or gated reafferent neck inputs cancel the 8th nerve input during active head movements. (Supported by 1P60 HD02072)

113.6

EFFECT OF AGE ON THE VESTIBULO-OCULAR REFLEX (VOR) AND VISUAL-VESTIBULAR INTERACTIONS DURING NATURAL MOVEMENTS. B. T. Crane*, E. S. Viirre, and J. L. Demer. Jules Stein Eye Institute and Dep't. of Neurology, Univ. of California, Los Angeles, CA 90095-7002.

The effect of aging on gaze stabilization by the VOR is not well characterized for head movements encountered during natural activities. We studied the VOR and visually-enhanced VOR (VVOR) in 9 young (29 ± 9 yrs, mean ± SD) and 11 elderly (78 ± 4 years) subjects during standing, walking in place (2 steps per second), and active sinusoidal head motion at 0.8 Hz in pitch and yaw. Gains were recorded separately for the pitch and yaw axes using magnetic search coils. Recordings were made with a visible target 6 - 10 m distant both with and without 2X binocular telescopic spectacles, and were repeated in darkness with remembered targets.

Measurable VOR and VVOR responses were present during all activities studied. For both subject groups, gains were <1.0 for normal vision and <2.0 for magnified vision, and were generally lower in darkness than in light. While VVOR gains during unmagnified vision were identical for the elderly and young during active head rotations and during natural activities with unmagnified vision, the elderly tended toward lower VOR and VVOR gains under all other conditions. Averaged across natural activities, VOR gains for the young were 0.93±0.02 (mean ± SEM) and 0.84±0.02 in pitch and yaw, respectively. For the elderly, corresponding gains were 0.91±0.02 and 0.72±0.03. Pitch gains were higher than yaw gains for both groups during natural activities ($P < 0.001$). Age-related gain differences were greater in yaw, during trials done in the dark or with magnification, and during walking. Gains measured during active head rotation were higher than during natural activities in both age groups for both pitch and yaw ($P < 0.001$).

While the VVOR performance of the elderly does not significantly differ from the young in the presence of normal vision, the elderly have lower VOR gain and reduced augmentation by magnified vision during natural activities. These findings imply that either the elderly have a VOR deficit during natural activities, or that differences in their patterns of head rotation and translation may require lower angular VOR gains.

Supported by MSTP, and EY-08656

113.7

ROTATIONAL AND TRANSLATIONAL HEAD STABILITY DURING NATURAL MOVEMENTS IN YOUNG AND ELDERLY SUBJECTS.

J. L. Demer*, B. T. Crane, and E. S. Viirre, Jules Stein Eye Institute and Department of Neurology, University. of California, Los Angeles, CA 90095-7002.

Natural activities such as ambulation produce head translations and rotations that must be compensated to stabilize gaze. We studied rotational motion using head-mounted magnetic search coils, and translational motion using a head-mounted flux gate magnetometer sensor, in 9 young (29 ± 9 yrs, mean ± SD) and 11 elderly (78 ± 4 years) subjects during quite standing, and walking in place (2 steps per second). Young subjects were also studied during running in place (3 steps per second). Recordings were made with a visible target 6 - 10 m distant both with and without 2X binocular telescopic spectacles, and were repeated in darkness with remembered targets. Rotational data were digitally sampled at 400 Hz and analyzed in the position and velocity domains, while translational data were sampled at 14 or 109 Hz and analyzed in the position domain by matrix transformation to obtain translation of the right eye.

Neither the presence of a visible target nor of spectacle magnification significantly influenced any aspect of head motion when data were averaged across young or elderly subjects. Root-mean-square (RMS, 0-20 Hz) head velocity was 15-20% greater in the elderly than the young subjects for both pitch and yaw during both standing and walking in place. Peak mediolateral (ML) displacement was similar in both groups during standing, but was about 50% greater in the elderly during walking ($P < 0.05$). Peak dorsoventral (DV) displacement was about twice as great in the elderly as the young during both standing and walking ($P < 0.05$). In both subject groups during walking and running, upward translation was tightly phase-locked to downward pitch rotation, and rightward translation was tightly phase-locked to leftward yaw.

Geometric analysis indicates that head translation and rotation during ambulation are phase-locked so as to have offsetting effects on gaze. This reduces the angular VOR gain required for gaze stabilization in a manner depending on target distance. During natural activities the greater head translation of elderly as compared with young subjects would thus enable appropriate gaze stabilization at lower VOR gains.

Supported by USPHS grants EY-08656 and MSTP

113.8

THREE-DIMENSIONAL ORIENTATIONS OF ANGULAR EYE ROTATION AXES DURING VERTICAL VESTIBULAR NYSTAGMUS IN PATIENTS WITH CEREBELLAR ATROPHY.

D. Straumann*, D.S. Zee, D. Solomon. Neurology Dept., Johns Hopkins Hospital, Baltimore, MD 21287, USA.

In a previous study we demonstrated that angular rotation axes of the eyes during upward drift in patients with cerebellar atrophy are close to eye-fixed, i.e. the angular axis rotates by the same amount and in the same direction as the line-of-sight, when the patient is looking right or left. We asked whether in these patients we could find a similar pattern during upward slow phases of vestibular nystagmus. It is known that in normal subjects the angular rotation axis during vestibular nystagmus is close to head-fixed, i.e. only tilts by about ¼ in the direction of the line-of-sight (Misslisch et al. 1994). In 3 cerebellar patients with downbeat nystagmus and 2 control subjects, we recorded eye-in-space and head-in-space movements during vertical head oscillations in complete darkness. Eye-in-head angular eye rotation axes were computed off-line, and their orientations in the head-fixed horizontal-torsional plane were plotted as a function of horizontal eye position. The slopes in the patients were 0.48, 0.47, and 0.36 during upward vestibular slow-phases, thus steeper than in normals (controls: 0.12 and -0.09), but flatter than during the patients' downbeat nystagmus, with slopes of upward drift being 1.31, 1.19, and 1.11 (slope = 0: head-fixed, slope = 1: eye-fixed axis). One possible explanation of our data is that eye position feedback, used to compensate for the non-commutative property of three-dimensional eye movements (Tweed and Vilis 1987), is controlled by the cerebellum. In the case of cerebellar atrophy with a deficit in the upward direction, this hypothesis predicts eye-fixed angular rotation axes, when angular velocity signals comply with Listing's law (e.g. pursuit signals), and a tilt of angular rotation axes by ½ in the direction of the line-of-sight, when angular velocity signals are head-fixed (e.g. vestibular signals).

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113.9

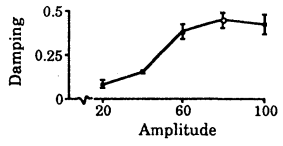
NONLINEAR DYNAMICS OF THE HEAD-NECK SYSTEM.

J. Goldberg* and Steve Lutes. OTO Dept, Baylor Col. of Medicine, Houston, TX 77030.

Reflexes controlling head stabilization are nonlinear at small movement amplitudes. The input/output properties of the human head-neck system have not been studied at stimulus amplitudes small enough to reveal such nonlinearities.

Seven healthy subjects were rotated about vertical axis in a servo-controlled chair, at frequencies and acceleration amplitudes observed during natural standing and walking. Sum-of-sines rotation waveforms collectively spanned a 0.2-10 Hz frequency range and 10 to 200 deg/sec^2 root-mean-square acceleration range. Head and trunk rotations in space were recorded with Watson velocity sensors while subjects performed mental arithmetic.

Head rotation exhibited resonance which was most conspicuous at the lowest stimulus amplitudes and least conspicuous at the highest. In contrast, resonant frequency did not change with amplitude, staying near 4 Hz. This implies that system damping is a function of amplitude, as the figure illustrates for one subject. The simplest model consistent with these data is a threshold nonlinearity in the vestibular rate feedback.



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113.10

RESPONSES OF SECOND-ORDER AND EFFERENT VESTIBULAR NEURONS TO FORELIMB SKIN AND MUSCLE STIMULATION IN THE DECEREBRATE DECEREBELLATE GUINEA PIG.

V. Marlinsky*, Dept. Physiol., Freie Universität Berlin, Arnimallee 22, 14195 Berlin, FRG.

The extracellularly recorded activity of medial vestibular nucleus neurons and efferent vestibular neurons was analysed in the decerebrate decerebellate guinea pig. Neurons were identified by means of electrical stimulation of the anterior semicircular canal. Thirty-six neurons were monosynaptically activated during semicircular canal stimulation and were regarded as second-order vestibular neurons. Thirty neurons were antidromically activated and therefore identified as efferent vestibular neurons. Both types of neurons investigated had spontaneous impulse activity and responded to sinusoidal roll tilt. All second-order vestibular neurons were excited during ipsilateral and inhibited by contralateral tilt. Eighteen efferent vestibular neurons also showed this pattern, while the remaining twelve were excited by contra-lateral and inhibited by ipsilateral tilt. Stimulation of high-threshold skin afferents evoked by the stance pressure of the plantar surface and stimulation of flexor muscle afferents evoked by passive extention at the elbow of forelimbs led to an increase in the impulse rate in 30% and to a decrease in the impulse rate in 20% of second-order vestibular neurons, while 50% of these neurons did not show any changes in activity. The majority of efferent vestibular neurons (67%) responded to the same stimulation with the increase in the impulse rate, the rest of these neurons (33%) did not. Neurons of both groups responded neither to stimulation of low-threshold skin afferents evoked by the plantar contact with the support surface nor to stimulation of extensor muscle afferents evoked by passive forelimb flexion.

VISUAL SYSTEM: DEVELOPMENT I

114.1

ACTIVITY-DEPENDENT REGULATION OF QUANTAL AMPLITUDE IN VISUAL CORTICAL CULTURES.

G.G. Turrigiano, K. Leslie, and S.B. Nelson*, Dept. of Biology and Center for Complex Systems, Brandeis University, Waltham, MA 02254.

The strength and number of synaptic inputs onto neurons can change dramatically during development or learning, thus altering the total amount of excitation received. How do neurons adjust their responsiveness to avoid firing rates that are too high or too low? One possibility is that ongoing activity can globally up or down regulate the strength of excitatory synaptic connections. Here we test this idea.

Whole-cell voltage clamp recordings were obtained from pyramidal neurons from cultures of P5-6 rat visual cortex after 7-9 DIV, in the presence of TTX and bicuculine. Miniature excitatory synaptic currents were recorded that could be blocked by the AMPA antagonist CNQX. Recordings were made from control cultures grown in normal medium or sister cultures treated for 48 hrs with TTX to block all spikes. In each of 5 experiments the quantal amplitudes from TTX treated cultures were larger than control cultures (32.8±1.6 and 16.5±1.1 pA, respectively; significantly different, $p<0.01$, Student's t test), whereas rise times and other kinetics were not different. Cumulative amplitude histograms from the two populations showed that the distribution was shifted to the right for the TTX-treated population (TTX different from control, Kolmagorov-Smirnof test, $p<0.001$). No differences in resting potential, series resistance, or whole cell capacitance were found between the two populations. These data indicate that blocking activity nearly doubles the quantal amplitude of AMPA-mediated synaptic transmission. Supported by NSF and the Whitehall Foundation (GGT), and NSF and the Sloan Foundation (SN).

114.2

ACTIVITY-DEPENDENT REGULATION OF NMDAR1 IN THE DEVELOPING VISUAL CORTEX

S. M. Catalano*, C. K. Chang and C. J. Shatz, HHMI and Dept. of Molecular and Cell Biology, University of California, Berkeley, 94720

NMDA receptors are known to make a significant contribution to the synaptic currents and activity of neurons in the mammalian visual cortex during development. We have examined the distribution of the NMDAR1 protein in cat visual cortex using a monoclonal antibody against the extracellular loop domain between transmembrane regions 3 and 4. This subunit is known to be required for channel function. Our previous results indicate that levels of immunostaining are relatively high in layers 2/3 throughout development and into adulthood. In contrast, immunostaining increases in layer 4 during development, reaching peak levels near the end of ocular dominance column formation (six weeks). Intensity of immunostaining then gradually declines in layer 4, reaching adult levels by about 15 weeks. Dark-rearing beginning at birth apparently does not affect the timecourse of this developmental decrease in layer 4 immunostaining. However, following two weeks of monocular tetrodotoxin (TTX) injections beginning at the sixth postnatal week, R1 staining is less intense in activity-blocked eye columns than in untreated eye columns. We conclude that 1) high levels of R1 are present in layer 4 during a time when anatomical rearrangements of axonal inputs can be rapidly induced by manipulation of neural activity, 2) levels of R1 gradually decline thereafter, reaching adult levels at the timepoint when anatomical rearrangements of geniculocortical axons can no longer be induced in layer 4 by manipulations of activity, 3) levels of immunostaining remain high in layers 2/3, where activity-dependent anatomical rearrangements are thought to persist into adulthood, 4) dark-rearing lowers, and TTX eliminates, incoming axonal activity. Results from these experiments indicate that while visual experience may not be responsible for the normal decline of R1 in layer 4, absolute levels of activity can affect R1 levels. Supported by grants from the NEI to CJS (EY02858) and SMC (EY06491).

114.3

CYCLIC AMP AS A MESSENGER OF METABOTROPIC GLUTAMATE RECEPTORS IN VISUAL PLASTICITY. S.N.M. Reid*, N.W. Daw, D.S. Gregory and H.J. Flavin. Dept. of Ophthalmology, Yale Univ. Sch. of Med., New Haven, CT 06510.

Deprivation of visual input from one eye can modify eye-specific connections in the visual cortex during a critical period. We have investigated the increase in cAMP produced by metabotropic glutamate receptors (mGluR) in cat visual cortex. The cAMP increase produced by the general mGluR agonist, trans-(1S,3R)-1-amino-1,3-cyclopentanedicarboxylic acid (ACPD), followed the critical period closely. When normal and dark-reared animals were compared, the increase was smaller in dark-reared animals at 5 weeks of age, similar at 8 weeks of age, and larger at 15 weeks of age, which is the same as the dark-rearing effect on ocular dominance plasticity (Mower, Dev. Brain Res. 58:151, 1991). The increase produced by a mixture of group I, II and III agonists is greater than the sum of cAMP levels increased by singly applied agonists, suggesting that there is an interaction between the G proteins and/or second messengers. A decrease in forskolin-elevated cAMP was seen by group II and III agonists, but these agonists also increase basal level of cAMP. The basal cAMP level also followed the critical period. That ACPD-elevated cAMP levels follow the critical period more closely than the basal levels can be accounted for by the fact that the number of mGluRs falls steadily between birth and adulthood (Reid et al., J. Comp. Neurol. 355:470, 1995). Since the major factor in the fit between ACPD-elevated cAMP and the critical period is basal levels, and the mGluR number is a minor factor, we suggest that the level of second messenger may be more important than the level of receptors in ocular dominance plasticity. Supported by RO1 EY 00053.

114.4

THE mGluR ANTAGONIST MCPG DOES NOT AFFECT GLUTAMATE-STIMULATED PHOSPHOINOSITIDE TURNOVER, LTP OR LTD IN DEVELOPING RAT VISUAL CORTEX K.M. Huber*, N. B. Sawtell and M.F. Bear Dept. Neuroscience, HHMI, Brown University, Providence, RI 02912.

Metabotropic glutamate receptors (mGluRs) coupled to phosphoinositide (PI) hydrolysis have been suggested to play a role in synaptic plasticity in the visual cortex. MCPG ((+)-α-Methyl-4-carboxyphenylglycine) reportedly can block many actions of the mGluR agonist ACPD (1-aminocyclopentane-(1S,3R)-dicarboxylic acid). Therefore, we investigated the effect of MCPG on long-term potentiation (LTP) and long-term depression (LTD) of field EPSPs (fEPSPs) in layer II-III elicited by stimulation of layer IV (L4) in slices prepared from mature (P35-50) rats and white matter (WM) of young (P14-28) rats. MCPG (0.25 mM) antagonized synaptic depression produced by ACPD (10 μM). However, LTP of fEPSP amplitudes was reliably induced by theta burst stimulation of L4 or WM in the presence of MCPG (0.25-1 mM; 118 ± 4 % of baseline values; n = 9 and 123 ± 8 %; n = 8, respectively) and was not different from controls (L4 117 ± 2 %, n = 10; WM 116 ± 3 %, n = 8). Low frequency stimulation (900 pulses at 1 Hz) of L4 or WM in the presence of MCPG (0.25 mM) induced LTD of fEPSP amplitudes (77 ± 3 %; n = 6 and 81 ± 8 %; n = 4 respectively) similar to control values (L4 78 ± 3 %, n = 8; WM 87 ± 4 %, n = 5). The significance of these results rests on the assumption that MCPG blocks glutamate-stimulated PI turnover in visual cortex. Therefore, we used a synaptoneurosome preparation of visual cortex to assess the effectiveness of MCPG in blocking mGluR-mediated PI turnover. We confirmed that half-maximal stimulation of PI turnover by ACPD (30 μM) was almost entirely blocked (16 ± 6 % of control) by MCPG (1 mM). Surprisingly, however, half-maximal stimulation of PI turnover by the endogenous ligand glutamate (200 μM) was still 82 ± 6 % (n = 3) of control in the presence of 1 mM MCPG. Substantial blockade of glutamate-stimulated PI turnover required concentrations of MCPG in excess of 5 mM. These results suggest that glutamate may stimulate MCPG-insensitive mGluRs which are not activated by ACPD or, as proposed by previous work (Brabet et al., Neuropharm., 1995), glutamate and ACPD stimulation of mGluR1 and mGluR5 are differentially affected by MCPG. We conclude that MCPG is not a suitable compound to test the role of mGluRs in glutamate-mediated synaptic plasticity. Support: HHMI

114.5

MONOCLONAL ANTIBODY CAT-307 RECOGNIZES PLC-β1 AND IDENTIFIES AN UNUSUAL ORGANELLE IN NEURONS OF THE DEVELOPING CAT CORTEX. P.C. Kind* and S. Hockfield, Sect. of Neurobiol., Yale Univ. Sch. Med., New Haven, CT., 06510

We previously reported that the Cat-307 monoclonal antibody recognizes a 165 kD protein that localizes to small, punctate "dots" in the cat visual cortex at 5-weeks of age, but not at 15-weeks of age. Immunoelectron microscopic analysis with Cat-307 identified a little described organelle, Spot, that has not been previously demonstrated in cortical neurons. The Spot organelle is situated between the endoplasmic reticulum and the Golgi apparatus. Biochemical analysis demonstrated that the Cat-307 protein associates with microsomal membranes, suggesting a role for the Cat-307 antigen in protein transport in developing neurons.

Purification and amino acid sequence analysis of the Cat-307 protein identify it as phospholipase C-β1 (PLC- β1). Amino acid sequence was obtained from two peptides, 8 and 17 amino acids in length. Both showed 100% amino acid identity with rat and bovine PLC-β1 sequences. On Western blots both Cat-307 and antibodies to PLC-β1 recognize a doublet with a major species at 165 kD and a minor species at 160 kD. Cross-immunoprecipitation using Cat-307 and antibodies to PLC-β1 confirm that the Cat-307 protein is PLC-β1. Finally, as we have shown for the Cat-307 protein, PLC-β1 associates with a microsomal membrane fraction.

PLC-β1 cleaves phosphatidyl inositol 4,5 biphosphate (PIP2) into two second messengers, inositol 1,4,5 triphosphate (IP3) and diacylglycerol (DAG). IP3 turnover has been linked to activation of the Q2 (or metabotropic) glutamate receptor and shows an increase in activity at the peak of the critical period in an experience-dependent manner (Dudek and Bear, Science, 246:673). Consistent with these findings, dark-rearing from birth delays the age-dependent reduction in Cat-307 immunoreactivity in primary visual cortex, but not in primary auditory or somatosensory cortices. Our data suggest a specialized locus of IP3 turnover in kitten, but not in adult, cortical neurons that may participate in protein transport. Supported by EY06511(SH) and EY06606(PK).

114.6

OCULAR DOMINANCE PLASTICITY IN MICE LACKING αCaMKII OR PKCγ. J.A. Gordon*[1], T.K. Hensch[1], D. Cioffi[2], C. Chen[3], A.J.Silva[2], S. Tonegawa[3], and M.P. Stryker[1]. [1]Keck Center for Integrative Neuroscience, Univ. of California, San Francisco, CA 94143; [2]Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724; & [3]MIT, Cambridge, MA 02139.

The recent characterization of plasticity in the mouse visual cortex permits the use of mutant mice to investigate the cellular mechanisms underlying activity-dependent development. As calcium-dependent signaling pathways have been implicated in plasticity, we examined plasticity in the visual cortex of mice with mutations in the genes encoding the α-isoform of calcium/calmodulin-dependent protein kinase II (αCaMKII) and the γ-isoform of Protein Kinase C (PKCγ).

In wildtype mice, brief occlusion of vision in one eye during a critical period reduces responses to that eye in the visual cortex. In half of the αCaMKII-deficient mice, visual cortical responses developed normally, but visual cortical plasticity was greatly diminished. Even after intensive training, spatial learning in the Morris water maze remained severely impaired in a similar fraction of mutant animals. These data indicate that loss of αCaMKII results in a severe but variable defect in neuronal plasticity.

Visual cortical plasticity was examined both *in vivo* and *in vitro* in PKCγ-deficient mice. Visual cortical responses developed normally in these animals, and ocular dominance plasticity in response to monocular deprivation remained intact. In visual cortical slices taken from these animals, LTP and LTD were also intact. These data indicate that PKCγ is not required for visual cortical plasticity. Since the refinement of climbing fiber innervation onto Purkinje cells is profoundly disrupted in these mice, the activity dependent organization of connections must occur via different molecular substrates in neocortical and cerebellar development. *Supported by HFSP, ARCS, & HHMI.*

114.7

NT-4/5 INFUSION ALTERS PLASTICITY AND RESPONSES OF CAT PRIMARY VISUAL CORTEX DURING THE CRITICAL PERIOD. D.C. Gillespie*, M.C. Crair and M.P. Stryker. Program in Neuroscience, Keck Center for Integrative Neuroscience, Dept. of Physiology, Univ. Calif., San Francisco, CA 94143-0444.

Neurotrophins have been implicated as signalling molecules in primary visual cortical development. To test for a role of NT-4/5 in physiological ocular dominance plasticity we infused NT-4/5 (0.2 mg/ml) into one hemisphere and vehicle control solution into the other hemisphere of four-week-old cats. After 2 days, monocular lid suture deprived the animal of pattern vision through one eye, a procedure that normally causes cortical cells to lose responsiveness to stimuli presented to the deprived eye. After 2 more days, orientation and ocular dominance maps were computed from intrinsic optical signals in response to blank and grating stimuli, and extracellular single-unit recordings were made near to and far from the infusion sites.

Optical images revealed a pattern dominated by the non-deprived eye in control areas, while signal near the infusion site was similar for the two eyes. Likewise, orientation columns were evident in control areas but absent close to the infusion site. Single units encountered within this area were largely binocularly driven, poorly selective or broadly tuned for stimulus orientation, and generally poorly responsive to visual stimuli. Cells at an intermediate distance from the infusion site were more responsive yet were still binocular and poorly tuned for stimulus orientation.

These results are consistent with a specific signalling role for NT-4/5 in normal development and plasticity, but they are also consistent with a less specific action such as induction of promiscuous sprouting in cortex.

Supported by NIH EY02874 (MPS), an NEI training grant (DCG) and an NIH postdoctoral grant (MCC).

114.8

AN ENDOGENOUS LIGAND OF TRKB IS REQUIRED FOR OCULAR DOMINANCE SEGREGATION. R. J. Cabelli*@ and C. J. Shatz#. @USC School of Medicine, Los Angeles, CA 90033 and #University of California, Berkeley, CA 94720.

The neurotrophins are a family of neurotrophic factors (incl. NGF, BDNF, NT-3, and NT-4/5) that regulate cell survival and other aspects of neuronal differentiation and function through interaction with their specific cellular receptors, trkA, trkB, and trkC, members of the trk family of receptor tyrosine kinases. We have been examining the hypothesis that neurotrophins can act as diffusible signals between pre- and post-synaptic nerve terminals to direct the modulation of synaptic efficacy and stability during development. The segregation of LGN axon terminals into ocular dominance (OD) columns in layer 4 of visual cortex is a particularly noted example of the activity and competition-based refinement of synaptic connections during development. We have shown recently that intracortical infusion of NT-4/5 or BDNF, but not NGF or NT-3, during the critical period for OD segregation inhibits the formation of eye-specific patches (Cabelli et al., 1995). This result would be predicted if OD segregation involved competition at the geniculocortical synapse between right and left eye-driven axons for limiting amounts of NT-4/5 and/or BDNF (both of which are ligands of trkB). However, this inhibition of OD segregation could instead be a pharmacological effect of infusing large amounts of these agents. To distinguish between these interpretations and determine whether OD segregation requires endogenous neurotrophins, we have infused neurotrophin antagonists into visual cortex during the critical period. These antagonists, constructed from the extracellular domain of each member of the trk family linked to the Fc tail of a human IgG, compete with native trk receptors for binding neurotrophins. Infusion of excess trkB-IgG protein, but not trkA-IgG or trkC-IgG, inhibits the formation of OD patches (assessed by ipsilateral intraocular injection of an anterograde transneuronal tracer) within 1-2 mm of the infusion site. Moreover, the density of transneuronal label is reduced in the affected region, in contrast to the robust increase in label density observed earlier with NT-4/5 infusion, consistent with NT-4/5 and/or BDNF being required for growth of terminal arbors. Together these two studies strongly support the hypothesis that OD segregation involves competition between LGN axons for an endogenous ligand of trkB, presented in limiting amounts within visual cortex. Supported by EY02858 (CJS).

114.9

ENDOGENOUS TrkB LIGANDS REGULATE DENDRITIC GROWTH IN DEVELOPING VISUAL CORTEX A.K. McAllister*, D.C. Lo, and L.C. Katz
Department of Neurobiology, Duke University Medical Center, Durham, NC, 27710.

Despite evidence that exogenous neurotrophins strongly influence structural plasticity in developing visual cortex, whether endogenous neurotrophins have similar functional roles is unclear. We have previously shown that exogenous TrkB ligands, especially BDNF, cause extensive growth of cortical dendrites in slices of developing visual cortex, implying that endogenous neurotrophins may be involved in regulating dendritic growth. To test this idea directly, we blocked endogenous TrkB ligands with receptor-bodies--chimeric proteins composed of Trk receptor ligand-binding domains fused to the human F_C IgG fragment--that bind BDNF and NT-4 with high specificity and affinity (Regeneron, unpublished data). Blocking endogenous TrkB ligands (20μg TrkB-IgG) for 36 hours in P14 slices caused a 50% decrease in complexity of basal dendrites of layer 4 pyramidal neurons (n=50 cells) due to decreases in length and number of basal dendrites and branches. This effect was dose-dependent: 10 μg TrkB-IgG caused no decrease compared to untreated cells. TrkB-IgG treatment had negligible effects on apical dendrites (<10% decrease). To control for potential non-specific effects of receptor-bodies, we treated similar cortical slices with 20 μg TrkA-IgG. Blocking endogenous TrkA ligands caused no decrease in dendritic complexity (n=40 cells). This is a direct demonstration of the presence of endogenous, bioactive neurotrophins in neocortex and supports a role for TrkB ligands in regulating dendritic growth of pyramidal neurons. Supported by NIH EYO7960 (L.C.K.), the Klingenstein Fund, the Alfred P. Sloan Foundation, NS 32742 (D.C.L.), and by a Broad Biomedical Research Foundation Fellowship (A.K.M.). Neurotrophins and receptor-bodies were generously provided by George Yancopoulos, M.D., Ph.D., Regeneron Pharmaceuticals.

114.10

ORIENTATION TUNING OF SINGLE CELLS AT "PINWHEEL" ORIENTATION SINGULARITIES IN KITTEN VISUAL CORTEX. E.S. Ruthazer*, M.C. Crair, D.C. Gillespie and M.P. Stryker. Keck Center, Dept. of Physiology, Univ. of California, San Francisco, CA 94143-0444

We studied the receptive field properties of primary visual cortical neurons in critical period kittens using a combination of optical imaging of intrinsic signals and extracellular single unit recording. At "pinwheel centers" of the orientation map, representations of the full range of orientation abut one another (Bonhoeffer and Grinvald, 1991). Optical imaging shows no or poor orientation tuning at these singularities, but it has not been clear whether this results from poorly tuned cells or the averaging of signals from well tuned cells of very different preferred orientations. In addition, both models of the development of orientation and ocular dominance columns (Miller, 1994) and recent findings localizing deprived-eye responses to pinwheel centers in monocularly deprived kittens (Crair et.al., in preparation) suggest that cells at pinwheel centers are broadly tuned, at least early in development.

Electrode penetrations targeted for pinwheel centers were more likely to encounter clusters of poorly tuned cells than were control penetrations. The localization of penetrations to pinwheel centers was confirmed by the abrupt change in preferred orientation of units immediately before and after a cluster of poorly tuned cells. For all kittens (n=5) in which pinwheels were targeted with multiple electrode penetrations, clusters of a similar nature were found. The regions of poor orientation tuning must be very small, since often multiple electrode penetrations guided by the optical map were necessary to find them. Results suggest that development of orientation selectivity in kitten visual cortex is delayed in regions of singularity in the orientation map.

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114.11

ANATOMICAL DEMONSTRATION OF OCULAR DOMINANCE COLUMNS IN STRIATE CORTEX OF THE SQUIRREL MONKEY. J.C. Horton*, D.R. Hocking. Dept. of Ophthalmology, UCSF, San Francisco, CA 94143-0730.

The squirrel monkey is the only primate reported to lack ocular dominance columns. Nothing anomalous about the visual capacity of squirrel monkeys has been found to explain their missing columns, leading to the suggestion that ocular dominance columns might be "an epiphenomenon, not serving any purpose" (Livingstone et al, 1995). Puzzled by the apparent lack of ocular dominance columns in squirrel monkeys, we made eye injections with transneuronal tracers in 4 normal squirrel monkeys. An irregular mosaic of columns, averaging 225 μm in width, was found throughout striate cortex. They were double-labelled by placing wheat germ agglutinin-horseradish peroxidase (WGA-HRP) into the left eye and [^{3}H]proline into the right eye. The tracers labelled opposite sets of interdigitating columns, proving they represent ocular dominance columns. The columns were much clearer in layer IVcα (magno-receiving) than IVcβ (parvo-receiving). The parvo laminae of the lateral geniculate showed extensive mixing of ocular inputs, suggesting that increased label spillover accounted for the blurred columns in cortical layer IVcβ. In the squirrel monkey the cytochrome oxidase (CO) patches were organized into distinct rows, but they bore no consistent relationship to the ocular dominance columns. These experiments indicate that striate cortex in squirrel monkeys differs in some respects from other primates, but it does contain ocular dominance columns. This fact is pertinent to a recent study reporting that ocular dominance columns are absent in normal squirrel monkeys, but induced to form by strabismus (Livingstone, 1996). Supported by NEI

DEGENERATIVE DISEASE: ALZHEIMER'S-BETA-AMYLOID—PROCESSING II

115.1

EFFECTS OF FAD-LINKED APP MUTATIONS ON THE SECRETION OF SOLUBLE FULL LENGTH APP. S. Efthimiopoulos*, S. Punj, A. Wu and N.K. Robakis. Dept. Psych. And Fishberg Res. Cntr for Neurobiol., Mount Sinai Schl. of Med. New York, N.Y. 10029.

The Alzheimer's amyloid precursor family of proteins (APP) are considered type I transmembrane glycoproteins. Cleavage of APP by secretases close to the junction of the extracytoplasmic and transmembrane sequence results in the secretion of APP (APPs) that does not contain the cytoplasmic domain. However, we identified a soluble APP species of about 130 kDa termed solAPPcyt, containing all of the cytoplasmic and Aβ sequence of APP. This observation suggested that in addition to the transmembrane topology, full length APP also assumes a soluble topology. We found that depolarization and cholinergic agonists stimulate secretion of the potentially amyloidogenic solAPPcyt from both mouse brain synaptosomes and primary chromaffin cells which display neuronal physiology. Furthermore we found that solAPPcyt is present in human CSF and, in lower levels, in human serum. These results suggest that solAPPcyt is secreted in neuronal synapses where it may exert its biological functions. We used CHO cells transfected with wild type or mutated APP carrying all FAD-linked mutations to determine the effects of these mutations on the secretion of the potentially amyloidogenic solAPPcyt. Our data showed that all these mutations increased secretion of solAPPcyt.

Our hypothesis is that FAD-linked APP mutations cause Alzheimer's Disease by increasing secretion of solAPPcyt and interfering with its synaptic function (Supported by NIH grant AG08200 and a Zenith Award from the Alzheimer's Association)

115.2

THE SWEDISH MUTATION OF ß AMYLOID PRECURSOR PROTEIN INCREASES Aß PRODUCTION IN GLIAL CELLS BUT NOT NEURONAL CELLS. M.S. Forman, D. Cook, S. Leight, D. Schenk, T. Iwatsubo, R. Doms and V.M.-Y. Lee*. Department of Pathology and Laboratory Medicine, University of Pennsylvania, Philadelphia, PA 19104

Missense mutations in the gene encoding the amyloid precursor protein (APP) have been identified in early onset Alzheimer's disease kindreds. In a large Swedish kindred, a double mutation was identified immediately N-terminal to the Aß peptide region. In various cell lines, this mutation causes a dramatic increase in the secretion of Aß. We examined the effect of overexpression of the Swedish mutation (APP^{SW}) relative to wild-type APP in glial cells and the neuronal cell line NT2/N, (a teratocarcinoma cell line terminally differentiated with retinoic acid). In the NT2/N cells, Aß was previously demonstrated to be detectable both intracellularly as well as in the media. Consistent with previous work from several laboratories, APP^{SW} causes a dramatic increase in the secretion of Aß in primary astrocytes. However, in the neuronal cell line NT2/N, APP^{SW} does not increase Aß secretion. Moreover, APP^{SW} does not cause an increase in intracellular Aß in this neuronal cell line; while, in several non-neuronal cell lines it increases intracellular Aβ to detectable levels.

To further assess the effects of the Swedish mutation, we examined the production of proteolytic fragments of APP during intracellular processing. In the NT2/N cells, primary astrocytes and several non-neuronal cell lines, APP^{SW} results in a large increase in APPsβ detected in the media with a relative decrease in APPsα. In addition, the mutation leads to the intracellular production of both APPsβ and C99. Thus, the γ-secretase activity is not increased in the NT2/N cells relative to the non-neuronal cells. This data suggests that in Alzheimer's disease involving Swedish family kindreds, the mutation exerts its effect on the glial cells.

115.3

EFFECTS OF PRESENILIN MUTATIONS ON THE DEPOSITION AND PRODUCTION OF AMYLOID ß PROTEIN.

T. Iwatsubo*[1], D.M.A. Mann[2], T. Tomita[1], T. Kosaka[1], S. Tokuhiro[1], R. Hosoda[1], K. Obata[3], T.C. Saido[4], K. Maruyama[3]. 1 Dept. of Neuropathol. & Neurosci., Univ. of Tokyo 2 Dept. of Pathol. Sci., Univ. of Manchester, UK 3 Lab. of Neurochem., N.I.P.S. 4 Tokyo Metr. Inst. Med. Sci., Japan

To clarify the pathomechanisms whereby presenilin (PS) mutations cause familial Alzheimer's disease (FAD), and especially their relevance to the production and deposition of amyloid ß protein (Aß), we have examined the frontal cortex of 8 cases of FAD with 5 different PS1 mutations and 6 cases with PS2 mutation (141N-I) using immunocytochemistry specific for the Aß C-terminus. The density and percentage area of Aß42(43)-positive (=total) senile plaques (SP) were both markedly increased in PS1 mutants, these being double those in sporadic AD (sAD) and elderly Down's syndrome (eldDS) cases and at comparable levels to those in FAD with ßAPP717 mutations (FAD717). However, the percentage of Aß40- to Aß42(43)-positive SP in PS1 mutants was 38% (on a density basis) and 28% (on and area basis), levels similar to those in sAD and eldDS, but far higher than those in FAD717 (8% and 2%, respectively). The amount of Aß42(43)-positive SP in PS2 mutants was slightly higher than or similar to that in sAD or eldDS; the percentage of Aß40-positive SP was also at similar levels. These results suggest that, as with ßAPP mutations, mutations in PS1 and PS2 may also enhance the deposition of Aß42(43) through an increase in production and thereby cause AD, although the pathomechanism and the C-terminal properties of deposited Aß seem different from those in FAD717. We have transfected PS1 and PS2 genes, with or without mutations, to cultured cells expressing ßAPP and quantitated the amount and C-terminal properties of secreted Aß by ELISAs. The results will be discussed in relation to our morphometric data on Aß deposition *in vivo*. (Supported by Grants from the Ministry of Education, Science and Culture, Japan)

115.4

Aß42(43) IS INCREASED *IN VIVO* BY THE *PS1/2* AND *APP* MUTATIONS LINKED TO FAMILIAL ALZHEIMER'S DISEASE.

D. Scheuner*[1], C. Eckman[1,11], M. Jensen[2], X. Song[3], M. Citron[4], N. Suzuki[5], T. D. Bird[6], J. Hardy[8], M. Hutton[8], W. Kukull[9], E. Larson[9], E. Levy-Lahad[9], M. Viitanen[2], E. Peskind[9], P. Poorkaj[7], G. Schellenberg[7], R. Tanzi[10], W. Wasco[10], L. Lannfelt[2], D. Selkoe[4], and S. Younkin[11]. Depts. [1]Neuroscience & [3]Pathology, Case Western Reserve U., Cleveland, OH, [2]Huddinge Univ. Hosp., Huddinge, Sweden, [4]Harvard Med. School and Brigham and Women's Hosp., Boston, MA, [5]Takeda Chemical Industries, Tsukuba, Japan, [6]Dept. of Neurology & [7]Geriatric Research, Education, and Clinical Ctr (182B), VA Med. Ctr, Seattle, WA, [8]Dept. of Psychiatry, Univ. of S. Florida, Tampa, FL, [9]Dept. of Medicine, Univ.of Washington, Seattle WA, [10]Dept. of Neurology, Massachusetts General Hosp., Charlestown, MA,[11]Mayo Clinic Jacksonville, Jacksonville, FL 32224

Amyloid ß protein (Aß) ending at Aß42(43) [Aß42(43)] is deposited early and selectively in the senile plaques that are an invariant feature of all forms of Alzheimer's disease (AD). To determine whether the presenilin 1 (*PS1*), presenilin 2 (*PS2*), and amyloid ß protein precursor (*APP*) mutations linked to familial AD (FAD) increase the extracellular concentration of Aß42(43) *in vivo*, we performed a blinded comparison of plasma Aß levels in carriers of these mutations and controls. Aß1-42(43) was elevated in plasma from subjects with FAD-linked *PS1* (8 symp, 1 presym; p<0.0001), *PS2*N141I (3 symp; p=0.003), *APP*K670N,M671L (7 presymp, 5 symp; p<0.0001), and APPV717I (one subject) mutations. Aß ending at Aß42(43) was also significantly elevated in fibroblast media from subjects with *PS1* (p<0.0001) or *PS2* (p=0.0005) mutations. These findings indicate that the FAD-linked mutations may all cause AD by increasing the extracellular concentration of Aß42(43) thereby fostering deposition of this highly amyloidogenic peptide. In 62 of 71 sporadic AD subjects plasma Aß1-42(43) was not elevated, but it was elevated into the FAD range in 9 of the subjects with sporadic AD.

115.5

STABLE EXPRESSION OF MUTANT PRESENILINS HAS DIFFERENTIAL EFFECTS ON Aß42 AND Aß40 PRODUCTION.

M. Citron,* T.S. Diehl, R. Sherrington[1], P. St. George Hyslop[1] and D.J. Selkoe. Harvard Medical School, Boston, MA; and [1]University of Toronto, Canada.

Humans harboring mutations in the presenilin genes undergo progressive cerebral deposition of the amyloid ß-protein (Aß), including Aß42, at an early age and develop a severe form of Alzheimer's disease (AD). A previous study (Scheuner et al., 1995) has shown that $A\beta_{42}$ is selectively elevated in the plasma of individuals with presenilin mutations, suggesting that these mutations influence ßAPP processing. We have extended this analysis by generating several stable 293 human embryonic kidney cell lines doubly transfected with ßAPP and a presenilin gene in its wild type or mutant form. The cell lines were analyzed for levels of all secreted ßAPP metabolites, full-length ßAPP, its C-terminal fragments and presenilin. Using end-specific antibodies in ELISA and immunoprecipitation assays, we examined the influence of presenilin mutations on ßAPP processing. We did not find a significant effect of presenilin mutations on the levels of full-length ßAPP, α-APP_s, ß-APP_s and Aß40. However, in all presenilin mutant cell lines analyzed the Aß42/Aß40 ratios were about twofold increased relative to untransfected or PS1 wild-type transfected cells. This effect occurred in cell lines transfected either with ßAPP wild-type, in which most Aß is produced in the endocytic pathway, or ßAPPsw, in which most Aß is produced in the secretory pathway. Variations in the presenilin expression levels had no effect on the extent of the increase. These data are consistent with the results of Scheuner et al. and suggest that (i) presenilin mutations cause a gain rather than a loss of function; (ii) they lead to increased cleavage by a 42-specific γ-secretase in both the secretory and endocytic pathways; and (iii) only catalytic amounts of mutant presenilins are needed to cause the increased Aß42 production. Supported by AG05134.

115.6

FAD-LINKED MISSENSE MUTATIONS IN PRESENILIN 1 INCREASE $A\beta_{42}$ PRODUCTION IN STABLY TRANSFECTED CHO CELLS.

W. Xia*, J. Zhang, E.H. Koo, D.J. Selkoe. Brigham and Women's Hospital and Harvard Medical School, Boston, MA.

The *presenilin 1* (*PS1*) gene on chromosome 14 was recently identified as the major cause of early onset familial Alzheimer's disease (AD). A previous study of fibroblasts obtained from FAD patients with *PS1* mutations indicates a selective increase of $A\beta_{42}$ in the media. To study the mechanism of the mutant gene product in the pathogenesis of AD, Chinese hamster ovary (CHO) cells stably expressing wildtype (wt) or mutant (M146L, C410Y) *PS1* genes were generated and the effects of wt and mutant PS1 proteins on Aß production were examined. Introduction of *PS1* genes into ßAPP-transfected CHO cells did not alter the synthesis of ßAPP, as determined by metabolic labeling and quantitation of ßAPP in these cells. Several clones with different levels of PS1 expression of each cell line stably expressing wt or mutant PS1 were randomly selected and tested for Aß production. Immunoprecipitation of conditioned media with antibodies specific for $A\beta_{42}$ and total Aß showed a relative increase of $A\beta_{42}$ levels in cells expressing mutant *PS1* genes, while total Aß remained unchanged. Mutant PS1-expressing cells had 1.4 to 2.5 fold increases of $A\beta_{42}/A\beta_{total}$ ratios when compared to wildtype PS1-expressing cells, and this increase was statistically significant. The level of $A\beta_{42}$ increase was not clearly correlated with the level of PS1 expression. Low MW oligomers (6-16 kD) of $A\beta_{42}$ were observed in immunoprecipitates of the conditioned media of some mutant PS1 cell lines having the largest increases in $A\beta_{42}/A\beta_{total}$ ratio. Taken together, our data support the hypothesis that mutations in the PS1 protein affect ßAPP processing in a way that leads to increased $A\beta_{42}$ production and deposition, thus causing the premature development of AD. Supported by NIH grant AG05134 (DJS), AG12376 (EHK).

115.7

ANALYSIS OF PRESENILIN 1 IN BRAIN AND CULTURED CELLS,

L.M. Refolo*, Y. Harigaya, L. Younkin, C.B. Eckman, K. Sambamurti, C-M. Prada, D. Yager, and S. G. Younkin. Mayo Clinic Jacksonville, Jacksonville, FL 32224

Our recent analysis of fibroblasts and plasma from subjects with 6 different presenilin 1/2 (PS1/2) mutations linked to familial AD indicates that these mutations increase the extracellular concentration of Aß1-42(43). As part of our effort to determine whether this effect is related to alteration(s) in PS1/2 processing, we prepared rabbit antisera to synthetic peptides corresponding to PS1 amino acids 1-14, 57-70, and 333-345. After affinity-purification, each antiserum was used to label immunoblots of lysates prepared from baby hamster kidney (BHK) cells or BHK cells infected with Semliki Forest Virus (SFV) expressing PS1 with a 9 amino acid HA tag inserted between amino acids 44 and 45 (generously provided by DG Cook, RW Doms, and VM-Y Lee, University of Pennsylvania). Anti-HA and each of the anti-PS1 antisera detected an ~46 kD protein that was markedly augmented in the SFV-PS1 infected cells. That this *augmented* protein was detected by antisera to 4 different epitopes in HA-tagged PS1provides strong evidence (i) that the ~46 kD protein is authentic PS1 and (ii) that each of our antisera to PS1 are useful for detecting PS1 on immunoblots. Using these antisera, we also detected several high molecular weight bands as well as putative N- and C-terminal PS1 derivatives that were augmented in SFV-PS1 infected cells and detected appropriately by the various antisera to PS1 peptides. Thus PS1 may normally be processed into smaller derivatives. In rat and human brain and in other cultured cells, proteins similar in size to those identified in BHK cells (e. g. the 46 kD protein) were detected by our antisera, with anti-PS157-70 showing the strongest labeling. In addition, a "non-augmenting" ~52 kDa protein was labeled by all 3 antisera to PS1 peptides. We are currently (i) using an immunoaffinity approach to isolate the various proteins recognized by our antisera for sequencing and specific identification and (ii) using our antisera to analyze Aß secretion and PS1 processing in transfected cells expressing mutant vs. wild type PS1.

115.8

PROTEIN CHARACTERIZATION OF THE EARLY ONSET FAMILIAL ALZHEIMER'S DISEASE GENE PRODUCTS, PRESENILIN 1 AND PRESENILIN 2.

D. J. Selkoe*, W. Xia, J. Zhang, M .B. Podlisny, P. Amarante, C. Haass, P. H. St. George-Hyslop, D.B. Teplow, E. H. Koo, and M. Citron. Brigham and Women's Hospital and Harvard Medical School, Boston, MA

The majority of cases of early onset familial Alzheimer's disease is caused by mutations in the *presenilin 1* gene (*PS1*) located on chromosome 14 and *presenilin 2* (*PS2*) gene located on chromosome 1. To gain insight into the biology of the PS1 and PS2 proteins, the *PS1* or *PS2* gene was expressed in transiently-transfected COS cells. Using immunoprecipitation and Western blotting with a C-terminal specific antibody, the PS1 protein was identified as a ~43-45 kDa doublet, and the *PS2* gene product had a similar apparent MW. ßAPP protein was not found to be directly associated with the PS1 and PS2 proteins, because preclearing cell lysates with ßAPP antibody did not change the band patterns of PS1 and PS2 upon subsequent immunoprecipitation with the PS1/PS2 antibody. Furthermore, no ßAPP was detected on Western blots of PS1/PS2 immunoprecipitates probed with ßAPP antibodies. Radiolabeled sequencing of the PS1 doublet immunoprecipitated by the C-terminal antibody showed that both species were intact PS1 protein starting at the first methionine residue. The half-life of both of these full-length PS1 proteins in COS cells was the same (<60-90 min), and they exhibited no clear precursor-product relationship. These data suggest that PS1 proteins do not undergo significant maturation after their synthesis. We have also generated CHO cells stably expressing both wildtype and mutant PS1 or PS2 proteins, and a comparison of the biosynthesis and processing of PS1 and PS2 proteins is in progress. Supported by NIH grant AG12749 (DJS).

115.9

ALZHEIMER'S AMYLOID PRECURSOR PROTEIN PROCESSING: CATHEPSIN D EXHIBITS γ-SECRETASE ACTIVITY

Harry F. Dovey*, Susanna Suomensaari, Patrice de St. Andrieu, Robin Barbour, Sukanto Sinha, Peter Seubert, Dale B. Schenk, Ivan Lieberburg, and Varghese John. Athena Neurosciences, Inc., 800 Gateway Boulevard, South San Francisco, California, 94080, USA.

The enzymes responsible for the proteolytic processing of the β-amyloid precursor protein (APP) to produce the β-amyloid peptide (Aβ), found in the senile plaques associated with Alzheimer's disease (AD), are potential targets for therapeutic intervention in AD. The protease responsible for the COOH-terminal cleavage has been termed γ-secretase, and has yet to be conclusively identified. In this study, an endogenous protease, found in the lysosomal/endosomal fraction of both normal and AD brain, has been shown to cleave an endogenous, amyloidogenic fragment of APP under acidic conditions to produce a COOH-terminal fragment (γ-CTF) consistent with γ-secretase activity. The pH and inhibitor profiles of this activity suggest that of an aspartyl protease. The γ-CTF generating activity could be removed by pepstatin-agarose chromatography, and an identical cleavage pattern restored by addition of cathepsin D (CD) (EC 3.4.23.5), suggesting that CD has γ-secretase activity. This suggestion was further supported by the ability of CD to appropriately cleave baculovirus-produced APP_{751} and *in vitro* translated, radiolabelled $APP_{589-695}$. Significantly, CD cleavage of a fusion protein, containing the COOH-terminal 125 amino acids of APP, at the COOH-termini of Aβ40 and Aβ42 was demonstrated with highly specific ELISAs. In addition, the aspartyl protease inhibitor pepstatin A and the Aβ COOH-terminus are homologous, and analogs of the Aβ COOH-terminus were nanomolar inhibitors of CD, further suggesting an affinity of CD for this region of APP. Based on these findings, CD appears to be a putative γ-secretase, and may be a therapeutic target for AD.

115.10

EVIDENCE THAT Aß42 AND Aß40 ARE GENERATED BY DIFFERENT PROTEASES. T.S. Diehl, M. Citron, Grace Gordon[1], D. Hartley*, P. Seubert[1], and D.J. Selkoe. Center for Neurologic Diseases, Brigham and Women's Hospital and Harvard Medical School, Boston, MA 02115; [1]Athena Neurosciences Inc., San Francisco, CA 94080.

Alzheimer's disease is characterized by the progressive formation in the brain of insoluble amyloid plaques consisting of the 4 kDa amyloid ß-peptide (Aß). Aß deposits show both N-and C-terminal microheterogeneity, and recent data indicate that the long tail $Aß_{42}$ form, although accounting for only 10% of secreted total Aß, is deposited in the earliest phase of the disease and remains the major constituent of most amyloid plaques throughout the disease. Thus, inhibition of $Aß_{42}$ production is a prime therapeutic goal. The proteases which cleave Aß from its precursor have not been identified, but the same γ-secretase is assumed to generate the C-termini of both $Aß_{40}$ and $Aß_{42}$.

We have analyzed the effect of the compound MDL28170 (gift of Marion Merrell Dow, Inc.), previously suggested to inhibit γ-secretase (Higaki et al., 1995), on ßAPP processing. By immunoprecipitating media of different cell lines with various 40 and 42 specific antibodies, we demonstrate a much stronger inhibition of the γ-secretase cleavage at residue 40 than of that at residue 42, stongly suggesting that two different proteases cleave at residue 40 and 42. Our data suggest that the initial steps of $Aß_{40}$ and $Aß_{42}$ generation occur in the same pathway, perhaps up to the point of γ-secretase cleavage. At this step, the same 12 kDa precursor can give rise to both $Aß_{40}$ and $Aß_{42}$ and the same 10 kDa precursor can lead to both p_{340} and p_{342}. Our findings have significant therapeutic implications, because they raise the possibility of identifying compounds which do not interfere with 90% of total Aß production and most steps of ßAPP metabolism but specifically block the generation of the pathogenic $Aß_{42}$ peptide. Supported:AG05134.

115.11

CELLULAR SYSTEM FOR THE ANALYSIS AND SCREENING FOR AMYLOID PRECURSOR PROTEIN (APP) SECRETASE ACTIVITIES. T. Dyrks, M. Härtel, D. Marmé, and J. Turner*, Research Laboratories of Schering AG, CNS-Research, D-13342 Berlin, Germany

There is good evidence for a central aetiological role for ßA4 deposition in Alzheimer's disease. Three distinct proteolytic activities (termed α-, ß- and γ-secretases, but not yet identified) are involved in APP processing resulting in the generation of secretory APP (APPsec) and amyloidogenic ßA4 peptide. Compounds which inhibit selectively ß- or γ-secretase, or stimulate α-secretase activity should inhibit ßA4 generation and thus deposition. The establishment of cell culture systems for the screening of each secretase activity is an essential step for the identification of selective secretase-inhibiting compounds.

To achieve this, APP reporter constructs, each containing the secretase recognition site of only one of the three APP-secretases are being generated. Neuronal cells which are known to process APP appropriately have been transfected with these constructs to produce stable cell lines expressing APP reporter constructs specific for each of the three secretase activities. The constructs are correctly processed and the released reporter into the medium indicates processing of the construct by the target protease. Pharmacological modulation correlates with that of APP processing.

115.12

METABOLISM OF AMYLOID β-PROTEIN IN CULTURED MAMMALIAN CELLS R. Wang*[1], S. Gandy[2], S.S. Sisodia[3], B.T. Chait[1]. [1]Lab. for Mass Spectrometry, The Rockefeller Univ., New York, NY 10021; [2]Lab. of Alzheimer Research, Cornell Univ. Med. Coll., New York, NY 10021, [3]Neuropathology Lab., The Johns Hopkins Univ. Sch. Med., Baltimore, MD 21205.

Amyloid β protein (Aβ) is the primary component found in senile plaques of Alzheimer's disease (AD) and is constantly produced and secreted from cells by proteolytic processing of its precursor protein, amyloid precursor proteins (APP). Various forms of Aβ peptides have been identified from plaques, neurofibrillary tangles and cerebrovascular amyloid fibrils of AD patients. Soluble Aβ (sAβ) species were also found in cerebrospinal fluid (CSF), blood and cultured cell media. Recently, we have investigated the sAβ peptide profile in conditioned media of mouse neuroblastoma cells (N2a) transfected with human APP cDNAs using a newly developed immunoprecipitation/mass spectrometry (IP/MS) method. The sAβ profile has revealed a complex mixture. More than forty different Aβ-related peptide species were identified. Time-course experiment indicated that these Aβ peptide fragment may generated either from metabolism of Aβ or from alternative proteolytic processing of APP or both. We further investigated the Aβ metabolism in cultured mammalian cells using IP/MS. The proteolytic cleavage products of synthetic Aβ in conditioned medium of N2a cells were characterized. The differences in both proteolytic cleavage pattern and degradation kinetics between $Aβ_{1-40}$ and $Aβ_{1-42}$ have been compared. The involvement of whole cell in the proteolytic degradation of Aβ were also examined. This study classified the origins and pathways for the observed Aβ fragments.

115.13

PKC REGULATED AMYLOID PRECURSOR PROTEIN PROCESSING IN YEAST, M. Seeger[1,2*], R. S. Fuller[3], H. Komano[3], H. Riedel[4], J. Greenfield[1], P. Greengard[2] and S. Gandy.[1] [1]Dept. of Neurology & Neurosci., Cornell Univ. Med. Coll., NY, NY 10021; [2]Lab of Mol. and Cell. Neurosci., Rockefeller Univ., NY, NY 10021; [3]Dept. of Biochem., Univ. of Michigan, Ann Arbor, MI 48109; [4]Joslin Diabetes Center, Harvard Med. School, Boston, MA 02215

Amyloid precursor protein (APP) is processed by a-secretase yielding a non-amyloidogenic secreted fragment (sAPP). This processing pathway can be stimulated by phorbol esters, which activate protein kinase C (PKC), resulting in increased secretion of sAPP and decreased secretion of the amyloidogenic peptide, Aß, a product of the ß-secretase pathway. The target upon which PKC acts to cause these effects is yet to be identified.

We have developed a genetically manipulable system in which to analyze the regulation of APP processing in response to stimulation of PKC Human APP has been expressed in the yeast, *Saccharomyces cerevisiae*. a-secretase cleavage of APP occurs in yeast, resulting in release of proteolytic fragments (including sAPP), and the retention of carboxyl terminal fragments which are identical to those formed in mammalian cells. In yeast strains that over-express both human APP and rat PKCa, there is an increase in the release of sAPP in response to PKC activation with phorbol esters. Since the secretory pathway of yeast has been well characterized, this host system should provide an excellent setting within which to gain insight into the mechanisms by which APP processing is regulated.

Supported by grants AG11508, AG09464, AG10491 and AG05689.

115.14

REGULATION OF ALZHEIMER ß-AMYLOID PROTEIN PRECURSOR METABOLISM IN CELL-FREE SYSTEMS. H. Xu*[1,2], D. Sweeney[1], R. Wang[3], G. Thinakaran[4], S. Sisodia[4], P. Greengard[2], and S. Gandy[1]. [1]Lab. of Alzheimer Research, Cornell Univ. Med. Coll., New York NY 10021; [2]Lab. of Molecular & Cellular Neuroscience; [3]Lab. for Mass Spectrometry, Rockefeller Univ., New York, NY 10021; [4]Neuropathology Lab., The Johns Hopkins Univ., Baltimore, MD 21205.

The ß-amyloid protein (Aß), a major component of parenchymal deposits in Alzheimer's disease, is generated by proteolytic cleavage from the ß-amyloid precursor protein (ßAPP). The relative utilization of alternative processing pathways for ßAPP can be regulated by the activation state of certain protein phosphorylation signal transduction pathways (e.g., activation of protein kinase C (PKC), or inactivation of protein phosphatases). Recently, we have demonstrated in a reconstituted cell-free system that PKC increases formation from the *trans*-Golgi network (TGN) of secretory vesicles containing ßAPP, revealing a major mechanism by which ßAPP metabolism is controlled. Unfortunately, from a therapeutic standpoint, PKC activation can stimulate ßAPP transcription, indirectly increasing Aß formation. Since there are many examples wherein multiple protein kinases can exert similar regulatory effects, we tested whether protein kinase A (PKA) might also regulate ßAPP metabolism. PKA-activating reagents increased sßAPP secretion from intact PC12 cells and, by using an *in vitro* reconstitution system, purified PKA also stimulated formation of ßAPP-containing vesicles from the TGN. Although PKA and PKC converge at the level of formation of ßAPP-containing vesicles, additional evidence (e.g., sensitivity to GTPγS or okadaic acid) indicates that the regulatory mechanisms involved are distinct. In addition, we have successfully reconstituted Aß formation using a cell-free system previously developed to reconstitute proteolytic cleavage of prohormones. This cell-free system can enable us to dissect as separate events, either Aß generation or the formation of nascent TGN vesicles containing Aß or ßAPP. This study should lead us to the identification of intracellular compartment(s) involved in Aß cleavage.

Supported by grants AG11508, AG09464 AG10491.

116.1

LESIONS OF THE CEREBELLAR NUCLEI DO NOT IMPAIR THE ACQUISITION OF A VISUAL-MOTOR CONDITIONAL LEARNING TASK. R.E. Passingham* and P.D. Nixon Department of Experimental Psychology, University of Oxford, Oxford OX1 3UD.

Several recent reports have claimed that patients with cerebellar pathology have cognitive as well as motor deficits. However, it is not certain that in these studies the pathology was confined to the cerebellum

We have addressed the issue by training monkeys on a task which requires the monkeys to learn an association between two coloured visual patterns and two movements of a joystick. Bilateral excitotoxic lesions were then made in the lateral cerebellar nuclei of 3 of the animals; the remaining 3 served as unoperated controls. When retested 3 weeks later, the operated animals made more errors than animals in the control group. However, this may have been the consequence of a motor impairment which gradually resolved with testing.

When the same animals were given two new visual stimuli to associate with the same two joystick movements, there were no significant differences between the groups on either the number of trials required to learn the new associations or the number of errors made. This result suggests that restricted cerebellar damage does not itself impair associative learning.

We are now checking whether there is a genuine retention impairment by making identical lesions in the control animals, and retesting them on both versions of the task. Any motor impairments should be resolved during the relearning of the first task, and thus not contaminate the retention scores for the second.

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116.2

IS THE HUMAN CEREBELLUM REQUIRED FOR THE STORAGE OF CONDITIONED EYEBLINK MEMORY TRACES? V. Bracha, D. A. Wunderlich, L. Zhao, L. Brachova* and J.R. Bloedel. Barrow Neurological Institute, Phoenix, AZ 85 013

Human subjects with cerebellar pathologies have greatly impaired capacity to acquire classically conditioned eyeblink responses (CR). Is the human cerebellum equally important also for retention of CRs? The answer to this question would require the identification of conditioned responses which are acquired naturally before the occurrence of any cerebellar abnormality. In the present experiments we examined visually-triggered anticipatory eyeblinks in a group of patients who in our previous study failed to acquire blinking responses in a traditional classical conditioning paradigm.

The cerebellar patients exhibited normal eyeblinks triggered by a ball quickly approaching and eventually hitting their forehead. These eyeblinks were generated well before the actual impact of the ball. The anticipatory eyeblinks also could be extinguished when the ball was repeatedly stopped before contacting the forehead and could be quickly reacquired when the ball impact was resumed. The possibility that this response extinguishes suggests that the visually-triggered eyeblinks are CRs for which the visual information serves as the conditioned stimulus while the cutaneous stimulation elicited by the object's impact serves as an unconditioned reinforcer. These results indicate that, unlike in the rabbit, the cerebellum of human subjects is not critical for the retention or extinction of conditioned eyeblinks and consequently is not required to store traces of conditioned eyeblink responses.

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116.3

IMPAIRED TRACE EYEBLINK CONDITIONING IN MEDIAL TEMPORAL AMNESIA

R. McGlinchey-Berroth*[1,2], M. Carrillo[3], C. Brawn[2], J.D.E. Gabrieli[4], L.S. Cermak[2], J.F. Disterhoft[3]. 1 Geriatric Research, Education and Clinical Center, Brockton/West Roxbury VAMC, W. Roxbury MA ; 2 Memory Disorders Research Center, Boston VAMC, Boston, MA; 3 Northwestern University, Chicago, IL; 4 Stanford University, Stanford, CA.

The precise role of the hippocampus in trace eyeblink conditioning has been difficult to resolve since animal studies have shown impaired acquisition in hippocampectomized rabbits (Kim et al. 1996; Moyer 1990; Solomon, 1986), while human studies have found intact acquisition in two bilateral human patients (Woodruff-Pak, 1994). In our study, acquisition of trace conditioning in human amnesics was tested in 3 experiments that differed only in the duration of the trace interval. Subjects were 7 bilateral amnesic patients (AM; 5 post-encephalitis, 2 anoxic) and age, educ. and VIQ matched control subjects (NC). All subjects participated in each experiment. Sixty conditioning trials were presented using a 100 msec, 1000 Hz, 85 db tone (CS) presented binaurally through headphones. The unconditioned stimulus (UCS) was a corneal airpuff (3 psi), 100 msec in duration delivered to the right eye. Trace interval from CS offset was 500 (T500), 750 (T750) or 1000 (T1000) msec, run in separate sessions at least 2 weeks apart. Following conditioning, subjects received 30 extinction trials during which the CS was presented alone. The mean %CRs for T500 were 40.5 for AM and 58.3 for NC; for T750 were 48.6 for AM and 71.4 for NC; and for T1000 were 40.1 for AM and 70.6 for NC. ANOVAs with Group and Trial Block (1-12) revealed significant effects of Group at each interval and a Block effect for T500. These data indicate that bilateral medial temporal lobe damage does disrupt acquisition of trace eyeblink conditioning. The magnitude of the impairment appears to vary linearly with trace interval. Supported by NIH 1P50NS26985 to LSC; AG08796 to JFD; GM17223 to MCC.

116.4

ORGANIZATION AND FUNCTIONAL ROLE OF MESENCEPHALIC DOPAMINERGIC PATHWAYS TO THE HIPPOCAMPAL FORMATION IN THE RAT. A. Gasbarri*, A. Sulli, A. Pompili and C.Pacitti. Department of Science and Biomedical Technology, Laboratory of Human Physiology, University of L'Aquila, 67100 L'Aquila (Italy).

The present study offers a description of the anatomical organization and functional role of the projections from the retrorubral field (RRF), substantia nigra (SN), and ventral tegmental area (VTA) to the hippocampal formation (HF) in the rat. Iontophoretically injected *Phaseolus vulgaris*-leucoagglutinin (PHA-L) in RRF, or SN, or VTA resulted in labeling primarily in the ipsilateral subiculum and adjacent CA1 field of both septal and temporal HF. These findings suggest that the general patterns of distribution of the RRF, SN and VTA efferents to the HF are similar. The percentage of mesencephalic dopaminergic (DA) neurons projecting to the HF was evaluated by combining the retrograde tracer Fluoro Gold (FG) with tyrosine hydroxylase (TH) immunohistochemistry. The evaluation of retrogradely FG and TH stained cells showed that the mesencephalic-HF projections are partially DA (10-18%). A topographical organization, and a more prominent input to the temporal than to the septal HF were also observed. Following bilateral 6-hydroxydopamine injection in the dorsal and ventral subiculum and adjacent CA1 field of HF, the effects on learning and memory of retrograde selective lesions of mesencephalic dopaminergic neurons were evaluated using the standard Morris water maze (MWM), cued and spatial versions. Though the lesioned rats were indistinguishable from sham-operated rats in performing the cued version of MWM task, in the spatial version of MWM, lesioned rats, compared to controls, exhibited significant differences, suggesting that the ability to acquire spatial learning and memory for place navigation in the MWM is dependent also on the integrity of meso-hippocampal DA connections.

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116.5

EXTENDING MODELS OF HIPPOCAMPAL FUNCTION IN ANIMAL CONDITIONING TO HUMAN AMNESIA,

M. A. Gluck *, B. R. Ermita, L. M. Oliver, and C. E. Myers.
Center for Molecular and Behavioral Neuroscience,
Rutgers University, Newark, NJ 07102.

Although most analyses of amnesia have focused on the loss of declarative memories following hippocampal-region damage, considerable insights into amnesia can also be realized through the study of simple procedural, or habit-based, associative learning tasks. In several recent papers we have described a computational theory of hippocampal function in classical conditioning, which argues that this brain region plays a critical role in the formation of new stimulus representations during learning; when applied to conditioning studies in both intact and hippocampal-lesioned animals, the model correctly accounts for a broad range of empirical data and makes numerous novel predictions (Gluck & Myers, 1993, 1995; Myers & Gluck, 1995; Myers, Gluck, & Granger, 1995). More recently, we have shown how this model can also be applied to studies of category learning in both normals and hippocampal-damaged amnesics (Gluck, Oliver & Myers, 1996/*in press*). This theoretical modeling integrates experimental studies of amnesic category learning (Knowlton, Squire, & Gluck, 1994) with theoretical accounts of associative learning, and builds on previously established behavioral correspondences between animal conditioning and human category learning (Gluck & Bower, 1988). We show here how the cortico-hippocampal model of Gluck and Myers (1993) can be applied to the processing of integral vs. separable stimuli in classification learning, and to the phenomena of categorical perception in learning.

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116.6

THE NEUROPHYSIOLOGY OF LATENT INHIBITION: A NEURAL NETWORK APPROACH N.A. Schmajuk*, J. Tye, V.C. Buhusi, and J.A. Gray. Deparment of Psychology: Experimental, Duke University, Durham, NC 27707, USA, and Institute of Psychiatry, London, United Kingdom.

Schmajuk, Lam, and Gray (JEP:ABP, 1996) offered a formal theory of latent inhibition (LI) in the context of a real-time, neural network model of classical conditioning. In the model, an attentional system enhances internal representations of conditioned stimuli (CSs) active at a time when the total environmental novelty is large, and decreases internal representations of those CSs active at a time when the total novelty is small. The magnitude of the CS internal representation controls both the storage and retrieval of CS-US associations. According to the model, LI reflects the decreased internal representation of a CS as a consequence of decreased attention after preexposure to that CS.

Computer simulations demonstrate that, due to its combined storage and retrieval attributes, the neural network correctly describes the effects on LI of numerous experimental manipulations (a) preceding CS preexposure, (b) during CS preexposure, and (c) during conditioning. Most important, the network correctly describes attenuation of LI (d) after different postconditioning manipulations.

After establishing the correspondence between nodes and connections in the model with different brain regions (e.g., hippocampus, subiculum, nucleus accumbens, ventral pallidum, colliculi, thalamus, cortex) and their interconnecting pathways and neurotransmitters (e.g., dopamine, glutamate, acetylcholine, serotonine, GABA), the model correctly describes the effect on LI of (1) amphetamine administration, (2) haloperidol administration, (3) nicotine administration, (4) scopolamine administration, (5) nonselective hippocampal lesions, (6) selective hippocampal lesions, (7) nonselective hippocampal lesions combined with haloperidol administration.

This study was supported by a grant from NATO.

116.7

LONG TERM MEMORY FOR VISUAL IMAGES IN THE HIPPOCAMPUS AND INFEROTEMPORAL CORTEX. JL Ringo*[1] and A Nowicka[1,2]. [1]University of Rochester, Rochester NY, [2]Nencki Institute, Warsaw, Poland.

Measures of memory in single unit responses often rely on averaging responses to different stimuli. However, if for some image(s) in a well-learned set the responses increase while for other images the responses decrease the average response may not change. Other measures of the response distribution, e.g., variance or higher order moments could prove more informative.

We have measured the variance of the unit responses (across images) for a group of images which were well-learned as members of a rewarded set, and compared that to the response variance measured across a group of novel images. The images were fully comparable as they were computer constructed images formed by the same algorithm. Responses from our sample of visually-excited hippocampal units (n=36) showed a greater variance across the well-learned set than across novel images. The average unit showed a 48% increase of across-image variance (statistically significant; $z=4.1$; $P<0.0001$). The mean response did not increase as much. The average response amplitude to the well-learned set was 11% higher ($z=1.8$; $0.05<P<0.10$). Extensive experience with a set of images appears to have remolded hippocampal unit responses in a soft winner-take-all fashion.

Our sample of visually-excited inferotemporal units showed similar behavior. The mean variance increase for the well-learned set over the novel set was 30% ($n=48$; $t=2.9$; $P<0.005$) while the mean response did not differ (within 1%; $z=0.5$; $P>0.2$). Supported by grant NS26526 from NINDS.

116.8

THE EMERGENCE OF STRUCTURE IN NEURONAL REPRESENTATIONS A.Treves*[1], S.Panzeri[1], R.G.Robertson[2], P.Georges-Francois[2] and E.T. Rolls[2] [1]SISSA, Cognitive Neuroscience, via Beirut 2, 34013 Trieste, Italy and [2]Univ. of Oxford, Expl. Psychology, OX1 3UD, UK.

Neurons from the hippocampus and adjacent cortex were recorded in freely locomoting monkeys, and procedures were used to *decode* from their activity which spatial view was concurrently being looked at by the animal. For this purpose, the position in the environment where the monkey was looking was binned into 8-16 discrete "views" and the number of spikes emitted in successive windows of 25, 50, 100 or 500ms was used to quantify the activity of each cell. The joint frequency of any pair of actual and decoded views was used to extract 2 measures: percent correct and information. As more (and/or more selective) cells are used for decoding, both measures rise, but in no fixed relation to each other; for a given percent correct, information values can range from a minimum, when all views but the actual one are decoded with equal frequency, up to a maximum, when one of a group of similar views including the actual one is always decoded, but the decoding is random within the group. An index λ running from 0 to 1 within the range reflects an increasing metric or semantic content of the set of views as perceived by these cells, since it measures the increasing relevance of relationships of similarity or closeness between views. It was shown that λ grows with the time window used to collect spike numbers, suggesting the gradual emergence of meaningful structure in neuronal activity. Values of λ were also compared across areas and with those derived from other experiments. A network model was used to explore the hypothesis that the perceptual structure extracted from the responses reflects the structure and metric content of the underlying synaptic connectivity.

116.9

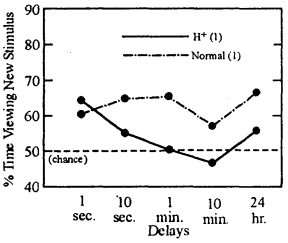
40-MINUTE VISUAL RECOGNITION MEMORY IN RHESUS MONKEYS WITH HIPPOCAMPAL LESIONS. E. A. Murray* and M. Mishkin, Lab. of Neuropsychology, NIMH, NIH, Bethesda, MD 20892 USA

Alvarez et al. (*J. Neuroscience*, 15: 3796, 1995) found that monkeys with selective hippocampal lesions were impaired in object recognition memory at long (10 and 40 min) but not short (8 sec to 1 min) delay intervals. To define the precise delay at which the hippocampus becomes important for recognition memory, we assessed memory for trial-unique objects at 1-min increments starting at less than 1 min and continuing until nearly 40 min. Eight naive rhesus monkeys were trained on 10-sec delayed nonmatching-to-sample, after which 4 Ss received an MRI-guided series of ibotenic-acid injections into both the hippocampus and amygdala (AH). All Ss were then retrained on the initial task, given performance tests with successively longer delays (up to 2 min) and lists (up to 10 objects), and, finally, given list lengths of 20 and then 40 objects with reverse-order testing on each. Both interstimulus and intertest intervals were 30 sec. Thus, in the final condition, delays between sample presentations and choice tests ranged from 30 sec to about 40 min. The Ss with AH lesions did not differ from the unoperated Ss on any delay at any stage (overall mean scores on final condition, tested for 40 days, were 69 and 71% correct, respectively). In *Alvarez et al.*'s study, the Ss were removed from the apparatus during the long but not the short delay intervals, which might have caused selective impairment at the long delays; also, the lesions were made with radio frequency and so might have damaged fibers of passage. Either or both of these factors could be responsible for the difference in the results of the two studies. Supported by the NIMH, NIH.

116.10

The Visual Paired-Comparison Task and the Medial Temporal Lobe Memory System. *R.E. Clark, E. Teng, L.R. Squire and S. Zola, University of California, San Diego. La Jolla, CA 92093 and VA Med. Center. San Diego, CA 92161.

The development of an animal model of human amnesia in the monkey has identified a neural system in the medial temporal lobe important for memory. To further clarify the role of the individual structures in this system, behavioral tests must be developed that are sensitive to even mild memory impairment and that can distinguish perceptual problems from problems in memory. In the visual paired-comparisons task, two identical stimuli are presented for viewing side by side (familiarization). Subsequently, the original stimulus and a new stimulus are presented side by side, and the experimenter records the time the animal spends looking at the new stimulus (test). We have modified this task, used previously in monkeys (Bachevalier et al. 1993), and have varied the interval between familiarization and test from very short delays, where presumably there is little or no memory demand (e.g., 1 sec) to very long delays, where long-term memory is required (e.g., 10 min). The figure shows that at a delay of 1 sec, the preference for novelty was similar in a normal monkey and in a monkey with damage to the hippocampal formation (H^+). Short-term memory was intact and there was no evidence of perceptual impairment. At longer delays (>10 sec), the preference for novelty was absent in the H^+ monkey. This task will be useful for clarifying the specific role in memory of the components of the medial temporal lobe memory system and to distinguish perceptual problems from problems in memory.



116.11

SOLVING THE RIDDLE OF THE LATE APPEARANCE OF SUCCESS ON THE DELAYED NONMATCHING TO SAMPLE TASK

A. Churchland[1] & A. Diamond[2]*. [1]Dept. of Psych., Wellesley College & [2]Dept. of Brain & Cognitive Sciences, MIT, Cambridge, MA 02139.

Delayed nonmatching to sample (DNMS) is a classic test of the recognition memory function dependent on the medial temporal lobe. Children cannot succeed on the standard DNMS task until about 21 months of age. Recognition memory cannot be the crucial factor in the late emergence of success on DNMS, however, since that ability is present much earlier. The late emergence of another ability, dependent on a neural system outside the medial temporal lobe, must account for infants' failure on DNMS. We report evidence suggesting the nature of that critical ability. We tested human infants, 9 & 12 months of age, on various conditions of DNMS. In all conditions, a sample object was presented, which the subject displaced. After a delay, the sample plus a novel object were presented. The subject was allowed to choose one object and, in conditions with a reward, was rewarded for displacing the novel stimulus. In the standard task, the reward is located in a well beneath the stimulus. In the critical condition here, the stimuli again sat atop wells, but rewards were attached by velcro to the base of the stimuli. Even the youngest infants tested (9-month-olds) succeeded in this condition. That is, when the reward and stimulus were physically connected, when the reward moved with the stimulus as the subject displaced the stimulus, the task was easy. The critical late-maturing competence is the ability to grasp the relationship between stimulus and reward, to understand the role of the stimulus as a marker or symbol for the reward's location. Supported by NIMH #MH41842.

116.12

EXCITOTOXIC LESIONS OF THE AMYGDALA FAIL TO PRODUCE IMPAIRMENT IN VISUAL LEARNING FOR AUDITORY SECONDARY REINFORCEMENT IN RHESUS MONKEYS. L. Malkova*, D. Gaffan and E. A. Murray, Lab. of Neuropsychology, NIMH, NIH, Bethesda, MD 20892, USA and Dept. of Experimental Psychology, Oxford University, OX1 3UD, UK

An earlier study showed that monkeys with bilateral aspiration lesions of the amygdala were severely retarded in learning visual discrimination problems based on auditory secondary reinforcement (Gaffan & Harrison, 1987). To determine if this finding would hold for more selective lesions, we measured the effects of excitotoxic lesions of the amygdala on the same task. Four monkeys were trained in an automated apparatus to solve a new set of 40 visual discrimination problems daily, with only auditory feedback providing information regarding the solution. On each trial, two visual stimuli were simultaneously presented on a color monitor. Correct and incorrect choices led to presentation of the auditory secondary reinforcer and nonreinforcer, respectively, and termination of the trial. To solve a problem, the monkey had to choose the positive visual stimulus four trials in a row, and only then did it receive a food reward, and a chance to learn a new problem. After learning the task, the monkeys received bilateral lesions of the amygdala made with ibotenic acid. Amygdalar lesions had no effect on the rate of learning of new visual discrimination problems. Thus the prediction that, for normal performance in this task, the amygdala is necessary for maintaining the value of the secondary reinforcer was not confirmed. By contrast, the same monkeys were impaired in a different task that involved devaluation of reinforcement. A set of 60 pairs of object discriminations was presented once per daily session until the monkeys performed at 90% correct responses over five consecutive days; half of the positive objects were rewarded consistently with one food (A) and the other half with another equally palatable food (B). Positive objects from the 60 pairs (one "A" and one "B") were then randomly paired for 30 critical choice trials following: 1) selective satiation of either food A or B; or 2) no satiation. Normal monkeys decreased their choices of the objects covering the devalued food to a significantly greater extent than monkeys with amygdala lesions. Supported by the Human Frontiers Scientific Program Organization and the NIMH, NIH.

116.13

VISUAL NEGLECT IN THE MONKEY: REPRESENTATIONAL IMPAIRMENT AND DISCONNECTION. D. Gaffan* and J. Hornak. Exp Psychology, Oxford University, Oxford OX1 3UD, U.K.

Macaques searched for a target pattern in a horizontal array of 5 patterns (1 target and 4 foils on each trial, chosen from 30 possible targets and 120 possible foils). Neglect after a unilateral lesion was measured by the failure to find targets that were contralateral to the lesion. Neglect was caused by optic tract section combined with forebrain commissurotomy (n = 6), and by parietal leucotomy (n = 3). It was not caused by posterior parietal cortical ablation, or by optic tract section alone, or by forebrain commissurotomy alone, or by posterior parietal ablation plus frontal eye-field ablation, or by frontal lobectomy plus forebrain commissurotomy (n = 3 in each group). We propose:- (1) The posterior cortex of each hemisphere maintains a retinotopically organized representation of the visible half-world that is contralateral to the animal's current point of fixation (2) This representation is based not only on analysis of the current retinal input, but also on memory (3) One of the uses to which this representation is put is the generation of contralaterally directed saccades. Neglect is not caused by hemianopia (optic tract section alone), because in hemianopia the affected hemisphere can use memories of visual inputs which arose from the then ipsilateral visual field in previous fixations to build a representation of what is contralateral to the current point of fixation. Neglect does follow, however, when hemianopia is combined with forebrain commissurotomy, even though the cortex is intact, because the affected hemisphere is then deprived of all visual information and cannot therefore maintain even a memory-based representation of the currently contralateral visible world. Neglect is also caused by parietal leucotomy, because this disconnects the posterior, retinotopically organized part of the visual cortex from its subcortical motor output. Supported by UK MRC.

VISUAL CORTEX: STRIATE I

117.1

PLASTICITY OF SYNCHRONIZATION AND OSCILLATORY MODULATION OF VISUAL RESPONSES AFTER BRAINSTEM RETICULAR ACTIVATION. S. Herculano*, M.H.J. Munk and W. Singer. Max-Planck-Institut für Hirnforschung, Deutschordenstraße 46, Frankfurt a.M. 60528, Germany.

It has been proposed that responses of neuronal populations coding for relared features are dynamically selected and bound together for joint processing by transient synchronization of their discharges. To examine the plasticity of neuronal synchronization in the visual cortex, we conditioned several simultaneously recorded units by pairing their light responses for 30-60 minutes with stimulation of the mesencephalic reticular formation (MRF). Activation of this ascending modulatory system desynchronizes the EEG, facilitates neuronal synchronization in the gamma-frequency range and enhances use-dependent synaptic plasticity.

Baseline variations of the oscillatory patterning and the correlation of multi-unit visual responses were measured in areas 17 and 18 of anesthetized adult cats over several hours of recording. The oscillatory modulation and correlation strength of visual responses varies widely over time, accompanied by changes in the power spectrum of the EEG. Activation of the MRF 100 ms prior to visual stimulation results in EEG desynchronization and strong oscillatory modulation of visual responses (14 out of 26 units). In the absence of MRF activation, the oscillation frequency of multi-unit responses can vary over a broad range (30- 100 Hz). In contrast, in responses conditioned with MRF activation, the oscillation frequency stabilizes in a range from 30 to 53 Hz (n= 5). MRF stimulation enhances the correlation of visual responses, both locally (14 out of 26 cases) and across the hemispheres (9 out of 21 pairs). Most strikingly, the enhanced correlation and oscillatory modulation persist in some cases for as long as 40 minutes after the end of MRF stimulation (local correlation, 7 out of 14; long-distance correlations, 2 out of 9 pairs). Our results suggest that the connections underlying cortical neuronal synchronization are plastic. Their modulation during attentive states, facilitating the formation of neuronal ensembles, can become long-lasting, and could therefore constitute a key mechanism in perceptual learning during development and adult life. *Supported by the Max-Planck-Gesellschaft*

117.2

INNATE MICROSTRABISMUS AND SYNCHRONIZATION OF NEURONAL ACTIVITY IN PRIMARY VISUAL CORTEX OF CATS. P.König[1,2*], P.R.Roelfsema[2], A.K.Engel[2] and W.Singer[2]. [1]The Neurosciences Institute, 10640 John J Hopkins Drive, San Diego CA 92121, USA. [2]Max-Planck-Institute for Brain Research, Deutschordenstr.46, 60528 Frankfurt, D

It has been proposed that synchronization of neuronal activity serves to integrate distributed cortical representations. Experiments in the visual system show that synchronization of neurons is stimulus specific, compatible with Gestalt laws and dependent on the functional architecture of tangential intracortical connections. Surgically induced strabismus has been shown to cause correlated changes of cortical connections and synchronization. Here we investigate the effects of innate strabismus on neuronal synchronization. Multi-unit responses to light bars and gratings were recorded under general anesthesia with multiple electrodes in area 17.

In accordance with previous results from such animals (Distler & Hoffmann, Visual Neuroscience, 6:25-41, 1991) the incidence of binocular cells was more reduced in the central than in the peripheral representation of the visual field. The incidence of synchonization was similar to that in normal cats for neurons with like ocular dominance but drastically reduced for neurons with different ocular dominance. Furthermore, in the latter the phase shift of synchronization often deviated from zero by up to 10-20ms. The incidence of oscillatory activity was similar to that found in normal cats. However, the frequency distribution shows a tendency towards the lower range, covering not only gamma but also the upper beta range.

In conclusion the alterations in cats with innate squint are qualitatively similar but quantitatively not as extreme as those in cats with surgically induced squint. This research has been supported by the NSF and the MPG.

117.3

SYNCHRONIZED GAMMA FREQUENCY OSCILLATIONS CORRELATE WITH PERCEPTION DURING BINOCULAR RIVALRY IN AWAKE SQUINTING CATS. P. Fries *, P.R. Roelfsema, A.K. Engel, P. König and W. Singer. Max-Planck-Institut für Hirnforschung, 60528 Frankfurt, Germany.

The neuronal correlates of visual perception can be discovered by producing ambiguous visual stimulation conditions. When two dissimilar images are presented to the two eyes, rivalry is experienced: At any moment only one of the images dominates perception while the other one is suppressed. What are the differences in the processing of information that lead to perceptual dominance or suppression? To address this issue we have studied cats with a surgically induced squint, a condition leading to strong rivalry due to the permanent incoherence of the information coming from different eyes. Neuronal activity was recorded from awake animals with chronically implanted wire electrodes. For visual stimulation, wholefield gratings moving in different directions were presented dichoptically. We computed auto- and crosscorrelation functions and spike triggered averages for all combinations of action potential or local field potential recordings, respectively. In primary and secondary visual cortex of these cats synchronization of action potentials was strongly enhanced between neurons encoding the dominant stimulus. In contrast, synchronization was reduced between neurons encoding the suppressed stimulus. Synchronized action potentials occurred in oscillatory patterns with frequencies of 40 to 60 Hz. The strength of the oscillations was correlated with perceptual dominance in the same way as synchronization strength. There was a strong synchronization between spikes and the 40-60 Hz component of the local field potential also showing this positive correlation to perception, while this was not the case for other frequency ranges. Interestingly, the average firing rates of the neurons did not differentiate between dominance and suppression. We hypothesize that synchronization of responses to the dominant stimulus is enhanced by locking discharges to the phase of oscillatory fluctuations of membran potential in the gamma frequency range. This enhanced oscillatory synchronization makes the dominant subset of responses more salient for coincidence detecting neurons in other areas and, thus, favours selection of these responses for processing by subsequent stages in the visual pathway.

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117.4

BINOCULAR INTERACTIONS EXHIBITED BY NEURONS IN THE CAT'S STRIATE CORTEX: NONLINEAR ANALYSIS. A. Anzai, I. Ohzawa, and R. D. Freeman*. Group in Vision Science, School of Optometry, University of California, Berkeley, CA 94720-2020

We have previously reported that binocular simple cells combine signals from the left and right eyes linearly, and then exhibit a threshold nonlinearity (Ohzawa and Freeman, 1986). In addition, we have proposed an energy model as part of a binocular disparity encoding system in which complex cells detect a stimulus energy associated with binocular disparity (Ohzawa et al., 1990). In both studies, we were not able to determine the specific nature of nonlinearities present in response patterns. These nonlinearities could be of prime importance since they may represent computations underlying binocular spatial processing. In this study, we have measured spatial characteristics of nonlinear binocular interactions exhibited by neurons in the striate cortex of anesthetized and paralyzed cats. We consider possible roles of nonlinearities in the binocular representation of three-dimensional space.

Extracellular recordings were made from single neurons. Dynamic noise patterns, generated according to binary m-sequences, were presented to receptive field (RF) locations for each eye to map left and right eye RFs and the binocular interaction RF for each cell. Responses of cells to the noise patterns were cross-correlated with the stimulus sequence to obtain each RF.

Both simple and complex cells exhibit significant nonlinear binocular interactions. Binocular interaction RFs of simple cells are well described by the product of left and right eye RFs. This is consistent with a model for these cells of a linear filter followed by a static nonlinearity. Binocular interaction RFs of complex cells are fit well by a Gabor function oriented in the fronto-parallel plane. This finding is consistent with the prediction of a binocular disparity energy model.

Since the binocular interaction RFs of simple cells are proportional to the product of left and right eye RFs, it is computationally equivalent to interocular correlation at a particular interocular phase disparity. The energy model eliminates monocular phase dependency of the simple cell output. Therefore, a population of binocular complex cells with various interocular phase disparities could compute the complete interocular correlation function which represents the distribution of stimulus energy in depth. This in turn would be useful for solving the stereo correspondence problem. (EY01175)

117.5

DISPARITY DETECTING NEURONS IN VISUAL CORTICAL AREA V1 GIVE SELECTIVE RESPONSES TO ANTI-CORRELATED RANDOM DOT PATTERNS THAT ARE IMPOSSIBLE TO FUSE PERCEPTUALLY.

B.G. Cumming, W.T. Newsome* and A.J. Parker. University Laboratory of Physiology, Parks Road, Oxford, OX1 3PT, UK

Random dot stereograms can readily be constructed in which half of the dots are white and half of the dots are black. An *anti*correlated version of the same stereogram can then be constructed by assigning a black dot in the left eye to match a white dot in the right eye and the converse. The perceptual consequences of anti-correlation are dramatic, making binocular fusion impossible (except at low dot densities). Here, we tested the responses of single neurons in V1 of awake, behaving macaque monkeys to both correlated and anti-correlated dynamic random dot stereograms. Monkeys were trained to fixate, and the binocular eye position was monitored using the scleral search coil technique. Standard (correlated) random-dot patterns generated the typical tuning functions for binocular disparity previously identified in V1. In the majority of neurons tested, anti-correlated patterns gave responses that resembled the inverse of the response to correlated patterns. Thus, tuned-excitatory cells would exhibit a tuned-inhibitory function and so on. This inversion of the tuning function is well predicted by a local filtering model for binocular combination within the cortex, but is not expected if the neurons in V1 are directly responsible for global cyclopean stereopsis.

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117.6

INCIDENCE OF OSCILLATIONS AND SYNCHRONY AS A FUNCTION OF BURSTING PROPERTIES OF MACAQUE STRIATE NEURONS. S.R. Friedman-Hill*, C.M. Gray, and P.E. Maldonado Center For Neuroscience, University of California, Davis, CA 95616

The interspike interval histograms (ISIH) of 201 well-isolated single units, from the striate cortex of macaques trained to maintain fixation, were classified by their peaks. The ISIH peaks were clustered into three categories: 1-2 ms, 3-10 ms, and 10-40 ms. These categories may correspond to "chattering" (C) intrinsic bursting (IB), and regular spiking (RS) cells, respectively. (See abstract by Azouz, et al. for details on this cell classification.)

We examined the incidence of oscillatory activity, as measured by a significant peak/d.c. ratio in the power spectrum of the autocorrelogram, in relation to the firing properties of the units. This analysis revealed that 83% of the significant autocorrelograms were C cells; the remaining units were RS.

We also looked at the incidence of significant synchronization as reflected in the cross-correlogram. For units with non-overlapping or sparsely-overlapping receptive fields (RFs), 80% of the significant cross-correlograms involved pairs of "bursty" cells (IB-IB, C-C, or IB-C). For units with largely-overlapping RFs, only 36% of the significant crosses involved pairs of bursters. All of the remaining significant cross-correlograms were the result of the pairing of an IB or C cell with a RS cell; no significant synchrony between RS-RS pairs were found.

These results indicate that bursting is an important factor in the establishment of oscillations and synchrony. Oscillations are exhibited by neurons with very high intraburst firing rates--namely, C cells. Bursting cells (IB and C) participate in all incidences of significant synchrony and pairs of bursting cells are important for long-distance synchrony.

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117.7

THE ROLE OF V1 IN SCENE SEGMENTATION AND SHAPE REPRESENTATION T.S. Lee*[1,2] and D. Mumford[3] Dept. of Brain and Cognitive Sciences[1], MIT; Div. of Applied Sciences[2] and Dept. Mathematics[3], Harvard University.

We studied the sensitivity of V1 neurons to globally-defined visual shapes in two awake rhesus monkeys. Stimuli were presented for 350 msec each trial while the monkeys fixated. The responses of 223 V1 neurons to different large figural shapes defined by texture and/or luminance contrast were analyzed at different time intervals. We found that when the figural boundaries are aligned with the orientation of the cells, there is a strong and persistent enhancement at the figural boundaries for most texture-responding neurons regardless of the orientation of the contrasting textures. The texture boundary response sharpens 80 msec after stimulus onset, later than the response to luminance boundaries. 30 % of the texture-responding cells also showed an enhanced response peak at the center/axis of the figures, suggesting a possible medial axis representation. The 'computation' of the central peaks seem to be sensitive to high-level segmentation results. Moreover, the neurons located at the center of shapes are sensitive to the distance as well as the shape of the global contours far away from the classical receptive fields. The simultaneous sensitivity of V1 neurons to multiple global perceptual variables such as the shape and the size of a figure and the location of its boundaries and symmetry axes in the later part of their responses suggests V1 is not just the first stage of visual processing, but is active throughout high as well as low visual algorithms. Any reasoning that requires high acuity, resolution and spatial specificity such as finding object boundaries and symmetry axes would refer back to V1 for image details and spatial registration. The evidence that individual cells in V1 are sensitive to many kinds of global perceptual structures suggests that different types of information might coexist or be multiplexed in the spike trains of individual neurons through concerted firings with different neuronal assemblies.

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117.8

FUNCTIONAL ARCHITECTURE OF V1, V2, AND LGN FOR PERCEPTUAL GROUPING. S. Grossberg[1]*, E. Mingolla[2], and W.D. Ross[3], Department of Cognitive and Neural Systems, Boston University, Boston, MA 02215.

A detailed neural model links the laminar and map cortical organization of V1 and V2 to context-sensitive visual percepts. This linkage suggests how cortical designs achieve the context-sensitivity characteristic of visual percepts. The model uses circuits in V1 and V2 that are homologous but of different scale to simulate neurophysiological data on spatial and orientational integration in V1 (Grosof *et al.*, 1993; Kapadia *et al.*, 1995) and long-range illusory contour formation in V2 (von der Heydt & Peterhans, 1989). Model interactions across layers 3, 4, and 6 simulate how long-range cooperative and short-range competitive interactions (Eysel, 1995; Fitzpatrick, 1995; Gilbert & Wiesel, 1990; McGuire *et al.*, 1991) generate context-sensitive perceptual groupings. Model cortical maps with recurrent excitatory and inhibitory feedback efficiently organize these interactions. Other simulations demonstrate that contours formed parallel and perpendicular to inducers have the strengths reported psychophysically (Kellman & Shipley, 1991; Lesher & Mingolla, 1993) as inducer numbers increase. Endstopped V1-LGN feedback strengthens groupings perpendicular to line ends and selects and synchronizes cortically consistent signals (Sillito *et al.*, 1994). The model shows how psychophysical measures can be used to study the balance between cortical cooperation and competition. [1, 2, 3] Supported in part by the ONR (N00014-94-1-0597, N00014-95-1-0409, and N00014-95-1-0657).

117.9

ORGANIZATION OF STRIATE VISUAL CORTEX: BALANCE BETWEEN FUNCTION AND WIRING COST

R. Everson,[1,2] D. Orbach,[1,3] E. Kaplan,[1,3] B. Knight,[1] J. Gordon*[1,4] Biophysics[1], Rockefeller University; Applied Math[2], Ophthalmology[3], Mt. Sinai Medical School; Psychology, Hunter College[4], New York, NY.

We seek to understand the functional architecture of the primary visual cortex in terms of a competition between the functions that the cortex must perform (comparing the input from neurons whose receptive fields are close) and the cost of making connections between neurons on the cortex.

To that end, the cortex was simulated as an array of "neurons" each of which has a receptive field location, an ocular preference and an orientation preference. The cost of a particular cortical configuration increases if neurons with nearby receptive fields are distant on the cortex, if neurons receiving input from the same eye are distant or if neurons with similar orientation preferences are distant. Starting from a random configuration, simulated annealing was used to find the configuration that minimizes the total cost.

Optical imaging of the intrinsic signal from the exposed cortex of anesthetized, paralyzed cats and monkeys was used to reveal the ocular dominance, orientation tuning and retinotopic maps. Flickering dartboard patterns were presented as visual stimuli to image the retinotopic map.

Simulations reproduce the retinotopic mapping of the visual hemi-field on the cortex. Optimal configurations for smaller cortical patches reveal ocular dominance columns and orientation pinwheels. In agreement with the optical imaging results, the centers of orientation pinwheels are located in the middle of ocular dominance columns. The relationship of ocular dominance columns to the retinotopic map was investigated at low and high eccentricity and compared to the simulated layouts.

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117.10

RESPONSE VARIABILITY OF CELLS IN V1 OF ALERT MONKEYS IS LOW

M. Gur*[1,2], A Beylin[1,2] and D. M. Snodderly[2,3], [1]Dept. of Biomedical Engineering, Technion, Haifa; Israel; [2]Schepens Eye Research Institute, Boston; [3]Dept. of Ophthalmology and Program in Neuroscience, Harvard Medical School, Boston,.

Response variability of single neurons limits the reliability and resolution of the sensory system. Understanding the nature and origin of this variability allows one to relate performance of single cells to models of neural circuitry and to behavioral tasks. It has been suggested that response variability increases at cortical levels thus rendering individual responses less reliable. Since anesthesia and fixational eye movements can affect response variability, we studied the question in the alert monkey with compensation for fixational eye movements.

We recorded from 83 single cells and 4 multiunit sites in all V1 layers using dye marks and/or physiological criteria to assign recording loci. In addition, 8 single parvo LGN cells were studied. All cells were stimulated with a bar of near-optimal orientation, color, and dimensions.

The relationship between response magnitude and response variance was similar in our V1 cells and LGN cells. The variance as a function of response amplitude was almost an order of magnitude lower than previously reported for V1 cells in both alert and anesthetized monkeys. The coefficient of variation was also smaller than previously reported.

We conclude that cortical cells are more reliable than previously thought and their ability to encode information is not degraded relative to cells earlier in the pathway.

117.11

THE SPATIAL EXTENT OF CONTEXTUAL RESPONSE MODULATIONS IN CAT STRIATE CORTEX.

S. Kastner*, H.-C. Nothdurft and I.N. Pigarev. Dept. of Neurobiology, Max-Planck-Institute for biophysical Chemistry, P.O. Box 2841, 37018 Goettingen, Germany.

Recently, we reported response modulations induced by moving and static texture patterns presented outside the classical receptive field (RF) (*Soc. Neurosci. Abstr., 1995).* In order to investigate the spatial organization of such contextual effects, we analyzed in the present study neuronal responses to texture rings at different distances from the RF-center in anaesthetized and paralyzed cats using standard extracellular recording techniques.

Contextual effects were strongest for texture elements presented close to the cell's excitatory RF and decreased with increasing distance from the RF-center. The spatial extent over which significant effects were seen was, in average, 4.4° for static texture rings (range: 1.4°-10.2°) and 3.9° for moving texture rings (range: 1.5°-7.8°). There was considerable cell-to-cell variability, so that no clear relationship between the spatial extent of interactive effects and the eccentricity of a cell's RF was seen (recording sites up to 12° eccentricity). Neurons exhibiting interactive effects were, on average, more strongly end-inhibited than neurons that did not show contextual response modulations.

The large spatial ranges of such effects suggest that they are mediated by long-range intrinsic horizontal connections of striate cortex. (Supported by DFG-grant No 180/8-1).

117.12

TRANSIENT AND SUSTAINED FACILITATION OF VISUAL RESPONSES BY NITRIC OXIDE IN CAT STRIATE CORTEX. P. Kara* & M.J. Friedlander. Neurobiology Research Center and Dept. of Physiology & Biophysics., Univ. of Alabama at Birmingham, 35294-0021.

We previously reported that transient local inhibition of endogenous nitric oxide (NO) production in the visual cortex of anesthetized cats reversibly reduces the number of spikes and peak firing frequency of visually-evoked responses in cortical neurons by 20-80% (Kara & Friedlander, Soc. Neurosci. Abstr., 21, 1995). We have extended these studies to include elevation of local NO levels by iontophoresis of (a) diethylamine-NONOate (D-NO), a pH sensitive NO-generating compound or (b) L-arginine (LArg), the natural substrate for the NO producing enzyme nitric oxide synthase (NOS). Using extracellular single-unit recording and microiontophoretic methods identical to that of our previous report, NOS blockade via L-nitro-arginine (LNA) or L-mono-methyl-arginine (LMMA) significantly inhibited the visual response in 37 of 58 neurons by 15-90% ($p < 0.05$, KS or *t*-tests). In 10 of 58 neurons, NOS blockade facilitated the visual response by 22-105%. The remaining 11 of 58 cells were not significantly affected. The effects of enhancing intra-cortical NO levels with either L-Arg or D-NO while simultaneously presenting optimally orientated visual stimuli in both preferred and null directions of motion were tested in 27 cells. In 9 of 27 cells, the visual response was potentiated by 34-125%. In 6 of these 9 cases, enhancement of the visual response decayed and returned to control firing levels within 5-10 minutes of cessation of the L-Arg or D-NO application. However, in the remaining 3 cells, persistent enhancement of the visual response was observed for a period greater than 20 minutes from the termination of the iontophoresis of the NO generating compound. In 2 of these 3 cells, the pairing of exogenous NO application and null direction visual stimulation unmasked a response to the previously ineffective stimulus. In 4 of 27 cells tested with L-Arg or D-NO application, the visual responses were inhibited by 20-86%. Twelve of 27 cells were not significantly affected. These findings suggest that NO might act as a synaptic amplifier, increasing the dynamic range of operation of neocortical microcircuits.

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117.13

OBSERVATION OF POSITIVE BOLD CHANGES CORRELATE TO NEURONAL DEACTIVATION DURING SACCADIC EYE MOVEMENTS. W. Chen, S.G. Kim, T. Kato, X.H. Zhu, R. Menon[+], T.M. cook*, S. Ogawa[#], K. Ugurbil, Center for Magnetic Resonance Research, University of Minnesota, School of Medicine, Minneapolis, MN55455, [#]Bell Labs, Lucent Technologies, Murray Hill, NJ 07974, *CIBA, Summit, NJ 07901, [+]Robarts Research Institute, London, Ontario, Canada.

fMRI has been extensively used for mapping of human brain activity. Most fMRI maps generated by positive BOLD response caused by the decrease of the deoxyhemoglobin concentration are considered to be correlated to regional CBF increase and the neuronal activation. However, the absence of extensive observations on negative BOLD response to tasks raises the question about the possibility of the correlation between negative BOLD effect to neuronal deactivation. It was reported by PET and clinical studies that the saccadic eye movements reduced the CBF in primary visual cortex (V1), in order to suppress neuronal activity in V1. We have used fMRI (4 tesla) to test whether saccadic eye movements cause BOLD change in V1 areas as well as CBF changes using flow sensitive MRI technique. The experiments were performed in a completely dark room and the subject's eyes were closed during both of control and task periods. A beep sound with selected frequency (from 40 min^{-1} to 120 min^{-1}) was sent to subjects during control and task periods. Therefore the auditory influence from the beep sound could be canceled by the subtraction between control and task images. Positive BOLD changes were observed in V1 during all saccade experiments. The average BOLD increase (n=8) for 120 min^{-1} saccade frequency (1.3%) is more than that for 40 min^{-1} saccadic frequency (1.0%). These results suggest for the first time that BOLD response can be positive under condition of deactivation where regional CBF appears to decrease.

117.14

TEMPORAL INTEGRATION WINDOWS FOR NEURONS IN THE FEEDFORWARD PATHWAYS OF VISUAL CORTEX J.B.Colombe* and P.S.Ulinski. Committee on Neurobiology and Dept. Organismal Biology and Anatomy, University of Chicago, Chicago, IL 60637

Recent analysis of the processing of dynamic sensory signals by neurons has focused on the range of past timescales that affect present neuron output. This range is partially determined by axonal and synaptic delays in feedforward pathways. The visual cortex of the pond turtle, *Pseudemys scripta*, is an ideal preparation for studying the contribution of axonal delays to temporal integration because the geniculate afferents form a set of parallel, horizontal delay lines across the cortex. *In vitro* studies of the geniculate pathway show that pyramidal neurons in the cortex have response latencies to retinal light pulses that depend on their positions in the cortex. We used the distribution of latencies to estimate conduction velocity at 0.01 mm/msec for geniculocortical afferents within the cortex. We could then calculate the rostrocaudal distribution of afferent delays prior to entering the cortex as 80 to 470 msec along the rostral to caudal axis of the cortex. To characterize the morphology of neurons in the path, we examined Golgi-impregnated material. Particular attention was paid to the spatial intersection of dendritic arbors with geniculate afferents. The horizontal dendrite dimensions were divided by the conduction velocity of the afferent axons to generate local windows of temporal integration, which ranged from 7 to 80 msec, depending on the type of neuron. The combination of delay and dispersed temporal integration of visual input is expected to play a major role in the processing of dynamic visual stimuli by the cortex. (JBC is supported by P32GM07839.)

GENESIS OF NEURONS AND GLIA

118.1

COUPLING BETWEEN NEOCORTICAL PROGENITORS CHANGES DURING PROGRESSION THROUGH THE CELL CYCLE. A.R. Kriegstein* K.S. Bittman, D. Olsen, L.H. Boyce, D.F. Owens, J.J. LoTurco Dept. of Physiol. & Neurobiol. University of Connecticut, Storrs 06269 and Dept. Neurol., Columbia P&S, NY, NY 10032

Throughout neurogenesis, cells in the ventricular zone (VZ) of developing neocortex are coupled together by gap junction channels. As cells leave the cell cycle and migrate out of the VZ they uncouple from VZ cell clusters. In the present study we have sought to determine the cell cycle phase of coupled cells, and whether coupling between cells in the VZ is dynamic with respect to the cell cycle. BrdU was injected into timed pregnant rats and mice 2, 8 and 16 hours before E16, at which time explants of embryonic neocortex were prepared for intracellular injection of biocytin. Biocytin injected into single cells within the VZ labeled discrete clusters of cells. A double-labeling procedure was carried out for biocytin and BrdU imunohistochemistry, and the percentage of biocytin labeled cells that were also labeled with BrdU was determined. Two-four hours following BrdU injection, 14% of biocytin labeled cells in coupled clusters were also labeled by BrdU. In contrast, eight-twelve hours following BrdU injection 47.8% of biocytin labeled cells were also labeled with BrdU. The average number of cells per cluster (45) was similar at both time points. This greater than 3 fold increase in the percentage of double labeled cells indicates that an increase in intercellular coupling occurs between S and G1 phase of the cell cycle.

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118.2

INTRACORTICAL DISTRIBUTION OF A COHORT OF CELLS ARISING IN THE PVE. T. Takahashi[1,2], T. Goto[1,2], S. Miyama[2], R. S. Nowakowski[3] & V.S. Caviness, Jr.* [1]Neurology, Massachusetts General Hospital and Harvard Medical School, Boston, MA 02114, [2]Pediatrics, Keio University School of Medicine, Tokyo 160, Japan, [3]Neuroscience & Cell Biology, UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ 08854.

Neocortical neuronogenesis occurs in the pseudostratified ventricular epithelium (PVE) at the ventricular margin of the embryonic cerebral wall. In mouse, neocortical neurons arise in the course of 11 cell cycles over 6 embryonic days. Throughout the neocortical PVE the length of G1 phase (TG1) and the Q fraction (Q: the fraction of postmitotic cells which exit the cycle) advance through an identical range of values and are the only developmentally regulated proliferative parameters. At any given moment in the course of neuronogenesis, neurons destined for different cortical layers are being formed along the lateral to medial axis of the PVE. Thus, both Q and TG1 are distributed as a lateral to medial gradient with values laterally greater than those medially. The distributions of cohorts of neurons arising over 2 hr intervals on each of embryonic days E12-E16 were determined at postnatal day 22 in medial, intermediate and lateral cortical zones of a coronal plane of the neocortex at midhemisphere. Independently of the cortical zone examined, cells arising from the first 8 of the 11 cell cycles are distributed within the infragranular layers (V-VI). This early period of neuronogenesis, when the numbers of neurons formed is relatively small, corresponds to the ascent of Q from 0 - 0.5 and a 4 fold increase in TG1. Cells arising from the last 3 cell cycles (9 through 11) are distributed within the granular (IV) and supragranular layers (II-III). TG1 and Q are the same in a zone of the PVE which is giving rise to cells destined for a specific cortical layer, whether this layer is medially or laterally in the cortex. Thus, this analysis establishes an invariant relationship in the neocortical PVE between the patterns of progression of Q, TG1 and the sequence of cell cycles, on the one hand, the cortical layer (and principal cell class) which is being formed, on the other. *Supported by NINDS grants NS12005 and NS28061.*

118.3

VIRALLY TRANSDUCED EGF-Rs MODULATE CELL FATE AND MIGRATION IN CORTICAL CULTURES. L. Lillien*[1], D. Wancio[1], and P. Levitt[2]. [1]Department of Neurobiology and Anatomy, Medical College of Pennsylvania, Philadelphia, PA 19129. [2]Department of Neuroscience and Cell Biology, UMDNJ, Robert Wood Johnson Medical School, Piscataway NJ. 08854.

The phenotypic potential of progenitor cells in the developing cerebral cortex was assessed in explants by modifying their responsiveness to TGFα/EGF with a retrovirus transducing extra copies of wild-type EGF-Rs. Previous studies with retinal progenitor cells demonstrated that this virus confers ligand-reponsiveness and increased ligand sensitivity. Introducing extra copies of EGF-Rs into cortical progenitor cells at 2 different embryonic ages had significant effects on cell fate and migration in explants. More than 70% of progenitor cells infected with EGF-R virus at E15 differentiated into astrocytes within 4 days. In contrast, 20-40% of progenitor cells infected at E12 in explants differentiated prematurely into astrocytes; instead, the probability of their expression of stem-cell properties increased over 100-fold. Analysis of the migratory behavior of infected cells revealed a ligand-dependent modulation of movement away from the ventricular edge of the explants at both ages. Differentiation was assessed further in monolayer cultures in which cell-cell signalling and normal migration were disrupted. Clonal analysis revealed that in the presence of TGFα, 60-70% of the clones expressing extra EGF-Rs from either E12 or E15 contained astrocytes after 3 days. Clone size was also increased compared to cultures grown without ligand. Both cell type choice and proliferation were therefore affected in a ligand-dependant manner in cells expressing extra EGF-Rs. The data also show that while progenitor cells infected at E12 and E15 respond differently in explants, they behave similarly under conditions in which cell-cell interactions are disrupted. These observations suggest that the fate and proliferation of progenitor cells in the early embryonic cerebral wall change in a temporally regulated fashion, but are not intrinsically restricted. They also suggest that changes in cell-cell signalling during different periods of cortical development are likely to play an important role in modulating responses to EGF-R activation.
Supported by March of Dimes.

118.4

Retrovirally mediated ectopic expression of the epidermal growth factor-receptor (EGF-R) during cortical development results in abnormal cell migration in vivo. RC Burrows*[1], L Lillien[2], P Levitt[1] Dept. of Neurosci. and Cell Biol. UMDNJ-RWJMS, Piscataway, NJ 08854[1]. and Dept. Neurobiol. Med. Col. of PA, Phila. PA 19129[2]

Our studies in vitro have shown that the EGF-R signaling system dramatically alters cell migration and phenotypic fate of cortical progenitor cells. Here, we extend these studies in vivo, using a retroviral vector that encodes the histochemical marker *β-geo* and wild-type *egf-r*, or the *β-geo* gene alone as a control. Fetuses were injected intraventricularly, *in utero*, at either embryonic day 14 (E14) or E15, and sacrificed on E18 or E20, respectively. In E18 control animals, approximately 25% of the labeled cells were in the ventricular zone (VZ), 50% in the subventricular zone (SVZ), 20% in the intermediate zone (IZ), 3% in the cortical plate (CP) and 2% in the marginal zone (MZ). In E18 EGF-R infected animals, in contrast, infected cells migrated more robustly, with only 1% of the labeled cells in the VZ, 22% in the SVZ, 50% in the IZ, 22% in the CP and 4% in the MZ. In control animals sacrificed at E20, approximately 65% of the cells remained within the proliferative layers of the developing cortex. In contrast, only 2% of the EGF-R-infected cells were found in the proliferative layers. The profound affect on migration was also evident in the CP and MZ, where only 10% of the control infected cells resided, compared to approximately 75% of the EGF-R infected cells. Comparison of the migration patterns of EGF-R infected cells at E18 and E20 showed a 2 fold increase in the number of labeled cells in the IZ and CP at E20 and a 5 fold increase in the number of labeled cells in the MZ. These results suggest that constitutive expression of the EGF-R causes cells to leave the proliferative zones and migrate faster and farther than normal. The abnormal settling patterns of the EGF-R infected cells may be associated with an altered phenotype consistent with observations in explants. This work supported by NIMH MH45507 (PL), March of Dimes (LL) and NRSA MH10777-02 (RCB).

118.5

TRANSPLANTATION TO THE NEONATAL RAT BRAIN OF CORTICAL PROGENITOR CELLS GENETICALLY MODIFIED TO OVEREXPRESS EGF-RECEPTORS. M.F. Barbe*, L. Lillien. Dept. of Physical Therapy, Temple Univ., Philadelphia, PA, 19140 and Dept. of Anatomy and Neurobiology, Medical College of Pennsylvania, Philadelphia, PA 19129.

We investigated the influence of different regions within the developing cortex in determining the fate of cortical progenitor cells with properties of stem cells. Cortical cells from embryonic day (E) 12 and E15 rat donors were marked by infection with either control virus expressing histochemical marker β-geo or the wild type human EGF-receptor (EGF-R virus), and exposed to TGF-α or EGF in culture to generate spheres (Reynolds BA, et al, 1992, J Neuroscience, Vol 12: 4565). In vitro, these spheres generated both neurons or glial cells. For transplant studies, spheres were labeled with a fluorescent marker and grafted into neonatal rat cerebral cortex. The cells integrated, survived for up to 5 weeks postsurgery, and expressed neuronal and glial markers. Overall, it was determined that over 80% of the EGF-R infected spheres (identified immunohistochemically with an antibody to the virally transduced human EGF-R) integrated into gray matter, and that a mixture of cell types developed from the EGF-R positive progenitors: many of the grafted cells expressed neuron-specific antigens (MAP2, NF200), while others expressed glial markers (GFAP, S100). Moreover, different proportions of MAP2-positive cells were observed in the different regions analyzed ranging from 30% to over 90%. Similar experiments are in progress to compare the fate and/or survival of spheres with or without virally transduced EGF-receptors in the cerebral cortex. Thus, the fate of cortical progenitor cells is in part determined by local environmental cues in vivo, which may be temporally and spatially regulated in the cerebral cortex.
Supported by Temple University (MB).

118.6

FGF2 CONCENTRATION-DEPENDENT NEURON/GLIA CELL FATE SWITCH BY MULTIPOTENTIAL NEURAL PROGENITOR CELLS

X. Qian*, A.A. Davis, S.K. Goderie, and S. Temple Dept. of Pharmacology and Neuroscience A-136, The Albany Medical College, Albany, NY 12208

To understand the factors regulating multipotential neural progenitor cell development, we examined the influence of cloned growth factors on the proliferation and differentiation of cortical ventricular zone (CVZ) cells in vitro. FGF2 elicits a dose-dependent proliferative response from CVZ cells with an ED50 of approx. 0.11ng/ml. In addition, FGF2 produces a dramatic concentration-dependent cell fate change in multipotential cells developed from E10 or E12 CVZ. In low FGF-2 concentrations (0.1ng./ml.), single CVZ cells generate proportionally more neuron-containing clones than at higher concentrations, (1-10ng./ml.), when more glial-containing clones are generated. E10 CVZ cells generate more neurons and fewer glia than E12 CVZ cells at each FGF-2 concentration tested, demonstrating a difference in responsiveness consistent with the normal timing of cell differentiation in vivo, where neurogenesis precedes gliogenesis. 40% of E10 CVZ cells responding to 0.1ng/ml of FGF-2, (when normally 80% generate neuron-only clones), can generate both neuronal and glial progeny after switching to 1-10ng/ml. FGF-2. This demonstrates that the cell fate switch results from changes in the differentiation of single multipotential neural progenitor cells, rather than selective stimulation of different neural progenitor cell classes. The neuron-to-glial fate switch exhibited by multipotential CVZ cells, along with the known FGF-2 up-regulation in embryonic cerebral cortex, suggests a mechanism for generating neurons and glia on schedule during development.
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118.7

DIFFERENTIAL REGULATION OF RADIAL GLIA AND ASTROGLIAL LINEAGE ELABORATION FROM EMBRYONIC SUBVENTRICULAR ZONE (SVZ) PROGENITOR CELLS BY LEUKEMIA INHIBITORY FACTOR β RECEPTOR (LIFβR) ACTIVATION AND BONE MORPHOGENETIC PROTEINS (BMPS).

M. F. Mehler*, P. C. Mabie, R. Marmur, and J. A. Kessler. Neurology and Neuroscience, Albert Einstein College of Medicine, Bronx, NY 10461.

The epigenetic signals and lineage intermediates involved in gliogenesis from multipotent progenitors within CNS generative zones are poorly defined. Clonal density analysis of epidermal growth factor (EGF)-generated embryonic (E17) SVZ progenitor cells demonstrates that activation of the LIFβR in concert with BMP2 or basic fibroblast growth factor(bFGF) potentiates the expression of RC2-immunoreactive radial glia with subsequent enhancement (BMP2) or inhibition (bFGF) of expression of mature astrocytes. Single cell analysis further suggests that astroglial lineage elaboration occurs through radial glial-dependent and independent lineage pathways regulated by distinct cytokines. Immunoselection using CD44, a marker selective for radial glial and early astroglial lineage species, was performed to further define the cellular targets of the differential cytokine actions. CD44-negative progenitor cells displayed selective proliferative responses to EGF and formed qualitatively distinct astroglial clones following application of LIF or BMP2. By contrast, CD44-positive cells were unresponsive to EGF or LIF, but showed identical cellular responses to the BMPs as those of CD44-negative SVZ-derived progenitor species. These observations suggest the presence of separate BMP-responsive astroglial progenitor populations that are differentially regulated by distinct early-acting cytokines and may be associated with sequential stages of neurogenesis and gliogenesis during mammalian CNS development.

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118.8

REGULATION OF NEUROGENESIS BY NEURONAL COLONY-FORMING PROGENITORS ISOLATED FROM MOUSE OLFACTORY EPITHELIUM. A.L. Calof*, J.S. Mumm, P.C. Rim & J. Shou. Dept. of Anatomy & Neurobiology & Developmental Biology Center, Univ. Calif. College of Med., Irvine, CA 92717.

In mammalian olfactory epithelium (OE), generation of olfactory receptor neurons (ORNs) takes place continuously throughout life, suggesting that a neuronal stem cell exists in this system. We have developed methods to isolate neuronal progenitor cells from OE, to identify neuronal stem cells and define conditions supporting their survival and generation of ORNs. Neuronal progenitor cells are purified by immunological panning from a neuronal cell fraction isolated from embryonic mouse OE. When grown in defined, serum-free culture, the majority of purified progenitors behave as Immediate Neuronal Precursors of the ORN lineage: they rapidly give rise to ORNs, which die shortly thereafter. However, if purified progenitors are co-cultured with stroma cells derived from olfactory turbinates, a small subpopulation of them gives rise to proliferative colonies. One morphologically identifiable subset of these colonies continues to generate both ORNs and undifferentiated progenitors as late as 7 days *in vitro*. The frequency at which such colonies arise suggests they are the clonal progeny of an undifferentiated cell which, when cultured in the presence of stroma cells, exhibits the capacity for sustained neurogenesis. This "neuronal colony forming cell" may be the neuronal stem cell of the OE. Significantly, development of neuronal colonies can be specifically inhibited by plating purified progenitors onto stromal feeder cells in the presence of a large excess of ORNs. This result suggests that differentiated neurons provide a signal that feeds back to inhibit production of new neurons by their own progenitors. *Supported by grants to ALC from NIH (DC02180), the Council for Tobacco Research (3663), and a March of Dimes/Basil O'Connor Scholar Award.*

118.9

LONG-TERM SURVIVAL OF NEW NEURONS GENERATED IN VITRO BY PRECURSORS DERIVED FROM ADULT HUMAN EPILEPTIC TEMPORAL NEOCORTEX. D. Pincus[1,2], C. Harrison[2], R. Goodman[1], D. Labar[2], R. Fraser[2], M. Nedergaard[3], S. A. Goldman[2*]. [1]Depts. Neurosurg., Columbia U. Col. P. & S., NY; [2]Neurology, Cornell Univ. Med. Col., NY; [3]Cell Biol., New York Med. Col., NY.

The adult mammalian forebrain harbors neuronal precursor cells throughout the rostral subependymal zone (SZ). In the human SZ, precursors capable of producing neurons in vitro have been harvested from the temporal lobes of adults with refractory epilepsy (Cereb. Cortex 4:576, 1994). In adult rodents, the proliferation of analogous precursor cells is promoted in vitro by FGF-2; thereafter, the survival of their neuronal daughters is supported by BDNF (PNAS 92:210, 1995). In this study, we sequentially treated adult human brain tissue with FGF-2 followed by BDNF, to promote the clonal expansion and long-term survival of precursor-derived neurons. Temporal lobe resections were divided into SZ and cortical samples, all of which were raised as explants. For the first week, half of the cultures were grown in DMEM/F12/N2/5% FBS, and the remainder supplemented with 20 ng/ml FGF-2. During this time, the cultures were exposed to ^{3}H-thymidine (^{3}H-dT) as a marker of cell division. All cultures were then switched to media containing 20 ng/ml BDNF. SZ explants derived from several of these brains generated small numbers of MAP-2$^+$ neurons, as we previously reported. In addition, *neocortical* cultures derived from at least two brains, one prepared as noted and the other as a trypsin-dissociate, were found to have generated neurons. These cells expressed MAP-2, and many incorporated ^{3}H-dT during the first week in culture. Confocal imaging confirmed that the new neurons were functional; they responded to KCl-depolarization with large increments in calcium. In the presence of BDNF, arrays of up to several dozen neurons were observed as long as 9 weeks in vitro. Thus, the adult epileptic temporal cortex may harbor residual neuronal precursors analogous to those of the SZ. These may be parenchymal precursors, or aberrant migrants with neuronal potential in epileptic subventricular heterotopias. The proliferation and survival of their neuronal daughter cells suggests the promise of generating new neurons from adult human brain tissue.

Support by the Mathers and Lookout Foundations, Hirschl Trust, and NINDS.

118.10

JAGGED DECREASES *IN VITRO* DIFFERENTIATION OF PHOTORECEPTORS IN RAT RETINA. K.L. McCabe*[1], G. Weinmaster[2], and T.A. Reh[1]. [1]Prog. in Neurobiology, Dept. of Biostructure, University of Washington, Seattle, WA, 98195. [2]Dept. Biol. Chem., University of California at Los Angeles, Los Angeles, CA, 90095-1737.

Cell-cell interactions are thought to play an important role in the development of the central nervous system. In C. elegans, Xenopus, and Drosophila, the Notch-Lin12 family of transmembrane receptors have been shown to be integral for the decision of a progenitor cell to differentiate, such that activated Notch delays progenitor cell differentiation. In Xenopus and chick retina, activated forms of Notch have been shown to decrease the number of newly differentiated neurons. A newly identified vertebrate ligand, Jagged, has been shown to activate Notch1. The presence of Jagged should decrease the number of newly differentiated neurons and increase the number of progenitor cells. Dissociated postnatal rat retinal cells were cultured with membrane prepared from Jagged transfected L cells to test the potential function of this Notch ligand. On postnatal day 2 the majority of cells generated in the retina are rod photoreceptors, while many other cell types including ganglion and amacrine cells have already been generated. Therefore, we predicted that photoreceptor differentiation should be inhibited by culturing P2 rat retinal cells on Jagged-L cell membrane. Consistent with this prediction we found a 30% decrease in photoreceptors and an 80% increase in progenitor cells. Ganglion and amacrine cell numbers were unchanged as predicted. Jagged and activated Notch may be critical in the timing and decision of progenitor cells to differentiate in the mammalian retina.

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118.11

biparous: A NOVEL BHLH GENE EXPRESSED IN NEURONAL AND GLIAL PRECURSORS IN *DROSOPHILA* A. Bush, J. Kanki*, Y. Hiromi, M. Cole. Dept. of Molecular Biology, Princeton University, Princeton, N.J. 08544

We have recently discovered a novel bHLH gene which may be involved in neuronal and glial specification. Based on its expression in progenitor cells that give rise to two types of cells, neurons and glia, we have named this gene *biparous* (pronounced bip-er-es), which means producing two offspring. *Biparous* is expressed in a dynamic and complex pattern during embryogenesis. The pattern is simplest during stage 14 and 15 when *BIPAROUS* transcript is found in cells at the lateral edge of the nervous system. These cells express neither the glial specific marker *repo* nor the neuronal specific marker *elav*, suggesting that they are progenitor cells which are not yet post-mitotic. Consistent with this, BrdU incorporation studies indicate that *BIPAROUS* positive cells are still synthesizing DNA.

Staining of embryos expressing a *biparous* promoter/lacZ fusion gene for ß-galactosidase immunoreactivity revealed a recapitulation of the late expression pattern of endogenous *biparous*. In addition, anti-ß-galactosidase staining persists beyond the time when the *BIPAROUS* transcript is no longer detectable by *in situ* hybridization. Double-labeling of these embryos for ß-galactosidase and either *elav* or *repo* revealed doubly positive cells, suggesting that the *BIPAROUS* positive cells are pre-mitotic and can generate both glial and neuronal progeny.

Preliminary misexpression studies have revealed no obvious effect on the number of neurons or glia, suggesting that *biparous* may play a role in cell specification rather than cell type determination. We are isolating strains lacking *biparous* in order to test this hypothesis.

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118.12

A NOVEL CELL-SURFACE GLYCOPROTEIN EXPRESSED BY NEURAL PRECURSOR CELLS J.Trotter* and A. Niehaus
Dept. of Neurobiology, University of Heidelberg, Im Neuenheimer Feld 364, 69120 Heidelberg, Germany.

To identify new cell-surface molecules regulating early events of myelination such as precursor cell migration and recognition of axons, we have generated monoclonal and polyclonal antibodies against cell surface glycoproteins isolated from oligodendrocyte precursor cells. As immunogen, an immortalised murine line of oligodendroglial precursor cells that ensheathes axons upon *in vivo* transplantation was used. We have identified a new cell-surface glycoprotein of 330kD expressed by a subset of proliferative oligodendrocyte precursor cells. The antigen shows a partial overlap of expression with O4 and GD3 antigens. It is down-regulated as the cells mature and express the myelin-associated glycoprotein and is not in myelin. Neurons and astrocytes do not express the antigen. The protein expresses the HNK-1 carbohydrate epitope and binds Peanut Lectin. Metabolic radiolabelling experiments with ^{35}S sulphate as well as enzmatic digestion of the carbohydrate epitopes indicate that a subpopulation of the antigen molecules carries glycosaminoglycan chains. In Western Blots of the developing mouse brain, expression of the protein is first seen at E13, peaks at P10 and is down-regulated in the adult. In addition to oligodendrocyte precursor cells, the antigen is expressed by Schwann cells and proliferative nestin-positive neural precursor cells that are found in embryonic cortex and hippocampus. The antigen may be involved in regulating precursor cell behaviour such as migration and adhesion.

Financed by the Deutsche Forschungsgemeinschaft.

CELL DIFFERENTIATION AND MIGRATION II

119.1

EMBRYONIC DEVELOPMENT OF GnRH NEURONS IN SYRIAN HAMSTERS. S.J. Berriman*, H.T. Jansen, J. Schaeffer, K. Humbaugh, and M.N. Lehman. Dept. Cell Biology, Neurobiology, and Anatomy. Univ. Cincinnati Coll. Med., Cincinnati, OH 45267.

The Syrian hamster is an important model for the study of photoperiodic and hormonal influences upon the developing GnRH neuroendocrine system (Jansen et al., Soc. Neurosci. Abstr., 1996). To define the development of the GnRH system in this species, we harvested embryos from pregnant hamsters beginning on embryonic day 11 (E0=day of mating) and each day until birth (E16), as well as on post-natal (P) days 1 and 5. Single-label immunocytochemistry was performed on thin frozen tissue sections to localize GnRH. Adjacent sections were double-labelled to visualize both GnRH and the intermediate filament, peripherin, since GnRH cells have been shown to be associated with peripherin-positive fibers in the developing mouse. At E11-E12, GnRH cells are found exiting the olfactory placode and crossing the presumptive cribiform plate into the primordial forebrain. GnRH cells and fibers are also seen within the forebrain, but no GnRH fibers are seen in the median eminence. At these ages, GnRH cells within the nasal cavity and brain are closely associated with peripherin-positive fibers that form part of olfactory and vomeronasal nerves. In addition, peripherin-positive fibers are located at base of hypothalamus. By E14, very few GnRH cells are seen in the nasal cavity, the majority being located in the septal region and preoptic area. From E15 to P5, there is very little change in the distribution of GnRH cells or fibers; few if any GnRH fibers are present in the median eminence at these ages. We are currently analyzing older animals to determine when GnRH fiber innervation of the median eminence is complete, and to see whether there is any change in GnRH cell number during this period. Supported by F32 NS09558 to SJB and NIH HD07841 to HTJ.

119.2

NEONATAL HYPOTHYROIDISM INCREASES GNRH CELL NUMBER IN SPECIFIC REGIONS OF THE ADULT MALE HAMSTER BRAIN. H.T. Jansen*, A. Khosla, S.J. Berriman, G.A. Iwamoto, and M.N. Lehman. Dept. Cell Biology, Neurobiology, and Anatomy, Univ. Cincinnati Coll. Med., Cincinnati, OH 45267 and Dept. Vet. Biosciences, Univ. Illinois, Coll. Vet. Med, Urbana, IL 61801.

Hypothyroidism affects the reproductive axis of males and females in a number of species but it is not known whether these effects are mediated via a direct action on the GnRH neuroendocrine system. The current study addressed this question in the seasonally breeding Syrian hamster using a previously validated rat neonatal hypothyroid model. In order to produce hypothyroid pups, lactating female hamsters were given 6-N-propyl-2-thiouracil (PTU) in the drinking water (0.1%) and feed (.4%). At 25 days of age male pups were weaned, allowed free access to tap water and lab chow, and housed under long-day photoperiod (14L:10D) until 5 months of age. Animals were killed and paired testes, body, and brain weights were recorded. Brains were sectioned coronally and processed for GnRH immunocytochemistry. The number of GnRH neurons (unipolar and bipolar) in every fourth section from the rostral forebrain to the mamillary bodies was counted by an investigator blind to the treatment regimen. Body and brain weights did not differ between groups. Testes weights were increased in hypothyroid animals by 26% ($p<0.001$), confirming earlier reports in rats. Compared to controls, hypothyroid animals possessed significantly greater numbers of GnRH neurons in the anterior hypothalamic area, lateral hypothalamus and mediobasal hypothalamus (overall increase of nearly 60%; $p<0.02$). This difference was seen for both unipolar ($p<0.001$) and bipolar ($p<0.05$) GnRH neurons. By contrast, the number of GnRH neurons located more rostrally in the tenia tecta, lateral septum, diagonal band of Broca, and preoptic area did not significantly differ between hypothyroid and control animals. In summary, transient hypothyroidism in the neonatal Syrian hamster increases the number of immunoreactive GnRH neurons located in caudal regions of their normal distribution. This may be due to either an increase in GnRH gene expression, or the interruption of a normal process of GnRH cell loss, in these regions. Supported by NIH HD07841.

119.3

ANALYSIS OF NASAL MIDLINE CUES WHICH INFLUENCE AXONAL OUTGROWTH AND LHRH CELL MIGRATION IN OLFACTORY EXPLANTS. S. Wray* and S. M. Fueshko. Lab of Neurochemistry, NINDS, NIH, Bethesda, MD 20892.

During development, luteinizing hormone releasing hormone (LHRH) neurons migrate from the olfactory epithelium into the diencephalon in association with a bilateral subset of axons of the olfactory complex. It is likely that guidance cues on cellular surfaces and/or substrate pathways are involved in directing outgrowth of olfactory axonal fibers, and subsequent neurophilic migration of LHRH neurons. Utilizing an *in vitro* explant system, we are examining cell surface mechanisms involved in both of these events. Previously, we showed that inhibition of N-glycosylation dramatically altered olfactory axonal outgrowth. Our results suggested that a midline-localized, N-glycosylated signal(s) directs outgrowth and/or turning of axons of the olfactory complex. To assay for directional cues from midline nasal structures, we have removed midline tissue or reoriented olfactory pit-midline relations in explant cultures. In unilateral olfactory pit cultures containing negligible midline tissue, peripherin axons and NCAM axons (two markers for the olfactory axon complex) exhibited oriented and directional outgrowth, and LHRH neuronal migration occurred. These results indicate that by E11.5, appropriate cue(s) for initial olfactory axon outgrowth are already established, or maintained, in explanted, unilateral olfactory pits. Currently, manipulations of the orientation and relation of the cartilagenous midline tissue to the olfactory pit are in progress to determine whether the midline region provides signals for the bilateral turning of olfactory axons towards the developing brain. Supported by NINDS, NIH Intramural Program.

119.4

OLFACTORY PLACODE DERIVED GALANIN NEURONS: A SUBPOPULATION OF LHRH CELLS? S. Key, Veeranna* and S. Wray. Lab. of Neurochemistry, NINDS, Bethesda, MD 20892.

Postnatally, a subpopulation of LHRH neurons has been shown to express galanin. Prenatally, LHRH neurons originate from the olfactory pit (OP) and during development migrate through nasal regions into the forebrain. Whether LHRH neurons co-express galanin outside the CNS is unknown. Thus, the spatiotemporal pattern of cells expressing mRNA encoding galanin and the relation of these cells to LHRH neurons was investigated in prenatal mice using *in situ* hybridization histochemistry (ISHH). Cells containing galanin mRNA were detected at E10.5 in the OP. At this age, no LHRH cells were detected. At E11.5, LHRH mRNA, like galanin mRNA, was expressed in cells in the OP. A day later, at E12.5, both galanin cells and LHRH cells dramatically increased in number. At this stage, galanin expressing cells and LHRH cells were located in nasal tracks extending from the OP to the telencephalon. However, galanin expressing cells were fewer in number, and showed decreased expression compared to LHRH cells in tracks or galanin cells in the OP. Thus, a subpopulation of cells expressing galanin showed a similar spatiotemporal pattern during development to that of LHRH cells. The location and apparent migration of these galanin cells suggest either co-localization or directly adjacent cell populations. We are currently using double labeled ISHH to test the hypothesis that all or a subpopulation of migrating LHRH neurons co-express galanin. Supported by NINDS, NIH intramural program.

119.5

ROLE OF *PAX-6* GENE IN LHRH AND OLFACTORY SYSTEM DEVELOPMENT STUDIED IN *SEY* MOUSE PHENOTYPE. T.L. Dellovade*, D.W. Pfaff, and M. Schwanzel-Fukuda Lab. of Neurobiology and Behavior, The Rockefeller University, New York, NY 10021.

Pax-6 is a member of a murine family of developmental control genes important for CNS development. A point mutation in the *Pax-6* gene is responsible for the Small-Eye (*Sey*) phenotype in mice. Homozygotes (*Sey/Sey*) lack both eyes and nasal structures, and die neonatally because they can not breathe. Heterozygotes (*Sey/+*) have a variety of eye abnormalities, and we recently found that both main olfactory bulb volume and mucosal surface area are decreased compared to wild-type litter mates by postnatal day 42. However, there was no effect on either the vomeronasal organ or accessory olfactory bulbs. In this study we examined the effect of this *Pax-6* point mutation on LHRH neuronal migration and olfactory system development in *Sey/Sey* and *Sey/+* embryos. *Sey/+* females were time-mated with *Sey/+* males and embryos were collected from day 10 postconception through postnatal day 1. Paraffin sections were examined for LHRH immunoreactivity in combination with NCAM, L1 and S-100 immunoreactivity. In *Sey/Sey* embryos the olfactory placodes failed to develop and there were no LHRH immunoreactive neurons observed in either "nasal regions" or in the brain at any age. In contrast, there does not appear to be any effect on LHRH migration or prenatal olfactory bulb development in *Sey/+* embryos. These data confirm the olfactory placode orgin of the LHRH neurons and suggest marked specificity of the role of *Pax-6* during olfactory system development. This research is funded by NIH grant NS 09632-01A1 (T.L.D.).

119.6

VASCULOGENESIS IN THE NASAL MESENCHYME OF THE EARLY HUMAN EMBRYO DURING LH-RH NEURONAL MIGRATION. M. Schwanzel-Fukuda*, D.W. Pfaff and T.L. Dellovade. The Rockefeller University, New York, NY. 10021.

In human embryos, at about 28 days, we have noted an extensive and distinct focus of vascular development in the nasal mesenchyme between the epithelium of the newly-formed olfactory pit and the developing forebrain. Neural cell adhesion molecule (N-CAM)-ir cells are seen in the epithelium of the olfactory pit at this age. These "pioneer" N-CAM-ir cells migrate out of the epithelium of the olfactory pit (Schwanzel-Fukuda et al., 1992; JCN; 321:1-18) and in close association with the developing blood vessels form a "cellulovascular aggregate" or strand either side of midline in the nasal mesenchyme between the olfactory pit and the anlage of the forebrain. This "cellulovascular aggregate"not only receives the ingrowing N-CAM-laden central processes of the olfactory, vomeronasal and terminal nerves but also by day 42 forms a scaffold along which the luteinizing hormone-releasing hormone (LH-RH)-ir neurons can migrate into the brain. The LH-RH cells, together with the vomeronasal and terminal nerves, originate from the medial part of the olfactory pit (Schwanzel-Fukuda et al., 1996, JCN; 366:547-557). The basal lamina of the rostral-medial forebrain, which is in contact with the "cellulovascular aggregate", is breached by a number of ingrowing blood vessels. The LH-RH neurons characteristically enter the forebrain along with these blood vessels. Therefore, the developing cerebral vasculature in this region may provide an important structural support facilitating LHRH neuronal migration into the brain. Supported by NIH grant DC 00880 (M.S.-F.)

CELL DIFFERENTIATION AND MIGRATION III

120.1

MIGRATION OF CEREBELLAR GRANULE CELLS WITHIN THE INTERNAL GRANULAR LAYER. H. Komuro* and P. Rakic, Section of Neurobiology, Yale University School of Medicine, New Haven, CT 06510.

In the developing cerebellum, granule cells migrate from their place of origin in the external granular layer, through the expanding molecular layer along Bergmann glial processes until they enter the internal granular layer (IGL). However, upon entering the IGL and detaching from the Bergmann glial processes, a subset of granule cells does not stop movement, but rather continues to move within the IGL. Here, we examined the migrating behavior of granule cells within the IGL, and the role of intracellular calcium ion as a messenger for regulation of the rate and cessation of their movement. Sagittal and coronal cerebellar slices from 7-13 day-old mice (CD-1) were labeled either with carbocyanine dye (DiI, 4 μg/ml) for monitoring cell movement, or with a mixture of Fluo-3, AM (3 μM) and Fura-Red, AM (4 μM) for ratiometric measurements of Ca^{2+} fluctuations using laser-scanning confocal microscopy. Dual-emission images were detected simultaneously at 540 ± 15 nm for Fluo-3 and >600 nm for Fura-Red. We found that a number of cells in the IGL displayed bipolar shape with voluminous leading and thin trailing processes characteristic of migrating neurons. The majority (~75 %) of these cells moved toward the deep strata of the IGL at an average rate of 10.1 μm/hr. This rate is slightly lower than the movement of granule cells in the molecular layer. Interestingly, about 20 % of cells moved tangentially, parallel to Purkinje cell layer, at a similar average rate (9.7 μm/hr). However, about 4 % of cells migrated in the opposite direction, toward the upper strata of the IGL at an average rate of less than 5 μm/hr. Fluo-3/Fura-Red ratio images revealed that all migrating cells in the IGL exhibit fluctuations of intracellular Ca^{2+} levels during their movement, similar to those observed in migrating granule cells in microexplant cultures. The Ca^{2+} fluctuations may potentially be regulated by contact with the afferent mossy fiber system, and thereby provide a signal for cessation of movement. Thus, the final stage of migration may play an important role in the functional specification of granule cell. (Supported by NS22807 and NS14841)

120.2

GRANULE NEURON MIGRATION IS RETARDED IN THE DEVELOPING CEREBELLUM OF MICE LACKING THE TISSUE PLASMINOGEN ACTIVATOR GENE. N. W. Seeds* and S. P. Haffke. Dept. Biochem/Biophys/Genetics, Univ. Colorado H.S.C., Denver, CO 80262.

In the developing cerebellum, granule neurons migrate from their site of origin in the external granule cell layer(EGL) through the molecular and Purkinje cell layers to find their niche in the internal granule layer(IGL). We have shown that tissue plasminogen activator (tPA) gene expression is turned-on as these granule cells begin to migrate from the EGL. tPA mRNA expression and tPA protein levels are highest in granule neurons as they migrate through the molecular and Purkinje cell layers. However, tPA gene expression is down-regulated after granule cells reach the IGL. To explore the role of tPA in granule cell migration, we have compared the number of granule cells in the molecular layer during the second postnatal week in mice containing null mutations in the gene encoding tPA and their wild-type counterparts. As reported (Nature 368:419,1994) these null mutants have a normal phenotype. Cerebellar weight and folia size were the same in both mice. Similarly, DNA levels and total cell number showed no difference between the two types of mice. However, an analysis of migration in more than thirty mice consistently showed two-fold more migrating granule neurons in the molecular layer of tPA null mutants at postnatal days 7, 10 & 13 than in wild-type mice or in urokinase null mutants. Migration was complete during the third postnatal week in all mice. ^{3}H-thymidine birthdating also showed a two-fold increase in labeled cells in the molecular layer on each of these days. These findings suggest that the absence of tPA protease leads to a retarded rate of granule neuron migration. (Supported by NIH grant NS-09818)

120.3

TARGETED DISRUPTION OF ASTROTACTIN, A GENE THAT FUNCTIONS IN CNS NEURONAL MIGRATION ALONG GLIAL PROCESSES G. P. Dietz*, C. Zheng and M. E. Hatten, Lab. of Developmental Neurobiology, The Rockefeller University, New York, NY 10021.

The directed migration of young neurons along a system of glial fibers is fundamental to the establishment of the architectonics of cortical regions of the vertebrate brain. The neuronal gene *astrotactin* functions in migration of neurons along glial fibers, by providing a ligand for neuronal binding and a signal that maintains glial differentiation. In *in vitro* studies, antibodies raised against the protein prevent the formation of stable neuron-glia contacts and migration of neurons along glial cells. *Astrotactin* is expressed by neurons in the CNS that migrate on glial processes or assemble in neuronal layers, including the cerebellar cortex, cerebrum, hippocampus and the olfactory bulb. In the cerebellum, *astrotactin* expression is highest between postnatal day 2 (P2) and P10, the time when massive migration of granule cell precursors from the external germinal layer (EGL) to the internal granular layer occurs. *Astrotactin* encodes a protein with an apparent molecular weight of 100 kD, containing 3 EGF and 2 fribronectin III repeats. The unique structure and expression pattern of *astrotactin* and its activity in *in vitro* studies suggest that the *astrotactin* gene plays an interesting role in the development of the central nervous system. To further examine the function of Astrotactin, a null mutant will be generated by targeted disruption of the gene. 2.8 kb of the locus containing the putative translation start codon were replaced with a neomycin resistance gene by homologous recombination in ES cells. Positively targeted clones were injected into blastocysts and used to generate chimeric mice. Chimeras in which the mutant clone contributed to 90% of the coat are bred for germline transmission of the ES cell genotype.(Supported by NIH Grant NS 15429 awarded to MEH.)

120.4

CONTROL OF TAG-1 EXPRESSION IN DEVELOPING CEREBELLAR GRANULE NEURONS. M.E. Dandenault*, R.W. Stottmann, and R.J. Rivas. Dept. of Zoology, U. of Maryland, College Park, MD 20742.

The GPI-anchored cell adhesion molecule, TAG-1, is transiently expressed during the initial stages of axon outgrowth of a number of CNS neuronal cell types. In developing postnatal cerebellar cortex, TAG-1 is expressed on granule cell parallel fiber (PF) axons in the deep external granule layer (EGL); TAG-1 positive axons sequentially accumulate over older PF axons in the molecular layer (ML) that no longer express TAG-1 (Yamamoto et al., 1986. *J. Neurosci.* 6: 3576-3594). Maturing PF axons form synapses with Purkinje cell dendrites in the ML. To determine the spatial relationship between TAG-1 expressing PF axons and Purkinje cell dendrites, sagittal sections of P2, P5, P10, P15, and P20 rat cerebellar cortex were double stained for TAG-1 and the Purkinje cell specific marker, calbindin, using indirect immunofluorescence. Confocal microscopy revealed that the layer of TAG-1 expressing axons in the deep EGL was localized above the furthest superficial extent of the Purkinje cell dendritic tree at all ages examined. To test if increased synaptic activity could turn off cell surface TAG-1 expression, purified P4-P6 mouse granule cells were cultured in serum-free medium containing control (5 mM) or chronically-depolarizing (25 mM) levels of KCl. Cells were immunostained for TAG-1 at 2 or 4 days in vitro (DIV). At 2 DIV, TAG-1 appeared to be expressed at equivalent levels on both control and chronically depolarized cells. At 4 DIV, control cells continued to express TAG-1. Chronically depolarized cells at 4 DIV showed higher rates of survival and more extensive neurite outgrowth than did control cells, yet showed a striking decrease in cell surface TAG-1 expression as compared to controls. Thus, the onset of synaptic activity between PF axons and Purkinje cell dendrites in the ML could provide a signal to turn off TAG-1 expression at the deep EGL/ML junction. Supported by American Paralysis Association grant RB1-9501.

120.5

MIGRATION OF GRANULE NEURONS IN CEREBELLAR ORGANOTYPIC CULTURES IS IMPAIRED BY METHYLMERCURY. M. Kunimoto, H. Asou#* and T. Suzuki. Environ. Health Sci. Div., Natl. Inst. Environ. Studies, Tsukuba, Ibaraki 305, and #Tokyo Metropolitan Inst. Gerontol., Itabashi, Tokyo 173, Japan.

To examine whether abnormal migration of granule neurons during cerebellar development is in part of the etiology of Minamata disease, organotypic culture of rat cerebellar slice was established according to the method of Tanaka *et al.* (*Brain Res.* 641, 319-327 (1994)). External granule-layer neuroblasts were pulse-labeled with bromodeoxyuridine (BrdU). Incubation of cultures in the presence of methylmercury (1.0 - 10 μM) resulted in dose-dependent inhibition of labeled neuroblast migration toward the internal granule layer. At 10 μM methylmercury neuroblast migration was inhibited almost completely. Moreover, indirect immunofluorescence using anti-BrdU antibody disclosed nuclear condensation typical of apoptosis in a large fraction of methylmercury-treated cells. Thus methylmercury inhibits the migration of cerebellar granule neurons in a model system for neural development. As demonstrated previously, methylmercury induces neuron apoptosis (M. Kunimoto, *Biochem. Biophys. Res. Comm.* 204, 310-317 (1994)), a phenomenon which now appears associated with methylmercury impairment of granule neuron migration. (This work was supported by the Grant for Encouragement of Promising Scientists from Natl. Inst. Environ. Studies, 1994 & 1995.)

120.6

CR-50 ANTIGEN, A REELER GENE-RELATED MOLECULE, IS RESPONSIBLE FOR THE ARRANGEMENT OF PURKINJE CELLS. T. Miyata*†‡, K. Nakajima†, K. Mikoshiba†‡, and M. Ogawa. Dept. Physiol., Kochi Med. Sch., Kochi 783; †Mol. Neurobiol. Lab., RIKEN, Ibaraki 305; ‡Dept. Mol. Neurobiol., IMSUT, Tokyo 108, JAPAN

To investigate the lamination mechanism in the developing cerebellum, we focused on an immunologically discovered molecule (CR-50 antigen) absent in reeler mice, in which morphological abnormalities occur in several brain regions, especially in the cerebellar cortex. This antigen is recognized by a monoclonal antibody (CR-50) produced by immunizing reeler mice with brains of normal mouse embryos, and transiently detected in developing brain regions where morphological abnormalities have been reported in this mutant (Ogawa et al. Neuron 14: 899, '95) and the mRNA of reelin, the candidate reeler gene, is expressed (D'Arcangelo et al. Nature 374: 719, '95). In the cerebellum, CR-50 antigen is produced during the period from E13 to P14 by non-Purkinje neurons, including early born neurons which subsequently form deep nuclei and late generated granule neurons. They present it extracellularly on their somas and processes that consist of superficial cortical structures, such as the nuclear transit zone and external granule layer along which Purkinje cells are aligned.

Further functional assays with E13 cerebella cultured as explants in collagen gels yielded the following findings: 1) Purkinje cells' distinct arrangement patterns between the in vivo cerebella of normal and reeler mice, i.e., multilayers vs. clusters recognized by P1-2, were almost reproduced by 7 day in vitro. 2) When exposed to CR-50, normal-derived Purkinje cells were forced to be clustered. 3) When reeler explants were covered with granule cells purified from normal mice and presenting CR-50 antigen extracellularly, Purkinje cells were aligned along the artificial granule cell layer, but remained clustered when granule cells for overlays were prepared from reeler mice. These blocking/rescue experiments in vitro suggest that the extracellular CR-50 antigen is crucial for the arrangement of Purkinje cells. [Supported by Ministry of Education, Science, Sports and Culture of Japan, and Science and Techinology Agency of the Japanase Government.]

120.7

REELIN IS AN EXTRACELLULAR MOLECULE THAT CONTROLS THE LAMINAR ORGANIZATION OF THE BRAIN. G. D'Arcangelo and T. Curran*. Department of Developmental Neurobiology, St. Jude Children's Research Hospital, Memphis, TN 38105.

Reeler (rl) is a mouse autosomal recessive mutation that causes impaired motor coordination, tremors and ataxia. This behavioral phenotype results from widespread disorganization of the cerebellar and cerebral cortices and other laminated regions of the brain. In our laboratory, a transgenic rl allele, rl^{tg}, was generated by insertional mutagenesis[1]. We cloned rl locus regions flanking the transgene, and screened them for coding sequences by exon trapping. A cDNA library was probed with trapped exons and a full-length clone, named *reelin*, was produced by a combination of library screening and PCR RACE techniques[2]. Coding regions of *reelin* are partially deleted in both the rl and rl^{tg} mutant strains, resulting in the lack of mRNA expression. The predicted Reelin protein (388 Kda) contains a signal peptide and several features of extracellular proteins, such as EGF-like repeats and potential glycosylation sites. The N-terminus is similar to F-spondin, a protein secreted by the floor plate that is involved in neuronal adhesion. In the embryonic cerebral cortex, *reelin* is expressed selectively by the Cajal-Retzius cells, the earliest-generated neurons that occupy the marginal zone. In the postnatal developing cerebellum, *reelin* is expressed by granule neurons. Other cell types express *reelin* at specific stages of development in many brain structures. The mechanism by which Reelin controls neuronal migration during development of each of these structures is not yet understood. To address this question, we have generated recombinant plasmid constructs encoding full-length or truncated Reelin. The biochemical and functional properties of recombinant and native Reelin will be analyzed using in vitro systems. Hopefully, these studies will shed light on the molecular events that control neural cell migration.

1. Miao, G.G. et al. *Proc Natl Acad Sci U S A*, 91 (1994) 11050-11054.
2. D'Arcangelo, G. et al. *Nature*, 374 (1995) 719-723.

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120.8

DISRUPTION OF HIPPOCAMPAL DEVELOPMENT IN VIVO BY A CR-50 MONOCLONAL ANTIBODY, WHICH RECOGNIZES CAJAL-RETZIUS NEURONS. K. Nakajima[1]*, K. Mikoshiba[1,2], T. Miyata[1,2,3], C. Kudo[1], and M. Ogawa[3]. [1]Molecular Neurobiology Laboratory, The Institute of Physical and Chemical Research (RIKEN), Ibaraki 305, Japan; [2]Department of Molecular Neurobiology, Institute of Medical Science, University of Tokyo, Tokyo 108, Japan; [3]Department of Physiology, Kochi Medical School, Kochi 783, Japan.

The events of hippocampal development have been described extensively in various species. However, little is known about the molecular mechanisms. The *reeler* mutant mouse provides a good model to approach this problem, because in these mice neurons are aligned abnormally in various cortical structures including the hippocampus, although the neurons are generated normally. In the hippocampus of *reeler*, the pyramidal neurons are known to be less densely packed and, most prominently, the stratum pyramidale of the regio superior is interrupted by cell-poor rifts resulting in irregular and fragmented cell laminae.

We recently obtained alloantibodies by immunizing *reeler* mutants with normal embryonic brains. The monoclonal alloantibody, CR-50, specifically recognizes Cajal-Retzius neurons in the neocortex of normal embryos but not those of *reeler* embryos. Here we show that the epitope of CR-50 is localized on Cajal-Retzius neurons also in the hippocampus and that intraventricular injection of CR-50 at the embryonic stage disrupts the organized development of the hippocampus in vivo. Thus, Cajal-Retzius neurons play a crucial function in hippocampal development and the CR-50 epitope plays a central role in this function. This is the first demonstration of the role of the CR-50 epitope in the *reeler* phenotype in vivo.

Supported by Science and Technology Agency of the Japanese Government, and the Ministry of Education, Science, and Culture of Japan.

121.1

IDENTIFICATION OF GENES WITH DISTINCT SPATIAL EXPRESSION PATTERNS IN THE DEVELOPING RAT CEREBRAL CORTEX. A. Okada, M. E. Levin and S. K. McConnell*. Dept. of Biological Sciences, Stanford University, Stanford, CA 94305.

Transplantation studies of cortical progenitor cells have shown that early and late cortical progenitor cells have different fate potentials: early progenitors are multipotent and can give rise to neurons of all layers of the cortex depending on their environment at the time just prior to their last division in the ventricular zone (VZ), while progenitor cells from late stages of development are restricted to upper layer fates. We have applied differential screening techniques (differential display PCR) on VZ and subventricular zone (SVZ) cells of the developing cerebral cortex at early (E14) and late (E19) stages in an effort to identify genes that might distinguish progenitor cells with different fate potentials. In the process of our screen, we isolated six cDNAs whose expression patterns intriguingly correlate with the spatial position of the two mitotic zones (VZ, SVZ) or newly differentiated cells. Three of the genes are expressed strongly in the VZ but not SVZ, and are not expressed or expressed at relatively low levels in other regions of the developing brain; one of these genes is the rat homologue of the p190-B gene, encoding a putative GTPase activation protein. Two genes are expressed specifically in the SVZ but not VZ, and the expression of one appears to correlate with the position of cells that migrate into the olfactory bulb. The rat homologue of NeuroD was also isolated as a gene expressed in the intermediate zone of the developing cortex. We are currently interested in further characterizing these genes. Supported by American Cancer Society Post-doctoral Fellowship PF B77942 and a McKnight Scholar Award.

121.2

EFFECT OF ECTOPIC EN-2 EXPRESSION IN PURKINJE CELLS ON DEVELOPMENT OF THE CEREBELLUM. S. Sanlioglu-Crisman and J. Oberdick*. Department of Cell Biology, Neurobiology and Anatomy and the Neurobiotechnology Center, The Ohio State University, Columbus, OH 43210.

Mammalian homologs of several Drosophila segment-polarity genes appear to be critically important for early cerebellar development, including the genes *wnt-1*, *en-1*, and *en-2*. Here we describe preliminary observations of En-2 overexpression in the cerebellum using the Purkinje cell-specific promoter, *pcp-2*(L7). Nine transgenic lines were obtained. The two highest copy number founders, and their offspring, show a mild ataxia which is detectable after postnatal day 20. Both lines show a decrease in cerebellar mass of 30-40% by at least P15 (the earliest time point examined to date), with no effect on the rest of the brain. This loss in mass is accompanied by a 15-25% decrease in size along both the rostrocaudal and dorsoventral axes; there is little change along the mediolateral axis. The decrease in size is especially apparent in the vermis, but seems to affect all cerebellar regions. Foliation is only subtly affected. In particular, folium X appears slightly larger in absolute terms than in wild-type controls and is clearly much larger relative to all other folia, which are rather uniformly decreased in size. In addition, folium IX is no longer distinguished by division into two subfolia on its ventral aspect. These latter observations suggest that some folium IX cells have been subsumed into folium X. Since Purkinje cell density and arrangement appears normal overall, some number of these cells must be lost during development to account for the decrease in cerebellar size. This is consistent with a rather substantial decrease in the density of synaptic terminals in the deep nuclei. The precise period of Purkinje cell loss and the effect on cerebellar sagittal banding in this transgenic mutant remain to be determined. These observations support the view that the *en* genes are part of a developmental switch that must be deactivated in Purkinje cells during late embryogenesis for normal cerebellar development to ensue. (Supported by NSF #IBN-9309611).

121.3

REGION-SPECIFIC GENES EXPRESSED IN THE PRESUMPTIVE GLIAL PRECURSOR CELLS IN THE EMBRYONIC RAT BRAIN. Y. HATANAKA*, Lab. for Neural Systems, Frontier Res Program, The Inst of Phys & Chem Research, RIKEN. Wako, Saitama, 351-01, Japan

Recently, two types of cDNA fragments termed M1 and M2, expressed in the dorso-medial telencephalon, have been isolated from the embryonic day (E)13 rat by the use of mRNA differential display method. In the present study, their expression in mid- to late-gestation embryo was analyzed in detail by in situ hybridization to clarify what kind of cells are expressing these region-specific genes. M1 expression was observed in a most ventral portion of the hippocampal rudiment with a sharp dorsal boundary from E13 through E17. Its expression domain corresponded to a region lacking neuron-specific class III beta-tubulin immunoreactivity, suggesting that the germinal matrix producing fimbrial glia expresses M1 gene. M2 expression in E13 telencephalon was confined to a medial cerebral wall, including the presumptive septum and hippocampus with a more diffuse dorsal boundary than that of M1. At the same stage, its expression was also observed in a most anterior part of the medial diencephalon with a caudal extension along the dorsal midline and in the zona limitans intrathalamica. At E15, M2 expression was observed in axon tracts, including stria terminalis, stria medullaris, mammillothalamic tract and habenulopeduncular tract. At E19, its expression was also apparent in cells surrounding the hippocampal commissure and fimbria, as well as in migrating cells in these tracts. These results suggest that certain types of glial and glial precursor cells, which are located initially in presumptive axon tracts and then migrate away from their original locations, express M2 gene.

121.4

RETINOIDS UP-REGULATE HOX GENE EXPRESSION IN NOVEL TERRITORIES OF THE HINDBRAIN AFTER THE FORMATION OF RHOMBOMERE BOUNDARIES. J.C. Glover*, F. Hoover, and K. Røhne. Dept. of Anatomy, Univ. of Oslo, 0317 Oslo, Norway

We have previously described the expression of retinoid X receptors in the hindbrain of the chicken embryo over a protracted period of development that extends well into the stages of neurogenesis and neuronal differentiation. To assess the role these receptors might play at such late stages, we have exposed chicken embryos to 9-cis retinoic acid (9-cis RA) under controlled conditions in an in vitro system. We focus here specifically on the effects on expression of the Hox B1 gene.

Exposure to 10-7M 9-cis RA led to an up-regulation of Hox B1 expression in the hindbrain. The up-regulation occured within 2 hours and was manifested by an increase in the intensity of in situ hybridization signal in all the normal regions of expression characteristic of each stage of development. It could also be demonstrated quantitatively using RNase protection assays on whole embryo tissue extracts.

The normal Hox B1 expression pattern is dynamic: it is initially continuous from hindbrain rhombomere (r)4 into the spinal cord but eventually diminishes below the level of detectability in r5 and r6, leaving a conspicuous stripe in the the domain of r4. In addition to up-regulating Hox B1 expression within this normal pattern, 9-cis RA up-regulated expression in specific domains within more rostral rhombomeres, where Hox B1 expression has never been detected previously. This indicates that the normal segmental pattern of Hox gene expression in the hindbrain is not strictly autonomous, but is subject to the effects of exogenous retinoids. Supported by Jahres and Nansens Funds.

121.5

CADHERIN-8 EXPRESSION IN SEGMENTAL AND FUNCTIONAL SUBDIVISIONS OF THE DEVELOPING MOUSE BRAIN. C. Redies* and K. Korematsu. Max-Planck-Institute for Dev. Biology, Spemannstrasse 35/II, D-72076 Tübingen, Germany.

We cloned full-length cDNA of a novel mouse cadherin (mCad8) which is highly homologous to human cadherin-8. The expression of mCad8 was studied by in situ hybridization. Results show that mCad8 is expressed in the CNS and thymus. At embryonic day E12.5, expression in the CNS is confined to particular embryonic subdivisions, e.g., the ventral thalamic neuromere and the cortical plate. Moreover, in the rhombencephalon, two large groups of mCad8-expressing cells are seen which form the anlagen of the pontine and facial nuclei at E14. From E16 to postnatal day P6, expression is restricted to particular developing brain nuclei and cortical areas in all major subdivisions of the CNS. The cerebellar cortex shows parasagittal stripes of mCad8 expression in Purkinje cells. In cerebral cortex, expression becomes restricted to limbic areas at P6. The mCad8-positive neuroanatomical structures can be grouped according to their known functional connections. They form particular parts of the limbic system, the basal ganglia and related nuclei, and the cerebellum and related nuclei.

These observations suggest that mCad8 plays a role in brain segmentation, in the morphogenesis of brain nuclei, in the functional regionalization of gray matter, and in the formation of neuronal circuitry. The results support the idea that cadherins are a family of molecules whose ontogenetic expression reflects the neuromeric organization of the early embryonic brain and functional neuroanatomy of the mature brain (see Redies, *Exp. Cell Res.* 220:243, 1995).
(Supported by the Max-Planck-Society and the Land Baden-Württemberg.)

121.6

A NOVEL bHLH PROTEIN EXPRESSED IN DISCRETE DOMAINS OF THE DEVELOPPING RAT NERVOUS SYSTEM P. Ravassard, F. Chatail, J. Mallet* and C. Icard-Liepkalns.
LGN CNRS (UMR C9923), Hôpital de la Pitié Salpêtrière, 75013 PARIS, France

Here we report the isolation of a rat cDNA which encodes a new bHLH protein of 204 amino acids. The bHLH domain of this protein shares high identity with the bHLH domain of the proneural gene products Mash1, NeuroD and *atonal*. We show, by electrophoretic mobility shift assay, that this protein binds specifically a consensus E-box (CAGGTG). Using a transient expression system in the PC12 cell line this protein can transactivate in a dose dependant manner the transcription from an E-box containing promoter.
Using *in situ* hybridization experiments we show that this transcript is expressed in the nervous system during development. Transcripts are detected in E11.5- E19.5 embryos in the diencephalon and from the anterior part of the hindbrain all the way down the spinal cord. In the spinal cord, transcripts appear as bilaterally symmetrical stripes extending from the lumen to the lateral margins of the ventricular zone at two dorsoventral levels: just below the dorsoventral midpoint of the spinal cord and lateral to the floor plate. The latter stripe stops at the anterior part of the spinal cord whereas the former ends at the anterior part of the hindbrain. In the diencephalon the transcripts are detected in the ventral region of the third ventricule with the strongest expression in the caudal part of the diencephalon.
The discrete expression domains of this new bHLH protein do not correspond to the expression patterns of any known protein but overlap those of other proteins implicated in the regulation of neural cell fate. Taken together these data participate to the understanding of the molecular mechanisms regulating neural development.
(Supported by CNRS, Rhône-Poulenc-Rorer, Institut de formation supérieure biomédicale and Institut de recherche sur la moelle épinière)

121.7

INSERTIONAL MUTATIONS THAT AFFECT CRANIOFACIAL DEVELOPMENT IN THE ZEBRAFISH EMBRYO

T. Becker*, M. Allende, N. Gaiano, A. Amsterdam, K. Kawakami and N.Hopkins.
Center for Cancer Research, MIT, Cambridge MA 02139.

Organization of the zebrafish head involves formation of the cartilaginous pharyngeal skeleton, which is subdivided into mandibular and hyoid arches, as well as five gill-bearing branchial arches. These structures, as well as anterior parts of the neurocranium and sensory ganglia of the head, are derived from the cranial neural crest. In a mutagenesis screen utilizing a pseudotyped retrovirus to create proviral insertions into the zebrafish genome, we have identified two mutations resulting in abnormal development of the pharyngeal skeleton. Both mutations show strong linkage to integrated proviral sequences. In insertion 67D, the mandibular and hyoid arches are strongly reduced, and all five branchial arches are absent. Insertion 38M results in deletion of anterior parts of the neurocranium, ventral structures of the mandibular and hyoid arches, and strong reduction or absence of all branchial arches. In addition, both mutants exhibit reduced eyes and shortened pectoral fins. In both mutant lines, the provirus lies within a transcription unit, immediately upstream of the putative initiation codon. We are currently further characterizing these mutants, and further investigating the consequences of these putative genetic lesions.

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121.8

EXPRESSION OF THE HOMEOBOX GENE OTX2 ENHANCES NEURITE OUTGROWTH-SUPPORTING PROPERTIES AND CELL ADHESION MOLECULES EXPRESSION OF STABLY TRANSFECTED 3T3 CELL LINES.

K.T. Nguyen Ba-Charvet, Y. von Boxberg, S. Guazzi#, E. Boncinelli# and P. Godement*. Institut Alfred Fessard, CNRS, 91198 Gif-sur-Yvette, France and #DIBIT, Istituto Scientifico H.S. Raffaele, Via Olgettina 60, 20132 Milano, Italy.

Otx2 is an homeodomain protein expressed early in the embryonic forebrain of the mouse (Simeone et al. 1992). Among its functions, it could be involved in regulating the expression of substrate- and cell- adhesion molecules in a regional manner during forebrain development. In situ hybridization and immunocytochemistry show that, at relatively late embryonic ages (>E13), it is expressed in several areas of the mouse forebrain, and that within these areas, its expression varies over time. In particular, at E13, Otx2 mRNA and protein are expressed in cells scattered throughout the superior colliculus (a main target of retinal fibers) , and are strongly expressed in the inferior colliculus. Strong expression of Otx2 appears in the developing stratum griseum superficiale at the surface of the SC by E15, and is maintained after birth. At this age, several other targets of the retina strongly express Otx2, among them the vLGN (but not the dLGN). To investigate whether Otx2 could regulate the expression of molecules involved in axonal growth, we transfected NIH3T3 cells with Otx2 cDNA, and tested retinal neurite outgrowth in vitro on membrane carpets prepared from control or transfected cells. We obtained several stable clones of cells, exhibiting different levels of expression of Otx2 protein. Neurites extended from retinal explants (from E13 and E14 embryos) are longer, less fasciculated, and more numerous on carpets of membranes of transfected cells than on control carpets. We then tested expression of known cell adhesion molecules by these cell lines using Western blots and by immunocytochemistry. We found that N-CAM 140 and Thy-1 were regulated in correlation with the level of Otx2 expression. These results suggest that Otx2 could affect axon growth by regulating cell adhesion molecules in a regional-dependent manner during brain development.

121.9

PAX-6 REGULATION OF FOREBRAIN DEVELOPMENT IN EARLY MOUSE EMBRYOS

G.S. Mastick*, N.M. Davis, and S.S. Easter, Jr., Dept. Biology., U. Michigan. Ann Arbor MI 48109

The *Pax-6* gene encodes a transcription factor that is expressed in regionally restricted patterns in the developing brain and eye. Here we describe *Pax-6* expression in the early forebrain on E8.5-E10.5 using both wholemount RNA in situ hybridization and antibody labeling. We make three close correlations between *Pax-6+* domains and initial neural patterns, and in each case identify defects in *Pax-6* homozygous mutants (*Small eye*). 1. Forebrain subdivisions are visible as six prosomeres (designated p1-p6, caudal to rostral), some of which are visible as bulges bounded by transverse constrictions. The edges of *Pax-6+* domains coincide with two prosomeric boundaries: p1/mesecephalon and p2/p3. Mutant embryos lack the first boundary, but form the second. 2. The first axons to cross the roof form the posterior commissure (pc) in caudal p1. The source of these axons are two groups of neurons that are *Pax-6-*, but lie within dorsal and ventral *Pax-6+* domains in p1. Mutant embryos have few pc axons, due to a lack of dorsal pc neurons. 3. The first forebrain tract, the tract of the postoptic commissure (tpoc), originates from *Pax-6-* neurons clustered at the base of the optic stalk. The pioneer tpoc axons project caudally, initially in a tight bundle, but fan out upon entering a *Pax-6+* domain in p4 and p3, and funnel together again upon exiting into p2, which is low-expressing. In the mutant, the initial section of the tract is normal, but the axons make dramatic errors in the p4/p3 domain, and fail to cross into p2. We conclude that *Pax-6* plays multiple roles in forebrain patterning, including regulating morphological segmentation, neuron specification, and axon guidance.

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121.10

MOLECULAR MARKERS WHICH IDENTIFY ROSTROCAUDAL BOUNDARIES IN THE MURINE CEREBELLAR CORTEX.

A. Frostholm*, D. Zdilar, P. McAndrew, A. Burghes and A. Rotter. Depts. Pharmacology and Neurology, The Ohio State University, Columbus, OH 43210.

In addition to the classical laminar and parasagittal organization of the cerebellar cortex, there is increasing evidence for rostrocaudal cerebellar compartmentalization. We have shown previously that acidic fibroblast growth factor (FGF-1) and novel protein tyrosine phosphatase (PTPλ) transcripts define a distinct rostrocaudal boundary in the cerebellar granule cell layer at the dorsal aspect of lobule VI, similar to that observed in the *meandertail* mutant. Here we show that the homeobox genes, Otx1 and Otx2, vertebrate homologues of the drosophila *orthodenticle* gene, also are differentially expressed in granule cells of anterior and posterior regions of the developing murine cerebellar cortex. Otx1 expression was highest in mitotically active granule cells of the perinatal external germinal layer (egl) in the anterior cortex. Conversely, the Otx2 transcript was expressed in proliferating and premigratory granule cells in the external germinal layer, and in postmigratory cells of the internal granular layer of the posterior cortex. There was a greater degree of overlap in the rostrocaudal distribution of the two Otx genes, which resulted in a less clearly defined boundary than that seen with the FGF-1 and PTPλ transcripts. The distribution and temporal expression of the four markers is consistent with the notion that FGF-1 and PTPλ are suppressed by Otx1 and 2; downregulation of Otx1 in the egl permits the expression of FGF-1 and PTPλ in mature granule cells in the anterior cerebellar cortex.

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121.11

EXPRESSION OF MOUSE *Zic1*, *Zic2* AND *Zic3*, THE HOMOLOGUES OF DROSOPHILA *odd-paired* DURING PATTERN FORMATION.

Takeharu Nagai, Jun Aruga*, Katsunori Nakata, Tsutomu Tokuyama and Katsuhiko Mikoshiba
Mol. Neurobiol. Lab., RIKEN, Tsukuba, Ibaraki 305, Japan

We have identified and characterized *Zic1* gene which encodes a zinc finger protein highly restricted in mouse cerebellar granule cell and medulloblastoma whose oncogenesis may be involved in the granule cell lineage. To examine the existence of *Zic1*-related genes, we performed a low stringency hybridization on mouse genomic library and could find novel structurally *Zic1*-related genes, *Zic2* and *Zic3*. Both *Zic2* and *Zic3* were expressed highly restrictedly in the cerebellum at adult stage and shared the highly conserved zinc finger motif and genomic organization with those of *Zic1* and *Drosophila* pair-rule gene, *odd-paired* (*opa*). *Zic* genes are thought to be the vertebrate homologues of *opa*, which is involved in the insect body pattern formation regulating timely activation of *engrailed* and *wingless*. Then, we examined expression of *Zic1*, *Zic2* and *Zic3* in mouse embryos to clarify their role in the vertebrate development. We found that the *Zic* genes are expressed in the multiple sites in developing mice, such as central nervous system, paraxial mesoderm and limb bud. The expression in the neural tube was dorsally restricted and, in other sites, the expression is also spatially restricted although there are significant differences in the expression patterns of the three gene. Our findings suggest that the *Zic* genes have multiple roles and may be involved in the vertebrate body pattern formation.

Supported by grants from Japanese STA and MESC.

121.12

CHARACTERIZATION OF THE VENTRAL RETINA IN TWO LINES OF ZEBRAFISH CYCLOPS MUTANTS.

E.A. Schmitt*, L. Meeks, P. McCaffery, M. Hammerschmidt, U.C. Drager and J.E. Dowling. Department of Molecular and Cellular Biology, Harvard University, Cambridge, MA 02138.

The ventral forebrain and optic stalk are deleted in two cyclopic lines of zebrafish, i.e., cyc^{b16}(Hatta et al., 1994) and oep^{tz257}(Hammerschmidt, personal communication). These effects are associated with a lack of *sonic hedgehog* (*shh*) expression in the ventral forebrain, and the loss of the transcription factor, *pax2*, which is normally localized to the optic stalk and ventral retina. The cyc^{b16} exhibits partial eye fusion with two retinas joined in the midline, whereas the oep^{tz257} exhibits complete eye fusion resulting in a single retina. To determine if a deletion of the ventral retina accompanies the deletion of the optic stalk, we examined ventral retinal markers in mutant larvae. First, the development of initial photoreceptors, normally located within a patch of the ventral retina (ventral patch), was examined using antibody markers for rods and cones. Second, the activity of the retinaldehyde dehydrogenase enzyme, localized to the ventral retina, was assessed by a zymography bioassay. Although neither mutant exhibited a ventral patch, (first photoreceptors appeared dorsally), both exhibited ventral retinaldehyde enzyme activity. As exogenous retinoic acid (RA) enhances ventral characteristics in the developing zebrafish retina (Hyatt et al.,1996), we next examined the effects of RA on both cyc^{b16} and oep^{tz257} mutant retinas. In the cyc^{b16}, RA induced an expansion of *pax2* expression and increased cell proliferation in the ventral retina eye, resembling the effect of RA in wildtypes. In addition, RA enhanced the activity of the ventral retinaldehyde dehydrogenase enzyme while suppressing that of the dorsal enzyme. In contrast, RA enhanced dorsal enzyme activity in the oep^{tz257} mutant. Our results suggest that the ventral retina is present in both cyclopic lines, and that unlike the optic stalk, it is not dependent on *shh* for its development. Although RA exerts expected effects in cyc^{b16} mutants, further analysis is required to explain the anomalous effect of RA in the oep^{tz257} line. *supported by NIH-EY00811.

121.13

THE DEVELOPMENT OF AMACRINE CELLS EXPRESSING THE LIM HOMEODOMAIN PROTEIN ISL-1 REVEALS MECHANISMS OF RETINAL MOSAIC FORMATION. L. Galli-Resta[#*] and G. Resta[§]. [#]Istituto di Neurofisiologia CNR, 56127 Pisa, Italy; [§]Istituto di Matematica Computazionale CNR, 56126 Pisa, Italy.

Modularity is a basic architectural principle of the nervous system. Many neuronal structures are organized in layers with neurons of the same type forming regular spatial mosaics. Little is known of the mechanisms generating ordered neuronal matrices given the difficulty of identifying the matrix elements before they attain their final position. The Lim homeodomain protein Isl-1 is expressed by a subset of retinal cells during migration, providing a unique opportunity to analyse how cells add to developing mosaics. Two mosaics of Isl-1+ amacrine cells are found in the retina, separated by the inner plexiform layer. The genesis of the two mosaics was analysed by the use of several mathematical techniques (Voronoi domains, Delaunay segments and nearest neighbour analysis) in whole mounted retinas of developing rats. A minimal spacing between adjacent cells characterizes both mosaics while they are forming. The growth of the retina allows newly arriving Isl-1+ cells to add to the pre-existing mosaic according to the minimal spacing rule. The minimal spacing is constant as long as cells are added to the mosaic, is the same for both mosaics and characterizes also the interactions between cells of the two arrays. No center to periphery gradient is observed as long as new cells arrive. A gradient appears later, following a phase of cell death and further retinal growth. We conclude that: 1) the phenotype of Isl-1+ amacrine cells is determined before migration to their final destination; 2) mosaics of Isl-1+ amacrine cells are generated by an active rule which guides these cells into positions compatible with a minimal spacing rule.

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121.14

NEUROBLAST PATTERN FORMATION: REGULATORY DNA THAT CONFERS THE *vnd (NK-2)* HOMEOBOX GENE EXPRESSION PATTERN ON A REPORTER GENE IN TRANSGENIC LINES OF *DROSOPHILA*. H.-M. H. Saunders* and M. Nirenberg. Laboratory of Biochemical Genetics, NHLBI, National Institutes of Health, Bethesda, MD 20892.

The *vnd/NK-2* gene is expressed in a pattern of > 52 clusters of neuroectodermal cells that give rise to a subset of neuroblasts arranged in a precise pattern in part of the CNS of *Drosophila* embryos. Expression of the *vnd/NK-2* gene initiates the neural pathway of development in part of the CNS; the *vnd/NK-2* homeodomain protein activates the proneural genes *ac*, *sc*, and *l'sc* in part of the ventrolateral neuroectoderm (J. B. Skeath, G. F. Panganiban and S. B. Carroll, Development 120, 1517-1524 (1994)) and therefore determines part of the pattern of proneural gene expression. DNA fragments - 0.57, -2.2, and -8.4 kb in length, from the upstream regulatory region of *vnd/NK-2* gene were subcloned in the 5'- flanking region of an enhancerless, promoterless ß-galactosidase (ß-gal) reporter gene in the P-element, pCaSpeR-AUG-ßgal, and the effects of the DNA inserts on the pattern and time of expression of ß-gal were determined in embryos from transgenic lines of *Drosophila*. ß-Gal patterns of embryos from transgenic lines of flies with P-elements containing - 0.57 or -2.2 kb of *vnd/NK-2* DNA inserts did not resemble the *vnd/NK-2* pattern of gene expression. However, embryos from 11 transgenic lines of *Drosophila* transformed with a P-element that contains -8.4 kb of *vnd/NK-2* regulatory DNA expressed ß-gal patterns that closely resemble the normal *vnd/NK-2* patterns of gene expression. We conclude that the -8.4 kb DNA fragment from the up stream regulatory region of the *vnd/NK-2* gene contains the regulatory elements required to generate the normal patterns of *vnd/NK-2* gene expression.

121.15

FUNCTIONAL ANALYSIS OF ISLET-3 IN THE ZEBRAFISH EYE DEVELOPMENT. Y. Kikuchi[1], M. Tokumoto[2], Y. Hotta[3], K. Uyemura[1], and H. Okamoto[1]*, 1.Dept. Physiol., KEIO Univ., Schl. Med., Shinanomachi 35, Shinjuku-ku Tokyo 160. 2.NIBB, Okazaki, Aichi 444. 3.Univ. Tokyo, Tokyo 113, Japan.

LIM domain/homeobox transcription factors play important roles in cell fate determination during development in vertebrates and invertebrates. Islet-3, which was identified by us in zebrafish embryos, belongs to this family. By 17 hr, Islet-3 expression in the CNS was restricted to the eyes and the tectum. We examined developmental defects caused by the ectopic overexpression of the Islet-3 LIM domains without homeobox. This variant protein could be expected to make a non-functional complex with a putative cofactor protein of Islet-3, and to decrease significantly the chance of the intrinsic Islet-3 making a normal functional DNA-complex. Embryos overexpressing the LIM domains of Islet-3 developed apparently normally except that they completely lacked the optic vesicles (OV). The lenses developed later in these embryos. It is possible that the presumptive OV cells may have differentiated to such an extent that they might induce the formation of lens placode in the ectoderm, but were unable to form the OV by themselves. To determine whether the complete deletion of the OV structure in the injected embryo was caused by an autonomous defect of the presumptive OV cells, we made mosaic embryos by transplanting the cells of injected embryos into the normal embryos at the blastoderm stage. The injected cells were incorporated into all parts of the host body, including the lenses, except the OV. This result demonstrated that the LIM-only variant directly inhibited the differentiation of the presumptive OV cells. Taken together, our results strongly suggest that Islet-3 and cofactor, which interact with each other through LIM domains, are directly involved in differentiation of the eye. (This research was supported by the Japanese government (MEC, AST) and the Uehara Foundation.)

121.16

ROSTRAL OPTIC TECTUM ADOPTS A CAUDAL PHENOTYPE FOLLOWING ECTOPIC ENGRAILED EXPRESSION

C. C. Logan[1], A. Wizenmann[1], U. Drescher[2], B. Monschau[2], F. Bonhoeffer[2] and A. Lumsden*[1]

[1]Department of Developmental Neurobiology, UMDS Guy's Hospital, London, SE1 9RT, UK; [2]Max-Planck-Institute for Developmental Biology, Department of Physical Biology, 72076 Tübingen, Germany

Expression of the homeobox-containing gene *Engrailed* (*En*) in an increasing rostral to caudal gradient in the dorsal mesencephalon is the earliest known marker for polarity of the chick optic tectum. In transplantation experiments, En protein expression correlates well with the subsequent gradient of cytoarchitecture as well as the pattern of retinotectal projections. To directly test the role En plays in determining tectal polarity, we have used the replication competent retroviral vector RCAS to mis-express mouse *En-1* throughout the chick tectal primordium and show that the rostral portion of the tectum adopts a caudal phenotype: the gradient of cytoarchitectonic differentiation is abolished and two molecular markers, RAGS and ELF-1, Eph-like receptor tyrosine kinase ligands that are normally expressed in an increasing rostral to caudal gradient in the tectum, are now strongly expressed rostrally. In addition, cell membranes from rostral tectum of RCAS *En-1* infected embryos preferentially repel temporal axons in *in vitro* membrane stripe assays. These results show that *En* can act as a determinant of rostrocaudal polarity within the developing tectum.

Funded by grants from the Wellcome Trust and Howard Hughes Medical Institute.

121.17

DIFFERENTIAL PROLIFERATION RATES DEFINE REGIONS WITHIN THE VENTRICULAR LAYER OF THE DEVELOPING CHICK HINDBRAIN. R.J.T. Wingate*, A.M. Myat† and A. Lumsden. Dept. Developmental Neurobiology, UMDS, Guy's Hospital, London SE1 9RT. †ICRF Developmental Biology Unit, Dept. Zoology, Parks Road, Oxford OX1.

Between E4 and E6, the chicken hindbrain loses its early segmental form. As rhombomere boundaries disappear, cell proliferation becomes limited to the ventricular layer, while a mantle of post-mitotic neurons is deposited to the pial surface. Although much of the basic hindbrain organisation is established during the rhombomeric phase of development (E2-E5), cell division continues at the ventricular surface until at least E8, and an adult complement of nuclei is only established at E10. The patterning of cell division after rhombomeres disperse is clearly of importance: some genes, e.g. *Hoxb-1* and *Hoxa-2*, retain a segmental expression within the ventricular layer. Others, e.g. *C-Serrate-1* and *Tlx-1*, develop longitudinal patterns of expression the formation of columnar sensory nuclei. We were interested to see the relationship between the changing patterns of gene expression and rates of proliferation in the ventricular layer. BrdU was injected *in ovo* into chick embryos of various stages between E4 and E9. After 30 minutes the embryos were decapitated and the hindbrains processed for gene expression by in situ hybridisation, using one of a variety of DIG-riboprobes, and for BrdU incorporation by immunohistochemistry. Surprisingly, cell proliferation rates are not uniform within the ventricular zone. Rather, longitudinal quiescent zones are defined by highly geometrical boundaries with sharp cut-offs. At each stage examined these boundaries are shared by boundaries of gene expression. Together, these results suggest that the ventricular layer of the hindbrain comprises geometric regions defined by differential gene expression and mitotic activity. Funded by the Medical Research Council.

121.18

DEVELOPMENTAL REGULATORY GENES CONTROL EMBRYONIC BRAIN REGIONALIZATION IN DROSOPHILA. F. Hirth, S. Leuzinger, B. Hartmann, E. De Battista, S. Richter, R. Finkelstein, A. Simeone, D. Acampora, U. Walldorf, W.J. Gehring, K. Furukubo-Tokunaga, and H. Reichert*. Institute of Zoology, University of Basel, CH-4051 Basel, Switzerland.

We have studied the functional role of a set of developmental control genes in building the embryonic brain of Drosophila. Among these are anterior HOM-C genes, head gap genes, as well as other regulatory genes involved in anterior head patterning. For all of these genes, we have carried out high-resolution expression studies using laser confocal microscopy together with molecular markers for neuromeric borders. In addition, we have analysed the mutant phenotypes that result in the developing brain when the genes are deleted. Our results indicate that all of these developmental regulatory genes are involved in brain regionalization, and that elimination of any one of the genes causes marked structural defects in defined brain regions. To analyse their functional role further, we are using genetic methods to overexpress the genes, as well as their mammalian homologs, with the goal of creating ectopic brain anlagen. Considering the expression, loss of function, and gain of function phenotypes of these genes in Drosophila, and in comparison with gene homologs in mammalian brains, we postulate that an evolutionarily conserved program underlies brain development in all higher animals. Supported by the Swiss NSF.

121.19

Development of whisker-related neuronal patterns in transgenic mice overexpressing human bcl-2 protein. Yuqing Li*[1,3], Weimin Zen[1], Marc Knight[1], Jean-Claude Martinou[2] & Susumu Tonegawa[3]. [1]Dept. of Molecular & Integrative Physiology, Neuroscience Program & Beckman Inst., Univ. of Illinois, Urbana, IL 61801, [2]Glaxo Inst. for Molecular Biology, Geneva, Switzerland, [3]H.H.M.I., Ctr. for Learning and Memory, MIT, Cambridge, MA 02139

We reported that the development of whisker-related neuronal patterns in brainstem trigeminal nuclei (barrelettes) is activity-dependent and requires NMDA receptors (Cell, 76:427, 1994). However, Henderson and colleagues reported that the development of barrelettes is regulated by trigeminal ganglion cell death and they could prevent barrelettes formation in neonatal rats by injecting NGF prenatally (J. Neurosci. 14:3389, 1994). To investigate the roles of NMDA receptor-mediated activity and cell death in whisker-related neuronal pattern formation, we examined whisker-related pattern formation and plasticity in transgenic mice overexpressing human bcl-2. In the bcl-2 transgenic mice, neuronal death is substantially reduced (Neuron, 13:1017, 1994). In adult transgenic mice (n=10), normal barrels and barrelettes could be detected by cytochrome oxidase staining. This is also true in neonatal transgenic animals (P4, n=4; P8, n=6). To examine whether lesion induced plasticity is affected in transgenic mice, C-row whiskers were cauterized on P0 and the barrels in these mice were examined on both P4 and P8. In both wild type (n=5) and transgenic (n=10) mice, expansion of neighboring row of barrels could be observed. Detailed quantitation of the barrels is underway to determine whether there are quantitative differences between wild type and transgenic mice. These results indicate that whisker-related neuronal patterns could still form when neuronal death is greatly reduced. Supported by HHMI, Shionogi Inst. for Medical Sci., NIH and Lucille P. Markey Charitable Trust.

121.20

A 1.1 Kb GOLLI-MBP PROMOTER DIRECTS EXPRESSION DURING EARLY NEUROGENESIS. T.M. Pribyl, C.F. Landry, V.W.Handley, and A.T. Campagnoni* MRRC/UCLA School of Medicine, Los Angeles, CA 90024.

The Golli-mbp gene overlaps the myelin basic protein (MBP) gene, shares the MBP exons, and produces a unique family of alternately spliced mRNAs and proteins. Golli transcripts have been identified in the CNS, the PNS, and cells and tissues of the immune system. In the CNS, Golli-mbp mRNAs are found in both neurons and oligodendrocytes, and expression of the gene in these cell-types is under strict developmental and temporal regulation. To identify promoter elements which control this complex pattern of expression, 1.1 Kb of Golli promoter sequence was linked to the β-galactosidase (β-gal) gene and used to create transgenic mouse lines. Interestingly, this promoter directs expression of the transgene to specific populations of neurons. Expression of the transgene was noted first in early developing cortical neurons at E11, during the initial stages of neurogenesis. By E13, β-gal stained cells were concentrated in the subcortical plate of the forebrain and within early forming neurons of the olfactory system. Expression of the transgene was also quite evident in the early forming dorsal and ventral root ganglia. By P0, a clear demarcation of the subcortical regions and the olfactory system were evident by β-gal staining. This pattern persisted throughout postnatal development (to P90). These results indicate: 1) The golli promoter is complex and contains elements which direct expression to specific subsets of neurons 2) the onset of expression controlled by the 1.1 Kb promoter involves neurons during the early stages of neurogenesis and axonal elongation, and 3) one function of the golli proteins may be required in the formation/maintenance of cytoarchitectural structures. (Supported by NS23022, NS23322, HD25831 and RG2233A1 from the NMSS)

FORMATION AND SPECIFICITY OF SYNAPSES II

122.1

CRITICAL EVALUATION OF SYNAPTOPHYSIN-BASED METHODS FOR SYNAPSE QUANTIFICATION. M.E. Calhoun[4], M. Jucker[5], D.L. Price[1-4], and P.R. Mouton[1-4]* Departments of Pathology[1], Neurology[2], and Neuroscience[3] and the Neuropathology Laboratory[4], The Johns Hopkins University School of Medicine and the Gerontology Research Center[5], NIA, Baltimore, MD.

Synaptic changes underlie several degenerative neurological disorders and are important parameters for assessing animal models of human disease. Synapses can be visualized and accurately quantified at the ultrastructural level although the techniques are technically difficult and labor intensive. To evaluate more efficient techniques, we compared three methods based on synaptophysin immunoreactivity (IR) (quantitative Western blot, optical densitometry, and unbiased stereology) in hippocampal subregions of rat brain. Quantitative Western blotting, which measures synaptophysin protein levels, showed high variability between animals and low sensitivity for detecting regional differences in hippocampus. Optical densitometry and unbiased stereology on systematically sampled synaptophysin-immunostained sections allowed greater delineation of hippocampal subregions than Western blots, although densitometry failed to discriminate qualitatively different patterns of synaptophysin IR between dentate gyrus molecular layer and stratum oriens. Using stereology (optical disector/Cavalieri method), the mean total number of synaptophysin-IR presynaptic boutons in dentate gyrus was found to be statistically greater than that in stratum oriens (7.4×10^8 vs. 5.1×10^8, $p < 0.01$). We report that all three synaptophysin-based methods offer technically straightforward and efficient alternatives to synapse quantitation using EM; however, the stereological approach was the most reliable technique quantifying differences in synapse numbers. Direct comparison of synaptophysin immunostaining between light microscopy and EM revealed comparable bouton visualization and number. This work was supported by a grant from the NIH (AG 05146).

122.2

MODULATION OF PRESYNAPTIC VARICOSITY DISTRIBUTION BY POSTSYNAPTIC NEURON IN METACEREBRAL GIANT CELLS OF *HELIX POMATIA*. G. Naretto, A. Casadio, M. Ghirardi and P.G. Montarolo*. Dept. of Neuroscience, Univ. of Turin, Cso Raffaello 30, Turin, Italy.

The presence of a target cell represents one of the factors regulating synaptic formation during development and regeneration. As previously shown, the presence of the target cell B2 modifies the distribution of synapsin-like immunoreactivity and the action potential-evoked Ca^{++} influx in cultured metacerebral giant cells (MGC) of *Helix pomatia*. By using the serotoninergic MGC with its bifurcated axon, we have now investigated the influence of the target neuron B2 in the distribution of varicosities in the two main branches (cbc and cc) of the presynaptic neuron MGC. After 5 days in culture, we identified the varicosities of MGC by immunostaining with an anti-5HT antibody and we counted the varicosities in the neurites outgrown from the stumps of the two axon branches. The MGC cultured alone showed a slightly higher number of varicosities in the cbc branch (131.33±23.85 in cbc versus 78.33±13.86 in cc, n=6), with an average cbc versus cc varicosity ratio of 1.71 (±0.18, n=6). The presence of the target cell B2 at the cbc branch significantly modified the distribution of the varicosities by reducing the number of varicosities in the cc branch (43.37±7.48, n=8, $p<0.03$), with an average varicosity ratio of 2.74 (±0.49, n=8). The presence of B2 at the cc branch induced a slight reduction of varicosities in the cbc branch with a ratio of 1.36 (±0.42, n=7) that is significantly different ($p<0.05$) from that obtained with B2 at the cbc branch. We next cultured the cbc branch of MGC in contact with the C3, a motoneuron that is not a target of MGC *in vivo*, and we found no change in the varicosity ratio (1.99±0.31, n=8) but a significant reduction in the total number of varicosities in the two branches compared to the isolated MGC (83.25±10.38 versus 209.66±35.98, $p<0.001$). Our preliminary data suggest that the number of varicosities in one branch of a neuron may be down regulated as a consequence of appropriate target interaction at another branch and that interaction with a wrong target can down regulate the varicosities at both branches. This work was supported by MURST and CNR grants.

122.3

NEW VARICOSITIES ARE ESTABLISHED PRIMARILY BY GROWTH CONE EXTENSION AND NOT BY DE NOVO EXPANSION OF PRE-EXISTING NEURITES IN *APLYSIA* SENSORY-MOTOR NEURON CULTURES. F. Wu*, R. Silverman and S. Schacher. Cntr Neurobiol. & Behav., Columbia Univ. Coll. of P & S, NYSPI, New York, NY 10032.

Sensory neurons (SNs) of *Aplysia* reliably form specific chemical connections with motor cell L7 in cell culture, which increase in efficacy during the first 4 days. The change in efficacy is correlated with increases in the number of SN growth cones and neurites fasciculated with and extending along the major axons of L7 and the number of SN varicosties contacting the axons of L7. To determine the cellular and molecular steps involved with the formation and stabilization of SN synapses, we combined light and electron microscopy to examine some of the properties of SN growth cones, neurites and varicosities in contact with the motor axon and substrate at different time points over 24 hrs during the initial phase of synapse formation in culture (20-44 hrs after plating). Although SN growth cones prefer to extend along the major axon of L7, their rate of growth was 62% slower than companion growth cones extending on the substrate or regenerated distal motor neurites. The growth cones extending along the L7 axon have smaller lamellipodia and fewer filopodia than their counterparts extending on the substrate. As the amplitude of the EPSP increased over 24 hrs, more than 80% of new SN varicosities were established by SN neurites that had extended along the L7 axon, with less than 20% of new varicosities formed as expansions at pre-existing neurites. At the three hr time point however, almost 50% of the new varicosities were formed by expansions of varicosities at pre-existing neurites. Our results are consistent with the idea that growth cone extension on a preferred substrate for synapse formation is slowed as it forms new synaptic contacts with the target. Moreover, SN varicosities formed on the shafts of pre-existing SN neurites are less stable than those formed by extending SN growth cones. Analysis of these structures for the presence of transmitter release sites will provide important insights into the formation of stable synaptic contacts. Supported by NS27541

122.4

DISTRIBUTION OF FUNCTIONAL RESPONSES TO GLUTAMATE ALONG THE SURFACE OF A POSTSYNAPTIC NEURON IS REGULATED BY SPECIFIC INTERACTIONS WITH THE PRESYNAPTIC NEURON. H. Zhu* and S. Schacher. Cntr Neurobiol. & Behav., Columbia Univ. Coll. of P & S, NYSPI, New York, NY 10032.

Synapse formation involves a series of reciprocal interactions leading to the proper alignment of a presynaptic structure capable of evoking release of neurotransmitter and a postsynaptic structure with a high density of receptors that transduce the binding of neurotransmitter. Previous studies using dissociated cell culture of identified neurons of *Aplysia* indicated that interaction with the appropriate postsynaptic target evoked a group of changes in the presynaptic neuron: increases in neurite branching and fasciculation, formation of varicosities with active zones, and expression of neuropeptide in varicosities contacting the target, and altering the overall functional distribution of receptors to neuromodulators of synaptic efficacy along the surface of the presynaptic neuron. To begin to explore how the postsynaptic neuron responds to interactions with the presynaptic neuron during synaptogenesis, we correlated the physiological responses to local applications of the sensory neuron (SN) neurotransmitter (glutatmate) along the surface of the postsynaptic neuron L7 with the local structural properties of the presynaptic SN. Brief focal applications of glutamate along the surface of the major axons of L7 grown alone in culture evoked membrane depolarizations of decreasing amplitudes as applications moved distally from the initial segment. In the presence of an SN that formed connections with the target, larger responses to glutamate were evoked in local regions that contained SN varicosities apposed to the major motor axon. Significantly weaker responses to glutamate were evoked in the adjacent areas that were closer to the initial segment of L7 and contained SN neurites, but had few or no varicosities. Because the overall response to glutamate did not appear to change with the presence of the SN, the results suggest that glutamate receptors on L7 may be redistributed in response to the distribution of apposed SN structures containing transmitter release sites. Supported by NS27541.

122.5

CONVERSION OF DREBRIN ISOFORMS AND THE FUNCTION OF ADULT TYPE DREBRIN IN MORPHOLOGICAL CHANGES IN THE DENDRITIC SPINE. K.Hayashi and T.Shirao*. Department of Neurobiology and Behavior, Gunma Univ. Sch. of Med., Maebashi, 371 Japan

Morphological development and changes in the dendritic spines have been postulated to participate in expression of synaptic plasticity. Drebrin is an actin-binding protein mainly expressed in neurons. First we examined the participation of drebrin in the mechanisms responsible for the changes in spine morphology. We found that drebrin was localized at spines and bound to spine cytoskeleton. Immunoprecipitation with an antibody against drebrin revealed that drebrin, actin, myosin and gelsolin formed a complex. In vitro, drebrin reduced the sliding velocity of actin filaments on immobilized myosin, and inhibited the actin-activated ATPase activity of myosin. These results suggest that drebrin may modulate the actomyosin interaction within spines and plays a role in the structure-based plasticity of synapses.

We next examined the developmental changes in the drebrin isoforms with RT-PCR. During the postnatal development, an embryonic type of drebrin is replaced by an adult type by alternative RNA splicing mechanisms. We found that the conversion of the isoforms occurred coincidentally with the formation of synapses both *in vivo* and in primary cultured cortical neurons. The *in vitro* system provides a useful model system for studying the activity dependency of neuronal maturation or the formation of postsynaptic structure.

This research was supported in part by Grants-in-Aid from the Ministry of Education, Science and Culture of Japan.

122.6

α1-ADRENOCEPTORS MEDIATE NUMERICAL MODULATION OF SYNAPSES BY NORADRENALINE IN THE RAT VISUAL CORTEX. K. Nakadate, M. Matsukawa* and N. Okado. Dept. Anat., Inst. Basic Med. Sci., Univ. Tsukuba, Tsukuba, Ibaraki 305, Japan.

In previous studies serotonin (5-HT) and noradrenaline (NA) have been suggested to facilitate synapse formation and maintenance in the central nervous system (J. Neurobiol. 24: 687-698; Neurosci. Res. 19: 111-115; Soc. Neurosci. Abst. 21: 445). Although 5-HT$_{2A}$ receptors have been identified mediating the facilitatory role (Neurosci. Lett. 195: 159-162), nothing was known on the mechanism by NA.

The present study was undertaken to identify receptor subtype(s) of NA mediating the facilitatory role by using selective adrenoceptor antagonists. For α1, α2 and β-adrenoceptor antagonists prazosin, rauwolscine and alprenolol were injected in 6 week-old-rats (N=4) for a week, and the density of axodendritic synapses in lamina I of the visual cortex was determined from electron micrographs. The density of synapses decreased in a dose-dependent manner following injections of prazosin: compared to the value of control animals 1mg and 10mg/kg weight of prazosin decreased synaptic density by 15% ($p<0.0001$) and 20% ($p<0.0001$), respectively. The magnitude of reduction in the density of synapses by the maximum dose of prazosin was similar to that by NA neurotoxin, DSP-4. It is interesting to note both 5-HT$_{2A}$ and NA α1 receptors are coupled to inositol phospholipid hydrolysis.(Supported by grant-in-aid for Scientific Reseach on Priority Areas from Ministry of Education, Science and Culture, Japan.)

122.7

Trophic-like role of serotonin (5HT) and noradrenaline (NA) in the rat spinal cord. T.Yajima, N.Suzuki and N.Okado* Dept. Anat., Inst. Basic Med. Sci., Univ. Tsukuba, Tsukuba, Ibaraki 305, Japan

Previous studies have shown that 5HT is the major component of monoamines , and that 5HT facilitates synapse formation and maintenance in the anterior horn of chicken spinal cord: at the maximum nearly 70 % of synapses decrease following to deplete 5HT (J. Neurobiol. 24:687-698). However, there are large species differences in the distribution pattern of monoamines in the spinal anterior horn (Neurosci. Lett. 132:155-158). Different from the chicken, both 5HT and NA fibers distribute similarly in the anterior horn of the rat. Compared to those in the chicken we expected to find out different mechanisms to facilitate synapse formation and maintenance by monoamines in the rat. Following intracisternal injections of 5,7-dihydroxytriptamine (5,7-DHT), 5HT neurotoxin, and/or 6-hydroxydopamine(6-OHDA), NA neurotoxin, the density of synapses was quantitatively examined with an electron microscopy. One week after simultaneous injections of 5,7-DHT and 6-OHDA on either at postnatal (P) 0 day or P3 weeks the density of axosomatic synapses decreased about 20% compared to that of control animals. The density of axosomatic synapses significantly decreased (-14%) by one week following an injection of 6-OHDA on P3 weeks. Although both 5HT and NA were suggested cooperatively to facilitate synapse formation and maintenance, the magnitude of monoamine-mediated synaptic plasticity in the anterior horn of rat spinal cord appeared smaller than that in the chicken. (Supported grant-in-aid for Scientific Research on Priority Areas from the Ministry of Education, Science and Culture, Japan)

122.8

SYNAPTIC LOSS IN THE PREFRONTAL CORTEX FOLLOWING INJECTIONS OF DOPAMINE RECEPTOR ANTAGONISTS IN THE RAT M.Sugahara[a,*], H.Shiraishi[a], N.Okado[a], [a]Dept. Anat., Inst. Basic Med. Sci., and [a] Dept. Psychiatry Inst. Clin. Med. Sci, Univ. Tsukuba, Tsukuba, Ibaraki 305, Japan.

Previous studies have shown that aminergic neurons (serotonin, noradrenaline, acetylcholine) have a trophic-like role to facilitate synapse formation and maintenance in the developing and adult brain (J. Neurobiol. 24: 687-689; Neurisci. Res. 19: 111-115; Neurosci. Abstr. 21: 445, 1217). The present study was undertaken to clarify whether the trophic-like role is also mediated by another aminergic system, dopamine (DA) neurons. D1 antagonist (SCH23390) or D2 antagonist (YM09151 nemonapride) was injected in 6 week-old rats for a week, and synaptic density in laminae I and V-VI was quantitatively determined from electron micrographs. Following injections of YM09151 (D2 antagonist; 1, 5 and 10 mg/kg weight) synaptic density decreased in a dose-dependent manner, and compared to values of saline injected control animals reduction rates of synaptic density by the highest dose were 18% ($p<.0001$) and 30% ($p<.0001$) in laminae I and V-VI, respectively. By injections of 10 mg/kg weight of SCH23390 (D1 antagonist) synaptic density decreased by 8% ($p<.0001$) and 20% ($p<.0001$) in laminae I and V-VI, respectively. Although DA has been suggested also mediating the trophic-like role for synaptic structures, we presently examine underlying mechanisms of synaptic loss by DA antagonists. (Supported by grant-in-aid for Scientific Research on Priority Areas from Ministry of Education, Science and Culture, Japan.)

122.9

ACTIVITY-DEPENDENT SYNAPSE REDUCTION (ADSR) IN MOUSE VENTRAL HORN AND MUSCLE CO-CULTURE MEDIATED THROUGH THROMBIN RECEPTOR. M. Jia, MX. Li, V. Dunlap and P.G. Nelson*. Lab of Developmental Neurobiology, NICHD, NIH. Bethesda, MD 20892

The ADSR was studied in mouse ventral horn and muscle co-cultures in a multi-compartment chamber. Unilateral bursts of electrical stimulation at 30 Hz, 2 seconds duration and 5 volts amplitude were delivered every 10 seconds for different periods of time in a 37°C incubator to ventral horn neurons in the side chambers synapsing with muscle cells in the center chamber. Twitch responses and excitatory postsynaptic potentials (EPSPs) of doubly innervated muscles were observed. After unilateral electrical stimulation for 20 hours, twitch responses from the unstimulated side only were lost in 21% of the muscles cells, compared with 3.5 % synapse loss from the stimulated side only. The ratio of the synapse loss from the unstimulated side vs. that from the stimulated side was about 6:1. Previous work has shown that the ADSR required thrombin proteolytic action since the loss is completely blocked by nanomolar concentration of the specific thrombin blocker, hirudin. In the present experiments a synthetic receptor peptide SFLLRNPNDKYEPF or SFLL, mimicking the newly exposed amino terminus of the thrombin receptor after it has been cleaved by thrombin, was applied to our vitro neuromuscular system. After incubating our preparation in 10^{-6}M SFLL for 1 day, synapse loss was 30%, and after 3 days 61%. The concentration of SFLL at 10^{-9}M induced 23% synapse loss, at 10^{-8}M induced 31% synapse loss and at 10^{-7}M induced 32% synapse loss after 3 days incubation. The inactive peptide FSLLRNPNDKYEPF at a concentration of 10^{-6}M did not induce synapse loss in our model. These results suggest that the synapse loss induced by electrical stimulation is mediated through the action of thrombin on the thrombin receptor in the cell membrane.

122.10

CONVERSION OF PARASYMPATHETIC NERVE FUNCTION FROM PREJUNCTIONAL INHIBITION TO POSTJUNCTIONAL SMOOTH MUSCLE EXCITATION FOLLOWING SYMPATHECTOMY IS ASSOCIATED WITH FORMATION OF PRESUMPTIVE NEUROEFFECTOR CONTACTS F. Marzban* and P.G. Smith. Department of Physiology, University of Kansas Medical Center, Kansas City, KS 66160

Contraction of the tarsal smooth muscle of the rat eyelid normally is mediated by sympathetically released norepinephrine acting on adrenergic receptors. However, 5-6 weeks following sympathectomy, prejunctional inhibitory parasympathetic nerves which usually do not directly affect smooth muscle tone now cause a large contraction via excitation of muscarinic smooth muscle receptors. This does not appear to be associated with muscarinic receptor supersensitivity or diminished acetylcholine degradation. The objective of the present study was to determine if the parasympathetically mediated smooth muscle contraction after sympathectomy is associated with the establishment of presumptive neuroeffector contacts.

In tarsal muscles with intact sympathetic excitatory innervation, quantitative EM analysis revealed that 45% of all varicosity profiles are located within 1μm of smooth muscle cell membranes. Numbers of varicosities were decreased by 86% 2 days following superior cervical ganglionectomy, coincident with the degeneration of the excitatory input to this muscle. Numbers of varicosities did not change significantly between 2 days and 6 weeks post-sympathectomy. At two days post-sympathectomy, when cholinergic excitation is low, only 25% of varicosities were located within 1μm of smooth muscle. However, at 6 weeks following sympathectomy, when parasympathetic excitation is well established, 62% of varicosities were within 1μm. These findings indicate that the establishment of atypical parasympathetic excitation of tarsal smooth muscle is associated with an increase in presumptive neuroeffector contacts between residual parasympathetic nerves and smooth muscle cells. Supported by NIH grant HD33025

122.11

DEPRESSION AND PAIRED-PULSE FACILITATION OF EPSP AMPLITUDE DURING EMBRYONIC DEVELOPMENT IN THE CERCAL SENSORY SYSTEM OF THE COCKROACH. M.A. Sosa* and J.M. Blagburn. Institute of Neurobiology, University of Puerto Rico School of Medicine, San Juan, Puerto Rico, 00901.

The cerci of the first instar cockroach (*Periplaneta americana*) bear two filiform hairs each, one lateral (L) and one medial (M), which are innervated by individual sensory neurons. These sensory neurons project to the CNS, where they synapse with giant interneurons (GIs) in the terminal abdominal ganglion. Cockroach embryos kept at 30°C take approximately 30.5 days to develop and hatch. The synapse between L and the contralateral GI3 forms between embryonic days 16 and 18. Here we characterize some of the properties of this synapse as it develops, specifically the phenomena of depression and short-term facilitation of EPSP amplitude.

Intracellular recordings of contralateral GI3 EPSPs, in response to direct electrical stimulation of L, were made in 16-23 day old cockroach embryos. The L-GI3 synapse of first instar nymphs is known to normally operate at a depressed level. Estimates of this steady-state level of depression were made for the embryos by stimulating L action potentials at a frequency of 2 Hz and measuring the GI3 EPSPs. Once a steady EPSP amplitude was reached, L was stimulated with pairs of pulses delivered at the same frequency. Paired-pulse facilitation was estimated at different stimulus intervals.

We have found that both depression and paired-pulse facilitation are present from when the L-GI3 synapse is first formed. However, while depression of EPSP amplitude (at 2Hz) stays relatively constant throughout embryonic development, facilitation was larger initially, during embryonic days 16 to 18. Afterwards, it remained relatively constant at a smaller level, at least up to day 23. These results indicate that the transmitter release machinery required for depression and short-term facilitation are present immediately upon formation of a functional L-GI3 synapse.

Supported by NIH Grant NS07464, with additional support from NIH RR03051 and an NSF Minority Postdoctoral Research Fellowship.

NEUROTROPHIC FACTORS: EXPRESSION AND REGULATION—DEVELOPMENT AND AGING

123.1

DYNAMIC EXPRESSION OF TRK RECEPTORS DURING SENSORY NEURON DIFFERENTIATION. Jason Rifkin, Kirk Danielson and Frances Lefcort*. Dept. of Biology, Montana State University, Bozeman, MT 59717. Recent evidence has pointed to novel roles for neurotrophins in the developing nervous system prior to the period of target-mediated cell death. As a first step towards identifying these early neurotrophin activities, we are determining the expression patterns of trk receptors, *in vivo*, during sensory neuron differentiation within the avian dorsal root ganglion (DRG) using a panel of highly specific anti-avian trk receptor antibodies (Lefcort et al., 1996). We find that trkA and trkC, but not trkB, are first expressed on a discrete subset of migrating trunk neural crest cells at St.18 (E 2.75) in the area of the future DRG. These early trk+ cells also express neuron-specific β tubulin (TuJi+) and typically, but not always, express the 160 kd neurofilament protein and BEN/DM1-GRASP. We find that all cells positive for any of the antigens mentioned above are also trkC+ whereas all HNK-1 positive cells are trkA and C-negative. Thus these trk+ cells may be the earliest (future) DRG neurons to differentiate or alternatively, given that sensory neuroblasts can express this β-tubulin isoform (Memberg&Hall, 1995), they may be a subpopulation of neuronal progenitors. To determine whether mitotically active neuronal progenitor cells express trk receptors, we are injecting BrdU *in ovo*, fixing at various time points and staining embryonic sections with BrdU and trk antibodies. By St.24 (E4) all β-tubulin+, 160 kd neurofilament+ and/or BEN/DM1-GRASP+ DRG neurons are also trkC+ while only a subset are trkA+ or trkB+ suggesting that all DRG neurons and/or progenitor cells, at some early stage in their differentiation express trkC. By E6 trkC expression is restricted to a minority of DRG neurons, primarily located in the lateroventral region of the DRG, while the majority of DRG neurons express trkA and reside in the dorsomedial region of the DRG. This same pattern is evident at E13, the end of target-mediated cell death, although by this time point, the most prominently expressed neurotrophin receptor in the DRG is p75. Supported by MONTS/EPSCoR.

123.2

NORMAL PAX-6 EXPRESSION IS REQUIRED FOR TRKC RECEPTOR EXPRESSION IN THE DEVELOPING CORTEX J.A. Clausen*, D. Caric† J. E. East, R. E. Hill† and D. J. Price. Dept. of Physiology, Univ. Med. Sch., Teviot Place, Edinburgh, EH8 9AG; †MRC Human Genetice Unit, Western General Hospital, Crewe Raod, Edinburgh, EH4 2UX, U.K.

We are studying the mechanisms involved in the development of the murine thalamo-cortical tract with emphasis on the role of diffusable neurotrophic molecules. We investigated the in vivo distribution of Trk receptor protein using routine immunocytochemical techniques in the developing cortex of Balb/c and homozygous Small-eye mice. The Small-eye mutation results in loss of function of the Pax-6 gene product and causes abnormal CNS development due to a delay in neuronal migration and impairment to axonal growth, neuronal differentiation and innervation. During normal cortical development, the deep layer neurons expressed TrkC receptor protein strongly from the time of their birth to adult (P21), whereas neurons in superficial layers did not express strongly until P21. In the homozygous state, we observed a reduction in the TrkC receptor protein in the cortical plate and subplate. TrkB receptor protein was expressed in the Balb/c cortical plate, subventricular zone but not the ventricular zone. TrkB receptor was similarly well expressed in the homozygote cortex. To study this further we co-cultured wild type E15 thalamus with E19 homozyogous cortex and found poorer cortical innervation when compared to controls. Taken together, these results suggest that the Pax-6 gene abnormality results in altered expressions of TrkC receptors, which may be a consequence of reduced thalamic innervation.

This work was supported by MRC and Wellcome Trust. JAC was supported by a University of Edinburgh Faculty of Medicine Fellowship.

123.3

REGULATION OF NERVE GROWTH FACTOR- GENE EXPRESSION IN VIVO IN MICE. K.Müllhofer, M.Meyer*, E.Wolff §, G.Brehm § and H.Thoenen. Max-Planck-Institut für Psychiatrie, 82152 Martinsried, FRG; § LMU, 81375 München, FRG

Nerve growth factor (NGF) is essential for the development of the peripheral nervous system. Temporal and spatial expression patterns as well as strength of expression are precisely controlled. However little is known about the mechanisms regulating NGF expression in developmental contexts. To approach this question, we fused 5 kb and 2 kb fragments of the NGF promoter including the first exon and part of the first intron of the NGF gene to a lacZ-reporter gene. These constructs were used to generate transgenic mice by pronucleus injection. By comparing the lacZ expression pattern with that of the endogenous NGF gene in these mice, we could show that both fragments of the NGF promoter contain several elements needed for correct temporal and spatial expression of the NGF-gene during development. However, except for the submandibular gland, the precise pattern of lacZ-expression was highly influenced by the site of transgene integration in the mouse genome. To avoid this problem, in a second approach we directly introduce alterations in the NGF promoterregion using the double replacement strategy in transgenic mice. Analysis of the effects different mutations have on the regulation of NGF gene expression will allow us to define DNA sequences which are important for the correct temporal and spatial expression of the NGF gene in various tissues. In addition, this approach is expected to generate partial NGF knock-out mice.

Supported by the Max-Planck-Gesellschaft, Germany

123.4

DEVELOPMENTAL EXPRESSION OF NGF, TrkA AND THE P75NGFR IN THE RODENT TRIGEMINAL SYSTEM. S. Jhaveri*, Dept. Brain & Cognitive Sciences, Mass. Institute of Technology, Cambridge, MA 02139.

Embryonic rat trigeminal ganglion (Vg) cells are dependent on NGF for survival. Message for NGF is present around whisker follicles; developing Vg cells express mRNA for TrkA, the NGF receptor. It has been proposed that during embryonic life, processes of Vg cells which project outside the whisker follicles are unable to access NGF and their cells die; others which project to whisker follicles, where NGF is expressed, survive. However, little information is available about actual projection patterns of embryonic Vg cells, about whether there is a regressive stage in whisker pad innervation which matches the period (E15-E18) of naturally-occurring cell death, and about specifically which trigeminal axons express TrkA. We have used immunohistochemical techniques to address some of these issues.

Five ridges are recognized on the E13 rat whisker pad; 24 h later, individual follicles begin to differentiate. NGF is expressed in epidermis, and along emerging follicles. TrkA-positive Vg axons are present under maxillary ridges by E13, and begin to innervate newly differentiated follicles. Initially, there is little invasion by Vg axons in the regions between whisker follicles, but with further peripheral maturation (by E18), epidermal innervation is initiated. The innervation pattern becomes progressively more dense over the next few days, with little indication of regression of axon populations. Immunoreactivity for p75NGFR is similar to that for Trk A, but has a more complex expression pattern: this low affinity receptor is found not only in Vg axons, but also on clusters of cells in the whisker pad.

Our results suggest that the targeting of Vg axons to their peripheral target occurs by a specific matching of neurotrophin (e.g., on follicle cells) and its receptor (e.g., on afferent fibers).There is no evidence of an exuberant projection followed by withdrawal of a portion of the peripheral trigeminal innervation.

SUPPORT: NIH Grant NS27678; TrkA antibody, courtesy of L. Reichardt.

123.5

MEASUREMENTS OF BDNF PROTEIN DURING CNS DEVELOPMENT J.E. Johnson*, M. E. Forbes, K. Drum and J. Anstrom Dept. of Neurobiology and Anatomy, Bowman Gray School of Medicine of Wake Forest University, Winston-Salem, N.C. 27157

BDNF (brain-derived neurotrophic factor) is a member of the neurotrophin family of factors that appear to regulate the development and maintenance of specific neuronal populations. Different CNS and peripheral tissues express different size variants of BDNF mRNA. In addition, the relatively widespread distribution of BDNF receptors on CNS neurons suggests that the resulting protein may be used for autocrine and/or paracrine activity or for retrograde and/or anterograde axonal transport.

We have recently developed a sensitive and highly specific immunoassay capable of detecting BDNF concentrations below the physiological levels required for trkB receptor signaling. This Electro-Chemi-Luminescent-Immunoassay (ECLIA) utilizes affinity purified polyclonal IgG antibodies (Amgen) in a single step sandwich assay measured with an Origen Analyzer (Igen, Inc.). It has a very broad linear dynamic range (4-5 log units) capable of detecting an increase in either physiological or pharmacological changes in BDNF protein concentrations. The assay has been optimized to improve the extraction and recovery of both bound and free BDNF from CNS tissues. We have applied this method to measure BDNF protein levels in tissues that differ in the quantity and type of BDNF mRNA variants. In addition we have measured developmental changes in BDNF protein levels in a variety of developing tissues in both mammalian and avian species. While some CNS regions (ie. hippocampus and cortex) appear to show a continual developmental increase in BDNF, other tissues (ie. visual system) appear to experience a transient BDNF peak during periods of connectivity. Measurements of changing BDNF protein levels are likely to be useful in characterizing the functional role of this factor in both development and plasticity. Sponsored by 5R01 EY-11127 (JJ)

123.6

EXPRESSION OF VEGF AND ITS mRNA IN THE NEURONAL CELLS DURING THE DEVELOPMENT OF POSTNATAL RAT BRAIN. W. L. Bao, L. M. Zhang, J. Y. Hua, B. S. Pan*, and F. Y. Sun. State Key Lab. of Medical Neurobiology, Shanghai Medical University, Shanghai, 200032, China.

In our previous study, VEGF-positive staining cells were observed high expression in the neuronal cells of adult rat brain which was time-dependantly increased after a transient cerebral ischemia. In the present study, *in situ* hybridization and immunohistochemistry were used to study the expression of VEGF mRNA and its protein in the different age of postnatal rat brain. The results showed that VEGF mRNA was highly expressed in the neuronal cells during the first three days except expressing in the vascular endothelium. The expression of VEGF mRNA in the neuronal cells started to decrease following the third days after the birth. However, VEGF-like protein positive staining neuronal cells were low density during the first seven days and higher density following the second to eighth weeks after the birth when VEGF mRNA was low expressed in the neuronal cells. The distribution of VEGF protein was well-negatively correlated to that of its mRNA in the neuronal cells of rat brain. Furthermore, fluorescence-double staining combined with a confocal laser scanning microscope analysis showed that VEGF-positive staining was located mainly in neurons and a little in glial cells of adult rat brain. The results suggest that VEGF may play an important role in the development of neurons in the postnatal rat brain.

123.7

INTERLEUKIN-1ß IS EXPRESSED IN THE DEVELOPING NERVOUS SYSTEM OF THE FROG *XENOPUS LAEVIS*. A. M. Jelaso* and C.F. Ide. Tulane/Xavier Center for Bioenvironmental Research and Neuroscience Program, Tulane University, New Orleans, LA 70118.

Expression of the cytokine interleukin-1ß (Il-1ß) in the developing frog *Xenopus laevis* was analyzed with immunocytochemistry. Il-1ß immunoreactivity was present in a stage dependent manner throughout the developing nervous system. From day 2 (stage 32) of development through day 6 (stage 50+) immunoreactivity was observed in large sensory and motor neurons, and in the muscles that control the early swimming response of frogs. Specific immunopositive structures include; distinct giant cells in the midbrain, Mauthner's giant neuron in the hindbrain, the Rohan-Beard cells in the dorsal spinal cord, motoneurons in the ventral spinal cord, dorsal root ganglia, cranial ganglia, the muscles of the eye and the myotomal muscles in the tail. Immunocytochemistry for the Il-1 type 1 receptor revealed that labeled cells are located in close proximity, but not coincident with those stained for Il-1ß. These data are compatible with the idea that Il-1ß may serve as a survival factor during larval development because for example, Mauthner's neurons persist until metamorphosis, a full eight weeks after stage 32. Supported by DoE grant #DE-FG01-93-EW532023.

123.8

AGE-RELATED CHANGES IN SENSORY CEREBRO-VASCULAR AXONS AND THEIR RESPONSIVITY TO NERVE GROWTH FACTOR. L.G. Isaacson[1]*, J.R.Tompkins[1], K.McNeely[1], W.L. Nixdorf[1], and K.A. Crutcher[2]. [1]Center for Neurosci, Dept Zoology, Miami Univ, Oxford, OH 45056; [2]Dept Neurosurg, Univ of Cinci Coll of Med, Cinci, OH 45267.

Previously we provided ultrastructural evidence that the aged sympathetic nervous system retains responsivity to exogenous nerve growth factor (NGF). The finding that young adult sensory cerebrovascular axons respond to exogenous NGF with increased neurotransmitter content provides the opportunity to determine whether aging affects such neuronal plasticity. Young adult (3 mo) and aged (24 mo) Fischer female rats received 2 week intracranial infusions of NGF (15 µg) or VEH and were perfused for CGRP immunocytochemistry. A significant decline (52%; $p < .05$) was observed in the perivascular density of immunoreactive fibers associated with aged anterior cerebral artery (ACA) when compared with the ACA from the young adult. Slight declines in the density of CGRP fibers from aged internal carotid (ICA) (34%) and middle cerebral (MCA) (32%) arteries were not significant. NGF resulted in a significant increase (110%; $p < .05$) in ACA perivascular density in both young and aged rats. In contrast to significant increases in the CGRP density of ICA (200%) and MCA (189%) observed following NGF infusion in the young adult, no changes were observed in the aged ICA and MCA, suggesting an age-related reduction in responsivity of sensory axons to NGF. (Supported by NS17131 to KAC and NS32876 to LGI)

123.9

THE RELATIVE ABUNDANCE OF NGF PROHORMONES (NGF-PRS) TO MATURE BETA-NGF IN HIPPOCAMPUS AND CORTEX IMPLIES A TROPHIC FUNCTION FOR THE NGF-PRS. Lakshmanan J, Reingshagen M, Geerling I, Eysselein VE and Huff K.* Harbor UCLA Med. Ctr., Torrance, CA 90509 and Univ. of Ulm, Germany.

In the present study, we examined the presence of beta-NGF in hippocampus and cerebral cortex, the CNS regions reported to express the NGF transcript in very high concentrations. Freshly dissected brain regions of adult mice and rats were frozen on a piece of dry ice and then immediately weighed and homogenized (250mg/ml) in 20mM Tris-HCl buffer, pH8.8 containing NaCl (10mM), EDTA (1mM), NP-40 (0.5%) and Na-deoxycholate (0.5%) using a tissuemizer. The homogenates, diluted (1:5) with Laemmli's electrophoretic sample buffer were processed for Westernblot analysis and the immunoreactive bands were identified with chemiluminescence using the ECL reagent. The polyclonal antiserum to 2.5S NGF (Sigma) identified 6 immunoreactive proteins ranging in molecular weight from 53 to 22 kDa. The intensity of the 53 and 32 kDa bands were strongest in hippocampal extracts of both species. Monoclonal antibodies to human beta-NGF (Promega) identified the 53kDa NGF-PR in hippocampal extracts of both rodent species while the monoclonal antibodies to mouse NGF (Boehringer-Mannheim) identified the 53kDa NGF-PR in mouse but not in rat hippocampal extracts. The two prepro-NGF domain-specific polyclonal antisera (Pro-Hormone Science) corresponding to the larger and smaller NGF precursors strongly immunoreacted with 32kDa NGF-PR while they weakly immunoreacted with the 53kDa NGF-PR. Mature beta-NGF was present in barely detectable quantity that could only be identified by the monoclonal antibody to human beta-NGF(Promega). The cerebral cortical extracts contained the 53 and 32kDa NGF-PRs but not the mature NGF. The relative abundance of the 53 and 32kDa NGF-PRs in hippocampus and cortex suggests that they may mediate neuronal trophic functions in the CNS. Support: IBD Ctr; DFG 789/2-2

123.10

CHARACTERIZATION OF A MURINE ACIDIC FIBROBLAST GROWTH FACTOR PROMOTER (FGF-1B) AND ITS EXPRESSION IN THE DEVELOPING BRAIN. A. Rotter*, K. Alam, A. Frostholm, K. Hackshaw, J. Evans and I-M. Chiu. Departments of Pharmacology and Internal Medicine, The Ohio State University, Columbus, Ohio 43210.

The structure and developmental expression of the murine FGF-1B promoter are described. The human FGF-1 gene contains four upstream untranslated exons (A-D) alternatively spliced to the first protein coding exon. Within the brain, the FGF-1B form predominates. A cDNA clone coding for the FGF-1B transcript was isolated from a mouse brain cDNA library. The clone contained an untranslated exon 34bp upstream of the translation start codon. The sequence upstream from the transcription initiation site of the 1B promoter was mapped by RNase protection assay. The upstream sequence lacked consensus TATA or CAAT boxes, but contained regulatory sequences including an AP1 element, an SP1 binding site and an RR2 enhancer region. In situ hybridization with probes specific for the 1B transcript showed the message to be restricted largely to neurons in discrete nuclei in the spinal cord, brainstem, thalamus and cerebellum. With few exceptions, the FGF-1B signal became detectable during postnatal weeks one and two. Maximal grain density was reached by postnatal weeks three and four, primarily in nuclei involved in processing information from exteroceptive sensory mechanoreceptors, and in motor control. The relatively late developmental expression suggests a role for FGF-1 in neuronal maturation, rather than in neurogenesis.

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123.11

EFFECT OF AGING AND DIETARY RESTRICTION ON GENE EXPRESSION IN DENERVATED STRIATUM H.W.Chen, T.Jiang and T.H.McNeill. Andrus Gerontology Center and Department of Biological Sciences, University. of Southern California. Los Angeles, CA 90089-0191

Previous studies have demonstrated that reactive synaptogenesis is diminished or impeded with aging and may be, in part, related to astrocytic hypertrophy and decrease of growth associated protein (**GAP**) genes expression. Chronic dietary restriction (**DR**) retards age-related gliosis and ameliorates age-related DNA damage. However, it is unclear if the DR effects will enhance the synapse replacement in aged brain. We therefore used in situ hybridization analysis to assess changes in the prevalence for astrocytic mRNAs (GFAP, clusterin, ApoE) and GAP mRNAs (SCG-10, GAP43) expression in denervated striatum (**ST**). Measurements were made from ad libitum (**AL**) fed young adult (3 mo.) and old (24 mo.) as well as diet-restricted old (24 mo.) rats.

We found that 1) DR fed 24 mo. rats vs AL fed 24 mo. rats, DR retards the age-related increase in the prevalence of GFAP, clusterin and ApoE mRNA expression in aged ST, and 2) DR fed 24 mo. rats vs AL fed 3 mo. rats, DR modulates the ability of astrocytes to regulate their genes transcription in response to a deafferentation lesion, which is compatible with that from young adult rats. In addition, we also found that diminished regulation of GAP mRNAs expression, such as SCG-10, in aged contralateral cortex in response to a unilateral cortical lesion was resumed in DR fed aged rats. These data suggest that the effect of DR on the astrocytic and GAP mRNAs in response to a lesion will modulate the effect of aging on the reactive synaptogenesis. Supported by NIH grant AG-09793.

123.12

DEVELOPMENTAL CHANGES IN MOTOR NEURON VULNERNABILITY TO INJURY. B.J. Wilcox* and V.E. Koliatsos. Division of Cytokine Biology, CBER, Food and Drug Administration, Bethesda, MD and Neuropathology Laboratory, The Johns Hopkins University School of Medicine, Baltimore, MD.

Adult motor neurons are less vulnerable to axotomy-induced death than neonatal motor neurons. We have investigated the hypothesis that this difference in vulnerability is due to increased local availability of neurotrophic factors (NTFs) in the adult resulting in decreased motor neuron dependence on target-derived NTFs during postnatal development. We have shown that selected NTFs are differentially regulated during development and suggest that this developmental alteration in NTF regulation may contribute to changes in motor neuron vulnerability. The neurotrophins, BDNF and NT4/5, GDNF and cardiotrophin-1(CT-1) are all present in skeletal muscle during the period of developmental death of motor neurons (E13-E15). Postnatally, BDNF is expressed in neonatal and adult facial motor neurons, whereas, NT4/5 is expressed in neonatal but not adult facial motor neurons. Following axotomy in the adult, BDNF is upregulated in muscle, whereas NT4/5 is downregulated. TrkB receptors remain expressed in both neonatal and adult facial neurons after axotomy but there is a progressive increase in receptor subtypes which lack catalytic properties. We are currently investigating the developmental pattern of GDNF and CT-1 expression in the environment of the facial motor nucleus.

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123.13

DIFFERENTIAL REGULATION OF HIPPOCAMPAL NEUROTROPHINS DURING RAT AGING Mako Saito and Hiroyuki Nawa* Dep. of Molecular Neurology Brain Reserch Inst., Niigata Univ. Niigata, Japan

Neurotrophins, a family of neurotrophic factors, exert a variety of trophic effects on neurons. We employed sensitive and specific two-site enzyme immunoassay (EIA) to assess age-associated changes of the three neurotrophins, nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF) and neurotrophin-3 (NT-3), in the hippocampus of Fisher 344 rats. Expression of these proteins and their mRNAs were compared in the same animals. Hippocampal extracts from two-month-old rats contained >200 ng BDNF per gram of tissue. This amount was 2 and 100 times greater than that of NT-3 and NGF, respectively. The levels of BDNF and NT-3 remained high 2-6 months after birth, whereas, NGF declined during this period and the altered protein levels of all three neurotrophins were maintained 6-18 months postnatally. mRNA levels of BDNF increased during both of these periods but the NT-3 mRNA appeared to decline. Change in the expression of BDNF protein was opposite to the data obtained from Alzheimer's patients. These results suggest that, during normal aging in rats, neurotrophin expression is regulated independently both at the mRNA and post-translational levels. Any deficiency in their regulation might contribute to neurodegenerative disorders.

NEUROTROPHIC FACTORS: EXPRESSION AND REGULATION—PHYSIOLOGIC AND PATHOPHYSIOLOGIC MECHANISMS I

124.1

UPREGULATION OF BRAIN-DERIVED NEUROTROPHIC FACTOR (BDNF) IN THE HIPPOCAMPUS AND CAUDAL CORTEX WITH PHYSICAL ACTIVITY. N.C. Berchtold*, H.S. Oliff, G.M. Deng, F. Gómez-Pinilla, C.W. Cotman. *Institute for Brain Aging and Dementia, University of Irvine, CA 92697-4540 USA*

Brain-derived neurotrophic factor (BDNF) supports the function and survival of many types of neurons which are vulnerable to aging and degenerative conditions. Ample evidence indicates that expression of BDNF mRNA is regulated by neuronal activity, suggesting that BDNF may be involved in neuronal plasticity.

Previously we reported that physical activity - voluntary wheel running - increases BDNF mRNA expression in rat hippocampus and caudal 1/3 of the neocortex. Since wheel running is a reproducible and quantitatible behavior, it is a useful paradigm for examining the regulation of BDNF in context to behavior. Prerequisite to understanding the initial events of BDNF induction by physical activity, the circadian running pattern of rats must be examined in order to tightly couple the running behavior with mRNA values. Running behavior of rats tends to fall into 2 general categories - either consistent running throughout the 12 hr. dark cycle, or intermittent bursts of activity. Interestingly, once a running pattern is established, it is maintained throughout the duration of the experiment. Two days of running has been shown to induce BDNF mRNA. Here, the time course for induction between 0 and 2 days is examined in more detail to determine the earliest timepoint (6 or 12 hours of running) at which a significant induction is seen, and to determine the stability profile of the induction over the next 24 hours.

Physical activity may provide a simple means to increase the level of BDNF and other neurotrophins in the brain, particularly, in highly plastic areas of the brain. In this way, exercise-induced upregulation of BDNF could help increase the brain's resistance to damage and degeneration, through BDNF's support of neuronal growth, function and survival.

(Supported by NIA, MacArthur Foundation to C.W.C.)

124.2

REGULATION OF FGF-2 BY SENSORIMOTOR EXPERIENCE. F. Gómez-Pinilla*, V. So, L. Dao, and C.W. Cotman. Dept. Neurology, and Inst. Brain Aging & Dementia, U. of California, Irvine, CA 92697-4540.

FGF-2 has well-recognized roles in promoting neuronal survival and growth and enhancing astrocyte function. Recent evidence indicates that FGF-2 is regulated by neural activity suggesting an involvement in molecular events underlying behavioral function. We have examined a potential regulation of FGF-2 by sensory experience including physical activity and visual input. For physical activity experiments, rats were exposed to running wheel cages connected to a computer that recorded distance for periods of 2, 4, or 7 days, and then brain tissues were processed for nuclease protection assay, *in situ* hybridization and immunohistochemistry. Results showed that FGF-2 mRNA and immunohistochemistry started to increase by two days of running in selected subregions of the hippocampus. For visual sensory experiments, rats were kept in darkness for 7 days, then re-exposed to light for periods between 1-6 hr and quickly sacrificed. Results showed a significant decrease in FGF-2 mRNA in the caudal cerebral cortex of rats kept in darkness, but levels progressively increased starting at 1 hr of light re-exposure reaching nearby normal levels by 6 hr. FGF-2 immunostained astrocytes showed a significant decrease in rats reared in darkness and a tendency towards an increase after light re-exposure. Results indicate that the FGF-2 system is regulated by physiological types of neural activity, suggesting that FGF-2 may have subtle roles in sensory transmission and processing (supported by grants from the American Paralysis and Alzheimer's Associations).

124.3

EXPRESSION OF NEUROTROPHIC FACTORS IN THE QUINOLINIC ACID MODEL OF HUNTINGTON'S DISEASE IN MICE. M. G. Zurich* and M. Blum, Fishberg Ctr. for Neurobiology. The Mount Sinai School of Medicine, New York, NY 10029.

Classically, trophic factors have been found to be provided to the neurons by their target cells. Therefore, a nigrostriatal dopaminergic neurotrophic factor would be expected to be synthesized within the striatum. But, the extensive neuronal loss in the striatum occuring in Huntington's disease (HD) does not result in transsynaptic degeneration of nigrostriatal dopaminergic neurons (DA). Thus, DA neurons may not be totally dependent on neuronal target-derived neurotrophic support. Instead of, or in addition to this putative target-derived trophic support, DA neurons may derive their trophic support in an autocrine manner. Alternatively this support may be provided by astrocytes either in the midbrain or in the striatum. FGF is present in the striatum and in the midbrain and may act as trophic factors for the DA neurons. These factors may be upregulated to sustain DA neurons in case of loss of their neuronal target.

To determine whether there is an upregulation of DA trophic factors within DA neurons or astrocytes, C57BL/6 mice received an unilateral striatal injection of quinolinic acid (QA, 75 nmoles), which has been shown to produce axon-sparing lesions similar to those observed in HD, or vehicle (PBS). They were sacrificed 6, 24, 72 hours or 7 days later. In situ hybridization was performed on the striatum 7 days after the QA lesion. RNA was isolated from the midbrain and was quantitated for aFGF. We found no regulation of aFGF in the midbrain at any time point, nor in the striatum after 1 week. This could signify that physiologically aFGF is derived in an autocrine manner by the DA neurons themselves and/or is provided to them by the astrocytes, and thus its expression is not affected by the loss of the neuronal target of the DA neurons. Alternatively, it could signify that aFGF is not involved in the survival of the DA neurons. Ongoing studies will reveal whether bFGF or TGF-α are regulated in this model. (Supported by Swiss National Foundation for Scientific Research and Lowenstein Foundation).

124.4

NEUROTROPHIC FACTORS IN NEURODEGENERATIVE DISEASES. R.W. Dorsey, P.G. Sarnacki, L. Radhakrishnan, C.L. Achim and C.A. Wiley*, Div. of Neuropathology, Univ. of Pittsburgh, Pittsburgh, PA 15213.

Neurotoxic factors secreted by immune activated cells like microglia are frequently invoked in the pathogenesis of neurodegeneration. We hypothesized that abnormal expression of neurotrophic factors (NTF) produced by microglia may also be involved in neurodegenerative disorders. To test this hypothesis we studied expression of NTF genes in autopsy brain tissues from Alzheimer disease, HIV encephalitis and control patients. Expression of BDNF, NT-3, GDNF and HGF genes was assessed by quantitative RT-PCR and compared to expression of monokines TGF-b, IL-6 and TNF-a. We found that expression of NTF genes was increased in brain tissues in areas with significant neurodegeneration. These *in vivo* findings of altered expression of NTF were confirmed in an *in vitro* model of HIV encephalitis. Interestingly, we found a specific distribution of NTF expression in resident microglia compared to infiltrating macrophages. Differential expression of NTF was also observed *in vitro* following various immune, viral or differentiating treatments. Increased expression of NTF in neurodegenerative diseases may begin as a protective response to neuronal injury, but when chronically expressed in the adult brain, NTF could mediate neurotoxicity. (This work was supported in part by an ADRC Pilot Grant to CLA)

124.5

HIPPOCAMPAL CONCENTRATION AND IMMUNO-EXPRESSION OF NERVE GROWTH FACTOR ARE REDUCED IN DEVELOPING HYPOTHYROID RATS AND THE PROSPECTS FOR RECOVERY. A. Farahvar* and E. Meisami (Dept. Molecular & Integrative Physiology, University of Illinois, Urbana, IL 61801).

We have previously shown that early thyroid deficiency in postnatal rats results in decreases in the growth of hippocampus and dentate gyrus which was found to be markedly reversible (Farahvar & Meisami, *Soc. Neursoci. Abst.* 18: 228, 1992; 19:1312, 1993). Paradoxically hypothyroidism resulted in *increased* expression of the low affinity NGF receptor (p75) in the hippocampus and cholinergic neurons of the rat basal forebrain (Farahvar & Meisami, *Soc. Neursoci. Abst.*; 20: 34, 1994; 21: 2014, 1995). In this study, concentration and immuno-expression of NGF were determined by ELISA and immuno-histochemistry (ICC) methods in the 25-, 50- & 90-day-old rats. Thyroid deficiency was produced from birth by adding propyl-thiouracil (PTU) to the drinking water (0.1% w/v); this suppresses plasma thyroid hormones as well as body and brain growth. Some hypothyroid rats were allowed to recover by withdrawal of PTU at day 25. The ELISA results revealed reductions of about 25% in NGF concentration and about 50% in total NGF content in hippocampus of hypothyroid rats. ICC results localized the NGF-immunoreactive cells in the CA1 and CA3 regions, with marked reductions in immunostaining in the hypothyroid animals. Hypothyroidism resulted in a marked decrease in the intensity of NGF immunostaining in the hippocampus. Recovery of thyroid function resulted in marked restoration of hippocampal NGF immunostaining and content as determined by the ICC and ELISA results. *Supported by NIH (GM07143) and UIUC Research Funds.*

124.6

DIVERGENT CHANGES IN INSULIN-LIKE GROWTH FACTOR-1 (IGF-1), ITS RECEPTOR AND IGF-BINDING PROTEIN-2 (BP-2) IN THE HYPOTHALAMUS AND CEREBELLUM OF STREPTOZOTOCIN INDUCED DIABETIC RATS. S. Busiguina, J.A. Chowen*, J. Argente, and I. Torres-Alemán. Instituto Cajal, C.S.I.C. and Hospital Niño Jesús, Madrid, Spain 28002.

Changes in the systemic insulin-like growth factor-1 (IGF-1) system have been well described in both insulin dependent diabetic humans and laboratory rats. However, whether the IGF-1 system of the CNS is affected remains to be illustrated. We have assessed the protein and mRNA levels of IGF-1, its receptor (IGFR), and IGF binding protein-2 (IGFBP-2) in the hypothalamus and cerebellum, areas where IGF-1 modulates various neuroendocrine systems and acts as a neurotrophic factor, respectively. As previously described in poorly controlled diabetics, serum IGF-1 and liver IGF-1 mRNA levels were decreased and returned to normal with insulin. The changes found in the cerebellum and hypothalamus are summarized below.

Area	+ or - insulin	IGF -1 protein	IGF-1 mRNA	IGFR mRNA	IGFBP-2 protein	IGFBP-2 mRNA
Cerebellum	- insulin	↓*	no Δ	no Δ	↓*	↓*
Cerebellum	+insulin	no Δ	no Δ	no Δ	↓*	↓*
Hypothalamus	- insulin	n.d.	↓*	↓*	n.d.	no Δ
Hypothalamus	+insulin	n.d.	no Δ	no Δ	n.d.	no Δ

Table 1. Modifications in the IGF-1 system of diabetic rats (↓* = significant decrease compared to controls; no Δ = no significant change; n.d.= not done).

These results indicate that anatomically specific changes occur in the brain IGF-1 system in response to diabetes and that all changes are not normalized by insulin. Furthermore, these modifications may be associated with known modifications of the hypothalamic neuroendocrine system and the more recently reported CNS neurodysfunction seen in insulin dependent diabetes. *Supported by grants from Fundación Endocrinología y Nutrición, Fundación Salud 2000 and the DGCYT.*

124.7

CHANGES OF BRAIN-DERIVED NEUROTROPHIC FACTOR (BDNF) IMMUNOREACTIVITY AFTER MIDDLE CEREBRAL ARTERY OCCLUSION.

Zaal Kokaia*, Gunnar Andsberg, Qui Yan† and Olle Lindvall.
Restorative Neurology Unit, Department of Neurology, University Hospital, S-221 85 Lund, Sweden; †Amgen Center, Thousand Oaks, California 91320, USA.

In the present study, we have analyzed changes of immunoreactivity for BDNF in a rat model of transient focal cerebral ischemia, produced by middle cerebral artery occlusion (MCAO). This model gives a densely ischemic core in the lateral caudate-putamen and overlying parietal cortex and a perifocal penumbra zone in adjacent neocortical areas. Twenty-seven adult male Wistar rats weighing 265-320 g were used. Prior to the MCAO or sham-procedure the animals were fasted overnight, but allowed free access to water. Five groups of rats (n=3-5 in each group) were subjected to MCAO for 2 h and were then perfused at 0, 2, 6, 16 and 24 h after the start of reperfusion. One sham-operated control animal was added to all groups. After perfusion of the brains, 40 µm thick free-floating sections were processed for immunohistochemistry using affinity-purified BDNF anti-rabbit polyclonal antibody at a dilution of 1:10 000.

The MCAO induced changes of BDNF immunoreactivity which largely corresponded to the alterations of BDNF mRNA levels observed in our previous studies. An increased number of BDNF immunopositive neurons was observed in the ipsilateral cingulate and frontal cortices at 2 h after MCAO. At the same time-point, strongly increased BDNF staining was detected bilaterally in the hippocampal formation. Interestingly, the BDNF immunoreactivity was mostly confined to mossy fibers. In the ischemic core of the parietal cortex only few scattered neurons were BDNF immunopositive. The observed pattern of BDNF immunoreactivity after MCAO is consistent with the hypothesis of a neuroprotective role of BDNF. However, the elevated levels of BDNF protein could also increase synaptic efficacy in the postischemic phase, which may promote epileptogenesis (supported by a grant from Swedish MRC B96-14X-0866-08C).

124.8

CHRONIC HYPOXIA INCREASES THE LEVELS OF FGF2 *IN VIVO*. S. P. Soni, M. L. Schwartz and F. M. Vaccarino*, Child Study Center and Section of Neurobiology, Yale University School of Medicine, New Haven, CT 06520.

The susceptibility of children to hypoxia is particularly pronounced during the newborn period, at a time when selective decreases in cell number and axonal/synaptic pruning occur in normal development. We have developed an animal model of chronic hypoxia in which rats are raised under low O_2 pressure for different periods after birth. Rats raised under chronic hypoxia show a decreased cerebral cortical volume, an increased cell number and an abnormally widespread distribution of callosal projections (Stewart et al, Soc. Neurosci. Abstr, page 2111, 1995). These abnormalities could be due in part to indiscriminately high levels of growth factors involved in the regulation of cell number and axonal/synaptic connectivity. To test this hypothesis, we measured growth factor levels in rats raised under hypoxia and age matched controls. Chronic hypoxia lasting 14 and 30 days resulted in a dramatic increase in levels of basic fibroblast growth factor (FGF2) in the cerebral cortex and hippocampus. All three forms (18, 21 and 21.5 kDa) of FGF2 were increased, as assayed by Western analysis. Using immunocytochemical analysis, we determined that all areas of the hypoxic cortex and hippocampus exhibited a significantly higher number of FGF2 positive cells and a greater intensity of staining respective to control normoxic animals. In the cerebral cortex of control animals, FGF2 staining was localized primarily to the cytoplasm of cerebral cortical neurons. In contrast, chronic hypoxia for 14 days caused an increase in FGF2 immunostaining which was localized both to the cytoplasm and to the nucleus of cerebral cortical cells. After 30 days of chronic hypoxia, FGF2 staining in the cerebral cortex was almost exclusively in the nucleus. Our data support the hypothesis that FGF2 is involved in activity dependent plasticity and that the delay in regressive phenomena (increase in cell number and exuberant connections) demonstrated in hypoxia could be due to the over-expression of FGF2 in the hypoxic brain.

Supported by NIH grant #1P2O-NS32578.

124.9

EXPRESSIONS OF PDGF B-CHAIN AND β-RECEPTOR IN NEONATAL HYPOXIC/ISCHEMIC ENCEPHALOPATHY. M. Ohno*, S. Narumiya, N. Tanaka, T. Yamano, M. Shimada. Dep. of Pediatrics, Shiga Univ. of Medical Sci., Otsu, Japan 520-21.

Platelet derived growth factor B-chain (PDGF B-chain) is considered to be a potent neurotrophic factor in the central nervous system. We have investigated the expressions of PDGF B-chain and its specific receptor, β-receptor, in the neonatal rat brain with hypoxic/ischemic injury. The left common carotid arteries of anesthetized 7-day-old rats were ligated, and pups were then placed in a hypoxic chamber. The expression in protein and mRNA of both PDGF B-chain and β-receptor were examined using an immunocytochemistry and a northern analysis, respectively. The spatial distribution of the transcripts of B-chain was also studied by an in situ hybridization. Three hours after hypoxia, expressions in both protein and mRNA of B-chain were ubiquitously elevated above the control levels, but foci with decreased expression were also seen. Distribution of the decresed expression corresponded to the infarct areas. Strongly enhanced signals of B-chain protein and mRNA occurred in the neurons surrounding the infarct. The enhancement of β-receptor protein expression was seen in some neurons in the peri-infarct af 3 hours after hypoxia and marked upregulation was seen at 16 hours. The β-receptor mRNA remained at the control level at 3 hours and markedly increased after 16 hours of hypoxia. This enhanced expression was not detected at 72 hours. These results indicate that neonatal hypoxic/ischemic insult induces the upregulation of the platelet derived growth factor B-chain and β-receptor expressions in neurons immediately after hypoxia. PDGF B-chain may act as a neuroprotective factor in developing brain with hypoxic/ischemic injury through autocrine and/or paracrine mechanisms. Supported by 06670785, 5A-5 and 7A-4 (the Ministry of Education, the Ministry of Health and Welfare, Japan)

124.10

REGULATION OF NGF SECRETION IN SMOOTH MUSCLE CELLS FROM HYPERTENSIVE AND HYPERACTIVE RATS. D.B. Clemow*, J.M. Spitsbergen, R. McCarty, W.D. Steers, J.B. Tuttle. Depts. Neurosci., Psychol., Urol., Univ. of Virginia, Charlottesville, VA 22908.

Cultured vascular (VSMC) and bladder (BSMC) smooth muscle cells from Spontaneously Hypertensive Rats (SHR: hypertensive, hyperactive) secrete more NGF than cells from Wistar-Kyoto rats (WKY: normotensive, normoactive). Regulation of NGF output differs in VSMCs from SHRs and WKY rats. We examined NGF secretion by VSMCs and BSMCs from two inbred strains (WKHT: hypertensive, normoactive; WKHA: normotensive, hyperactive) to assess phenotypic association with altered NGF metabolism. Basal NGF secretion was similar for VSMCs in both strains, and BSMCs secreted at higher basal rates than VSMCs. WKHT cultures of both tissues grew to higher density than those from WKHA. Data was analyzed as NGF secretion per cell. Angiotensin-II (1*u*M) and PMA (10nM) had little effect on BSMCs, but increased NGF secretion in VSMCs of both strains. PDGF (350pM) and carbachol (100*u*M) increased NGF secretion from BSMCs and VSMCs. Isoproterenol (10*u*M) and forskolin (10*u*M) decreased the rate of NGF output similarly in both tissue types. Neuropeptide-Y (1*u*M) and phenylephrine (10*u*M) increased NGF secretion in WKHT VSMCs, but had little effect on BSMCs and WKHA VSMCs. Stimulation of NGF secretion rate over control was greater in WKHT VSMCs than WKHA, in response to PMA, PDGF, angiotensin-II, isoproterenol, carbachol, NPY, and phenylephrine. Therefore, NGF metabolism is altered in the hypertensive WKHT. However, β-adrenergic and PKC signaling differences between SHR and WKY are not preserved in the WKHT and WKHA comparison, nor is elevated basal secretion of NGF associated with hypertension in WKHT. These results suggest that disturbances in α-adrenergic and peptidergic control of NGF output are features common to both genetic models of hypertension. Supported by grants from NIH.

124.11

INCREASED EXPRESSION OF ACTIVIN/INHIBIN FOLLOWING HYPOXIC-ISCHEMIC BRAIN INJURY IN THE INFANT RAT. M.Lai, E.Sirimanne, D.Wu, K.Mountjoy*, C.Williams and P. Gluckman. Research Centre for Developmental Medicine & Biology, School of Medicine, University of Auckland, New Zealand.

Growth factors and cytokines produced by both neurons and glia are recognised as the major mediators of cell survival and functional recovery following injury to the CNS. Recent studies by us have demonstrated marked upregulation of activin/inhibin mRNA levels following transient unilateral hypoxic-ischemic (HI) brain injury in the 21 day old Wistar rat (Lai et al. 1996 Neurosci. 70: 1013-1024). This is the first evidence for a response by the activin/inhibin system after brain injury and provides a uniquely suitable model to fully establish an *in vivo* role for these polypeptide factors in the CNS. Here the expression of the βA subunit and the specific type II receptor ActRII, were examined by immunohistochemistry. Distinct βA and ActRII immunoreactivity were found in many structures known to be α and βA mRNA positive after HI injury including the hippocampal formation, choroid plexus, piriform cortex, thalamus and region of the infarct. Immunostaining in most cases was confined to neurons. Immunoblot analysis of cerebrospinal fluid (CSF) identified a 66Kd protein with an α-subunit specific antiserum but no bands were identified using the βA subunit specific antiserum. This suggests that an inhibin precursor protein circulates in the CSF. These findings demonstrate a novel neural activin/inhibin axis whereby interplay of activin and inhibin may regulate the responses to CNS injury including roles in neuronal survival and/or wound repair. Supported by the Health Research Council of New Zealand.

124.12

NEUROTROPHIC RESPONSE IN NEURODEGENERATIVE DISEASES. P.G. Sarnacki, K. Sadler, G. Wang, E.A. Bonaroti, C.A. Wiley and C.L. Achim*. Div. of Neuropathology, Univ. of Pittsburgh, Pittsburgh, PA 15213.

Neurotoxins are often involved in the pathogenesis of chronic brain degeneration. Frequently, these neurotoxic factors are produced by immune activated glial cells. We hypothesized that in response to chronic injury, human microglia and astrocytes may also produce abnormal levels of neurotrophic factors (NTF) that could be involved in neurodegeneration. To test this hypothesis we studied expression of NTF genes in an in vitro model of chronic neurodegeneration (i.e. HIV encephalitis). Human fetal and adult brain cells were cultured in the presence or absence of HIV infected macrophages. Production of BDNF, NT-3, GDNF, HGF and the receptors trk and c-met was studied in purified or mixed neuroglial cultures using immunofluorescent laser confocal microscopy and ELISA. Abundance and cellular distribution of these NTF were compared to production of the monokines TGF-b, IL-6 and TNF-a. We found that levels of some NTF were increased in microglial and astrocytic cultures exposed to HIV. Neuronal damage measured by immunostaining for dendritic and synaptic markers paralleled also microglial activation. We conclude that abnormal production and distribution of NTF in neurodegenerative diseases may be initially protective, but become neurotoxic when chronically secreted. (This work was supported in part by MH46790-07 to KS).

124.13

INCREASED NGF EXPRESSION IN CULTURED HIPPOCAMPAL NEURONS RECOVERING FROM ANOXIA: POSSIBLE INVOLVEMENT OF CYTOKINES. S. Di Loreto[1], D. Piancatelli[1], F. Di Marco[1], L.Corvetti[1], P. Sebastiani[2], P. Romano[2], E. Del Giudice[2], A. Buriani[2]*, A. Leon[2], D. Adorno[1] and C.U. Casciani[1]. [1]CNR, 67100 L'Aquila and [2]Research*life*, 67100 L'Aquila, Italy.

Inflammatory cytokines like IL-1β and TNF-α are known to be involved in the pathogenesis and development of several inflammatory and anoxic disorders occurring in the CNS. In addition, they might play a role in the recovery of injured neurons. Given the neuroprotective potential of NGF, its expression and regulation by cytokines after neuronal injury was examined. A nitrogen chamber was used to induce anoxia in rat hippocampal cultures, which were then transferred to a CO_2 incubator to recover. The expression of NGF mRNA, using PCR, was examined at various times after recovery. After 6 hr of anoxia NGF expression was poor, but 3 hr following recovery it increased, reaching a peak at 6 hr and decreasing thereafter. Anoxic treatments longer than 6 hr gave poorer NGF expression during recovery. Cytokines are known to increase during CNS damage. NGF expression was thus examined in IL-1β and TNF-α treated hippocampal cultures. Both cytokines, used at 1 ng/ml for 3 hr, induced NGF expression in the cultures, while longer treatments (24 hr) resulted in NGF downregulation. Taken together, these results suggest the existence of an initial reaction by hippocampal neurons in culture to protect NGF-dependent cells from anoxic insult, a process probably overcome at later times by NMDA-mediated cytotoxic events. This neural behaviour can be reproduced in vitro by treatment with inflammatory cytokines, suggesting that they might be involved in CNS-injury-induced NGF expression.
Supported by M.U.R.S.T. contract, National Research Program on Neurobiological Systems, Consorzio - NIRECO - L'Aquila.

124.14

LOCALISATION OF NERVE GROWTH FACTOR PROHORMONES ISOFORMS IN THE INFLAMED GUT OF PATIENTS WITH CROHN'S DISEASE AND ULCERATIVE COLITIS

M. Reinshagen*, I. Geerling, J. Lakshmanan, C.v. Tirpitz, H. Rohm, G. Adler
Department of Medicine I, University of Ulm, Germany, Dep. of Biochemistry, Vysal College, Salem, India

Utilizing a panel of polyclonal and monoclonal antibodies to mature NGF, we previously reported immunoblotting evidence for the localisation of 53 and 73 kDa NGF prohormones in the inflamed colon of a rat model of experimental colitis (Soc. Neurosci. 21:23.3, 1995). In the present study we examined the presence of NGF prohormone isoforms in the inflamed ileum or colon of patients with Crohn's disease or ulcerative colitis compared to normal human gut by immunoblotting and immunohistochemical analysis. The tissues were obtained by surgical resection. A panel of polyclonal and monoclonal antibodies to beta-NGF and two prepro-NGF specific domains from commercial vendors (Sigma, Promega, Boehringer, Pro-Hormone Science) were employed. In Crohn's disease and in Ulcerative colitis we found intense expression of 53 kDa NGF prohormone in the inflamed ileum and colon whereas the uninflamed control gut tissue showed only a slight intensity of the band. The two prepro-NGF domain specific antibodies identified a 73 kDa NGF prohormone isoform in the human inflamed tissues. In the immunohistochemical analysis using NGF prohormone staining was observed in the epithelium and in the myenteric plexus using polyclonal antibodies to beta-NGF and prepro-NGF specific domains. In summary, the marked increase in 53 kDa and 73 kDa NGF prohormone levels in the inflamed bowel of patients with Crohn's disease and ulcerative colitis suggests an important role for both prohormones in the pathophysiology of inflammatory bowel disease.
This study was supported by the Deutsche Forschungsgemeinschaft (DFG)Re789/2-2.

124.15

CHANGES IN HIPPOCAMPAL NGF EXPRESSION FOLLOWING EARLY MATERNAL SEPARATION IN DEVELOPING RATS.
F. Cirulli,[1]* A. Micera,[2] E. Alleva[1] and L. Aloe[2]. [1]Lab. Fisiopatologia O. S., Istituto Superiore di Sanità, I00161 Rome, Italy; [2] Institute of Neurobiology, C.N.R., I00137 Rome, Italy.

Nerve Growth Factor (NGF) is a neurotrophin affecting neuronal differentiation and remodelling of axonal and dendritic arborization, thus modifying CNS connectivity. As a consequence, changes in NGF expression at an early developmental stage might have long lasting effects on brain activity. In this study a maternal separation paradigm was used to test whether brief manipulations of rat pups early during ontogeny might influence NGF synthesis in the hippocampal formation. The expression of NGF mRNA was examined in 3 day-old Sprague-Dawley rat pups following 45 min maternal separation using in situ hybridization. Early maternal separation in neonatal rats resulted in increased expression of NGF mRNA in the dentate gyrus and the hilus of the hippocampus. NGF protein levels measured (by means of a sensitive 2-site ELISA assay) in the whole hippocampus the day following the separation procedure did not differ in separated vs non-separated pups. These data indicate that a single brief separation performed early during development can affect hippocampal NGF expression. Growth and neurologic development in premature human infants are improved by active tactile stimulation. Although this hypothesis is at this time only speculative, our model suggests the possibility that some of the mental disabilities or psychiatric disorders described in humans who experienced maternal separation or not adequate stimulation might be associated with early changes in NGF synthesis and release.

124.16

SPECIES SPECIFIC TRANSFER OF ALBUMIN FROM BLOOD TO CSF (CEREBROSPINAL FLUID) IN POSTNATAL *MONODELPHIS DOMESTICA*.
G. W. Knott, K. M. Dziegielewska, M. D. Habgood, Z. Li, C. F. L. Hinrichsen* and N. R. Saunders. Department of Anatomy & Physiology, University of Tasmania, GPO Box 252C, Hobart, Tasmania, 7001, Australia.

The mechanisms by which peptides and proteins are transported into and out of CSF are fundamental to understanding brain development. CSF of fetal eutherians and neonatal marsupials contains a high concentration of proteins (15-40 times adult) which appear to originate from plasma (Saunders, 1992, Handbook Exper. Pharm. 103, 327-369). In marsupials the highest CSF protein concentration occurs in the early neonatal period, rather than *in utero* as in eutherian fetuses. The aim of present experiments was to measure the transfer of various exogenous albumins from plasma to CSF in *Monodelphis* pups aged 5-36 days postnatal (P). After intraperitoneal injection of human (HSA), bovine (BSA) and succinylated BSA (succ-BSA) serum albumins, the steady state ratios (CSF/plasma) for each injected albumin were compared with endogenous *Monodelphis* albumin (MSA). The highest CSF/plasma ratios occurred in the youngest animals (P5) and were highest (over 40%) for MSA and HSA. At later stages, from P7 to P24, the BSA ratios were significantly below those for MSA; the succ-BSA ratios were even lower. By P32-36 the ratios for all albumins had fallen to less than 3%. Immunocytochemical double staining for albumins showed that MSA, HSA and succ-BSA were localized in the same choroid plexus epithelial cells, but only about 1/3 of the MSA positive cells contained succ-BSA. Similar experiments in other species have shown that succinylation disrupts specific albumin transfer. It is suggested that the decline in CSF/plasma ratio for succ-BSA in *Monodelphis* with age reflects a reduction in passive transfer, which is superimposed on a developmentally regulated decline in specific transfer of albumin, which has disappeared by P32-36. The approach outlined here provides a way of determining both the passive contribution to plasma-CSF exchange and more specific, developmentally regulated transfer of proteins that are likely to be important for brain development.

Supported by the Australian Research Council.

NEUROTROPHIC FACTORS: EXPRESSION AND REGULATION—SYNTHESIS, EXPRESSION, AND TRANSPORT

125.1

Narp , a Novel Member of the Pentraxin Family of Proteins that is Synthesized, Secreted, and Active as a Monomeric Molecule,
C.C. Tsui,* B.A. Pierchala, and P.F. Worley, Department of Neuroscience, Johns Hopkins School of Medicine, Baltimore MD 21205.

Narp , Neuronal activity-regulated pentraxin, is an immediate-early gene product that has been demonstrated to have dendritic-outgrowing activity using *in vitro* cortical explant cultures (Tsui et al., 1996.) Recent studies with primary cortical neurons have shown that *Narp* protein expression is induced and regulated through the activation of NMDA receptors. Analysis of its deduced amino acid sequence has indicated its homology to the pentraxin family of secreted lectins such as C-reactive protein (CRP) and serum amyloid P component (SAP). These pentraxins have in common an eight amino acid "pentraxin signature" sequence and a specific Ca^{2+}-dependent affinity to sulfated galactose sugar moities. Using FPLC Superose 12 gel filtration size fractionation, *Narp* in both the presence and absence of Ca^{2+} eluted from the column in a single peak that corresponds to a ~46 kDa protein. This finding demonstrates that *Narp*, unlike CRP and SAP which aggregate to form functionally active pentameric molecules, functions as a monomeric protein.

Tsui, C.C., Copeland, N.G., Gilbert, D.J., Jenkins, N.A., Barnes, C., Worley, P.F. *Narp*, a Novel Member of the Pentraxin Family, Promotes Neurite Outgrowth and Is Dynamically Regulated by Neuronal Activity. Journal of Neuroscience 16(8):2463-2478

125.2

CLONING AND CHROMOSOMAL LOCALIZATION OF THE MOUSE VGF GENE.
S. Hahm, K. Kelley, C. Kozak# and S.R.J. Salton*. Fishberg Research Center for Neurobiology, Mount Sinai School of Medicine, New York, NY, 10029 and #National Institutes of Health, Bethesda, MD 20892.

Our overall goal has been to investigate the functional role of VGF, a nervous system-specific mRNA that is rapidly and selectively induced by neurotrophic factors in vitro, and is expressed by developing and adult central and peripheral neurons in vivo. We have cloned the mouse *Vgf* gene from a lambda dash genomic library. Preliminary analysis indicates that the mouse and rat *Vgf* coding and regulatory sequences are highly conserved as is the intron/exon structure. The 5' flanking regions of the mouse and rat *Vgf* genes each contain CRE, NGFI-A and CCAAT elements that regulate VGF induction by NGF and Ras in PC12 cells. Expression patterns of two constructs that contain either 2.5 kb or 0.3 kb of mouse *Vgf* 5' flank driving synthesis of a tagged VGF mRNA are being analyzed in transgenic mice. The mouse *Vgf* gene has been mapped to the distal end of the mouse chromosome 5 in a region of defined homology to human chromosome 7.

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125.3

BDNF EXON 1E PROMOTER ANALYSIS IN C6 GLIOMA CELLS.
J. F. Bishop, G. P. Mueller* and M. M. Mouradian. Genetic Pharmacology Unit, Experimental Therapeutics Branch, NINDS, and Department of Physiology, USUHS, Bethesda, MD 20892.

Brain-derived neurotrophic factor (BDNF) has established neuroprotective effects on several neuronal populations and is therefore an important candidate for treatment of neurodegenerative diseases. Since this gene contains multiple alternate promoters that are active in a cell-type and injury-specific manner, targeted regulation of BDNF expression is an attractive possibility. Mounting evidence suggests that calcium-dependent processes are involved in both the regulation of BDNF gene expression and its neuroprotective actions. In previous studies, we had determined that BDNF mRNA expression in rat C6 glioma cells is controlled primarily by the exon 1e promoter, which is also responsive to calcium. To localize important basal and calcium responsive regulatory elements, C6 cells were transfected with CAT reporter constructs containing full-length and truncated segments of the exon 1e promoter and treated 48 hours later with either 0.01% DMSO (vehicle) or 10 μM A23187 (calcium ionophore). After a 4 hour incubation period, CAT assays were performed and the results suggest that prominent basal regulatory elements are located within 174 bp of the transcription start site and that a calcium responsive element is located within 379 bp of this site. Further studies are in progress to localize basal and calcium responsive elements in the BDNF exon 1b and exon 1d promoters.

125.4

EXPRESSION OF EPH-FAMILY MEMBERS IN EMBRYONIC RAT GANGLIA.
L.M. Bianchi*, N.W. Gale ,L. Pan, and G.D. Yancopoulos. Medical University South Carolina, Charleston, 29425; Regeneron Pharmaceuticals, Tarrytown, NY 10591

The largest known family of receptor tyrosine kinases is the Eph-family, (originally isolated from an erythropoietin producing hepatocellular carcinoma). Ligands of the Eph family activate multiple receptors, and appear to do so only when attached to the cell membrane (Davis et al., 1994, Science, 266, 816-819). Ligands can be functionally divided into two binding subclasses based on whether the ligands are membrane bound by linkage to glycophosphatidylinositol (GPI) or through a transmembrane domain (Gale et al., submitted). The prominent expression in the nervous system, particularly early in development, has led to speculation that these family members play crucial roles in tissue segmentation, neuronal migration, and axonal outgrowth.

To investigate the expression of Eph family members in the developing sensory ganglia, northern blot analyses of dorsal root, trigeminal and statoacoustic ganglia were conducted. RNA from E15 rat ganglia revealed the presence of several Eph-related ligands and receptors within each neuronal population. The transmembrane ligands ELK-L and Htk-L were detected in all three ganglia. ELK-L3 and ELK receptor were not detected. Several GPI anchored ligands were also detected in these ganglia at various intensities. ELF-1 and LERK 4 were expressed in all three ganglia, whereas Ehk-1 L and AL-1/RAGS were more prominently expressed in trigeminal and dorsal root ganglia. Ehk-3 receptor was present in all ganglia, but Ehk -1 and SEK were below the level of detection. These results suggest that several members of the Eph family may interact to regulate development of sensory neurons.

125.5

DISTRIBUTION OF HEK-LIGAND mRNA IN DEVELOPING AND ADULT CENTRAL NERVOUS SYSTEM: A QUANTITATIVE IN SITU HYBRIDIZATION STUDY, A.A. Welcher*, V. Cummins, and L.R. Williams, Depts. Neuroscience and Immunology, Amgen, Inc., Thousand Oaks, CA 91360.

The Human EPH-like receptor tyrosine kinase (HEK4) is one member of the large Eph/Elk/ECK/ subfamily of receptor tyrosine kinases. HEK4 was used as an affinity reagent to identify a binding protein, referred to as HEK-ligand (Fox et al., in prep.). The HEK-ligand sequence shows homology to the expanding family of proteins related to B61, and is identical to Al-1. For the present work, the distribution of HEK-ligand mRNA was determined in the developing and adult rat nervous system using quantitative in situ hybridization using an oligonucleotide procedure (Wisden, 1993). During development HEK-ligand mRNA is expressed at high levels throughout the embryo. In the prenatal brain, higher levels are expressed in the brain stem than in the developing cortices. In the neonatal brain, the highest levels of HEK-ligand are observed in the cerebral cortex. In the adult brain, the abundance of HEK-ligand mRNA is reduced from that observed in the developing CNS, but still is in highest abundance in the cerebral cortex, particularly layer 4. Abundant message is also observed in the dentate gyrus. Kainic acid - induced, excitatory amino acid stress to the brain results in significant (30%) increases in message in the superior colliculus and caudate nucleus, and 30% decreases in message in the layer 4 cortex and pyramidal neurons of the hippocampus.

125.6

DISTRIBUTION OF VASCULAR ENDOTHELIAL GROWTH FACTOR B IN THE RAT AND MOUSE CENTRAL NERVOUS SYSTEM. G. von Euler*[a], R. Zetterström[b], B. Olofsson[a], L. Olson[b] and U. Eriksson[a]. [a]Ludwig Institute for Cancer Research, Stockholm Branch, Box 240, S-17177 Stockholm, Sweden, and [b]Dept. of Neuroscience, Karolinska Institutet, S-17177 Stockholm, Sweden.

We have recently discovered a novel growth factor termed vascular endothelial growth factor B (VEGF-B) which has sequence similarities to VEGF and stimulates endothelial cell growth in vitro (Proc. Natl. Acad. Sci. 93, 2576-2581. 1996). Human and mouse VEGF-B are found in two isoforms, 167 and 186 amino acids long, that are generated by alternative splicing of mRNA. The alternative splicing produces a frame shift which gives rise to entirely distinct amino acid sequences in the carboxy terminal regions of the two VEGF-B isoforms, while the amino terminal regions are identical.

VEGF-B is detected in many tissues including brain, by Northern blot analysis. *In situ* hybridization with cDNA probes against various regions of VEGF-B mRNA showed a high general signal in the grey matter of the CNS. Random probes caused little or no labeling. Certain regions expressed more VEGF-B mRNA than others; the mitral cell layer in the olfactory bulb, globus pallidus, the CA2 and CA3 regions of hippocampus, the Purkinje cell layer in cerebellum, various nuclei in pons, and grey matter of the spinal cord. Similar results were observed for two non-overlapping probes and both in rat and in mice. Work is in progress to assure the cellular identity of VEGF-B positive signals.

In conclusion, the novel growth factor VEGF-B has a widespread distribution in the rat and mouse central nervous system and is enriched in certain regions. Supported by the Ludwig Institute for Cancer Research, the Swedish MRC, and USPH funds.

125.7

ACTIVIN AND FOLLISTATIN EXPRESSION AND FUNCTION IN THE DEVELOPING MOUSE RETINA. D. Srivastava, W.-F. Lau and R. Adler*. Johns Hopkins University, The Wilmer Eye Institute, Baltimore, MD 21287.

We are investigating the role of activin, inhibin and follistatin in the regulation of mouse retinal cell survival and differentiation; activins and inhibins are dimeric signaling peptides known to affect these processes in other systems, while follistatin can prevent activin binding to its receptor. Using the reverse-transcription polymerase chain reaction (RT-PCR), the expression of genes encoding inhibin and activin subunits, activin receptor and follistatin was demonstrated in retinas from embryonic, neonatal and adult C57Bl/6J mice. Biological effects of activin and follistatin were examined using glia-free, low density cultures of neonatal mouse retinal cells, which differentiate *in vitro* as photoreceptors and non-photoreceptor neurons. Activin produced time- and dose-dependent decreases in the number of photoreceptors present in these cultures; these effects were preventable with follistatin which, however, did not appear to have effects of its own. RT-PCR analysis using RNA from purified cultures of either retinal neurons/photoreceptors or retinal glial cells demonstrated that, while activin and inhibin subunits were expressed by both cell populations, follistatin mRNA was detected exclusively in glial cells. These data are consistent with the hypothesis that a homeostasis between activin and follistatin may regulate the survival and/or differentiation of retinal photoreceptor cells.
Supported by NIH Grant EY05404 and Research to Prevent Blindness, Inc.

125.8

AMPHIREGULIN, AN EGF-RELATED GROWTH FACTOR, IS EXPRESSED IN DISCRETE REGIONS OF MOUSE NERVOUS SYSTEM. K.B. Seroogy*[1], L.A. Opanashuk[1], B.M. Davis[1] and H.I. Kornblum[2]. [1]Dept. of Anatomy & Neurobiology, University of Kentucky, Lexington, KY 40536 and [2]Depts. of Pediatrics and Pharmacology, UCLA, Los Angeles, CA 90024.

Amphiregulin (AR) is a recently identified member of the epidermal growth factor (EGF) family of mitogenic polypeptides, which all mediate their actions by binding to the EGF receptor (EGF-R). Although AR has been examined in certain peripheral tissues, its presence and actions within the nervous system are unknown. To determine whether AR is expressed in neural tissue, sections throughout the mouse central and peripheral nervous systems were processed for the *in situ* hybridization localization of AR mRNA at neonatal and adult ages using ^{35}S-labeled cRNA probes (cDNAs kindly provided by D.C. Lee, UNC-Chapel Hill; S.K. Das, U. Kansas Med. Ctr.; and G. Plowman, Sugen, Inc.). Expression of AR mRNA was also examined in brains of mice that lack transforming growth factor-α (TGFα, another EGF-R ligand). AR cRNA-hybridization was observed in subpopulations of cells distributed throughout the forebrain including the olfactory bulb, prefrontal cortex, rostral septum, striatum, amygdala, and in scattered cells of the hippocampus, hypothalamus and neocortex. In brainstem, expression was detected in midline raphe nuclei, nucleus tractus solitarius, and in regions of the ventrolateral medulla including the facial motor nucleus. Peripherally, AR cRNA labeling was localized to numerous superior cervical ganglion neurons as well as within a few dorsal root ganglion cells. Expression levels were usually higher neonatally than in adulthood, suggesting a role for AR in neural development. Patterns and levels of AR mRNA expression appeared unaltered in TGFα knockout mice, indicating that AR expression is not upregulated in brain in compensation for a TGFα deficiency. Overall, these results provide evidence that the EGF-R ligand AR may have functional roles in select brain regions as well as in sympathetic and primary sensory systems. Supported by UK Med. Ctr. (KBS), DOE (HIK) and NIH fellowship NS10007 (LAO).

125.9

THE INTERLEUKIN-6 RECEPTOR IS PRESENT ON PRIMARY SENSORY NEURONS P.G. Murphy* and P.M. Richardson, Dept. of Neurology and Neurosurgery, Montreal General Hospital. 1650 Cedar Ave. Montreal Quebec. H3G 1A4

Previous results have indicated that axotomized sensory neurons express the interleukin-6 (IL-6) mRNA. This cytokine message is synthesized for several days after axotomy in medium to large sensory neurons. IL-6 might be important in neuronal survival and gene activation after injury. To indicate possible functions of IL-6 after injury, the IL-6 receptor was localized on cellular components of the dorsal root ganglion (DRG). Embryonic DRG were separated into a neuronal and non-neuronal population by centrifugation through BSA and percoll gradients in combination with pre-plating. Cell purity was 90-95% assessed by morphology and immunocytochemistry with glial, neuronal, and fibroblast markers. RT-PCR assays indicated presence of the IL-6α receptor mRNA in the neuronal cell population. A subpopulation of non-neuronal cells also express the message for the receptor. Current cell fractionation studies will identify which non-neuronal cells have the receptor mRNA. The alpha receptor protein also is present in neuronal cells as demonstrated with immunocytochemistry. An autocrine mechanism may exist as the receptor is present in neurons which express IL-6 mRNA. Preliminary immunocytochemistry results suggest the IL-6α receptor is on subpopulations of neuronal and non-neuronal cells in vivo. Infusion of rIL-6 into the subarachnoid space resulted in an increase in the neuropeptide galanin compared to saline infusion while not affecting NPY, GAP-43, and VIP. These results suggest that IL-6 might induce some injury associated genes after axotomy.
Funded by MRC Canada and the Rick Hansen Man in Motion Foundation

125.10

ANTEROGRADE TRANSPORT OF BRAIN DERIVED NEUROTROPHIC FACTOR RA Rush* and X-F Zhou Department of Human Physiology and Centre for Neuroscience, Flinders University of South Australia, GPO Box 2100 Adelaide, 5001, Australia

Neurotrophins are a family of proteins which act as survival and differentiative factors in the developing and mature nervous system. Extensive evidence has been provided for their retrograde action following incorporation into nerve terminals and transport to the cell body. In contrast, we now demonstrate that one neurotrophin, brain derived neurotrophic factor (BDNF) is transported anterogradely via both peripheral and central processes of spinal sensory neurons. Using newly generated antisera, we have examined the distribution of BDNF and found it present within a subpopulation of sensory somata, primarily those of a small to medium diameter. Within the L_5 dorsal root ganglia (DRG), the granular appearing BDNF-immunoreactive (ir) neurons comprised approximately 20% of the total and ranged in area from 105 to 3,188 μm^2 with an average of 774 ± 37 μm^2. Within the spinal cord, varicose nerve terminals in laminae I and II were intensively labelled, with a few BDNF-ir nerve fibres present in laminae II, IV and V. No BDNF immunoreactivity was detected in dorsal or ventral roots or peripheral nerves unless these nerves had been previously ligated or crushed. Nerve ligation studies indicate the factor is transported away from the spinal ganglia to terminals in the periphery and spinal cord. Spinal cords were also examined in animals with ligated dorsal roots revealing a heavy reduction of the immunoreactive nerve terminals within the ipsilateral, but not the contralateral spinal cord. The findings add a new dimension to the role of neuronal growth factors since anterograde transport has not previously been observed for any endogenous survival factor.

125.11

RETROGRADE TRANSPORT OF ENDOGENOUS NT3 BY SPINAL SENSORY NEURONS IN THE CHICK EMBRYO. P. Jiang, R. A. Oakley, and E. Frank*. Department of Neurobiology, University of Pittsburgh, Pittsburgh, PA 15261.

The distribution of neurotrophin-3 (NT3) in developing chick embryos was studied using a monoclonal antibody directed against recombinant NT3. This antibody intensely labels a subpopulation of large diameter sensory neurons that are mainly located in the ventrolateral region of the DRG. The antibody also labels spinal motoneurons less intensely.

Because the pattern of labeling in the DRG is similar to that of trkC expression, the localization of NT3 in sensory neurons might result from retrograde transport from peripheral tissues rather than from local synthesis within the DRG. To investigate this possibility, we used two independent manipulations to block retrograde transport: ligation of peripheral nerves and colchicine injection. Nerve ligations led to a dramatic decrease in NT3-immunoreactivity within DRG neurons and to an accumulation of immunoreactivity distal to the ligation. Similarly, colchicine injections led to a loss of NT3-immunoreactivity in the DRG. These results indicate that the NT3 detected in DRG neurons is mainly derived from peripheral sources via retrograde transport.

To determine the source of endogenous NT3 in peripheral tissues, we used injections of monensin to block the secretion of newly synthesized proteins. In vehicle-injected control limbs, NT3-immunoreactivity is virtually undetectable. Monensin injection to the hindlimb resulted in a dramatic increase in NT3-labeling in developing limb muscles. The labeling was differentially distributed, with some muscles being intensely stained while others were only weakly stained. These results suggest that individual muscles may differ in their production of NT3 during this stage of development, which coincides with the period of cell death in the DRG.

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125.12

DIFFERENTIAL EXPRESSION AND RETROGRADE TRANSPORT OF NEUROTROPHINS BY THE LOCUS CERULEUS. S Borson*, MA Bothwell, M S Butler, and B Kim. Depts of Psychiatry & Behavioral Sciences and Physiology & Biophysics, Univ Washington Sch of Med, Seattle WA 98195.

In various neuronal systems, neurotrophins may have anterograde, autocrine, and paracrine effects, in addition to their more widely-appreciated retrograde effects. The adult rat locus ceruleus (LC) is believed to be responsive to only one member of the neurotrophin family, NT-3. We compared the expression and retrograde transport of NT-3, BDNF, and NGF by LC neurons in adult Sprague-Dawley rats, using *in situ* hybridization histochemistry for mRNA, and I^{125}-labeled peptides (courtesy of R. Lindsay, Regeneron) prepared in our lab by a modification of the lactoperoxidase method, purified by centrifugal filtration (Millipore Ultrafree-MC), and injected stereotaxically into forebrain sites (cortex, hippocampus, and lateral ventricle), for transport studies. RESULTS. Under basal conditions, most neurons of the adult rat LC expressed NT-3 mRNA at low to moderate, uniform levels; BDNF expression was much more variable and NGF expression was negligible. Neurotrophin transport was similarly specific: 1^{125}-NT-3 was transported ipsilaterally by LC neurons after a forebrain injection in 15/19 animals, BDNF in 1/16, and NGF in 0/4. NT-3 and BDNF appear to be differentially regulated in LC neurons; the preferential synthesis and retrograde transport of the same neurotrophin, NT-3, suggests the need for new experiments to identify its role in LC-related neuronal pathways.

Supported by the Alzheimer's Association, IIRG 93-116 (S Borson).

125.13

BRAIN-DERIVED NEUROTROPHIC FACTOR (BDNF) IN THE RAT CEREBRAL CORTEX: MISMATCH BETWEEN SITE OF SYNTHESIS AND ACCUMULATION M.P.Berzaghi[1]*, T.F. Freund[2], E.Cs.Papp[2], D.Lindholm[1], U.Zirrgiebel[1], E.Castrén[1],& H.Thoenen[1] 1. Dept. Neurochemistry, Max Planck Institute for Psychiatry, 82152 Martinsried, FRG. 2.Institute of Experimental Medicine, Hungarian Academy of Science Budapest, P.O.Box 67, H-1450 Hungary

The expression and release of brain-derived neurotrophic factor (BDNF) are regulated by neuronal activity in the rat brain. Cholinergic activity has been shown to be an essential constituent in the regulation of BDNF expression. The aim of the present study was to analyse both the localization of BDNF mRNA expression and the distribution of BDNF immunoreactivity in the adult rat cerebral cortex before and after cholinergic stimulation. Pilocarpine, a muscarinic cholinergic agonist, markedly increased BDNF mRNA expression in scattered neurons in layers II to VI throughout the cerebral cortex. The increase in BDNF mRNA levels was paralleled by an increase in the number of BDNF immunoreactive neurons in all cortical areas examined. BDNF immunoreactivity was localized within a subset of neurons present in cortical layers II to VI. Using "mirror technique" we demonstrated that the vast majority of BDNF immunoreactive cells were also positive for the calcium binding protein parvalbumin, which is expressed by a subpopulation of GABAergic interneurons. Although BDNF immunoreactivity was localized within parvalbumin positive neurons, double *in situ* hybridization experiments demonstrated that parvalbumin-containing interneurons do not express BDNF mRNA in the adult rat neocortex. Thus, BDNF is not synthesized by parvalbumin-containing interneurons, but is instead upregulated within, and released from, a distinct neuronal population in an activity-dependent manner, and subsequently accumulates in parvalbumin containing GABAergic interneurons.

This work was supported by the Max-Planck-Gesellschaft and the Howard Hughes Medical Institute.

125.14

BDNF SECRETION IS MEDIATED BY PERTUSSIS TOXIN-SENSITIVE G-PROTEIN Erik C. Gunther, Christopher von Bartheld, Laura Goodman **, and Mark Bothwell*. Department of Physiology and Biophysics, University of Washington, Seattle, WA 98195; ** Genentech, Inc., Department of Neuroscience, South San Francisco, CA 94080

Mechanisms governing neurotrophin secretion are undefined. We present in vitro and in vivo evidence that BDNF release can be blocked by pertussis toxin (PTX), which specifically inactivates Gi and Go G-proteins. AtT20 cells transfected with a BDNF expression vector release BDNF in an activity-dependent manner. Preliminary results indicate that 1.0 hr. pretreatment of these cells with 2.0 μg/ml PTX decreases BDNF release. The developing chick optic system was used to assay the effect of PTX on endogenous neurotrophin supply. Developing neurons of the Isthmo Optic Nucleus (ION) project from the midbrain to BDNF-expressing retinal target cells, on which they depend for trophic support from embryonic day 13 to 17 (E13-17). Intraocular injection of 175 ng PTX resulted in a 42% increase in normal ION neuron death during, but not after this period of neurotrophin dependence, consistent with an inhibitory effect of PTX on trophic factor secretion. Co-injection of exogenous BDNF with PTX rescued 50% of ION neurons which would have died with PTX injections alone. Retrograde transport of exogenous ^{125}I-BDNF co-injected with PTX was increased over ^{125}I-BDNF injected alone, suggesting decreased competition from endogenous BDNF for receptors mediating retrograde transport, and consequent enhanced transport of exogenous BDNF.

125.15

CO-LOCALIZATION OF NEUROTROPHINS AND THEIR CORRESPONDING HIGH AFFINITY RECEPTORS IN MATURE RAT NEOCORTEX. A.F. Pitts* and M.W. Miller. Neuroscience Prog., Depts. of Psychiatry & Pharmacology, Univ. of Iowa Coll. of Medicine, Iowa City IA 52242 and Research Serv., V.A.M.C., Iowa City IA 52246.

We determined whether individual cortical neurons can elaborate both a neurotrophin [nerve growth factor (NGF), brain-derived neurotrophin factor (BDNF), and neurotrophin-3 (NT-3)] and its preferred high affinity receptor [*trk*A, *trk*B, and *trk*C, respectively]. A double-labeling immunofluorescence procedure was used. Sections of fixed rat somatosensory and motor cortex were incubated with a primary antibody (Ab) directed against NGF (goat polyclonal Ab, E.M. Johnson), BDNF, or NT-3 (guinea pig polyclonal Ab, F. Hefti) and then with a fluorescence-labeled (FITC) secondary Ab (Vector). Subsequently, the sections were incubated with an anti-*trk*A, *trk*B, or *trk*C Ab (rabbit polyclonal Ab, Santa Cruz) and then with a secondary Ab conjugated to Texas Red (Vector). A labeling index was determined by counting 5 fields in 4-5 rats. Neurotrophin-positive somata were distributed largely in layers II/III, V and VI of cortex; e.g., ⅓ of the layer V neurons were neurotrophin-positive. In contrast, *trk*-positive neurons were mostly in layer V where each *trk* isoform was expressed by about ⅓ of the neurons. Nearly ⅔ of the *trk*-positive neurons co-expressed their associated ligand, i.e., *trk*A and NGF, *trk*B and BDNF, and *trk*C and NT-3. Thus, the results provide a morphological basis for autocrine regulation within the cortex.

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125.16

EVIDENCE FOR AUTOCRINE INTERACTIONS AND REDUNDANCY OF NEUROTROPHIC SUPPORT IN THE ADULT RAT TRIGEMINAL BRAINSTEM SYSTEM. J. Jacobs* and M.W. Miller. Neuroscience Prog., Depts. of Psychiatry & Pharmacology, Univ. of Iowa Coll. of Med., Iowa City IA 52242 and Research Serv., V.A.M.C., Iowa City IA 52246.

The retrograde transport of neurotrophins influences neuronal survival and differentiation, however, there is increasing evidence that retrograde transport does not account for all of the trophic interactions in adult animals and that CNS neurons may participate in an autocrine system. To resolve this issue, we investigated the expression of nerve growth factor (NGF) and its receptors in primary and secondary trigeminal nuclei. We used antibodies against NGF (E.M. Johnson, Wash. Univ.), p75 (Oncogene), generic *trk* (Oncogene), and *trk* A, *trk* B, and *trk* C (Santa Cruz). We also performed *in situ* hybridizations using a full length rat preproNGF cRNA (S. Whittemore, Univ. Miami). Neurons in the trigeminal ganglion (77%), mesencephalic nucleus (87%), the principal sensory nucleus (PSN; 74%), and the motor nucleus (86%) expressed NGF (based on the immunoreactions and hybridizations). More than 80% of the first order and motor neurons expressed a *trk* isoform, whereas in the PSN only ¼ of the neurons were *trk*-positive. Thus, every first order neuron expresses NGF, p75, *trk* A and another *trk* isoform. In the PSN, every trk-positive neuron expresses *trk* A and *trk* B or *trk* C. Furthermore, the trigeminal system is capable of not only retrograde (e.g., from the PSN to the first order neurons), but also autocrine regulation (e.g., expression of NGF and *trk* A in the first order neurons).

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125.17

NGF, BDNF AND TRKB, BUT NOT TRKA, mRNAS ARE LOCALIZED TO THE DENDRITES OF RAT NEURONS.

E. Tongiorgi*, M. Righi and A. Cattaneo, Int. School for Adv. Studies (SISSA). 34013 Trieste, ITALY

The selective localisation of specific mRNAs into the dendrites, axons or the cell body is supposed to be an important mechanism to restrict protein synthesis at specific functional sites. The subcellular localisation and the regulation of the transport of mRNAs coding for proteins involved in synaptic plasticity is largely unknown.

In this study we used a sensitive non radioactive in situ hybridization method to examine the subcellular distribution of mRNAs specific for neurotrophins and their receptors in rat brain slices and in acutely dissociated hippocampal neurons in culture. In neurons of the basal forebrain NGF, BDNF and TrkB mRNAs are localised in the cell bodies and in the dendrites while trkA mRNA is restricted to the cell bodies. Dendritic expression of BDNF and TrkB mRNAs appears to be a general phenomenon in CNS, as confirmed by detailed studies performed on the visual cortex, hippocampus and substantia nigra. In order to facilitate the quantification and the experimental modulation of dendritic mRNA localization, in situ hybridisation was performed on acutely dissociated hippocampal neurons in culture. A somatic/dendritic localisation of BDNF and TrkB mRNAs was confirmed in these experimental conditions. Moreover, stimulation of such cultures with high potassium (10 and 20 mM) modulates the mRNA distribution in a subpopulation of neurons by increasing the amount of BDNF and TrkB mRNAs in the dendritic varicosities. We propose that the modulation of mRNAs for neurotrophins and their receptors in the dendrites might represent an important mechanism for achieving selective local responses at individual synapses.

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125.18

NT3, NT4 AND GDNF IN TOOTH DEVELOPMENT. C. A. Nosrat, K. Fried*, E. Lindquist, S. Lindskog and L. Olson, Dept. of Oral Diagnostics and Dept. of Neuroscience, Karolinska Institutet, Stockholm, Sweden.

Teeth have a rich innervation. Sensory stimuli originating in the dental pulp and the periodontal tissues are conveyed in nerve fibers which have their cell bodies in the trigeminal ganglion and, for some proprioceptive neurons, in mesencephalon. NGF mRNA seems to be expressed in developing teeth and has been reported to increase in pulpal fibroblasts after injury. In the present study, using *in situ* hybridization, we have investigated mRNA expression of neurotrophin 3, neurotrophin 4 and GDNF in developing and adult teeth and after injury (partial pulpotomy). NT4 mRNA had a predominantly epithelial expression during development but some labeling was also observed in ectomesenchymal cells. NT4 mRNA labeling was observed in high concentration in the oral and nasal mucosa, skin and vibrissae as well. NT3 mRNA labeling was also mainly epithelial. In the developing dental epithelium the expression pattern was related to the maturation gradient of the mitotic cells. NT3 mRNA seemed to be expressed in the adult tooth, and was also observed in oral and nasal epithelium, skin and vibrissae. GDNF mRNA was first seen in the ectomesenchymal cells of tooth primordiae, later in different parts of the epithelium and as the tooth developed, predominantly in the pulpal tissue. The possible effects on mRNA levels following injury is under investigation. We suggest that these factors not only might act as classical neurotrophic factors in the developing teeth and other oral tissues but also participate in the reciprocal mesenchymal-epithelial interaction during tooth development and morphogenesis. Supported by the Dental Faculty, Karolinska Institutet, Sv. Tandläkare-Sällskapet and the Swedish MRC (12X-08654).

125.19

EXPRESSION OF FGF-R ISOFORMS ON NEUROEPITHELIAL CELLS. A. Kalyani* and M. S. Rao, Dept. of Neurobiology and Anatomy, University of Utah School of Medicine, Salt Lake City, UT 84132.

Neuroepithelial (NEP) cells from E10.5 rat embryos are multipotent and can differentiate into neurons and glia (Kalyani et. al., unpublished results). NEP cells proliferate and maintain their pleuripotent character in culture when grown with either acidic or basic fibroblast growth factor (FGF). To characterize the receptors that mediate the proliferative response to FGF's, we have examined the expression of FGF receptors 1-4 and their splice variants IIIb and IIIc. Using reverse transcription polymerase chain reaction (RT-PCR), we show that undifferentiated neuroepithelial cells express a subset of FGF receptor isoforms. The IIIc isoforms of FGFR's1-3 are detectable in E10.5 neuropepithelial cells. The IIIb isoforms are not present though expression can be detected in control tissue. FGFR-4 expression is also detected in E10.5 rat neuroepithelial cells. We have used single cell PCR and RNA profiling techniques to show that individual NEP cells express multiple FGFR isoforms.

Comparison of the FGF receptor expression of undifferentiated NEP cells with that of differentiated neurons and glial cells suggests that FGFR3 is uniquely expressed on neuroepithelial cells. FGFR1 is expressed by neuroepithelial cells and its expression is maintained by neurons while FGFR2 expression is downregulated. The pattern of expression of FGF receptors in recently differentiated glial cells is distinct from the pattern in neuroepithelial cells and neurons. To determine if neuroepithelial cells synthesize FGF's we have analyzed FGF expression by PCR . We show that NEP cells synthesize FGF's and that NEP cells grown at high density can proliferate in culture in the absence of exogenously added FGF. E10.5 neuroepithelial cells may therefore maintain their proliferative state by using an autocrine mechanism. *Supported by the Muscular Dystrophy association and a University of Utah faculty award.*

125.20

IMMUNOHISTOCHEMICAL LOCALIZATION OF CNTF-Rα IN ADULT MOUSE RETINA *IN VIVO* AND *IN VITRO*. J.A. Miotke*[1], A.J. MacLennan[2], and R.L. Meyer[1]. [1]Dev. and Cell Biol., UC Irvine, Irvine, CA, 92717 and [2]Dept. of Neuroscience, University of Florida, Gainesville, FL, 32610.

CNTF has been shown to be a survival factor for early postnatal retinal ganglion cells *in vitro* (Meyer-Franke et al., 1995) However, attempts to demonstrate an effect on the survival and regeneration of axotomized adult retinal ganglion cells (RGCs) have shown conflicting results. Using our characterized *in vitro* system where the regencrating RGCs of adult retinal explants extend neurites onto a laminin substrate, we have shown that 100 ng/ml of rhCNTF significantly increases the number of neurites per explant after 3 days *in vitro* (Miotke and Meyer, 1995).

While the adult retina has been shown to have mRNA for CNTF-Rα, the receptor component of the tripartite CNTF receptor complex which binds CNTF, little is known about changes in the level or location of the protein *in vitro* or *in vivo* following axotomy. To address this, we are using an affinity-purified antiserum which has been shown to specifically recognize CNTF-Rα (MacLennan et al., 1996).

Immunohistochemistry on sections of normal adult mouse retina and optic nerve reveals high levels of CNTF-Rα in the photoreceptor layer. Neurons of the inner plexiform layer show low levels of immunorcactivity, with labeling of both perikarya and processes. Most neurons in the ganglion cell layer also show low to moderate levels of immunoreactivity. This labeling was localized to the perikarya. Similar low level immunolabeling of presumptive RGC perikarya was also seen in adult retinal explants after 5 and 7 days *in vitro*, but not on the neurites which had extended onto the laminin substrate.

Current work is focusing on the changes in the pattern of immunoreactivity *in vivo* following intraorbital optic nerve crush.

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125.21

SKIN AS A MODEL SYSTEM FOR STUDIES OF NGF-PROHORMONE EXPRESSION AND FUNCTION. Moore GPM, Yardley G, Isaacs K, Moore AG, Huff K and Lakshmanan J.* CSIRO Prospect, Australia, University of Western Sidney, Nepean, Australia, Harbor UCLA Medical Center, Torrance, CA. USA, 90509, Vysya College, Salem, India.

Utilizing antiserum to 2.5S NGF (Sigma, St. Louis) we previously reported NGF-immunostaining in the outer root sheath (ORS) cells, on the surface epithelium of apocrine sweat glands, and in the pilary canals of follicles near the epidermis in ovine skin (Soc. Neurosci. Abs. 21:22.5,1995). An investigation with the same antiserum revealed the presence of 143, 73, 63 and 53kDa immunoreactive proteins in skin (10th Internat. Cong. Endocrinol. 1996). In the present study we performed in situ hybridization on sections of ovine skin with a DIG-labeled antisense mouse cDNA probe to ascertain the sites of NGF expression. In addition, we examined the immunohistochemical localization of NGF-prohormone(PR) utilizing anti-prepro-NGF domain specific antibodies (Pro-Hormone Science, CA) that recognized the 53kDa NGF-PR present in the recombinant human NGF preparations on the Western blot (Reinshagen et al. submitted). In in situ hybridization experiments the NGFmRNA was localized to the follicle bulb and immunostaining with anti-prepro-NGF(-60 to -91) was found in the ORS. Immunoneutralization studies of litter mates treated with IgG fraction of antiserum to mouse 53kNGF-PR, 53-73kDa NGF-PRs and prepro-NGF specific domains from the day of birth (Pro-Hormone Science, CA) are in progress and the results will be reported in the meeting. In summary, the detection of NGFmRNA in follicular bulb and localization of NGF-PR in the ORS provide a mophological and biochemical basis for the developmental delay of hair growth reported in NGF geneknock out mice (Cell 76:1001, 1994).

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125.22

RESPONSE OF PC 12 CELLS TO MOUSE NGF PROHORMONE DIFFERS FROM THAT TO A COMMERCIAL HUMAN RECOMBINANT NGF.

S. Soinila, M. Reinshagen, I. Geerling, K. Huff, J. Lakshmanan. Dept. Neurology, Univ. Helsinki, Finland, Dept Internal Med I, Univ. Ulm, Germany, Dept Pediatrics, Harbor UCLA Med Ctr, Torrance, CA, Dept Biochemistry, Vysal College, India.

Using immunoblotting and a panel of polyclonal and monoclonal antibodies to β-NGF, we recently reported evidence for the presence of 53 kDa NGF prohormone (PR) and its isoforms in adult rat dorsal root ganglia as well as in normal and inflamed colon (Soc. Neurosci. Abstr. 12:23.3, 1995). To study the effects of 53 kDa NGF PR on neuronal cells, PC 12 cells were cultured in the absence and presence of mouse 53 kDa NGF PR (Pro-Hormone Science, CA) and the responses were compared to those of a commercial recombinant human NGF (R&D Systems, MN). Commercial polyclonal antibodies to 2.5S NGF (Sigma, MO) or β-NGF (Pro-Hormone Science, CA) were utilized to examine the purity of the preparations used for bioassay. Mouse 53 kDa NGF PR elicited consistently short neurite outgrowth from PC 12 cells at low serum concentrations and stimulated cell multiplication at high serum concentrations. In contrast, rhNGF preparations elicited robust neurite outgrowth and concomitantly inhibited cell multiplication. Western analysis revealed that mouse 53 kDa NGF PR was devoid of β-NGF but contained trace amounts of 73 kDa NGF PR. In contrast, rhNGF preparation contained as many as 8 high molecular weight NGF-immunoreactive proteins besides β-NGF. We conclude that PC 12 cells can be used as a model system to characterize the actions of NGF PR and its derivatives on neurons.

125.23

Adenovirus-Mediated Expression of Neurotrophic Factors; Biological Activity *In Vitro* and Retrograde Transport and Expression *In Vivo*

Shine, HD* and Baumgartner, BJ Department of Neurosurgery, Baylor College of Medicine, Houston, Texas 77030.

Replication-defective recombinant adenoviral (Adv) vectors were constructed carrying the neurotrophic factor (NF) genes BDNF, CNTF, GDNF, NGF, and NT3 under the control of the Rous sarcoma virus (RSV) promoter (Adv.RSV-*nf*). HeLa cells transduced with the Adv.RSV-*nf* vectors produced NFs which co-migrated with their respective recombinant NF standards on Western blots. Quantitative ELISAs showed that HeLa cells transduced with Adv.RSV-NGF (100 active viral particles per cell) produced β-NGF at a rate of approximately 5 $\mu g/10^6$ cells/ day. Estimates from Western blotting experiments suggest that the other NFs were produced from Adv.RSV-*nf* at similar rates. Conditioned media from Adv.RSV-*nf* transduced HeLa cells supported the *in vitro* survival of chicken sensory neurons at levels equal to or greater than the respective recombinant NFs. After injection of Adv.RSV-CNTF or Adv.RSV-GDNF into the facial muscles of neonatal rats, expression of Adv.RSV-CNTF or -GDNF mRNA in the facial nucleus was detected by RT-PCR. In addition, immunocytochemical analysis showed that high levels of CNTF were detected in spinal and facial motoneurons after injection of Adv.RSV-CNTF into their appropriate muscle groups. Taken together, the *in vivo* experiments show that motoneurons of the facial nucleus or spinal motoneurons retrogradely transport Adv.RSV-*nf* vectors and express the gene products. A portion of this work was supported by a grant to H.D.S. from the Texas Higher Education Coordinating Board Advanced Technology Program.

125.24

NERVE GROWTH FACTOR SOMATIC MOSACISM PRODUCED BY HERPES VIRUS-DIRECTED EXPRESSION OF *cre* RECOMBINASE IN MICE HARBORING A RECOMBINATIONAL SUBSTRATE. A.I. Brooks, N. Panahian, M.W. Halterman, D.F. Howard, G. Gianakakos, S.N. Haber* and H.J. Federoff, Departments of Microbiology, Immunology and Neurology: Division of Molecular Medicine & Gene Therapy, University of Rochester School of Medicine, Rochester, NY 14642.

To investigate NGF action in specific regions of the adult mammalian nervous system we have developed a somatic gene transfer approach to create genetic mosaics in mice. In this approach we use a binary system that involves an inactive germline-transmitted transgene that is activated by the somatic delivery and expression of *cre* recombinase. We have constructed an excision activated transgene (XAT) that is composed of the rat NSE promoter, an inactivating cassette, a bicistronic transcription unit consisting of a NGF minigene and IRES initiated reporter gene (NGF XAT). We have generated five independent lines of transgenic mice, two of which express the inactive transcript. Line #30 has been demonstrated by Northern analysis to express the inactive transgene at a level of ~ 30% of the amount of native NSE mRNA. Multiple independent bacterial plasmids revealed by Southern blot efficiently removed the inactivating cassette while NGF XAT was recovered "uncollapsed" from all bacteria that did not express *cre* recombinase. Recombination *in vivo* by somatic delivery of *cre* recombinase has been confirmed on the RNA, DNA, and protein levels. HSVcrelac (expressing both *cre* and ß-gal) and HSVlac were stereotactically injected unilaterally into the dorsal hippocampus of line #30 animals. Confirmation of recombination at the DNA level was determined by PCR-Southern blot analysis and nested PCR. Animals that received HSVcrelac produced a fragment consistent with the removal of the inactivating cassette (785 bp) while HSVlac injected samples did not. NGF ELISA and immunocytochemistry have demonstrated the activation of NGF XAT on a protein level. Direct quantitation of NGF expression by ELISA in the HSVcrelac injected area revealed a 13 fold increase of NGF over the HSVlac injected animals. NGF immunocytochemistry revealed a 49% increase in ß-NGF responsive cells in HSVcrelac injected animals. Ongoing experiments are investigating morphological changes associated with the focal upregulation of NGF in the hippocampus. Immunocytochemistry using antibodies against ChAT and p75 in combination with AChE staining of coronal sections from HSVcrelac injected areas will help investigate cellular networks that have been modified by the permanent focal increase of NGF. This work is supported by NSF IBN-9522307 (to HJF).

125.25

NERVE GROWTH FACTOR (NGF) EXPRESSION IN THE MOUSE PERIPHERAL NERVOUS SYSTEM AND SPINAL CHORD USING HERPES SIMPLEX VIRUS (HSV) VECTORS. W. F. Goins[1]*, J. Huard[1], M. Pike-Cavalcoli[1], S. DeKosky[2], D. J. Fink[1,3] and J. C. Glorioso[1]. Dept. of Molecular Genetics & Biochemistry[1], Western Psychiatric Institute & Clinic[2], Dept. of Neurology[3], University of Pittsburgh School of Medicine, Pittsburgh PA 15261

We have previously engineered replication competent and replication defective herpes simplex virus type 1 (HSV-1) vectors which express a functionally active form of mouse nerve growth factor (ß-NGF) in neuronal and non-neuronal cells in culture. High levels of ß-NGF were secreted into the medium from HSV-1 vector infected neuronal cells in culture and this media was capable of inducing PC-12 cell differentiation and neurite sprouting.

We have extended these in vitro studies to examine HSV-1 vector-mediated ß-NGF expression in mouse trigeminal and dorsal root ganglia during acute and latent infection using ELISA, immunohistochemistry, and RNA analyses for detection of the transgene. Vectors in which the strong human cytomegalovirus (HCMV) IE promoter was employed to drive ß-NGF expressed the neurotrophic factor at 3 days post-infection in both mouse trigeminal and dorsal root ganglia. However, ß-NGF expression from these vectors was transient in mouse trigeminal ganglia. In contrast, vectors containing the HSV-1 latency active promoter (LAP-2) driving ß-NGF were able to express the transgene at 28 days in mouse trigeminal ganglia, a time consistent with viral latency. The HCMV IEp-NGF vectors also expressed the transgene in both muscle and spinal chord during productive infection. Vector-mediated ß-NGF expression was confirmed using double-labeling studies to identify cells that expressed the neurotrophic factor and the lacZ reporter gene. We are currently evaluating the ability of these vectors to ameliorate peripheral nervous system disease.

This research was sponsored by grants from the NIH-GM34534 and -AG094701 as well as a grant from the NSF-IBN9222432

125.26

NEUROTROPHINS ARE EXPRESSED BY ADULT SPINAL CORD ASTROCYTES IN VIVO. C.F. Dreyfus*, B. Racey, G. Mendoza, X. Dai, W.J. Friedman and I.B. Black. Dept Neuroscience and Cell Biology, UMDNJ/ Robert Wood Johnson Medical School 08854 and Dept Pathology, Columbia University Coll Phys. & Surg.10032.

Recent work has indicated that exposure to exogenous neurotrophin molecules enhances regeneration of descending corticospinal neurons in the injured spinal cord (Schnell et al, 1994). To determine whether neurotrophins are normally expressed in the adult spinal cord in vivo, and to examine the cellular sources of these molecules, antisera developed against neurotrophin-3 (NT-3) and brain derived neurotrophic factor (BDNF) were utilized to detect these factors immunocytochemically in the adult rat spinal cord. The antisera used were the generous gift of David Kaplan. Robust expression of BDNF and NT-3 was evident in the spinal cord, particularly in small cells of the grey and white matter. To identify these cells, co-localization studies were performed utilizing a monoclonal antibody to the glial marker, glial fibrillary acidic protein (GFAP). Both BDNF and NT-3 were localized in GFAP+ cells, suggesting that astrocytes are a source of these trophins. To determine whether, in fact, spinal cord astrocytes elaborate trophic factors, cultured neurons derived from embryonic day 14 spinal cord were exposed for 7 days to spinal cord astrocyte conditioned medium (CM) produced by E-17 astrocyte cultures. Astrocyte CM elicited an approximate 2-fold increase in the numbers of acetylcholinesterase+ cells, suggesting that astrocytes may produce factors that enhance survival of the spinal cord cholinergic subpopulation. Our results indicate that BDNF and NT-3 are expressed by astrocytes in the normal adult spinal cord and suggest that trophic molecules elaborated by astrocytes enhance neuronal survival in culture.

Support: Amer. Paralysis Assoc. Consortium on Spinal Cord Research.

125.27

SIGNALING PATHWAYS INVOLVED IN THE REGULATION OF FGF-1, FGF-2 AND FGFR-1 IN RAT CORTICAL ASTROCYTES. M.A. Riva [a,b,*], R. Molteni [b], M. Roceri [b], B. Begni [b], and G. Racagni [b]. [a] Di.Bi.T., San Raffaele Hospital, and [b] Center for Neuropharmacology, University of Milan, Via Balzaretti 9, 20133 Milan, Italy.

The study of the molecular mechanisms involved in neurotrophic factor biosynthesis is of great importance to understand if an increased production of such factors may exert neuroprotection and contribute to the maintenance of cellular homeostasis. Astroglial cells in culture were used to investigate the signaling pathways involved in the regulation of FGF's and their receptor FGFR-1. These cells represent a source of different neurotrophic factors and may play important roles in physiological and pathological situations of the CNS.

Even though FGF-1 and FGF-2 are highly homologous, their regulation in cultured astrocytes is quite different. FGF-2 mRNA levels are increased by activation of the cAMP pathway or exposure to glucocorticoid hormones and the co-administration of these agents is synergic in the up-regulation of FGF-2. On the contrary, the gene expression for FGF-1 is decreased by both stimuli. We found that the cAMP elevating agent isoproterenol (1 μM) produced an elevation of FGFR-1 mRNA. The protein synthesis inhibitor cycloheximide decreased the expression of FGFR-1 and prevented its induction by isoproterenol, indicating significant differences with respect to FGF-2 regulation. The synthetic glucocorticoid dexamethasone, a potent inducer of FGF-2 expression, decreased FGFR-1 mRNA levels alone or in the presence of isoproterenol suggesting that this regulatory pathway may predominate over the cAMP-induced up-regulation of the receptor. Our data suggests that the response of a specific cell population to an external signal may be the result of changes at different levels involving the regulation of neurotrophic molecules and/or their receptors.

125.28

TRANSCRIPTIONAL AND TRANSLATIONAL REGULATION OF NERVE GROWTH FACTOR MRNA EXPRESSION IN GLIAL CELLS: ROLE OF AP-1. A.M. Colangelo, P. F. Johnson, M.A. DeBernardi* and I. Mocchetti. Dep. of Cell Biology, Georgetown Univ. Sch of Med., Washington DC, 20007, and ABL-BRP, NCI-Frederick Cancer and Research Center, Frederick, MD 21702.

Molecular mechanisms regulating nerve growth factor (NGF) gene expression in the CNS are still largely not well understood. The NGF gene contains an AP-1 consensus sequence which plays a role in basal and inducible transcription of the NGF gene in non neuronal cells. We have used C6-2B rat glioma cells, a line known to express and release NGF, to investigate the role of AP-1 in the transcription regulation of NGF mRNA. We have analyzed nuclear extracts from these cells following stimulation of various signaling pathways for changes in factors which bind to the AP-1 recognition sequence of the rat NGF gene. Gel mobility shift assays using an oligonucleotide homologous to the AP-1 responsive element of the rat NGF gene ($AP\text{-}1_{NGF}$) revealed that 12-O-tetradecanoyl phorbol-13-acetate (TPA), and to a lesser extent isoproterenol (ISO) stimulated binding to $AP\text{-}1_{NGF}$. The increase in $AP\text{-}1_{NGF}$ binding preceded the accumulation of NGF mRNA. Moreover, cycloheximide pretreatment blocked the TPA and ISO-mediated binding to $AP\text{-}1_{NGF}$ and inhibited the NGF mRNA accumulation by these stimuli suggesting that *de novo* synthesis of c-fos/c-jun may be required for the transcriptional regulation of NGF gene. Nuclear run-on assays and studies on NGF mRNA stability revealed that while TPA increases NGF transcription only, ISO affects NGF mRNA stability as well. We propose that different signal transduction mechanisms regulate the expression of the NGF gene in cells derived from the CNS, and that mRNA transcription and stability account for the cAMP-mediated accumulation of NGF mRNA. Experiments are underway to characterize whether other leucine zipper proteins might be involved in the transcription regulation of NGF gene. [Supported by HHS grant NS29664].

125.29

PREPARATION OF BRAIN-DERIVED NEUROTROPHIC FACTOR AND NEUROTROPHIN-3 SECRETING SCHWANN CELLS BY INFECTION WITH A RETROVIRAL VECTOR S. Sayers*, T. Khan, G. DeVries, R. Shahid, Y. Ahmed, N. Khan, T. Lopez and L. Liu. Rehabilitation R&D Center, Hines VA Hospital, Hines, IL 60141.

One of the reasons for the lack of regeneration in the CNS of adult mammals is the absence of neurotrophic factors. Recent studies have shown that BDNF and NT-3 encourage the regrowth of motor and sensory fibers after spinal cord injury. Preliminary studies from our laboratory have found that Schwann cells cultured on small diameter carbon filaments induced neuritic outgrowth from fetal rat spinal cord explants. The purpose of the present study was to genetically modify Schwann cells to produce increased amounts of BDNF or NT-3.

The cDNA for BDNF and NT-3 were obtained from Dr. George Yancopoulos of Regeneron Pharmaceuticals, Inc. and the retroviral vector LXSN cDNA was obtained from Dr. Dusty Miller at the Fred Hutchinson Cancer Research Center. Retroviral vectors were constructed by the ligation of the BDNF cDNA or the NT-3 cDNA to the LXSN retroviral vector DNA. Virus were generated from the plasmid forms of the retroviral vectors by transient transfection of PA317 amphotrophic packaging cells. The virus was harvested and used to infect the human Schwann cell line designated NF-1T. Poly (A^+) RNA was prepared from the infected Schwann cell cultures, subjected to electrophoresis, and then transferred onto membranes. The Northern blots were hybridized with ^{32}P-labeled riboprobes which were prepared to detect either BDNF or NT-3 mRNA.

The autoradiograms obtained from the Northern blots from Schwann cells which were infected with either BDNF or NT-3-containing virus clearly showed the presence of the BDNF or NT-3 mRNA signal. We are currently in the process of evaluating whether these genetically engineered Schwann cells are secreting biologically active BDNF and NT-3. Hopefully, these neurotrophin-secreting Schwann cells will act as effective stimulators of axonal regrowth *in vivo*.

(Supported by Rehabilitation R&D Center core funds, DVA)

125.30

BIOSYNTHESIS OF NGF IN NEURONS

S. J. Mowla, S. Pareek*, W. Sossin, S. Iacampo, N. G. Seidah[1] and R. A. Murphy. Montreal Neurological Institute, McGill University, and [1]Clinical Research Institute of Montreal, Montreal, Canada

In the CNS, neurotrophins (NTs) are produced by neurons, but the pathways by which NTs are synthesized and secreted is not well understood. In non-neuronal cells, we have detected two patterns for the processing of NGF that differ in constitutive and regulated secretory cells (Seidah *et al.*, 1996, Biochem J., 314, 951-960). Both types of secretory cells produce a 35 kDa precursor. Constitutive cells (BSC40, LoVo cells) modify the precursor by glycosylation and sulfation to yield a 42.5 kDa intermediate that is cleaved by co-expressed convertase processing enzymes to yield 16.5 and 13.5 kDa forms of NGF. Regulated secretory cells (AtT20) produce a 39 kDa intermediate that is processed into a 13.5 kDa form mature NGF. In this study, we investigated the processing of NGF within neurons. Primary cultures of rat sympathetic, sensory, and mouse hippocampal neurons infected for 1 hr with vaccinia viruses (VV) containing cDNA constructs coding for the ORF of NGF. The cells were incubated an additional 8 hrs and metabolically labelled with radioactive amino acids for 1 hr. Cell extracts and cell conditioned media were immunoprecipitated with antibodies to NGF and analyzed by SDS PAGE and autoradiography. Data indicate that the three types of neurons produce a 35 kDa form of the NGF precursor that is post-translationally modified (presumably by glycosylation) to yield three higher molecular species in the range of 39-42 kDa, as determined by pulse chase labelling. These species are processed by endogenous cellular enzymes to generate only the 13.5 kDa form of NGF that could be detected both in cell extracts and in conditioned medium. None of the 16.5 kDa form of NGF was evident. The results show that neurons have the cellular machinery to process NGF correctly and efficiently. Work is in progress to determine the secretory pathway of NGF in neurons.

This work is supported by NCE, MRC (RAM) and MCHE (JM).

NEURONAL DEATH: LESIONS

126.1

CONFOCAL IMAGING OF RAT RETINAL GANGLION CELLS AFTER OPTIC NERVE INJURY. R. Engelmann*, M. F. Humphrey and B. A. Sabel. Institute for Medical Psychology, Otto-von-Guericke-University Magdeburg, D-39120 Magdeburg, Germany.

We have developed a new *in vivo* method to repeatedly visualize retinal ganglion cells (RGC) labelled by retrograde transport of fluorescent markers, in adult rats. This was accomplished with an upright Confocal Laser Scanning Microscope (CLSM, Noran Instruments) with low aperture lenses, a special non-traumatic headholder, and an immersion plano-concave correction lens. Before and after optic nerve cut or crush fluorescent dyes were injected stereotaxically into the superior colliculus and the fluorescing RGCs were repeatedly visualised for up to five weeks. The population density of *in vivo* RGCs at 30° excentricity was 1800 cells/mm² in control retinae. After optic nerve crush the density dropped to 1550 cells/mm² at one week and 1100 cells/mm² at two weeks. At later time points the density remained at the same level but only a small proportion of fluorescing objects maintained the strong compact fluorescence, characteristic of RGCs. Here, the majority of the fluorescence was found to be limited to smaller, diffusely and faintly labelled objects. Conventional microscopic examination of retinal wholemounts revealed that these corresponded to label sequestered in microglial-like cells. Selective label of the surviving population of RGCs by colliculus injection *after* the crush showed a strongly labelled population of RGCs, with a density of 360 cells/mm² which did not change between 2 and 5 weeks after crush. No damage of the retinal tissue could be observed when the retinae were prepared as conventional wholemounts despite repeated illumination with the CLSM.

Supported by the DFG Innovationskolleg #15/TP-A6 and Noran Instruments.

126.2

APOPTOSIS IN RETINAL GANGLION CELLS AFTER A CONTROLLED CRUSH OF THE ADULT RAT OPTIC NERVE

A. Bien*, M.F. Humphrey, C. Seidenbecher, B.A. Sabel, M.R. Kreutz
Inst. of Med. Psychology, Otto-von-Guericke University, Magdeburg, Germany.

Apoptosis is a common type of cell death in the nervous system after neurotrauma. We investigated whether axonal trauma leads to changes in retinal ganglion cells (RGC) characteric for cells going into the cell cycle. As shown previously controlled optic nerve crush is followed by a massive retrograde cell death of RGC over a time course of several weeks. In the present study the optic nerve of hooded rats was crushed and at different time points after injury eyes were removed and processed for immunocytochemistry or DNA and protein extraction. Pyknotic cells were readily observable 72 hours, 1 week and 6 weeks after axonal trauma in the retinal ganglion cell layer of rat paraffin sections. At the same time points an increasing number of TUNEL-positive cells was found in parallel sections. DNA fragmentation 1 week post-injury was independently confirmed by agarose DNA-gel electrophoresis and TUNEL staining in retinal whole mounts. This study emphasizes that the massive cell death after axonal trauma (~70% of all RGC die within two weeks) is related to apoptotic processes. Since apoptosis is oberservable for a long period, this model of partial axonal injury provides key features for in vivo investigations on pharmacological and molecular underpinnings of neurotrauma-induced cell death.

Supported by BMBF Neurotrauma Magdeburg-Berlin TPA2 to B.A.S. and M.R.K.

126.3

SURVIVING RETINAL GANGLION CELLS DO NOT EXPRESS PHOSPHORYLATED NEUROFILAMENTS IN SOMA AND DENDRITES AFTER NERVE CRUSH. M.F. Humphrey*, R. Engelmann and B.A. Sabel. Institute of Medical Psychology, Otto-von-Guericke University, D-39120 Magdeburg, Germany.

Highly phosphorylated forms of neurofilaments (pNFs) are normally restricted to axons. Following optic nerve cut, where most retinal ganglion cells (RGCs) eventually die, pNFs are also found in the soma and dendrites. These abnormal pNFs may therefore be part of a cell death sequence. However, because altered pNFs are also seen in regenerating peripheral nerve cells they may also be involved in adaptive reorganization. Therefore, following optic nerve crush, we have compared pNF expression in RGCs connected to the visual centers with those that degenerate.

In adult PVG/c rats the optic nerve was crushed unilaterally using special cross-action forceps (Sautter and Sabel, 1993, Euro. J. Neurosci., 5, 680-690). At various times after crush Fluorogold (FG) was injected into the superior colliculus to retrogradely label the connected RGCs, and the retinae were then prepared for labelling with the 2F11 monoclonal NF antibody using a Cy-3 fluorescent labelled second antibody for detection.

pNF labelled RGCs were found at 7, 14 and 21 days post-crush although the proportion was low (up to 100 cells/mm²). A variety of soma sizes and primary dendritic types were labelled although most were in the medium to large range. Most of the strongly pNF labelled RGCs were not FG labelled, even when surrounded by many other FG labelled cells indicating that FG was available for uptake. Therefore, the cells which survive and remain connected with their target after nerve crush do not express pNFs in the soma and dendrites. We are currently examining the time of disconnection before pNF expression and the relation to apoptosis.

Supported by BMBF Verbund 07 NBL 04 TP B5 and Neurotrauma-Verbund TPA2.

126.4

INCREASE IN GENE EXPRESSION IN THE RETINA AFTER OPTIC NERVE CRUSH, L. A. Levin* and K. M. Geszvain. Department of Ophthalmology and Visual Sciences, University of Wisconsin Medical School, Madison, WI 53792.

Retinal ganglion cell death is the final common pathway of a variety of optic nerve disorders. We and others have shown that optic nerve injury induces apoptosis in retinal ganglion cells. To better understand the molecular program for the death of these cells, we attempted to identifiy genes associated with retinal ganglion cell axotomy.

Total RNA was isolated from Long-Evans rat retinas at 1 and 4 days after intraorbital crush of the ipsilateral optic nerve; the contralateral retinas were used as a source of control RNA. Reverse-transcribed (RT) cDNA was used in a refinement of the differential display technique, using long primers; all amplifications were done in duplicates. After screening of several thousand bands, a single reproducibly differentially expressed band was reamplified and cloned into a TA-cloning vector. Multiple clones from this band were screened, and one product found to hybridize with Northern blots of total RNA, with greater expression at 1 and 4 days after crush than control. The product was sequenced and used to design primers for PCR. These were then used to confirm differential expression of the product in additional groups of experimental animals. RT-PCR demonstrated mRNA in the retina and liver, but not the brain, lung, spleen, kidney, or thymus of rats. Western blots confirmed expression of the protein product in the retina, and immunohistochemistry demonstrated immunolabeling of ganglion cell layer cells in a human eye that had previously sustained ischemic optic neuropathy.

The possible role of this gene in retinal ganglion cell apoptosis will be discussed. Supported by NIH K11 EY00340 and Research to Prevent Blindness, Inc.

126.5

LIGHT EXPOSURE ENHANCES THE SURVIVAL OF ADULT AXOTOMIZED RETINAL GANGLION CELLS. L. J. Aigner, S. A. Singel, M. Rasminsky*, G. M. Bray and A. J. Aguayo. Centre for Research in Neuroscience, McGill University, The Montreal General Hospital Research Institute, 1650 Cedar Avenue, Montreal, PQ H3G1A4, Canada.

We have tested the hypothesis that regulation of survival of injured neurons may involve neuronal activity. To this end we investigated the effects of light on the survival of axotomized retinal ganglion cells (RGCs) in adult pigmented rats ten days after intraorbital optic nerve cut. Rats (n=6 per group) were kept for ten days after axotomy in the usual 12h light/dark cycle, under constant light, or were deprived of light by keeping them in complete darkness or by closure of the eyelid. 467±72 RGCs/mm^2 and 570±141RGCs/mm^2 survived axotomy in retinas exposed to the 12h light/dark cycle or to constant light, respectively, whereas only 185±113 RGCs/mm^2 and 191±26 RGCs/mm^2 survived axotomy in retinas deprived of light by complete darkness or by closure of the eyelid, respectively. These *in vivo* results indicate a significantly decreased survival ($p < 0.001$) of axotomized RGCs that were not exposed to light, suggesting that light stimulation of the retina might be beneficial for RGC survival after injury.

Supported by the Canadian Neuroscience Network, the Deutscher Akademischer Austauschdienst and by Boehringer Ingelheim.

126.6

AXOTOMY OF DORSAL LATERAL GENICULATE NEURONS (LGN) IN THE RAT AND CAT LEADS TO APOPTOTIC CELL DEATH. **R.E. Kalil*, M. Shively and J.P. Fedynyshyn. Center for Neuroscience and Department of Ophthalmology and Visual Sciences, University of Wisconsin, Madison, WI 53706.**

Although it has been known for some time that most neurons in the mammalian central nervous system die after axotomy, relatively few studies have explored the mechanisms by which these cells may die. In the rat and the cat, removal of visual cortex axotomizes neurons in the LGN. After two to three days of apparent stasis, large numbers of axotomized LGN neurons die quickly and are rapidly cleared. When stained with cresyl violet, dying LGN neurons display some of the cytological features e.g., condensation of the nuclear chromatin, that have been associated with apoptosis. To obtain further evidence about the mode of cell death involved, we have used the TdT-mediated dUTP Nick-End Labeling (TUNEL) assay to detect DNA fragmentation in dying LGN neurons. Sections through the LGN in newborn kittens and adult rats that had received a large lesion of visual cortex at three days (kittens) or seven days (rats) prior to perfusion with mixed aldehydes were labeled with fluorescein-12-dUTP by the TUNEL method and counterstained with propidium iodide. Fluorescently labeled LGN neurons were examined with a Bio-Rad MRC 1024 confocal microscope. In both rats and cats, fluorescein-stained, TUNEL+ LGN neurons were observed scattered among propidium-iodide stained cells with intact DNA. Although prior work indicates that LGN cell death is massive after axotomy, the number of TUNEL+ neurons in single sections through the LGN is relatively modest, in keeping with the belief that apoptotic death proceeds swiftly. The results from these experiments, in conjunction with the observations on dying neurons mentioned above, provide additional evidence suggesting that apoptosis is the mechanism by which axotomized LGN cells die.

We thank Promega Neurosciences for providing the TUNEL reagents. This work was supported by NIH grant EYO 1331.

126.7

NEURONAL DEATH IN THE LATERAL GENICULATE NUCLEUS OCCURS FOLLOWING TARGET DEPRIVATION. NA Al-Abdulla, C Portera-Cailliau, BJ Crain*, DL Price, and LJ Martin. Neuropathology Laboratory, The Johns Hopkins School of Medicine, Baltimore, MD 21205.

Mechanisms of neurodegeneration following axotomy and target deprivation remain poorly understood, and the presence of neuronal death in these models is unconfirmed. We used a unilateral occipital cortex ablation model in adult rats to identify the time course of neuronal injury and death in the lateral geniculate nucleus (LGN). To determine if neurons die in the LGN ipsilateral to the cortical lesion, we used the TUNEL method of in-situ labeling of nuclear DNA fragmentation. TUNEL-positive LGN neurons were present at 3 to 14 days post-ablation, peaking around 7 days. The morphological features of LGN neuronal death were evaluated by light microscopic analysis of Nissl-stained paraffin sections and semithin plastic sections as well as by electron microscopy. Vacuolation of neuronal cytoplasm occurred in the medio-ventral portion of the ipsilateral LGN by 3 days and peaked at 14 to 30 days. Neuropil vacuolation was found maximally at 3 to 14 days. In addition, neurofilament (NF) immunocytochemistry revealed irregularly shaped perikarya with abnormal NF accumulation initially, followed by NF loss. By 7 days, subsets of LGN neurons atrophied, and neuronal density as assessed by Nissl-stain decreased, confirming neuronal death in the medio-ventral LGN. Some dorsal ipsilateral LGN neurons survived but appeared hypertrophied by 3 months. We conclude that neuronal death in the adult LGN occurs following target deprivation. The morphological changes leading to neuronal death were highly organized and patterned but not apoptotic. Supported by NIH.

126.8

Identification of Genes Differentially Expressed in the Lateral Geniculate Nucleus Following Axotomy. J.S. Bhangav* and M.S. Cynader. Department of Ophthalmology, University of British Columbia, Vancouver, British Columbia, Canada. V5Z 3N9

It has been well established that lateral geniculate nucleus (LGN) neurons undergo retrograde degeneration following a visual cortex lesion. The death of these LGN neurons occurs by apoptosis which makes it distinct from the necrotic form of cell death that results from direct insult. Since apoptosis generally requires RNA and protein synthesis, it is believed that the process is dependent on active gene expression.

To investigate the genes involved in apoptotic neuronal cell death in the LGN, we have used the RNA arbitrarily primed polymerase chain reaction (RAP-PCR) technique for fingerprinting RNA transcripts. Briefly, a unilateral lesion of the visual cortex was made in two adult cats. After 48 hours, the animals were perfused and RNA was isolated from the LGNs ipsilateral and contralateral to the lesion. Total RNA from both the lesioned and control sides was used for cDNA synthesis using an arbitrary primer at low annealing stringency. The resulting cDNA was then amplified by high-stringency PCR. The PCR products were electrophoretically separated and a number cDNAs representing potentially differentially expressed mRNAs were isolated. Thus far, the degeneration association of several of these transcripts has been confirmed by Northern blot analysis. Characterization of these and other putative cell death genes is currently underway.

Supported by MRC and NCE of Canada.

126.9

AGE-AT-LESION REDUCTION IN BILATERAL THALAMIC VOLUME IN HEMINEODECORTICATE CATS. J.R. Villablanca*, H.M. Patel, T.D. Schmanke. Mental Retardation Research Center, UCLA, LA, CA 90024

We have used behavioral, anatomical and cerebral metabolism measures to establish that the outcome of cerebral hemispherectomy is better if the removal is sustained neonatally versus in adulthood. We are now investigating at which postnatal age the transition to poorer outcome may occur. Here we present relevant anatomical results. Groups (N=5) of cats that sustained hemineodecortication (HDec) at postnatal ages P5-15, P30, P60, P90, P120 days and in adulthood were compared to intact controls. With all cats as young adults, we measured the cross-sectional area of the thalamus bilaterally in 18 - 21 coronal sections per brain (50µm thick, 600µm apart) and used these values to calculate mean total thalamic volume per cat group and per right and left thalamus. Volumes are in µm3; left is ipsilateral to the lesion:

Age at HDec, days:	P5-15	P30	P60	P90	P120	Adult;	Intact
Ipsilat. Thal.,left	202	206	203	200	177	157	363
Contral. Thal.,right	361	373	338	350	323	341	394

Ipsilaterally: there was a large thalamic atrophy relative to controls ($P<.01$), Tukey's test, range 43.3% (P30) to 56.7% (adult-lesioned), for all lesion ages but this was less pronounced, compared to adult HDec cats ($P<.01$ to .05) for cats with HDec at ages P5-15, P30, P60 and P90 days. **Contralaterally:** there was also atrophy, range 5.5% (P30) to 18.2% (P120),but this was significant ($P<.01$) only for cats HDec at P60, P120 and in adulthood. Thus, for both sides the protective effect of young age lasted only through about 90 days of age. Grants USPHS HD-05958, HD-14612, NRSA T32HDO7416.

126.10

A NEOCORTICAL LESION SUSTAINED DURING THE LATE FETAL PERIOD INDUCES A WIDESPREAD ENDURING REDUCTION IN CEREBRAL METABOLISM IN CATS. D.A. Hovda*, J.R. Villablanca and T.D. Schmanke. Mental Retardation Research Center, UCLA, LA, CA 90024

When a unilateral frontal cortical lesion is sustained in utero, adult cats show substantial behavioral impairments while neonatal-lesioned cats exhibit almost none. Here we present physiological correlates of these effects using [^{14}C] 2-deoxy-D-glucose autoradiography . Groups of kittens (N=3 each) sustained resection of the pericruciate frontal cortex prenatally (E47-52) or neonatally (P6-10) or were used as intact controls (N=7). When all animals were young adults, local cerebral metabolic rates for glucose ($LCMR_{glc}$) were measured in 50 brain regions bilaterally. Taking all gray matter structures together, there was a bilateral decrease of $LCMR_{glc}$ which was greater in fetal-lesioned (FL) than in neonatal-lesioned (NL) cats. In terms of µg/min/100gr the mean (S.E.M.) $LCMR_{glc}$ rates were, for the hemisphere ipsilateral to the lesion: FL cats, 49.2 ± 5.7, NL cats, 57.5 ± 3.8, for the contralateral hemisphere: FL cats, 49.2 ± 6.7 , NL cats 55.7 ± 2.9, intact cats, 71.4 ± 5.5. Only FL cats show significant decreases versus controls ($P<.05$). Considering individual structures, in FL cats 22 regions showed significant decreases ($P<.01$ to $<.05$) while in NL cats only 5 (ipsi) and 6 (contra) regions showed significant reductions ($P<.05$). The areas with little or no bilateral changes in FL and NL cats were the visual cortex, hippocampus, most ventral- diencephalic sites, s.nigra, sup. and inf. colliculi, and cerebellum.. The widespread $LCMR_{glc}$ decrease in prenatal lesioned cats match the behavioral impairments and neocortical-thalamic atrophy seen in these cats. USPHS HD-05958, HD-14612, NRSA T32HDO7416.

126.11

EARLY-BRAIN LESION EFFECT: WHICH IS THE POSTNATAL AGE-AT-LESION LIMIT FOR GREATER RECOVERY/SPARING IN CATS?

P. Carlson-Kuhta, J.R. Villablanca, T.D. Schmanke and D.A. Hovda.
Mental Retardation Research Center, UCLA, Los Angeles, CA 90024

There is evidence that after a similar telencephalic resection neonatal-lesioned cats show a greater behavioral/sparing than adult cats, but there is little information regarding the age-at-lesion where the transition occurs. Here we report on behavioral effects of hemineodecortication sustained at postnatal ages P15,P 30,P60,P 90, P120 and in adulthood (N=5-6/group). An extensive behavioral battery of tests was applied starting at approximately 180 days post-lesion. For 8 tests assessed using a scoring system (limb paresis and posture, limb placing reactions-plank walking, face tactile responses), age-at-lesion accounted for significant differences (Kruskal-Wallis ANOVA) for all tests, and there was a tendency for a linear increase in impairment scores from the P5-15 through adult-lesioned groups. For 4 tests assessed with parametric measures (turning behavior, paw usage bias, pupil asymmetry and eye alignment), a regression analysis showed a significant linear trend for increased impairments in the P5-15 through the P120 (or adult-lesioned) groups. Finally, for 3 additional parametric tests (paw food retrieval, hind limb stride length, eye-opening asymmetry) there was a sharp decline in performance starting with the P60 group and continuing thereafter. Thus: a) age-at-lesion was important for the behavioral outcome during the P5-120 day period in cats; b) for the P5-15 and P30 groups the outcome was fairly similar with some performances declining sharply at about P60 while other declined progressively through P120. Grants USPHS HD-05958, HD-14612, NRSA T32HD07416.

126.12

DIFFERENTIAL SHRINKAGE OF THE REMAINING NEOCORTEX IN CATS WITH HEMINEODECORTICATION SUSTAINED NEONATALLY OR IN ADULTHOOD.

T.D. Schmanke*, J.R Villablanca and V. Lekht.
Mental Retardation Research Center, UCLA, LA, CA 90024

Using a developmental animal model of cerebral hemispherectomy and stroke, we reported that following the lesion in adulthood there were **bilateral** changes in thalamic size and cell numbers. Here we report on changes in the remaining neocortex. We used young adult cats with hemineodecortication performed neonatally (P8 to P15) or in adulthood and intact animals (N=6/group) to measure the cross-sectional area of the remaining neocortex in 42 to 63 coronal sections (AP +24 to -10) per brain (50μm thick at 600μm intervals in fresh-frozen brains, cytochrome oxidase staining). We then used these values to calculate the total cortical volume per group. The mean neocortical volume (S.E.) for intact brains was 4,165 ± 159 μm3, for adult-lesioned cats was 3,595 ± 194μm3 ($p<0.05$) indicating a 14% shrinkage, and for the neonatal-lesioned animals it was 3.690 ± 117μm3, indicating a tendency to cortical atrophy (12%). In addition, for sections between stereotaxic coronal planes +2 and +6 the mean cross-sectional areas were significantly larger in the neonatal vs. the adult-lesioned cats. Thus, in hemineodecorticated cats the remaining neocortex shrunk significantly in the animals with the lesion in adulthood but was somewhat spared in animals lesioned neonatally. This fits well with our finding of increased neocortical plasticity in neonatal animals. We suggest that these patterns of change should also apply to humans due to a relatively similar perinatal time course of brain maturation in man as compared to cats. (Grant USPHS HD-05958, HD-14612, NRSA T32HDO7416).

126.13

DEAFFERENTATION CAUSES APOPTOSIS: AN *IN VIVO* MODEL OF NEURONAL DEATH IN THE CEREBRAL CORTEX OF ADULT RATS.

S.A. Capurso[1,4], M.E. Calhoun[4], D.L. Price[1-4], V.E. Koliatsos*.
Departs. of Pathology[1], Neurology[2], and Neuroscience[3], and the Neuropathology Lab.[4], The Johns Hopkins Univ. Sch. of Med. Baltimore, Maryland, USA

The present study provides evidence that apoptosis is inducible in the cerebral cortex of mammalians by deprivation of sensory afferents. We propose an *in vivo* model of neuronal death produced in the piriform cortex of adult rats by unilateral total bulbectomy. We studied degenerating neurons with an *in situ* labeling method (TUNEL), electron microscopy (EM), and biochemical assays. TUNEL-positive neurons were mapped; total neuronal loss was estimated using stereology. The maximal *in situ* labeling of apoptotic neurons occurred in the olfactory cortex ipsilateral to the lesion at the commissural level 18-26 hours postbulbectomy; stereology estimated that an 18% neural loss occurred after survival for 48 hours. DNA gel electrophoresis yielded a ladder pattern of fragmentation. EM allowed visualization of ultrastructural features characteristic of apoptotic neurons in early stages of degeneration whereas synaptic structures and cytoplasmic organelles were still intact. Apoptosis was triggered by a massive loss of inputs rather than axonal transection. Injections of a retrograde fluorescent tracer (Fast Blue) in the olfactory bulb as well as in the posterior olfactory cortex, two days before bulbectomy, provided evidence that neurons undergoing apoptosis were pyramidal cells receiving inputs from, but not projecting to, the olfactory bulb. We have also evaluated the inflammatory reaction at the site of degeneration; immunolabeling of astrocytes and microglia localized glial cells in the olfactory cortex 22 hours postbulbectomy but failed to reveal macrophage-specific epitopes. The present model provides a tool to study signal transduction pathways that lead to apoptosis in the rat cerebral cortex *in vivo* and can be used to test the efficacy of treatment strategies. This work was supported by grants from the NIH.

126.14

LONG TERM CHANGES IN BRAIN CHOLINERGIC MARKERS AND NGF LEVELS AFTER PARTIAL IMMUNOLESION.

Z. Gu, J. Yu, J.R. Perez-Polo*. Dept. HBC&G, Univ. TX Med. Br., Galveston, TX 77555-0652.

There are deficits in cholinergic basal forebrain neurons (CBFNs) in aging and Alzheimer's disease. To mimic the partial loss of CBFNs in these situations and study the long term effects of the loss of CBFNs on remaining cholinergic activity and target-derived nerve growth factor (NGF) levels, we produced a partial immunolesion to CBFNs with 192 IgG-saporin, a well recognized toxin to low affinity NGF receptor-bearing neurons. We measured the cholinergic markers, choline acetyltransferase (ChAT) and acetylcholinesterase (AChE), and NGF protein levels at 10, 30, 70, 180, and 365 days. Ten days after 192 IgG-saporin treatment, ChAT activity decreased to 35-50% in the olfactory bulb, hippocampus, and cortex when compared to normal controls. At 6 months postlesion, ChAT activity in the olfactory bulb returned to near control values and then decreased to 56% of controls at 1 year postlesion. There was a slight but significant recovery of ChAT as early as 1 month in the hippocampus and at 6 and 12 months postlesion in cortex. Similar but less dramatic changes in AChE activity were observed in these areas. There were significant long-term increases in NGF protein levels in the olfactory bulb with the highest levels at 3 to 6 months after lesion. Relative short-term increases in NGF levels were present in the hippocampus and cortex with a return to control values at 6 and 12 months postlesion, respectively, and no further recovery of ChAT activity in these areas. In addition, graded changes in both ChAT and NGF levels were observed in all the brain regions examined with respect to a partial and total immunolesion 1 month postlesion. These results suggest that there is a region-specific and time-dependent recovery of cholinergic activity in the target areas of the basal forebrain after a partial elimination of CBFNs and comparative changes in NGF levels in these areas suggesting that both ChAT and NGF levels respond to the state of cholinergic innervation of target areas of the basal forebrain. This is publication 53A from USPHS P01-A610514 awarded by NIA. Also supported by NINDS NS 18708 and NS 33288.

126.15

ULTRASTRUCTURAL ANALYSIS OF THE HIPPOCAMPUS OF ADULT RAT FOLLOWING LONG-TERM ADRENALECTOMY.

A. Islam*, J. Westman, N. Bogdanovic, B. Winblad and A. Adem. Department of Clinical Neuroscience & Family Medicine. Geriatric Section. Karolinska Institute. Huddinge University Hospital. Huddinge. Sweden. Department of Anatomy. Bio-medical Center. Uppsala. Sweden.

Adrenalectomy (ADX) causes neurone loss in the hippocampus of the adult rat. We undertook this study on adrenalectomized rats 5 months after surgery to analyse the type of cell death in the hippocamal neurones. Bilateral adrenalectomy was done by dorsal approach in male Sprague Dawley rats at 10 weeks of age. Twenty weeks later, 3 ADX and 3 sham operated rats were perfused through the heart with a short rinse of 0.1M phosphate buffer (100 ml) followed by 300-400 ml of 2.5% gluteraldehyde and 2.0% formaldehyde in 0.1M phosphate buffer (38^{O}C, pH 7.4). After termination of the perfusion, the brain was left in situ for an hour, removed and left overnight in fresh fixative at 4^{O}C. The brain was then cut transversely on a vibratome (Oxford Instruments) into sections 100μm thick. The sections were post fixed for 20 minutes in 2% OsO_4 in cacodylate buffer, dehydrated in a graded series of ethanol and embedded in Epon between acetate foils. Light microscopic investigation was performed on 2μm thick sections recut from 100μm sections and stained with toludene blue. Electron microscopy was done on ultra thin sections picked up on one-hole grids and stained with uranyl acetate followed by lead citrate. Electron microscopy showed apoptotic changes both in the dentate and in the pyramidal areas. These results indicate that adrenalectomy produces neuronal death both in the dentate and the pyramidal areas which is mediated through apoptosis.

This study was supported by Swedish Medical Res. Council, Gamla Tjänarinnor Foundation & KI fonder

126.16

CHRONIC STRESS DOES NOT INDUCE NEURONAL DEATH IN THE HIPPOCAMPAL FORMATION OF AGED RATS.

N. Sousa, M.D. Madeira, L. Xue, O.F.X. Almeida† and M.M. Paula-Barbosa*. Dept. of Anatomy, Porto Medical School, Porto, Portugal and †Max Planck Institute of Psychiatry, Munich, Germany.

Sustained exposure of rats to elevated levels of corticosterone (CORT), as found during chronic stress, have been associated with behavioural impairments. Several authors have suggested that the behavioural deficits ensue from CORT-induced neuronal damage in the hippocampal formation (HF); further, the magnitude of neuronal death was shown to correlate positively with age of the experimental subject. All these previous studies assessed neuronal death using conventional, but biased, morphometric methods. In this study, we therefore applied unbiased stereological tools to evaluate the effects of chronic exposure to stress upon the volume of the HF divisions, using Cavalieri's principle, and its total number of neurones, using the optical fractionator. Male rats aged 17 months were submitted to the unpredictable stress paradigm for a period of 1 month, and sacrificed when 24 months old. One of several stressors (i.p.injections of saline, overcrowding, restraint, and placement on a rocking platform) were applied in a random order on a daily basis. The efficacy of this treatment was confirmed by elevated serum CORT levels measured at various intervals during exposure to stress. Stereological analysis of the various HF subdivisions (CA_{1-3}, dentate gyrus, hilus) failed to reveal any significant loss of neurones as result of the chronic stress treatment. The present findings, based on unbiased measurements, call for a reappraisal of the hypothesis that chronic hypercorticalism leads to behavioural impairments as a direct result of neuronal losses in the HF. Experiments are currently being undertaken to examine the possibility that the HF of younger rats may be more vulnerable to the effects of chronic stress. (Supported by JNICT)

127.1

NEURONAL PROTEINS AS MARKERS OF CNS DAMAGE. N.S. Deineka, C.M. Patanow and M.L. Billingsley*. The Pennsylvania State University College of Medicine, Department of Pharmacology, Hershey, PA 17033.

Physical and chemical insults to the CNS result in altered expression of neuronal and glial proteins. Damage can be assessed by measuring increases in injury response elements (i.e. GFAP) or losses in neuronal proteins. We used two proteins, SNAP-25 and stannin, as potential markers of CNS damage. SNAP-25 is a presynaptic "snare" protein which regulates vesicle docking and synaptogenesis. A developmentally-restricted isoform, SNAP-25a, predominates until post-natal day 7. Stannin, is expressed in cells sensitive to trimethyltin (TMT). Previous experiments suggest that stannin is necessary, but not sufficient for the manifestation of TMT toxicity. Exposure to TMT (8mg/kg) induced a marked loss of neuronal stannin immunoreactivity in hippocampus. SNAP-25b immunoreactivity decreased 3 days post-TMT but increased robustly after 7 days. Unilateral-mechanical transection of the fimbria-fornix and perforant paths caused a decline in SNAP-25b immunoreactivity in the hippocampus with no change in stannin. Notably, there was a marked increase in SNAP-25a during reactive synaptogenesis. Localized increases in GFAP were seen following both forms of damage. Current experiments are focused on assessment of changes in epitope-tagged neuronal and glial markers in transgenic animals following selective CNS damage. This work was supported by PHS grant ES-05450 and EPA grant R818002-01-0 to M.L.B.

127.2

IS THERE LIGHT AT THE END OF TUNEL IN THE DEVELOPING WHISKER-BARREL NEURAXIS? M.F. Jacquin*, H.-X. Dong, K.E. Good, P. Osborne, E.M. Johnson & T.A. Henderson. Neurology & the Center for the Study of Nervous System Injury, Washington University School of Medicine, St. Louis, MO 63110.

The terminal deoxyribonucleotidyl (TdT) mediated dUTP-digoxigenin nick-end labeling (TUNEL) assay is widely used to address issues related to programmed cell death. Several paradigms were used to study relationships between TUNEL staining, cell death, pattern formation, and injury-induced plasticity in the rat trigeminal (V) system: **1)** In normal development from E19-P10, TUNEL$^+$ cells were plotted relative to the whisker pattern revealed by cytochrome oxidase in V nucleus principalis. On P1-2, TUNEL$^+$ neurons were most frequent in septae between barrelettes; however, the numbers of stained neurons were low relative to the numbers of neurons known to be dying from E19-P10. **2)** Cutting the infraorbital nerve on P0-4 did not increase the number of TUNEL$^+$ cells in affected V ganglia and brainstem nuclei after 1-8 hrs and 1-4 days. This negative result is noteworthy because nerve injury kills thousands of ganglion/brainstem cells and obliterates whisker patterns during these intervals. **3)** After electrocautery of the S1 barrel cortex on P0, TUNEL$^+$ cells were numerous at the injury site; yet, there was no increase in the number of TUNEL$^+$ cells in the affected VPM thalamus and nucleus principalis where thousands of neurons die. **4)** Anti-NGF injections on E15-16 increased the number of TUNEL$^+$ cells in V, spinal and superior cervical ganglia. Thus, either injury-induced anterograde and retrograde cell death in early development does not occur by apoptosis, or TUNEL staining is not a reliable detector of such forms of cell death.

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127.3

ASP, A NOVEL MARKER FOR APOPTOTIC MOTONEURONS. J.A. Garrah, M.A. Bisby, A.C. Jackson[1]* and J. Rossiter[2], Departments of Physiology, [1]Medicine and [2]Pathology, Queen's University, Kingston, Ontario, K7L 3N6.

An intended c-jun antibody recognized a different protein, designated "ASP", by immunocytochemistry and Western blotting of apoptotic cultured cells (1) We investigated whether this antibody would also recognize dying neurons in vivo, and serve as a convenient and reliable marker for neuronal apoptosis. Following transection of facial nerve in rat pups, which causes >90% death of facial motoneurons, we performed immunocytochemistry with Oncogene Science c-jun antibodies Ab-1 and Ab-2 on frozen brainstem sections. While Ab-1-i.r. was found in the nuclei of the majority of the motoneurons, Ab-2-i.r. revealed only occasional cells, all with apoptotic morphology. Ab-2-i.r. was cytoplasmic, and included the dendrites. The peak number of Ab-2-i.r. neurons equalled the peak number of neurons showing DNA fragmentation, but the peak Ab-2 frequency preceded peak DNA fragmentation by 2 hours. Ab-2-i.r. cells were also observed in the cerebellum, where programmed cell death continues after birth. We conclude that Ab-2 marks cells at an early stage of apoptosis: its uniform distribution in long dendrites suggests that the epitope recognized by Ab-2 results from modification of an existing molecule, rather than the appearance of a novel death protein.

Reference (1) Grand et al. (1995) Exp. Cell Res. 218:439-51.

Supported by the NCE Neuroscience Network and ALS Society of Canada.

127.4

CELL DEATH AFTER SPINAL CONTUSION: LIGHT MICROSCOPY. H.-X. Dong, K.E. Good*, M.P. Goldberg, J.W. McDonald, X.Z. Liu, X.M. Xu[2], R. Hu[2], C. Du, S.X. Zhang[2], Y.J. Wu[2], G.S. Fan, C.Y. Hsu, D.W. Choi & M.F. Jacquin. Center for the Study of Nervous System Injury and Dept. of Neurology, Washington Univ. Sch. of Med., St. Louis, MO 63110; [2]Dept Anat. & Neurobiol, St. Louis Univ. Sch. Med., St. Louis, MO 63104.

We aim to characterize cell death following traumatic insults to the rat spinal cord. As a first step, anatomical studies were conducted to elucidate which cells die after severe or mild impact to the thoracic cord using the NYU impact device. After severe (50 g-cm) impact, cellular degeneration achieved a maximum rostrocaudal distribution of 7.5 mm after 3 d, associated with considerable TUNEL$^+$ staining in neurons and glia (the latter especially in white matter) apparent already at 6-8 hr. At 4-6 d, TUNEL$^+$ cells were similarly distributed, but after 7 d, their extent diminished to 2.5 mm. The density of TUNEL$^+$ cells was greatest at 4 d. TUNEL negative, swollen cells with intact cytoplasm were also observed bordering necrotic regions at 1-7 d. At 20-36 hr post-injury, many TUNEL$^+$ cells expressed c-jun or P53 at the borders of the injured zone. At 1 and 3 d, high levels of BAX expression were seen in cells at the impact site; Bcl-2 expression was low. After mild (6.25 g-cm) impact, TUNEL$^+$ neurons and probable glia were also prevalent already after 4 hr and associated with chromatin condensation and Hoechst 33342 staining, with maximum numbers by 8 hr. These TUNEL$^+$ neurons were confined within a well defined area of degeneration at the injury site. TUNEL$^+$ and Hoechst$^+$ glial cells were seen at 4 and 8 hr as well, but their numbers peaked at 24 hr. DNA laddering was prominent in injured tissue at 24 hr postinjury. Thus, apoptosis may occur in many neurons and perhaps glial cells after at least mild impact injury to the spinal cord. Support: NIH DE07734, NS32000, APA, Daniel Heumann Fund for Spinal Cord Research.

127.5

CELL DEATH AFTER SPINAL CONTUSION: ELECTRON MICROSCOPY (EM). J.A. DeMaro*, H.-X. Dong, X.Z. Liu, X.M. Xu[2], R. Hu[2], S.X. Zhang[2], G.S. Fan, C.Y. Hsu, D.W. Choi & M.F. Jacquin. Center for the Study of Nervous System Injury and Dept. of Neurology, Washington Univ. School of Medicine, St. Louis, MO 63110; [2]Dept Anat. & Neurobiol, St. Louis Univ. Sch. of Med., St. Louis, MO 63104.

Various markers are in widespread use to investigate programmed cell death. Yet, EM remains the most valid method for distinguishing apoptotis and necrosis. Accompanying light microscopic studies of TUNEL staining and cell death gene expression suggested that apoptosis may occur in neurons and glia after at least mild impact injury. The present study extended this evaluation to the EM level. The thoracic cord was exposed and injured by weight drop using the NYU device. 24 hr after a severe (50 g-cm) impact, TUNEL$^+$ neurons and glia were prevalent. Approximately 30 TUNEL$^+$ cells were visualized by EM. These cells were identified, drawn, and photographed, taking care to note landmarks to ensure cell correspondence between the light and electron microscopes. TUNEL reaction product consisted of electron dense spots in the nuclei of cells that were in a very late stage of necrosis degeneration. Indeed, cell death had progressed to a point where TUNEL$^+$ cell types could not be ascertained. After a mild (6.25 g-cm) impact, many apoptotic cells were observed by EM at 8 hr postinjury. Apoptotic cells typically showed the nucleus broken down into several membrane bound, highly condensed chromatin masses, cytoplasmic shrinkage and condensation with intact cytoplasmic membranes.

These data support the occurrence of apoptosis in spinal cord cells after mild impact injury, and provide further evidence for the non-specificity of TUNEL staining alone as a criterion for apoptosis. Support: NIH DE07734, NS32000, APA, Daniel Heumann Fund for Spinal Cord Research.

127.6

EXPRESSION OF MAP-2, SPECTRIN, AND TAU PROTEIN FOLLOWING TRAUMATIC INJURY TO THE RAT SPINAL CORD. J.E. Springer*, R.D. Azbill, S.E. Kennedy, R.G. Siman, N.S. Soultanian, and J.W. Geddes. Department of Anatomy and Neurobiology, University of Kentucky Medical Center, Lexington, KY, 40536-0084, and Cephalon, Inc. 145 Brandywine Parkway, West Chester, PA 19380-4245 (RGS).

Glutamate-mediated excitotoxicity is one of the primary mechanisms thought to contribute to delayed neuronal cell death following traumatic spinal cord injury (SCI). The excessive release of glutamate and the sustained stimulation of glutamate receptors following SCI promotes the influx of micromolar concentrations of calcium into the cell. High levels of intracellular calcium result in a series of biochemical events, one of which is the activation of destructive lipases and proteases that degrade structural proteins, including MAP-2, spectrin, and tau. In the present study, immunocytochemical and Western blot techniques were used to investigate the expression of these structural proteins at different times following SCI. Moderate spinal cord contusion injury resulted in a gradual loss of dendritic MAP-2 staining at all time points analyzed (1, 4, and 24hr). Spectrin degradation was apparent in neuronal cell bodies and fibers as early as 1 hour following SCI, with the most intense staining occurring within and adjacent to the injury site. The appearance of spectrin breakdown products declined at 4hr and was nearly undetectable by 24hr following injury. A non-phosphorylated tau epitope, detected with the tau-1 antibody, occurred over time in both the gray and white matter, and was restricted to small cells located in close proximity to the injury site. These results illustrate that calpain activation and cytoskeletal disruption are early events in SCI, and that treatments aimed at minimizing the breakdown of these proteins may provide a substantial degree of functional savings or recovery. Supported by a grant from the Kentucky Spinal Cord and Head Injury Trust (JES).

127.7

CAN DELAYED FETAL TRANSPLANT PREVENT DEATH OF CLARKE'S NUCLEUS NEURONS IN ADULT RATS? M.Shibayama,[1,2] S.Hattori,[2] B.T.Himes,[1,3,*] N.Matsui,[2] M.Murray,[1] and A.Tessler[1,3] Dept. of Anatomy & Neurobiology, Medical College of Pennsylvania and Hahnemann University, 3200 Henry Ave. Philadelphia, PA 19129. [2] Dept. of Orthopedic Surgery, Nagoya City Univ. School of Medicine, Nagoya, Aichi, Japan. [3] Philadelphia VA Hospital

Fetal spinal cord tissue grafted into a midthoracic spinal cord hemisection at the time of injury rescues Clarke's nucleus (CN) neurons which otherwise die following axotomy. (Himes et al., 1994). In the present study we examined whether transplants performed after a delay could keep injured CN neurons alive and, if so, for how long transplants could be delayed. Female Sprague-Dawley rats (250-300g) underwent laminectomy at the mid-thoracic level with interruption of the right dorsal spinal spinocerebellar (DSCT) tract by spinal cord hemisection and insertion of gelfoam into the cavity. This injury transects the axons of the right CN neurons and leaves the left CN as an intact control. At 1, 3, 7 and 14 days postoperative, the hemisection site was reexposed and the gelfoam replaced by a graft of embryonic day 14 (E14) spinal cord. Two months after the initial surgery CN neurons of the L1 segment were counted and measured. Hemisection only rats showed a 30% loss of CN neurons ipsilateral to surgery and a 40% reduction in average cell area. Rats receiving a transplant at 1, 3 and 7 days showed minimal cell loss; >90% of CN neurons survived. Rats which received transplants 14 days after hemisection showed a 30% cell loss, which was similar to hemisection only animals. Transplants did not prevent cell shrinkage in any group. Transplants can therefore prevent most CN neuron loss if provided within the first week after injury. This result may influence the timing of surgery if transplants become part of the treatment of clinical spinal cord injury. Supported by the VA Medical Research Service, NS 24707 and NS 10090 (BTH).

127.8

CEP-1347 RESCUES DRG NEURONS FROM AXOTOMY-INDUCED CELL DEATH. **R.V. Bhat*, M. Miller, C. Murakata# and P.C. Contreras. Cephalon, Inc., Dept. Pharmacology, West Chester, PA 19380 and #Kyowa-Hakko Kogyo Co. Ltd., Tokyo, Japan.**

Recent studies have demonstrated that CEP-1347, a structurally novel indolocarbazole, possesses neurotrophic properties since it rescues motor neurons from cell death *in vivo* and *in vitro*. In addition, CEP-1347 prevents death of dorsal root ganglion (DRG) cells *in vitro*. To test whether CEP-1347 is efficacious at rescuing sensory neurons from cell death *in vivo*, we have determined its effect on DRG neurons after axotomy of the sciatic nerve in rat neonates. Unilateral axotomy on rat pups was performed on postnatal day 1 and the time course of loss of the neuropeptides CGRP and Substance P were determined in DRG. A 40% depletion of these neuropeptides and a comparable loss of DRG neurons was observed 4 days post axotomy when compared to the contralateral (sham-operated) side. To determine the effects of CEP-1347 on neuropeptide levels and cell loss in the DRG, vehicle or 0.1, 0.3, 1 and 3 mg/kg of CEP-1347 was injected s.c., once daily, starting at the time of axotomy. Following sciatic nerve axotomy, CEP-1347 (0.3 and 1 mg/kg) significantly prevented the depletion of Substance P and CGRP by 70% in DRG. CEP-1347 (0.3 and 1 mg/kg) also significantly reduced the axotomy-induced neuronal loss by 50% in the DRG. Taken together, our results suggest that CEP-1347 is efficacious at rescuing sensory neurons *in vivo* from axotomy-induced cell death.

127.9

INDUCTION OF NITRIC OXIDE SYNTHASE (NOS) ACTIVITY IN IMMATUR AND MATURE FACIAL MOTONEURONS AFTER AXONAL AN FUNCTIONAL INJURY, R. Mariotti[1], Z.-C. Peng[1], K. Kristensson[2], Bentivoglio[1] (SPON: European Brain and Behaviour Society), [1]Inst. Anat. Histol Univ. of Verona, 37134 Verona, Italy; [2]Dept. Neurosci., Karolinska Institute, 1717 Stockholm, Sweden

We investigated the differences in the induction of NOS, the NO syntheti enzyme, between immature and mature motoneurons after axotomy and th relationship between NOS induction and apoptotic cell death in immatur motoneurons. The facial nerve was transected in rats at different postnatal ages, fro birth to early adulthood, and NADPH-diaphorase (ND) histochemistry w performed to analyze NOS induction in facial motoneurons. *In situ* nick-end labelin of DNA fragmentation (TUNEL technique) was performed after axotomy at birth. striking age-dependency was found in ND activity induction: ND positivity was n detectable in facial motoneurons 1 and 2 days after axotomy at birth, when apoptoti changes were evident and marked, and was hardly detectable 4 days after axotomy birth, when extensive motoneuron loss was evident. ND positivity was instea induced in facial motoneurons axotomized from the end of the first postnatal week t adulthood, when the nerve cell loss was less severe than in newborns. However, th time course of ND induction varied considerably in relation to the animals' ag These findings indicate that cell death of maturing motoneurons may not requir NOS activity. In another set of experiments, facial muscles were paralyzed wit botulin toxin injections in 3 week-old rats. Preliminary findings indicate that N was induced in some facial motoneurons in the first days after paralysis, indicatin that functional impairment may also induce NO production in motoneurons.

The financial support of Telethon-Italy (Grant no. 524) is gratefull acknowledged.

127.10

CHANGES OF p75 NGF RECEPTOR AND CHOLINE ACETYLTRANSFERASE IN AXOTOMIZED DEVELOPING MOTONEURONS. T. Zheng*, Y. Sun, R. G. Wiley, and D. M. Armstrong, Georgetown Univ. Sch. of Med., Washington D.C. 20007.

In order to understand the mechanism of motoneuron death or survival after axotomy in developing animals, we employed immunocytochemical techniques to examine the expression of p75 nerve growth factor receptor ($p75^{NGFR}$) and choline acetyltransferase (ChAT) in intact and axotomized hypoglossal motoneurons in rats 5 to 28 days of age. In intact developing rats $p75^{NGFR}$ is transiently expressed within the hypoglossal nucleus with intense immunolabeling occurring at P5, weaker staining at P7 and P11, and barely detectable immunolabeling at P14. Transection of the hypoglossal nerve in P5 and P7 rats results in the decreased expression of $p75^{NGFR}$. Transection during this time period produces death and/or degeneration of these motoneurons as revealed in Nissl stained preparations. In contrast, axotomy in rats 14 days and older (i.e., at a time when intact motoneurons are not longer expressing the receptor) results in the de novo expression of $p75^{NGFR}$. Examination of adjacent tissue sections stained for Nissl substance revealed that the majority of these motoneurons survived the transection. In contrast to $p75^{NGFR}$, axotomy of the hypoglossal nerve results in a decrease in ChAT immunoreactivity within motoneurons during all periods of development. This reduction of ChAT immunostaining, however, is transient and the enzyme returns to basal levels in surviving motoneurons. The normally intense expression of $p75^{NGFR}$ within the hypoglossal nucleus during early postnatal periods and the death of these immature motoneurons after axotomy suggests that maturation and survival after axonal injury may be related to a neurotrophin (supported, in part, by NIH grant AG08026).

GLIA AND OTHER NON-NEURONAL CELLS I

128.1

METABOTROPIC GLUTAMATE RECEPTORS MODULATE THE STIMULATION BY GTP OF RAT ASTROCYTE PROLIFERATION. M.P. Rathbone*, R. Ciccarelli, I. D'Alimonte, P. Di Iorio, P. Ballerini, P. Giuliani, A. Renzetti, and F. Caciagli *Dept. Biomed. Sci. McMaster Univ. Hamilton, Ont. L8N 3Z5 and Inst. Pharmacol. Univ. Cheiti, Italy.

In several brain disorders and injuries glutamate (Glu) release is enhanced and astrocytes undergo reactive gliosis. Glu activates release of purines (Ps) (e.g. adenine- and guanine-based compounds) from cells. Extracellular Ps can stimulate gliosis. Guanosine (GUO) and GTP, like Glu, increase NGF gene expression. Rat astrocytes have several subtypes of metabotropic glutamate receptors (mGluRs) whose stimulation modulates the outflow of Ps. We evaluated whether mGluR agonists influenced the effects of guanine nucleotides on rat astrocytic proliferation. 100-500μM GTP increased astrocyte proliferation in a concentration-dependent manner. HPLC analysis showed extracellular GTP was reduced by 50% and 75% after 15 and 60 min respectively, with a constant production of GUO (25 μM up to 4 hr) and progressive accumulation of other metabolites. The GTP hydrolysis-resistant analogue guanosine βγ imido-triphosphate (GMP-PNP) and GUO each also increased astrocyte proliferation; potency: GMP-PNP>GUO≥GTP. P_2 antagonists partly inhibited the nucleotide-induced effect whereas P_1 antagonists reduced the GUO activity. 3,5 Dihydroxyphenylglycine (DHPG), a selective class 1 mGluR agonist that increases phosphoinositide hydrolysis and cAMP formation, significantly increased astrocyte proliferation and enhanced the GTP or GMP-PNP-induced mitogenic effect. 2,3 Dicarboxycyclopropylglycine (DCG-IV) a class II mGluR agonist negatively coupled to adenylyl cyclase, produced opposite effects. Thus GTP and GUO stimulate rat astrocyte proliferation by different mechanisms. mGluR agonists may regulate gliosis both affecting astrocytes directly and indirectly through modulating the effects of GTP. This has potential therapeutic implications. (Support: OMHF and ALS Canada)

128.2

ETHANOL INHIBITS PROLIFERATION OF ASTROGLIA FROM THE ADULT HUMAN CEREBRUM IN CULTURE. F.A. Boop*, D.L. Davies and C.J.M. Kane. Department of Neurosurgery and Department of Anatomy, University of Arkansas for Medical Sciences, Little Rock, AR 72205.

Fetal exposure to ethanol can cause microencephaly, while chronic alcoholism is associated with atrophy of the adult brain. Ethanol can produce direct neuronal damage as well as indirect neuronal damage through detrimental effects on astroglia. Astroglial pathology is a common feature of ethanol exposure in humans and in animal models.

The influence of ethanol on proliferation of human astroglia from the gray and white matter of adult temporal lobe was determined and compared. Astroglial cultures were exposed to a constant concentration of ethanol at realistic social and clinical levels (0.1, 0.2 or 0.5%; w/v) for 1 to 5 days. Cellular proliferation was quantified by bromodeoxyuridine labeling and enumeration of replicating cells. Ethanol exposure significantly inhibited proliferation of both gray and white matter astroglia in a dose and duration dependent manner. Gray matter was slightly more sensitive than white matter to inhibition at moderate concentrations of 0.1 to 0.2% ethanol. In contrast, white matter was more sensitive at high concentrations of 0.5% ethanol. Maximum inhibition was 20% in gray matter and 25% in white matter. These results demonstrate that human astroglial proliferation is directly inhibited in the absence of neurons, microglia, neuronal degeneration or systemic factors that confound *in vivo* studies. Ethanol damage may impair the ability of astroglia to adequately maintain the parenchymal microenvironment or to properly respond to CNS insults. Restricted proliferation may underlie aspects of the astroglial pathology associated with ethanol exposure. (UAMS Foundation, UAMS Research Council, NIH AG12411, NIH AA07145).

128.3

NUTRITIONAL FACTORS MODIFY THE INHIBITION OF CNS DEVELOPMENT PRODUCED IN NEONATAL RATS BY EXPOSURE TO ETHANOL AND/OR METHADONE. K.E. Light*, D.R. Pierce, N.N. Nyamweya, H. Yang, Y. Kasmi, R. Mosby, D.C. Serbus and C.J.M. Kane+. Dept. of Biopharmaceutical Sciences, College of Pharmacy and +Dept. of Anatomy, College of Medicine, University of Arkansas for Medical Sciences, Little Rock, AR 72205.

The ability of nutritional status to alter the consequences of exposure to ethanol and/or methadone on the development of the rat CNS was determined. Rats were exposed to ethanol (3 g/kg) and/or methadone (0.25 or 0.5 mg/kg) via intragastric intubation at 8am and 4pm on postnatal days 6–10 inclusive. Rats were weighed, anesthetised and perfused on postnatal day 11. Overall growth was assessed by measure of body, heart, liver and kidney weight. Specific effects on CNS development were assessed by measure of whole brain, cerebrum, cerebellum and brainstem weight. Nutrition was modified by drug delivery in two intubation vehicles (20% Sustacal® or 75% Intralipid-II®) which differ in caloric density and composition. Neither ethanol, nor methadone, alone produced significant alteration in brain growth. However, significant dose-related inhibition of brain, cerebrum, cerebellum and brainstem growth was evident with combined drug exposures only with the Sustacal vehicle. No nutritional compromise was evident in intubated control rats compared to suckle control using either intubation vehicle. Neither drug alone, nor in combination, caused significant effects on liver, heart or kidney growth. These results indicate that nutritional effects can mask or enhance the determination of specific drug effects. Importantly, these results emphasize that nutritional effects must be considered, properly controlled, and monitored with quantitative measures in order to accurately quantify specific drug effects in rat models of fetal drug exposure. (College of Pharmacy, UAMS).

128.4

LONG-TERM POSTNATAL EFFECT OF PRENATAL IRRADIATION ON THE INJURY-INDUCED ASTROCYTE PROLIFERATION IN THE RAT BRAIN, K. Janeczko, M. Ziaja, Z. Sołtys, R. Pawliński, Z. Setkowicz, A. Ryszka. Department of Neuroanatomy, Jagiellonian University, 6 Ingardena St., 30 060 Kraków, Poland (Spon: ENA)

Pregnant Wistar rat were exposed to single doses of 1.0 Gy gamma-radiation on gestational days 13, 15, 17 or 19 (E13, E15, E17 or E19, respectively). Pregnant animals were allowed to give birth. When the irradiated offsprings were 30-day-old a lesion was made in the left cerebral hemisphere of male rats. The rats were injected with a single dose of [^{3}H]Thymidine on 1st, 2nd, 4th or 8th days following injury and sacrificed 4 hours later. Brain sections were immunostained for glial fibrillary acidic protein (GFAP) or S100beta protein (S100), autoradiographed and examined microscopically.

Statistically significant increases in the number of proliferating GFAP-immunopositive astrocytes were recorded on the 2nd day after injury in brains irradiated on E15 and E17. All the irradiated groups showed a significant global elevation in the proliferative activity of S100-immunopositive astrocytes.

The results suggest that prenatal low-dose irradiation has a long-term effect on the astrocyte ability to proliferate in response to postnatal brain injury. The effect appears to depend on the stage of embryonic development when the irradiation is performed.

The work was supported by the PECO grant of the European Commission, contract no. ERBCIPDCT930410.

128.5

GLIAL MATURATION, REACTIVE GLIOSIS AND THE TARGET OF THE ANTIPROLIFERATIVE ANTIBODY, rTAPA. E.E. Geisert, Jr.*, L.J. Yang and C.D. Sullivan. Department of Anatomy and Neurobiology, University of Tennessee Memphis, College of Medicine, Memphis, TN 38163.

The present study defines the expression pattern of the rTAPA in the rat brain. rTAPA is a member of the tetramembrane spanning family of proteins. Like other members of this family, rTAPA is associated with the stabilization of cellular contacts. We have examined the expression of rTAPA using immunohistochemistry on cultured cells and sections of the developing brain. The levels of rTAPA were defined on immunoblots of: muscle, tendon, peripheral nerve, cartilage, liver, kidney, brain, skin, and testicle. The highest levels of rTAPA are found in the central nervous system. rTAPA is expressed by: ependyma, choroid plexus, astrocytes and oligodendrocytes. At embryonic day 18 (E18) the levels of rTAPA on immunoblots is low, with virtually all of the immunoreaction product being localized to the ependyma, chorioid plexus and the glial limitans. As development continues the amount of rTAPA expressed in the brain increases and at postnatal day 14 (P14) the levels approach that observed in the adult. When the distribution of rTAPA is examined in sections of the developing brain, there is an increase in the intensity of immunoreaction product observed as a fine reticulated pattern within the parenchyma of the brain. At P7 there is a relatively even labeling of the cerebral cortex and the future white matter. By P14 the level of immunoreaction product in the gray matter appear to increase relative to that observed in the adjacent white matter. However, approximately equal level of rTAPA are found on immunoblots of white matter, gray matter and within purified myelin. Thus, rTAPA is found in all glial cells and the level of this protein correlate with the maturation of glial cells. To test the hypothesis that rTAPA is associated with glial maturation, the levels of rTAPA were examined following a cortical stab wound. As the glia mature they acquire the ability to enter a reactive state. We have found that the expression of rTAPA correlates with the ability of the cells to become reactive. Supported by the Spinal Cord Society.

128.6

GLIAL PROLIFERATION IN THE VENTRAL NERVE CORD OF CRAYFISH AFTER INJURY. M. González-del-Pliego[(1)], R. Gutiérrez[(1)], J. Hernández-Falcón[(2)], and B. Fuentes-Pardo*[(2)]. Departamento de Embriología[(1)] y de Fisiología[(2)]. Facultad de Medicina, UNAM. México.

In many invertebrates as the crayfish, some neural fibers degenerate very slowly when the ventral nerve cord (VNC) is transected. Many morfological studies of the transected VNC of crayfish correspond to the injury's zone and some micrometers around that zone but there is little information on the status of the surrounding tissue and its participation in the regenerative processes. The aim of this work was to characterize the time course of degeneration of the VNC of the crayfish, and the role of the connective tissue during this process. In a longitudinally-oriented section of VNC we found that degenerative changes can be detected since 10 days after the transection of the nerve cord. The small fibers show a loss of integrity with disruption in the arrangement of membranes and subcellular organelles, and a strong increase in glia. About 60-70th day, it could be detected the same changes but to a lesser degree in the giant fibers with a loss of the architectural arrangement of organelles. These process continue along the time with an increase in glial tissue both adaxonal and peripheral glia, covering the entire stumps (proximal and distal). Ninety days after transection the glial tissue reconnected both stumps and a great recovery in the arrangement of neural tissue can be observed. Supported by DGAPA IN202392

128.7

CYTOTOXICITY OF PROLIFERATING ASTROCYTES IN PRIMARY CULTURE MEDIATED BY HSV-TK/GANCICLOVIR. S. Audouy, F. Revah, J. Mallet, A. Privat and M. Giménez y Ribotta*. INSERM U. 336, Université Montpellier II, 34095 Montpellier and CNRS-Rhône-Poulenc Rorer UMR C9923, Hôpital de la Pitié Salpêtrière, 75013 Paris, FRANCE.

It is generally agreed that astrocyte proliferation is one element of CNS post-traumatic gliosis. In order to overcome this obstacle, we have used an adenoviral vector (ADV/RSV) to evaluate the ability of herpes simplex virus thymidine kinase (HSV-TK) coupled with the prodrug ganciclovir (GCV) to reduce the proliferation of cultured primary astrocytes, taken as a model of reactive gliosis.

Primary cultures of astrocytes were obtained from the cerebral cortex of 2-days postnatal rats. After mechanical dissociation a cell suspension was obtained and adjusted to 10^6 cells/ml. The Bromodeoxyuridine (BrdU) labeling index, measured by double immunocytochemical detection of BrdU/GFAP, was used to determine the time in culture at which the peak percentage of phase S-cells was achieved. Transfection efficiency of adenovirus was demonstrated by using ADV/RSV-βgal.

A peak percentage of BrdU+ cells is reached at 6 days in vitro and an optimal transfection efficiency at a multiplicity of infection (MOI) =16. One day after transfection with ADV/RSV-TK at MOI values of 8 or 16, the cultures were treated with either culture medium or GCV (10 or 50 µg/ml). Two or four days later, the surviving cells were counted. The maximum effect (88% of death) was obtained for a GCV concentration of 10 µg/ml and a survival of 4 days.

In conclusion, the HSV-TK/GCV system may constitute an efficient tool to investigate the influence of the elimination of proliferating cells on the regrowth of severed axons following traumatic injury of the CNS in vivo.

The ADV/RSV-TK and -βgal were kindly provided by Rhône-Poulenc Rorer (RPR). Supported by grants from RPR (Bioavenir Program), IRME, AFM, FRMF, DRET.

128.8

EFFECTS OF GANCICLOVIR ON TRANSGENIC ASTROCYTES EXPRESSING HSV-THYMIDINE KINASE FROM THE MOUSE GFAP PROMOTER

T.G. Bush[1]*, M.H. Johnson[1], L. Mucke[2] and M.V. Sofroniew[1]

[1]MRC Cambridge Centre for Brain Repair and Department of Anatomy, University of Cambridge, Forvie Site, Robinson Way, Cambridge CB2 2PY, UK and [2]The Gladstone Molecular Neurobiology Program and Department of Neurology, University of California, San Francisco, PO Box 419100, San Francisco, CA 94141-9100

To provide a model in which astrocytes can be selectively ablated, transgenic mice have been generated that express herpes simplex virus thymidine kinase (HSV-TK) from the mouse glial fibrillary acidic protein (GFAP) promoter (Bush et al. Soc. Neurosci. Abst. 21:814, 1995). Immunocytochemical double-labelling of astrocytes in the brains of these mice, demonstrates complete co-localization of HSV-TK and GFAP immunoreactivity. Manipulations that upregulate GFAP expression *in vivo* caused upregulation of HSV-TK. When cultured transgenic astrocytes were exposed to ganciclovir (GCV; 2µM) for one week their number was reduced by 80%. In contrast, GCV treatment caused no cell loss in astrocyte cultures from non-transgenic littermates. The effects of ganciclovir on astrocytes in normal and injured brain are being investigated. This transgenic model may be useful to investigate the role of astrocytes in a variety of processes.

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128.9

THE GLIAL-DERIVED PROTEIN S100β INDUCES APOPTOTIC CELL DEATH IN CULTURED ASTROCYTES. J. Hu* and L.J. Van Eldik, Dept of Cell and Mol. Biology, Northwestern University Medical School, and Northwestern Univ. Institute for Neuroscience, Chicago, IL.

S100β, a calcium binding protein expressed primarily by astrocytes in the brain, has been implicated in development and maintenance of the nervous system. However, S100β has also been postulated to play a role in mechanisms of neuropathology, because of its specific localization and selective overexpression in Alzheimer's disease. Recent studies have suggested that S100β may be beneficial to cells at low doses and detrimental at high doses. To begin to study the toxic signalling pathways activated by high concentrations of S100β, we have demonstrated (*Hu et al., J. Biol Chem. 271, 2543-2547, 1996*) that treatment of astrocytes with μM S100β results in a potent stimulation of the mRNA level and enzyme activity of inducible nitric oxide (NO) synthase, an enzyme previously implicated in glial pathology. We provide evidence here that S100β-stimulated NO formation can lead to cell death in astrocytes, with characteristics defined for apoptosis. Incubation of astrocytes with S100β for 48 hr results in an increased percentage of cells undergoing apoptotic cell death, as determined with TUNEL technique, DNA fragmentation assays and lactate dehydrogenase activity. The cell death induced by S100β addition correlates with the levels of NO production, and the specific NOS inhibitor L-NAME attenuates the NO formation elicited by S100β, as well as the cell death. Therefore, we propose that S100β has the potential to be trophic or toxic. Although S100β may be involved in development, homeostasis and repair, chronic overexpression of the protein may mediate toxic responses or even cell death (supported in part by NIH grants AG10208 and AG11138).

128.10

Programmed cell death (apoptosis) in the rat lumbar spinal cord after surgical isolation. D.L. Allen W.M. Walwyn*, E.J. Grossman, R.R. Roy and V.R. Edgerton. Department of Physiological Science and Brain Research Institute, UCLA, Los Angeles, CA 90095

Spinal cord isolation (SI) or complete spinal cord transection at a mid-thoracic and low lumbar level coupled with bilateral dorsal rhizotomy between the transection sites results in an electrically silent preparation producing dramatic alterations in the target muscle. However, little is known of the effects of spinal isolation on the cells of the spinal cord. SI was induced in adult female rats and the cellular changes in the spinal cord determined 4, 15, 30, 60 and 90 days after surgery. Histochemical staining of 12 μm sections for double stranded DNA breaks revealed increased DNA fragmentation indicative of apoptosis in the gray and white matter of the spinal cord. An increased number of apoptotic nuclei was evident by 15 days after surgery (24±6, mean±SD), peaked after 30 days (48±12), and decreased after 60 and 90 days. The number of apoptotic nuclei in the control rats was not different at any of the time points studied (15 days: 2±1; 30 days: 3±2). Dual fluorescent staining and confocal microscopy showed that the majority of apoptotic cells were astrocytes (positive glial fibrillary acidic protein immunolabeling). Apoptotic glial cells were shrunken and the normal fibrillary structure was dramatically reduced, consistent with morphological alterations characteristic of apoptosis. Spinal isolation also resulted in a progressive increase in the number of nuclei in the white matter as shown by cresyl violet staining. These data suggest that both cell death and proliferation are increased in the lumbar spinal cord following spinal isolation, and may play a role in spinal cord remodeling following removal of afferent, supraspinal and infraspinal input. Supported by NIH grant NS16333.

128.11

PHENOTYPE OF OLFACTORY ENSHEATHING CELLS IN CELL CULTURES INITIATED FROM NGF OVER-PRODUCING TRANSGENIC MICE. R. Doucette* and M. Kawaja. Dept. Anat. & Cell Biol., U. of SK, S'toon, SK Can. and Dept. Anat. & Cell Biol., Queen's Univ., Kingston, ON, Can.

We are studying the olfactory system in a transgenic mouse in which the mouse beta-NGF gene is being driven by the GFAP promoter. Olfactory ensheathing (En) cells are the glial cells that ensheath the primary olfactory axons in both the PNS and CNS portions of the pathway. Since these cells express GFAP they would presumably express the transgene as well. The objective of the present study was to compare the phenotype of En cells in neuron-free cultures with that of cells obtained from wild-type C57/black mice. Cell cultures of En cells were initiated from the olfactory bulbs of E15.5 mouse embryos. The cultures were stained with rabbit polyclonal antisera to the p75 neurotrophin receptor, as well as to GFAP and S100. A monoclonal antibody to the nestin intermediate filament protein (Rat 401) was also used. The cultures were stained using the Vectastain ABC Elite kit. The phenotype of En cells in both transgenic and wild-type cultures was the same: i.e. p75+ve, GFAP+ve, S100+ve & Rat401+ve. The addition of NGF to the wild-type En cell cultures did not alter the phenotype of cells in these cultures either. (Supported by grants from the NIDCD to R.D. and from the MRC of Canada to M.K.).

128.12

THE MIDLINE RADIAL GLIAL PROTEIN TRANSITIN DEFINES A NEW CLASS OF INTERMEDIATE FILAMENT PROTEIN. J.-A. Lee, Y. Yuan, A. Napier, and G.J. Cole*, Neurobiotechnology Center & Dept. of Cell Biology, Neurobiology and Anat., The Ohio State Univ., Columbus, OH 43215.

In the present study we describe the molecular cloning of a previously characterized radial glial protein, originally named EAP-300, that is expressed by a number of CNS midline radial glial structures. This protein is characterized by an intermediate filament (IF) core domain most closely resembling human nestin and Xenopus tanabin, and a novel central heptad amino acid repeat domain located in the variable tail region of the protein, which is comprised of multiple leucine zipper repeats that are predicted to form coiled-coils. Based on these structural motifs, but only limited homology to other IF proteins, we propose that a novel IF protein that is transiently expressed by radial glia during CNS development has been identified. We also show by molecular cloning and Western blotting that a rat homologue of EAP-300 is expressed in rat C6 cells. Based on its temporal and spatial expression pattern we have renamed this protein transitin (from the Latin word for transient and crossing). We also demonstrate the existence of splice variants of transitin with splicing giving rise to heptad repeats of different lengths, or even transitin isoforms that lack a central heptad repeat domain. By in situ hybridization analysis we show that transitin mRNA is expressed by midline glial structures that are thought to regulate axonal guidance, by glia in several axonal commissures, and by Bergmann glia of the developing cerebellum. Based on the structural properties of the transitin protein, and expression of its mRNA, we suggest that transitin may represent a new class of intermediate filament protein transiently expressed by radial glia, which may play a role in the morphological differentiation of these cells in the CNS. Supported by NIH grant NS29934.

128.13

GENERATION OF A RADIAL-LIKE GLIAL CELL LINE FROM RAT C6 GLIOMA. D.R. Friedlander*, T. Sakurai, G. Fishell and M. Grumet. Depts. of Pharmacology and Cell Biology, NYU Medical Center, 550 First Avenue, New York, NY 10016.

The rat C6 glioma cell line has been used extensively as a model of astroglia. We reported that C6 cells as well as developing glia including radial glia and mature astroglia express a receptor protein tyrosine phosphatase called RPTPβ (J Neurosci. Res. 43:694-706,1996). To analyze the function of this receptor in glia, we transfected C6 cells with a plasmid encoding this receptor lacking the intracellular phosphatase domains and established several stable transformants that expressed the truncated receptor. Many of the transformants assumed a more stellate morphology than the parental cells but the morphological changes did not strictly correlate with the level of expression of the truncated receptor. Interestingly, one cell line called C6#1 assumed a highly polarized morphology in culture that resembled radial cells, and at confluence, they aligned in a palisade-like orientation. This phenotype was stable after more than ten passages. The radial morphology was observed even at low cell density and conditioned medium from C6#1 did not alter the morphology of parental C6 cells indicating that this effect was unlikely to be due to secreted factors. Rat cerebellar granule neurons bound to the radial-like C6#1 cells and migrated along them in culture. The phenotype of Di-I-labeled C6#1 cells was compared to the parental C6 cells following injection into the developing forebrain. Three days later, histological analysis revealed that most of the C6#1 cells were in a radial orientation spanning the neuroepithelium, in contrast to the parental C6 cells which exhibited a much less polarized morphology and often formed aggregates The results suggest that we have generated a stable cell line from C6 glioma which resembles radial glia in morphology both in culture and in vivo, and that can support neuronal migration. *Supported by NS33921.*

128.14

ACTION OF GABA IN THE DEVELOPING RAT OPTIC NERVE. [1]A.G. Howd, [1]S. L. Kirvell, [2]M. Rattray, [3]D. Shewan* and [1]A. M. Butt, Divisions of [1]Physiology, [2]Biochemistry & Molecular Biology and [3]Developmental Neurobiology, UMDS, London, UK.

$GABA_A$-receptor mediated modulation of axonal conduction and astrocytic depolarization have been shown in the developing rat optic nerve, and both responses attenuated with age. The present study addresses the question of the site of GABA action. The effect of GABA (1mM) on $[K^+]_o$ was measured using single-barrelled K^+-selective microelectrodes in isolated optic nerves from rats aged P1-21. In nerves enucleated at P1 or P2, which contained astrocytes and degenerating axons, there was only a small GABA mediated increase in $[K^+]_o$ of 0.8 ± 0.2mM (mean ± SEM, n=10) one day after enucleation, compared to 4.8 ± 1.3mM (n=13) in normal nerves of the same age. RT-PCR indicated that in normal P10 optic nerves the mRNA for the α-2 subunit of the $GABA_A$-receptor was not expressed. These results suggest the presence of axonal $GABA_A$ receptors which are responsible for the GABA-mediated increase in $[K^+]_o$ and subsequent astrocytic depolarization in the neonatal rat optic nerve. Immunocytochemistry has identified astrocytes as the primary source of GABA and RT-PCR has shown that GAT2 and 3 but not GAT1 mRNA are present in normal P10 nerves, suggesting that glial cells in the optic nerve are capable of GABA uptake. Together, these results suggest a role for GABA and $[K^+]_o$ in axon-glial signalling which may be important in the control of axon-glial interrelationships during development in the rat optic nerve.

128.15

VOLTAGE-GATED CURRENTS IN THE GLIAL CELLS ASSOCIATED WITH OLFACTORY GLOMERULI DEVELOPING IN THE ANTENNAL LOBE OF THE MOTH. H.G. Marrero and L.A. Oland*. ARL Div. of Neurobiology, Univ. of AZ, Tucson, Arizona 85721.

In the developing antennal lobe, glomerulus development is initiated by olfactory receptor axons and proceeds only if glial cells, under the influence of the receptor axons, stabilize the newly forming glomeruli by forming a border around each one. The mechanisms by which the neurons and glial cells communicate and coordinate their roles during glomerulus formation are not known. In this study, we have begun to characterize the voltage-gated currents in glia engaged in the process of border formation. Whole-cell recordings were made from glia in acute slice preparations taken from antennal lobes throughout the period of glomerulus formation. Lucifer Yellow dye was included in the patch pipette to allow morphological verification of glial class and to indicate qualitatively the degree of dye coupling. At early stages, many border glia were dye-coupled to one another and often to antennal-nerve glia; at late stages, however, glia typically were dye-coupled to only a few adjacent border glia. At all stages, all cells showed an outward potassium current that could be blocked partially with external 4-AP and blocked, in some cases completely, by internal TEA. Blockade of the outward current unmasked a TTX-sensitive inward current with a slow rise time in glia at early stages of development and a faster rise time in glia from late stages. As early as stage 5, at the onset of glomerulus formation, extracellular stimulation of either the olfactory receptor axons in the antenna or one of the clusters of cell bodies of antennal-lobe neurons, elicited a relatively long-lasting (5-10 sec) depolarization in the glia. The observed developmental differences in coupling and in current profile provide the basis for exploring mechanisms of neuron-glia interaction in the formation of glomeruli. Supported by NIH grant NS28495.

128.16

WHOLE CELL RECORDING FROM HIPPOCAMPAL ASTROCYTES DURING ORTHODROMIC STIMULATION. R. D'Ambrosio*, G.M. McKhann and D. Janigro. Dept. of Neurosurgery, Univ. of Washington, Seattle, WA 98104

The application of the voltage-dependent ion channel blocker cesium (Cs^+) to rat hippocampal slices causes synchronous interictal-like bursting and prevents the maintenance of synaptic long-term depression (LTD). Cs^+ also potentiates epileptiform activity in neocortical slices treated with bicuculline and induces short-term potentiation. Since Cs^+ is known to block both neuronal I_h and glial potassium channels, we performed experiments designed to dissect out the relative contribution of glia in Cs^+-mediated epileptogenesis and reversal of LTD. Field and whole cell patch clamp recordings from glial cells were performed in the CA1 region of hippocampal slices during low- frequency orthodromic stimulation to induce LTD. During LTD induction (1 Hz, 15 min), a biphasic depolarization of glia followed by a return to pre-LTD was observed. Cesium (3 mM) decreased the early depolarization and prevented the following hyperpolarization consistent with a blockade of potassium uptake into glia. Cs^+ prevents glial uptake of potassium through voltage-activated astrocytic channels, thus causing an abnormal $[K]_o$ increase and depolarization of surrounding neurons during low-frequency activation. This depolarization may influence the maintenance of LTD by simultaneous induction of long-term potentiation resulting from pre- and post-synaptic depolarizations mediated by $[K_o]$.
Supported by NIH 51614 and NIEHS 07033

128.17

AN EXTRACELLULAR SIGNALLING COMPONENT IN PROPAGATING ASTROCYTIC CALCIUM WAVES. T.D. Hassinger*, P.B. Guthrie, P.B. Atkinson, M.V.L. Bennett, and S.B. Kater. Department of Neurobiology and Anatomy, University of Utah, Salt Lake City, UT 84132; Department of Neuroscience, Albert Einstein College of Medicine, Bronx, NY 10461.

Focally evoked astrocytic calcium waves are thought to propagate by gap-junction-mediated intercellular passage of chemical signal(s). In contrast to this idea we have observed isolated astrocytes, which had no physical contact with other astrocytes in the culture, participating in a calcium wave. This observation suggests a previously unrecognized extracellular route of astrocyte signalling.

To test for extracellular signalling we made artificial gaps 10-300 μm wide in confluent cultures by deleting a row of astrocytes with a glass pipet. Cultures were allowed to recover for 4-8 hrs before use at which time regions of confluent astrocytes separated by lanes devoid of cellular material were easily located. Electrical stimulation (17Hz for 2 sec.) by a fine bore extracellular pipet was used to initiate astrocytic calcium waves.

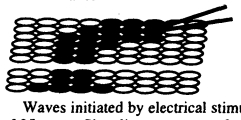

Figure shows results of one experiment. Filled cells indicate all cells participating in the astrocytic calcium wave.

Waves initiated by electrical stimulation crossed the artificially created gaps in 12 of 35 cases. Signaling was most often seen with gaps of less than 50μm and was not observed crossing gaps greater than 100μm. An extracellular signalling path is also supported by the finding that focal superfusion severely decreased the spread of the calcium waves.

Therefore, in addition to gap junctions mediating astrocyte-astrocyte communication, extracellular signals can also play a role.

Supported by Development funds from the Uni. of Utah and NRSA #NS09767.

128.18

THE FREQUENCY OF $[Ca^{2+}]_i$ OSCILLATIONS IN CORTICAL AND HIPPOCAMPAL ASTROCYTES IS MODULATED BY NEURONAL ACTIVITY. L. Pasti, G. Carmignoto, P. Giusti * and T. Pozzan. Dept. of Biomedical Sciences, University of Padova, 35127 Padova, Italy.

Astrocytes in culture respond to the excitatory neurotransmitter glutamate with regular $[Ca^{2+}]_i$ oscillations. We previously reported that astrocytes also display long lasting changes in their oscillatory response upon repetitive activation of the metabotropic glutamate receptor (mGluR). In the present study we investigated whether the stimulation of mGluRs induce a change in the $[Ca^{2+}]_i$ of visual cortical and hippocampal astrocytes in brian slices. Slices (100-150 μm) from 5-22 day old rats, were loaded with the fluorescent calcium indicator Indo-1 and the change of $[Ca^{2+}]_i$ analyzed by a real time confocal microscope (Nikon). Similarly to cultured astrocytes, the frequency of oscillations increased upon repetitive activation of mGluRs by *t*-ACPD (5-30μM) (0.75±0.04 and 1.38±0.01 peaks/min at the 1st and 3rd stimulation, respectively; mean change 102±18 %; $p > 0.001$; n= 52). Electrical stimulation of presynaptic afferents at 0.2 Hz (100-200 msec train at 20-50 Hz; 100 pA) evoked $[Ca^{2+}]_i$ oscillations in astrocytes surronding stimulated neurons. When the firing of presynaptic afferents was increased by changing either frequency or intensity of the electrical stimulus, the frequency of astrocytes $[Ca^{2+}]_i$ oscillations also increased. Neuronal stimulation induced oscillatory $[Ca^{2+}]_i$ elevations also at discrete loci along the astrocyte process. These Ca^{2+} hot spots were not synchronized indicating that each of them represent an independent compartment of Ca^{2+} signaling perhaps in close association with the synaptic cleft. The application of TTX (1-5μM) abolished the $[Ca^{2+}]_i$ in both neurons and astrocytes. Astrocytes are, therefore, sensitive to glutamate released by synaptic activity, in particular at discrete sites along their processes, and display the same long-lasting changes in the oscillatory response originally observed in culture.
Grants from CNR "Biotechnology" and Thelethon Italy.

128.19

LOCAL CHANGES IN $[K^+]_o$ ELICIT INWARD AND OUTWARD CURRENTS FROM THE SOMA AND ENDFOOT OF MÜLLER (GLIAL) CELLS ISOLATED FROM THE FROG RETINA. S. N. Skatchkov, J. Krůšek and R.K. Orkand* Inst. of Neurobiology, Univ. of Puerto Rico MSC, San Juan, PR 00901

Müller cells are glial cells that traverse the retina. They have an asymmetric distribution of K^+channels and function to equalize K^+gradients (Karwoski et al., Science 244:578 1989). Such a function requires these cells to carry K^+ inward (I_{KIN}) in regions where $[K^+]_o$ is raised and outward (I_{KO}) at more distant regions. We have used local changes in $[K^+]_o$ (from 3 mM to 1 or 10 mM), to elicit currents from Müller cells with either the endfoot or soma of the cell voltage clamped using the whole-cell variation of the patch-clamp technique. Two populations of cells were obtained by enzymatic dissociation from the retinas of adult frogs (*Rana pipiens*). Long cells, > 100 μm, were obtained from the central portion of the retina whereas short cells, <50 μm were obtained from the peripheral retina. Membrane currents were elicited by local perfusion with different $[K^+]$ from a fine theta pipette. In long cells, I_{KIN}/I_{KO} was 4.5 (± 0.5 SE, n=11) at the endfoot and 1.8 (±0.2 SE, n=10) at the soma. In short cells the ratio was 6.1 (± 0.2 SE, n=5) at the endfoot and 3.0 (±0.4 SE, n=5) in the soma. In contrast to the short cells, where currents readily spread from endfoot to soma, in long cells the soma and endfoot appeared to be electrically isolated.

The results indicate that there is less inward rectification of K^+ channels in the soma than in the endfoot. In addition, it appears that spatial buffer K^+ currents can equalize $[K^+]_o$ only over local regions of the central retina whereas in the periphery these currents can equalize $[K^+]_o$ over the entire thickness of the retina.

Supported by NIH (NINDS, RCMI, FIRCA, M-RISP) and NSF (EPSCoR, RIMI)

129.1

IN VITRO ANALYSIS OF GLYCINERGIC NEURON SURVIVAL IN THE POSTNATAL AUDITORY CNS: DEPENDENCE ON TARGET. L. Guo and D. H. Sanes*, Center for Neural Science, New York University, New York, NY 10003.

The regeneration of axonal projections depends critically on the ability of their cell bodies to survive axotomy. We investigated the survival of glycinergic medial nucleus of trapezoid body (MNTB) neurons, and the effect of transecting its projection to the lateral superior olive (LSO), using an organotypic culture preparation. Tissue slices were obtained from postnatal (P) day 12 gerbils, and cultured in serum-containing media for 6 days. Control slices exhibited robust survival of MNTB and LSO neurons, as assessed with immunocytochemical staining for Parvalbumin and GAP-43. In addition, a profuse MNTB projection to LSO was visualized in dextran-conjugated biotin labeled slices. When the MNTB was cultured in isolation, the number of Parvalbumin-positive cell bodies was severely decreased, suggesting MNTB cell death. There was also little evidence of neuron survival when the transected MNTB was grown adjacent to the LSO. In a few slices, MNTB neuron survival occurred in the presence of another target, the superior paraolivary nucleus (SPN). The apparent MNTB cell death was not prevented by growing the cultures in glutamate receptor antagonists (100μM AP-5 and CNQX) or brain-derived neurotrophic factor (BDNF, 40 ng/ml) and basic fibroblast growth factor (bFGF, 20 ng/ml). In contrast, LSO neurons did survive after transection of their axons, as well as the MNTB afferent projection, as assessed with GAP-43 immunocytochemistry. Taken together, these findings suggest that MNTB neuron survival is compromised when deprived of their targets, but LSO neurons do not appear to require afferent or efferent projections. (Supported by NIH and NSF.)

129.2

POSTNATAL DEVELOPMENT OF GABAERGIC AND GLYCINERGIC SYSTEMS IN THE AUDITORY PATHWAY OF THE RAT. C. De Cabo De La Vega*, M.L. Campos and J.M. Juiz. Instituto de Neurociencias, Campus de San Juan, Univ. Of Alicante, E-03080 Alicante, Spain.

Previous work has provided a detailed description of the distribution of both γ-aminobutyric acid (GABA)- and glycine- immunoreactivity (IR) in the adult mammalian auditory pathway. However, very little is known about the postnatal development of these two inhibitory neurotransmitters in the auditory pathway. We are currently investigating the development of GABAergic and glycinergic neuronal systems in auditory nuclei throughout postnatal maturation in the rat. Here we report data regarding the development of GABA and glycine-IR neurons in the inferior colliculus (IC). Neonatal pups were perfused with a fixative containing paraformaldehyde and glutaraldehyde. 50 μm thick serial sections were cut with a Vibratome. Sections were incubated in anti- GABA and -glycine antibodies and processed using either the ABC method or immunofluorescence with cyanine labeled secondary antibodies. Our results show that GABA-like IR is already present in the IC at P0 in neuronal bodies and in a few fiber systems. GABA-IR neurons and fibers increase during the first week reaching a peak between P3 and P5, with an apparent decrease at P7. The number of GABA-IR cell bodies and terminals in the neonatal IC seems higher than in the adult. Glycine-IR fibers are also present in the IC at P0 and increase steadily until P7. Although glycine immunorective cell bodies can not be observed at P0, a few very weakly labeled cell bodies are seen at later ages. These data suggest a differential development of the GABA and glycine neurons in the auditory midbrain during the first postnatal week. *Supported by Grants from the Spanish Government DGICYT PB93/0931 and FIS 95/1672 to JMJ.*

129.3

PROTEIN KINASES CONTRIBUTE TO THE EXPERIENCE-DEPENDENT FORMATION OF THE AUDITORY SPACE MAP IN THE GUINEA-PIG SUPERIOR COLLICULUS. D. McCrossan, B. Platt* and D.J. Withington. Department of Physiology. University of Leeds. UK.

The maturation of the map auditory space in the superior colliculus (SC) occurs in an activity-dependent manner that involves NMDA receptors. The formation of the auditory map occurs relatively late during development and requires auditory, visual and somatosensory experience. Spatially coincident activation of collicular loci, by more than one modality, is thought to be pivotal to the establishment of spatially tuned auditory responses.

Protein kinases (PKs) have been shown to be involved in modifications of synaptic efficacy and are, therefore, thought to play a key role in neuronal plasticity. The aim of the present study was to investigate whether PKs are important during the naturally occuring plasticity observed in the SC. Elvax polymer, containing the general PK inhibitor, K252a, was surgically implanted onto the SC, prior to normal auditory map emergence in one group of animals and in a second, at an age when the SC map is no longer disrupted by visual deprivation. Following a recovery period, the guinea-pigs were re-anaesthetised and the SC probed for auditory responses. Normalised polar plots were derived from the neuronal responses and indices of spatial tuning were determined. The tuning parameter values obtained from guinea-pigs implanted prior to normal map emergence were significantly greater than those obtained from control (implant prepared using water), and there was no correlation between the peak angle of each auditory response and the collicular position of the recording electrode. In contrast, the SC auditory responses obtained from the older guinea-pigs demonstrated a normal representation of auditory space. The present findings indicate that PKs are vital for the neuronal modifications underlying the formation of spatially tuned auditory responses in the guinea-pig SC. In contrast, the maintenance of the SC auditory space map is not susceptible to such an insult.

129.4

IMPROVEMENT IN AUDITORY SPATIAL ACUITY FOLLOWING EARLY VISUAL DEPRIVATION IN FERRETS. A. J. King* and D. J. Semple. University Laboratory of Physiology, Parks Road, Oxford OX1 3PT, UK.

Early loss of vision has been reported to lead to intermodal changes that are generally regarded as compensatory in nature. However, studies of the effects of blindness on auditory localization behaviour have produced conflicting results. We have examined the effects on auditory spatial acuity of depriving ferrets, either in infancy or adulthood, of patterned visual cues by binocular eyelid suture.

The animals were trained using a positive conditioning procedure to discriminate broadband noise bursts from two loudspeakers that were separated by various angles on the horizon. All animals achieved scores of more than 90% correct following a similar period of training. The minimum audible difference in speaker separation was measured for 500, 100 and 40 ms digitally-matched noise bursts at the midline and around 45° left and right. The ferrets that had been visually deprived since birth achieved significantly better scores in the lateral field, but not at the midline, than the normal-sighted controls. The lateral-field minimum audible angles attained by adult ferrets that were lid sutured 4-7 months before testing were not significantly different from either of the other groups, suggesting that their performance was intermediate between the normal and neonatally-deprived ferrets.

These data suggest that visual deprivation throughout life leads to an improvement in auditory spatial acuity, at least for lateral positions where localization accuracy is inferior to that at the midline, and that a more limited capacity for compensatory changes may persist into adulthood.

Supported by the Wellcome Trust and the Hearing Research Trust.

129.5

GLUTAMATE, GABA AND MUSCARINIC RECEPTORS ARE INVOLVED IN THE DEVELOPMENT OF THE AUDITORY SPACE MAP IN THE MAMMALIAN SUPERIOR COLLICULUS

N.J. Ingham, D.J. Withington*, S.K. Thornton & D. McCrossan, Dept. of Physiology, Univ. of Leeds, LS2 9NQ, UK.

The auditory space map in the guinea pig superior colliculus (SC) is a complex neural representation of the auditory field surrounding the animal, which is highly dependent on correct sensory experience during its post-natal development. As a start point for investigations into the cellular developmental processes involved with auditory space map emergence, the slow-release polymer Elvax was used to deliver various neurotransmitter antagonists to the SC for long periods, either during the post-natal development of the map, or in the adult animal, when the map is no longer susceptible to visual deprivation.

During early development, exposure of the SC to either AP5 (NMDA-type glutamate receptor antagonist), CNQX (non-NMDA-type glutamate receptor antagonist), or atropine (muscarinic ACh receptor antagonist) produced complete disruption of auditory spatial tuning in the deep layers of the SC, as seen using multi-unit recordings to free-field broad band noise stimuli. Blockade of $GABA_A$ and $GABA_B$ receptors (using bicuculline and hydroxysaclofen) produced partial disruption with responses exhibiting some topographical organisation. Blockade of NMDA receptors in the adult animal also produced severe disruption of the auditory space map. These results indicate that normal neurotransmission involving glutamate, ACh and GABA in the SC is required for development of a normal map of auditory space. The disruption produced by AP5 in older animal indicates that the guinea pig SC retains the ability to undergo plastic reorganisation in the adult.

Supported by the Medical Research Council.

129.6

HAIR CELL REGENERATION IN THE CHICK AUDITORY EPITHELIUM IS MEDIATED BY cAMP AND PKA. D.S.Navaratnam, H.Su, T.Bell, D. Kay, J.C.Oberholtzer *. Dept. of Path., Univ. of Penna. Sch. of Med., Phila., PA 19104.

Hair cells are the primary transducers of sound. These cells along with the surrounding supporting cells are the principal cells found in the auditory receptor epithelium. Hair cells are lost with increasing age, and as a result of insult brought about by exposure to intense sound or treatment with high doses of aminoglycoside antibiotics. That hair cells in vertebrates can be replaced in the adult animal has been a recent observation. However, the exact mechanisms that bring about regeneration in the auditory receptor epithelium remain indeterminate. Using explanted chick cochleas, we demonstrate here that pharmacological agents (forskolin, 8 Br-cAMP) that increase levels of cAMP cause proliferation in the undamaged chick auditory receptor epithelium. The proliferative response was measured by the incorporation of the S phase markers BrdU and ^{3}H-thymidine into the nuclei of cells. These markers were incorporated into both hair cell and supporting cell nuclei. This proliferative response was significantly reduced when the cells were pre-treated with inhibitors of protein kinase A before forskolin treatment. Furthermore, treatment with PKA inhibitors significantly reduced the proliferative response that follows damage to hair cells brought about by gentamicin, indicating that the cAMP-PKA pathway was also operational in this paradigm. The proliferative response to forskolin in the undamaged epithelium required serum, although serum alone could not induce cell division. Neither EGF, PDGF or insulin were able to supplant the permissive effect of serum. In a related observation, it was found that nuclei incorporating BrdU in response to forskolin treatment were clustered within the epithelium suggesting that these were clonal and possibly derived from a subpopulation of stem cells. In conclusion, the ability of agents which increase cAMP to induce proliferation in the normally-quiescent undamaged epithelium suggests approaches to the therapeutic stimulation of hair cell growth. Such approaches will depend upon the existence of a similar mechanism in the mammalian cochlea. *(Supported by grants from the NIH & the PA. Lions Hearing Res. Fndtn.)*

129.7

PROGRAMMED CELL DEATH IS INVOLVED IN PATTERNING THE SENSORY EPITHELIUM OF THE CHICKEN EAR. A. E. Riedl, D. A. Cotanche and D.M. Fekete* + Dept. of Anatomy and Neurobiology, Boston University School of Medicine, Boston, MA 02118; +Dept. of Biology, Boston College, Chestnut Hill, MA 02167.

The mature epithelium of the chicken basilar papilla (BP) is distinguished by a precise pattern of hair cells and their underlying supporting cells. These cells arise from common progenitor cells (Muthukumar and Fekete, Soc. Neurosci. Abstr. 20: 1079, 1994), although the mechanism by which their fates are determined is not known. It is also not known how the cell patterns are established, although it has been suggested that lateral inhibition is involved.

We propose that programmed cell death (PCD) plays a role in the formation of the highly organized pattern of hair cells and supporting cells in the chick sensory epithelium. We are testing this hypothesis by eliminating PCD in embryonic chicken ears and observing the effects on patterning in the BP. This is accomplished by retrovirus-mediated overexpression of human bcl-2, a gene that inhibits PCD in a variety of systems.

By the third embryonic day (E3) the presumptive ear has pinched off of the surface epithelium and formed a fluid-filled sac called the otocyst. The right otocysts of E4 embryos were injected with RCASBP(B)/bcl-2, a replication-competent retrovirus containing the human bcl-2 gene. On day E12-E19, the injected embryos were sacrificed and fixed. The cochlear duct was carefully removed and prepared for scanning electron microscopy. Many of the RCASBP(B)/bcl-2 injected ears showed a strong, localized phenotype, in which patches of cells, including hair cells, were being extruded from the epithelium. Control ears, which were either not injected or were injected with the parent retrovirus RCASBP(B), did not show this phenotype. Our interpretation of these results is that PCD is required for normal patterning of the chick sensory epithelium.

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129.8

A GENETIC SCREEN IN ZEBRAFISH FOR MUTATIONS THAT AFFECT HAIR CELLS IN THE EAR AND LATERAL LINE. R. Kollmar[1]*, J. M. Fadool[2], J. E. Dowling[2], and A. J. Hudspeth[1]. [1]Howard Hughes Medical Inst. and Lab. of Sensory Neuroscience, Rockefeller Univ., New York, NY 10021-6399; [2]Dept. of Molecular and Cellular Biology, Harvard Univ., Cambridge, MA 02138.

Hair cells, the sensory cells of the vertebrate auditory and vestibular systems, can detect motions of atomic dimensions, respond more than 100,000 times a second, and discriminate frequencies differing by only a few percent. These remarkable properties depend on precisely engineered cellular structures, such as the mechanosensitive hair bundle at the apex and fast synapses at the base.

Because intact single cells are easily isolated from their internal ears, the biophysical properties of hair cells have largely been characterized in frog and turtle. Similarly, the morphogenesis of the hair bundle has been described in the easily accessible chicken embryo. Identification of the hair cell's molecular components, however, has been hampered by the small number of cells per animal.

To investigate the development and regulation of hair-cell function, we are taking a genetic approach using the zebrafish, *Danio rerio*. We have developed a morphological assay that utilizes the specific staining of hair cells in the lateral-line organ by styryl-pyridinium dyes and a behavioral assay that exploits the role of hair cells in the fish's escape reflex. We are now conducting a pilot screen for recessive mutations in the F3 progeny of chemically mutagenized fish.

(Supported by National Institutes of Health grants EY00811 to J.E.D. and DC00241 to A.J.H. and by the Howard Hughes Medical Institute)

129.9

RECOVERY OF THE CHICKEN VESTIBULOCOLIC REFLEX FOLLOWING AMINOGLYCOSIDE OTOTOXICITY. C.T. Goode, J.P. Carey, A.F. Fuchs* and E.W Rubel. Regional Primate Center and Dept. of Physiology and Biophysics and Virginia Merrill Bloedell Hearing Research Center, University of Washington, Seattle, WA 98195

In birds, auditory and vestibular hair cells regenerate after damage by injury or treatment with ototoxic aminoglycoside antibiotics. Several studies have demonstrated that these regenerated auditory hair cells function normally in terms of evoked auditory potentials and behavioral detection thresholds. Recently it has been shown that the loss of vestibular hair cells following streptomycin treatment is accompanied by a substantial reduction in gain of the vestibuloocular reflex (VOR). The VOR returns to normal levels as vestibular hair cells regenerate over a period of several weeks. We examined another indicator of regenerated vestibular hair cell function, the horizontal vestibulocolic reflex (VCR), which produces head movements that compensate for rotational body motion. We treated young chickens with high doses of streptomycin and measured VCR gain at intervals after treatment. Some subjects were sacrificed at each interval to assess the degree of hair cell regeneration by means of electron and light microscopy. We report that both hair cell loss and the regeneration are reflected in the gain of the VCR and that the reflex recovers before regenerated Type I hair cells are present. We also show that VCR gain is correlated with hair cell density. Moreover, the time course of recovery of the VCR parallels that of the VOR.

129.10

TWO HOMEOBOX-CONTAINING GENES ARE EARLY MARKERS OF SEMICIRCULAR CANALS IN THE CHICKEN INNER EAR. A.E.Kiernan*[1], F.Nunes[2], D.K.Wu[2], and D.M.Fekete[1]. [1]Dept. of Biology, Boston College, Chestnut Hill, MA 02167. [2]National Institute on Deafness and Other Communication Disorders, Rockville, MD 20850.

The vertebrate inner ear is an elaborate organ which begins development as a simple ovoid vesicle. Complex morphogenesis results in a labyrinthine morphological structure complete with a cochlea, three semicircular canals (SSCs), and six to eight separate sensory areas necessary for the functions of hearing and balance. Although many genes are expressed in the ear, very little is known about how they may influence pattern formation. Using *in situ* hybridization, we present a detailed expression study of two related homeobox genes, *SOHo-1* and *GH6*. Adjacent sections were hybridized for direct comparisons. Results showed that their expression domains were identical although *GH6* was present at a lower level. Both genes were detected early, in the posteroventral and lateral portion of the otic cup. As the ear developed, the expression was associated with the SSCs in the lateral half of the otocyst. The SSCs consist of both nonsensory canals and associated sensory organs (cristae). We asked whether the cristae were included in the *SOHo-1/GH6* expression domain by using *BMP-4* as a marker for sensory areas. Results of these experiments showed that *SOHo-1* and *BMP-4* expression overlapped in the presumptive cristae at E3.5. At later time points (E5) *SOHo-1* message is no longer detectable in the developing cristae, indicating that these genes are likely not to be involved in later sensory organ differentiation. These data are consistent with early fate mapping experiments in which the lateral half of the otocyst—the region of *SOHo-1* and *GH6* expression—was shown to give rise to all three semicircular canals and their respective cristae (J. Morph., (1978) *157*: 249-268). A boundary model is proposed for cell fate specification in the early otocyst, which includes a possible role for GH6 and SOHo-1 in SSC formation. (Supported by NIH RO1 DC02756 and the Henry Luce Foundation.)

129.11

DOES REARRANGEMENT OCCUR IN THE PERIPHERAL RAT TASTE SYSTEM DURING DEVELOPMENT? Robin F. Krimm* and David L. Hill. University of Virginia, Charlottesville, VA 22903

Frequently during development, the morphology and function of a peripheral target becomes coordinated with its innervation. For example, we have demonstrated that the number of geniculate ganglion cells innervating a taste bud in adult rats is positively correlated with the size of the taste bud. Furthermore, this relationship is not established until postnatal day 40 indicating that it develops over a prolonged postnatal period. It is possible that rearrangement in peripheral innervation during development establishes the adult relationship. The present study was designed to test this hypothesis by determining whether the same geniculate ganglion cells innervate a taste bud on postnatal day 10 and 40. Individual fungiform papillae containing single taste buds were injected with a fluorescent tracer (true blue) on postnatal day 10 and a different fluorescent tracer (tetramethylrhodamine dextran) on postnatal days 15, 20 and 40. Preliminary results indicate that 90% of the geniculate ganglion cells labeled on postnatal day 10 were also labeled on postnatal day 40. Similarly, 73% and 81% of the geniculate ganglion cells labeled on day 10 were labeled on day 15 and 20, respectively. Therefore, it appears that individual taste buds remain innervated by the same geniculate ganglion cells during postnatal development. Thus, it is unlikely that rearrangement of peripheral innervation is responsible for the development of the correlation between taste bud size and number of innervating fibers. Supported by NIH Training Grant HD07323 and NIH DC 00407.

129.12

MAMMALIAN TASTE BUDS DEVELOP INDEPENDENT OF THEIR SPECIFIC INNERVATION. P.S. Sarai[#], B. Fritzsch[#], I. Silos-Santiago[@], M. Barbacid[@], D.H. Nichols[#]*. [#]Creighton University School of Medicine, Omaha, Nebraska, [@]B.M.S., Princeton, New Jersey.

The purpose of this study is to show that fungiform papilla taste bud formation occurs independent of gustatory innervation in embryonic mice. Such taste buds express neurotropin BDNF mRNA and the gustatory geniculate ganglion neurons innervating them express the corresponding tyrosine kinase B (*trk*B) receptor mRNA. Gustatory neurons lacking this receptor would thus lack their only known trophic support. Here we show that in experimental mice with a targeted disruption of their *trk*B genes, there is an almost complete loss of geniculate ganglion neurons and that their afferent gustatory fibers never reach the fungiform taste buds. Degeneration of some geniculate ganglion cells begins while others are still mitotic and a decline to 10% of control numbers is reached by 13.5 days post coitum (dpc). At birth this number has declined further to 5% and the chorda tympani shows an appropriate decrease in fiber number. The remaining cells in experimental ganglia are general somatic neurons innervating the external auditory meatus. The remaining fibers in the experimental nerves are preganglionic parasympathetic axons. Taste bud formation in experimental and control mice ranging from 13.5 dpc to 16 days postnatal (dpn) show that the onset of fungiform papilla differentiation, including taste bud formation, is, in most respects, identical. However, by 16 dpn experimental taste buds appeared less well organized than controls. Thus, innervation is not necessary to initiate taste bud formation, but may be required to complete maturation.

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129.13

ACTIVITY-DEPENDENT INCREASES IN DOPAMINE AND DOPAC IN THE OLFACTORY BULBS OF NARIS OCCLUDED RATS. B.D. Philpot*, D. Men, R. McCarty, and P.C. Brunjes. Dept. of Psychology, University of Virginia, Charlottesville, VA 22903.

Rodent olfactory bulbs contain numerous dopaminergic interneurons, known as periglomerular cells. Both dopamine and its metabolite, DOPAC, are dramatically reduced in these cells following either naris closure or deafferentation (Baker et al., J Neurosci, **3**, '83; Baker et al., Brain Res. **10**, '93). These studies collectively suggest that olfactory receptor neuron activation of periglomerular cells is necessary for dopamine production. We directly tested this hypothesis. On postnatal day 30, an external naris was closed via cautery. Thirty days later, rats were anesthetized and experimental bulbs (ipsilateral to closed nares) were carefully exposed. A bipolar stimulating electrode ($\sim$500μm tip separation) was placed on the olfactory nerve layer, and stimulus trains (100 ms, 500μA, 100Hz) were delivered every second for an hour. Sham animals received the same procedure but without current application. Animals were rapidly decapitated 6, 12, 24, 48, or 96 hours after surgery, and their brains were removed and frozen in isopentane. Olfactory bulb homogenates were prepared in perchloric acid and assessed for protein levels using the Lowry method. Catecholamine levels were determined using high performance liquid chromatography after alumina extraction from the perchloric acid supernatant. In animals receiving sham surgery, there was $\sim$60% less dopamine and $\sim$70% less DOPAC in deprived bulbs as compared to their contralateral control. Norepinephrine levels were similar between bulbs. Partial recovery of dopamine and DOPAC levels was observed between 48 and 96 hours following nerve stimulation. Ninety-six hours after stimulation, the difference between control and experimental bulbs for dopamine and DOPAC levels was reduced to only $\sim$25% and $\sim$35%, respectively. Thus, direct olfactory nerve activity transynaptically regulates dopamine in the bulb.

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129.14

ALTERATIONS OF NEST ODOR PERCEPTION IN NEONATAL RAT AFTER CAPSAICIN TREATMENT. Carrillo, P. ,* López, G., Camacho, M., García, L., Manzo, J. and Pacheco, P. Lab. de Neurobiología del desarrollo, INE., Univ. Veracruzana, Xalapa, Ver. Depto. de Fisiología, IIBM-UNAM,. México, D.F.

Trigeminal sensory receptors associated with odor perception are affected after neonatal Capsaicin (CAP) treatment, however, still is uncertain if CAP administration certainly produces alteration in the olfactory function. Thus, the present study was done in pups of Wistar rats (housed in inverted light-dark cycles 8:00-20:00), in order to analyze its odor orientation tests, after the sc injection of 50 mg/kg bw of CAP. Both, the experimental (CG) and control (VG) (vehicle solution) groups were injected at 2 day of life (day of Birth = Day 0) From 3 to 9 days of age two behavioral tests were done twice a day (9:00 and 17:00 h). The set designed for the tests included a plastic mesh arena that covered two separated wood shaving beds, one from the nest (ON) and the other from fresh wood shaving (OF). This design prevented physical contact between pups and bed, but permitted sniff the shavings. At the beginning of first behavioral test the pup was located on the upper border of the arena and in the second test it was placed at the inferior border. Once started, pups were allowed for 150 sec to move freely over beds. The time on and out beds area was scored. RESULTS. During tests performed at 9:00 h, CG pups expended less time over ON than VG ($p<0.005$). During the first week of life, VG increases their time over ON, then this time declined. In contrast CG pups did not present this time declination. The tests performed at 17:00 h showed that VG and CG did not present time oscillations on the ON bed. Again as in tests performed at 9:00 h, CG pups expend less time over ON than VG ($p<0.005$). With this results we can conclude that Cap neonatally injected produce an alteration in the olfactory perception of nest odor. (CONACYT PCC-92264)

129.15

PRE- AND POSTNATAL DEVELOPMENT IN THE RAT AUTONOMIC BRAINSTEM: CHANGES IN NEUROPEPTIDE-EXPRESSION AT BIRTH. H.-J. Rietzel[1], E. Guenther[2]* & H. Herbert[1], [1]Dept. Animal Physiol., Univ. Tübingen, [2]Dept. Exp. Ophthalmology, Univ. Eye Hospital, Tübingen, Germany.

Birth represents a time point were dramatic changes occur with respect to the control of various autonomic functions. To find out, if the changes in function involve modifications in the respective autonomic nuclei in the CNS we investigated the expression of neurotensin (NT), substance P (SP), leu-enkephalin (ENK), vasointestinal polypeptide (VIP), neuropeptide Y (NPY), angiotensin II (ANG) and dynorphin B (DYN) by employing immunocytochemical techniques. In the present study we concentrated on the nucleus of the solitary tract (NTS) and the parabrachial complex (PB), both are important nuclei in the central autonomic network controlling a variety of visceral, cardiovascular and respiratory functions. To study the pre- and postnatal development of these neuropeptides, we investigated brains from rats at embryonic day 18 (E18), E20, E21, postnatal days 0 (P0 = E22), P2, P4, P5, P8, P12, P20, P28 and brains from adults.

Our results demonstrate that in the NTS and in the PB neuropeptides are differentially expressed during pre- and postnatal development. For example, in the PB we observed at birth a strong increase in SOM-, ENK-, ANG- and SP-ir, reaching their maxima around P5. Thereafter, the staining became weaker but persisted into adulthood. For DYN-ir, moderate staining was present at E20 but the staining intensity did not show any dramatic changes during ontogeny. In contrast, NT-ir was most prominent already before birth (E20) and started to decrease after P5. In the NTS these neuropeptides show also distinct patterns of expression during development. The present study revealed that at birth dramatic changes occur in the PB and NTS with respect to the expression of specific neuropeptides. It is most likely that these peptides play crucial roles in the development of autonomic brainstem circuits which control autonomic functions immediatly after birth.

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129.16

INGROWTH OF MALE-SPECIFIC SENSORY AXONS INITIATES THE FORMATION OF PHEROMONE-SPECIFIC OLFACTORY GLOMERULI IN *MANDUCA SEXTA*. W. Roessler*, L.P. Tolbert, J.G. Hildebrand. ARL Div. of Neurobiology, University of Arizona, Tucson, AZ 85721.

Previous studies have shown that the construction of olfactory glomeruli in the antennal lobes (ALs) of the moth *Manduca sexta* depends upon the ingrowth of antennal sensory axons. To gain a more precise understanding of the formation of individual glomeruli with known odor specificity, we investigated the morphogenesis of the male-specific macroglomerular complex (MGC). It has been shown that an antennal nerve from a transplanted male antenna can induce an MGC-like structure in the AL of a host female's brain (Schneiderman et al. 1982, *Nature* 298:844). To understand this powerful role of male-specific axons, we compared the development of ALs of males and of female gynandromorphs by means of extra- and intracellular staining techniques and laser-scanning confocal microscopy. The MGC is located at the entrance of the AL and comprises 3 subdivisions, the globular cumulus and two toroidal glomeruli. Formation of the MGC in male ALs begins at the same time as the formation of the earliest "ordinary" glomeruli in female ALs. As soon as sensory axons and dendrites of projection neurons (PNs, with somata in the medial group of AL neurons) overlap at the entrance of the antennal nerve, glial cells outline a bulging structure and extend processes into this region. The glial cells migrate to form a complete matrix around the subdivisions of the emerging MGC, and processes of sensory axons and PNs become subdivided into 3 compartments within 2 days. Early development in female gynandromorphs shows very similar changes in glial cells and in the projection pattern of neurons. A significant portion of female gynandromorphs develops an MGC with 3 subdivisions. Our results indicate that axons of olfactory receptor cellss with a specificity for female pheromone initiate a complex sequence of interactions with glial cells and PNs of either gender that determines the formation of an anatomically and physiologically identified set of glomeruli. *[Supported by the DFG grant Ro 1177/1-1, and NIH grant NS-28495.]*

REGENERATION AND DEGENERATION

130.1

GELATINASE ACTIVITY IS INCREASED IN AXOTOMIZED PERIPHERAL NERVE OF ADULT RAT. J.S. Bains, C.A. Krekoski, K.R. Chapman, D.R. Edwards, A.W. Clark* Department of Pathology and Neuroscience Research Group and the Department of Pharmacology and Therapeutics, University of Calgary, Alberta, T2N 4N1 Canada

Matrix metalloproteinases (MMPs) are enzymes which degrade certain extracellular matrix proteins and thus facilitate extension and growth of cells through an extracellular environment. The gelatinase subgroup of MMPs includes gelatinase A (72 kDa, MMP-2) and gelatinase B (92 kDa, MMP-9), which act upon certain collagens, gelatins, and fibronectin. In regenerating peripheral nerve, neurites extend through the distal segment, along a path formed by the Schwann cell and its basement membrane. Macrophages and products of increased fibroblast activity also enter the regeneration route. Using gelatin zymography, we assayed activity of gelatinase A and B in adult rat sciatic nerve at 1, 3, 7, 14, 30, and 60 days (3 rats/timepoint) following sciatic nerve crush at midthigh. The contralateral sham-operated and sciatic nerve from unoperated rats served as controls. In unoperated animals, gelatinase A activity was constitutively expressed at moderate levels, whereas gelatinase B activity was trace or undetectable. One day after axotomy, gelatinase B activity was sharply increased at the crush site and extending about one centimeter into the adjacent distal segment; but subsided by three days. Gelatinase A activity also increased post-axotomy, but with a less pronounced and slower rise, subsiding by 30 days; and a wider distribution, beginning at the crush site and extending to the most distal segments and up to one centimeter into the proximal stump. Gelatinase A and B activities in peripheral nerve probably derive from different sources and may serve distinct functions after axotomy, gelatinase B facilitating an earlier phase of regeneration. (University of Calgary grant)

130.2

A RECOMBINANT Fab-FRAGMENT AGAINST MYELIN-ASSOCIATED NEURITE GROWTH INHIBITORS PROMOTES AXONAL REGENERATION OF LESIONED CORTICOSPINAL FIBERS IN ADULT RAT C. Brösamle, L. Schnell, A. Skerra°, and M. E. Schwab* Brain Research Institute, University of Zurich, Switzerland and °Department of Biochemistry, Technical University Darmstadt, Germany

Axons in the CNS of higher vertebrates are generally not capable of regeneration after injury. In CNS myelin, growth inhibiting proteins were identified that actively prevent regenerative fiber growth. A monoclonal antibody (IN-1) was raised that neutralizes these myelin-associated growth inhibitors (Schwab and Caroni, 1988) both, in vitro and in vivo, thus promoting long-distance growth of axotomized corticospinal fibers in adult rats (Schnell and Schwab, 1990, Bregman et al., 1995). The cDNA of this monoclonal antibody was cloned and a recombinant Fab-fragment expressed in *E. coli*. IN-1 Fab neutralizes the neurite growth inhibiting and growth cone collapse inducing effects of CNS myelin in vitro (Bandtlow et al., 1996).

Young adult rats underwent a laminectomy and dorsal hemisection of the spinal cord at a level of T8. The IN-1 Fab-fragment was delivered via a miniosmotic pump and a catheder into the lateral ventricle. Control animals received unspecific mouse IgG antibodies. The corticospinal tract was traced anterogradely with biotin dextran amine (BDA) and after 2 weeks survival time the animals were perfused and the spinal cord processed for the neuronal tracer. BDA tracing revealed detailled single fiber morphology. In IN-1 Fab fragment treated animals regenerated fibers far distal to the lesion were observed.

Supported by: Swiss National Science Foundation, International Research Institute for Paraplegia, Regeneron Pharmaceuticals (Tarrytown, NY).

130.3

REGENERATION OF THE AXOTOMIZED AUDITORY NERVE IN THE ADULT RAT PROMOTED BY INTRATHECAL APPLICATION OF A RECOMBINANT IN-1 Fab M. Tatagiba[+], C. Brösamle, C. E. Bandtlow*, A. Skerra[#], and M.E. Schwab Brain Research Institute, University of Zurich, Switzerland, [+]Nordstadt-Krankenhaus, Hannover, and [#]Technische Hochschule Darmstadt, Germany

We investigated whether axonal regeneration of injured auditory nerve of the adult rat can be promoted by the application of the antibody IN-1, which neutralizes the myelin-associated neurite growth inhibitory proteins. The adult rat auditory (cochlear) nerve is composed almost entirely of CNS glial tissue, and its injury normally leads to complete degeneration of the distal nerve portion, as well as severe loss of primary auditory neurons. Adult Lewis rats underwent crush lesion axotomy of the central auditory nerve at the internal auditory meatus. The lesion completely interrupted the cochlear nerve axons at the lesion site producing ipsilateral deafness in all rats. The animals were infused with a recombinant Fab fragment of the antibody IN-1 for one week via an osmotic pump into the ipsilateral cerebello-pontine angle cisterns. An age-matched control group was treated with unspecific mouse IgG. Cochlear nerve fibers were anterogradely traced by horseradish peroxidase (HRP) or biotinylated dextran amine (BDA) injected into the spiral ganglion. Following a two-days survival time a complete interruption of labelled axons at the lesion site was evident in all animals analyzed. After 9-10 days, untreated rats and rats treated with control antibody showed single axonal sprouts crossing the lesion site in half of the animals, and in 1/5 of the cases labelled axons were observed in the brain stem. In-1 fragment treated rats showed in 2/3 of the cases axons crossing the lesion and reaching the brainstem. In about half of these animals, fibres were seen to branch in the cochlear nuclei in the brainstem. Our results suggest that regenerating fibres navigate to correct targets, and are capable of establishing synaptic connections for functional recovery.

Sup. by: Europeen Science Foundation, Swiss National Science Foundation, IFP, Regeneron

130.4

X-RAY THERAPY OF THE LESION SITE IN TRANSECTED SPINAL CORD LEADS TO RECOVERY OF POSTURE AND WEIGHT SUPPORT IN ADULT RAT. N. KALDERON* AND Z. FUKS. ROCKEFELLER UNIV. AND MEMORIAL SLOAN-KETTERING CANCER CTR., NEW YORK, NY 10021.

WE FOUND THAT SOME OF THE PATHOLOGICAL CONSEQUENCES OF INJURY TO THE ADULT RAT SPINAL CORD CAN BE PERMANENTLY AVERTED BY ERADICATING CERTAIN CELLS AT THE LESION SITE WITH X-IRRADIATION, PROVIDED IT IS DELIVERED WITHIN A CRITICAL TIME-WINDOW (3RD WK) POSTINJURY (PI). X-IRRADIATION (SINGLE DOSE, 17.5-20 GY) OF LESIONED ADULT RAT SPINAL CORD (SECTIONED AT T12) RESULTED IN RESTITUTION OF STRUCTURAL CONTINUITY AND RE-GROWTH OF SEVERED CORTICOSPINAL (CS) AXONS INTO THE DISTAL STUMP. THIS TREATMENT RESULTED ALSO IN RESTITUTION IN AXOTOMIZED CS NEURONS OF THEIR ELECTROPHYSIOLOGIC CONTROL OF HINDLIMB (HL) MUSCLE ACTIVITY (*PNAS*, SUBMITTED).

HERE WE EXAMINED WHETHER THE RESTITUTION OF DISRUPTED CIRCUITRY INDUCED BY X-RAY THERAPY COULD BE DETECTED IN THE MOTOR BEHAVIOUR OF RATS THAT HAD SUSTAINED COMPLETE SPINAL CORD TRANSECTION. IRRADIATED (N=11) AND UNIRRADIATED (N=6) RATS WITH SEVERED CORDS WERE OBSERVED VISUALLY, 3-5 MO PI, FOR THE FUNCTION AND CONTROL OF THEIR POSTERIOR BODY. COMPLETE TRANSECTION OF THE CORD RESULTED IN COMPLETE LOSS OF FUNCTION IN THE LEGS; THE POSTERIOR BODY DISTAL TO THE CUT WAS PARALYZED AND LAY FLAT ON THE TABLE. IN COMPARISON, SOME OF THE IRRADIATED RATS (N=6) REGAINED PLANTAR FOOT CONTACT AND THE ABILITY TO SUPPORT WEIGHT AND STANDING POSTURE OF THEIR POSTERIOR BODY. FURTHER, UNILATERAL ABLATION OF THE HL AREA OF THE RIGHT MOTOR CORTEX IN THE IRRADIATED RATS LED TO DEFICITS IN THEIR LEFT LEG POSTURE; THEIR POSTERIOR BODY TILTED TO THE LEFT IN A SIMILAR MANNER TO NORMAL RATS WITH INTACT CORD WHICH HAD UNDERGONE IDENTICAL HL-CORTICAL ABLATION. THESE RESULTS INDICATE THAT X-RAY THERAPY LEADS TO MOTOR RECOVERY AND THAT THE RESTITUTION IN POSTURE OF THE POSTERIOR BODY IS DUE IN PART TO REGENERATION OF THE CS AXONS. NO GRANT OR COMMERCIAL FUNDS SUPPORTED THIS RESEARCH PROJECT.

130.5

INHIBITION OF AXONAL REGENERATION BY MYELIN INVOLVES A NEURONAL SIALOGLYCOPROTEIN. Maria E. De Bellard, Song Tang, John Rodert and Marie T. Filbin*. Biology Dept., Hunter College, CUNY, New York, NY 10021, USA, and † Mount Sinai Hospital, Toronto, M5G 1X5, Canada

Central nervous system myelin can inhibit axonal regeneration from adult neurons. Recently, we have shown that myelin associated glycoprotein (MAG), a component of CNS myelin, can inhibit neurite outgrowth when expressed in transfected CHO cells. We also established that MAG binds to a sialoglycoprotein on neurons, and that this interaction is involved in the inhibition of neurite outgrowth. Here we assess if the inhibition of neurite outgrowth by myelin, also involves a neuronal sialoglycoprotein and if MAG is involved in this inhibition. Neonatal cerebellar cells were cultured overnight on 1.5, 3 or 6µg of freshly prepared myelin, layered onto poly-L-lysine coated wells. Control tissue was freshly prepared membrane from neonatal liver plated at the same concentration. When desialyated neurons were grown on CNS myelin, with sialidase present in the culture media, compared to untreated neurons, a significantly greater number of neurons extended neurites. No such increase was observed with desialyated neurons on liver. Similarly, the presence of sialic acid oligosaccharides in the media increased significantly the number of neurons with neurites when grown on myelin, while neurite outgrowth on control membrane was not affected. To assess if MAG in CNS myelin contributes to the inhibition of neurite outgrowth, neurons were grown on myelin prepared from either MAG knockout mice, or from control mice. Neurons growing on MAG-/- CNS myelin, extended significantly longer neurites compared to neurites from neurons on myelin from control mice. These results suggest that inhibition of axonal regeneration by CNS myelin involves a neuronal sialoglycoprotein and that MAG may be partly responsible for this inhibition. Supported by NMSS RG2760 and AHA.

130.6

MAG EXPRESSED BY SCHWANN CELLS INHIBITS AXONAL GROWTH AND BRANCHING Y. Shen, F. Walsh*+, M. T. Filbin Biology Dept., Hunter College, CUNY, New York, NY10021; +Guy's hospital, London, UK.

Previously we showed that myelin-associated glycoprotein (MAG) is a potent inhibitor of axonal regeneration. When expressed in CHO cells, MAG inhibits neurite outgrowth from adult DRG neurons and other postnatal neurons including cerebellar, retinal ganglion, hippocampal neurons. In contrast, MAG promotes neurite outgrowth from neonatal DRG neurons. Non-myelinating Schwann cells are a permissive substrate for neurite outgrowth. They express the growth promoting molecules L1 and NCAM and secrete laminin and NGF. However, when non-myelinating Schwann cells are induced to express MAG by transfection, neurite outgrowth from cerebellar neurons is inhibited by 40%. Similarly, the number of adult DRG neurons with neurites longer than 3x diameter of the cell body decreased by 30%. These results confirm our previous findings and show that MAG can overcome the growth promoting factors of Schwann cells. In addition to its ability to inhibit axonal growth, MAG also inhibits neurite branching. The number of branches extended from DRG neurons decrease from scores to 3 or 4 per neuron when grown on MAG-expressing Schwann cells compared to Schwann cells not expressing MAG. The inhibition of neurite branching by MAG is apparent both in neonatal DRG's from which axonal elongation is promoted by MAG and in adult DRG's from which axonal growth is inhibited by MAG. These results suggest that the restriction of neurite branching by MAG is not simply related to the inhibition of neurite extension and that separate mechanisms could underlie these processes. In addition, MAG may also contribute to the limited collateral sprouting observed in myelinated regions. Supported by NMSSRG2760 and AHA.

130.7

ONLY MAG OF THE SIALOADHESINS INHIBITS AXONAL REGENERATION S. Tang, Y.J. Shen, M.E. deBellard, G. Mukhopadhyay P.R. Crocker[$], P. Doherty[*+] and M.T. Filbin . Biology Dept., Hunter College CUNY, New York, 10021, [$]Univ. of Oxford, [+]Guy's Hospital, London.

Myelin associated glycoprotein (MAG) inhibits axonal regeneration from all postnatal cerebellar and adult dorsal root ganglion (DRG) neurons. We have established that MAG binds to neurons via a neuronal sialo-glycoprotein. Together with CD22 and sialoadhesin(SN), MAG forms a sub-group of Ig-like molecules, the sialoadhesins, defined by their sequence similarity and sialic acid-dependent binding: MAG recognizes 2,3 O-linked sialic acid, CD22 recognizes 2,6 N-linked sialic acid and SN recognizes 2,3 N- or O-linked sialic acid. Using chimeric forms of these molecules, consisting of the extracellular domain of each fused to the Fc region of IgG, we studied their binding to neurons. Both MAG-Fc and SN-Fc bound specifically to neurons in a sialic acid-dependent manner, but there was no binding of CD22-Fc. Although SN-Fc binds to neurons, SN-expressing cells do not inhibit axonal regeneration. By aligning the extracellular domain of MAG, SN and CD22, we identified Arg118 of MAG as conserved in the same location in all three family members. Mutation of Arg118 to either Asp or Ala abolished binding of MAG-Fc to neurons. Furthermore, MAG inhibits axonal regeneration when either expressed by transfected cells and used as a substrate or when added to neurons in a soluble form as MAG-Fc, using immobilized L1-Fc as a substrate. However, MAG mutated at Arg118 still inhibits axonal regeneration when expressed by CHO cells but there is no inhibition when added in a soluble form to neurons. Taken together we suggest that, first, the sialic acid-recognition site on MAG is Arg118 and second that, the sialic acid-dependent binding of MAG to neurons is necessary, but is not sufficient, for inhibition of axonal regeneration. Supported by NMSSRG2760 and AHA.

130.8

MYELIN ASSOCIATED-GLYCOPROTEIN (MAG) INHIBITS NEURITE/AXON GROWTH. M.Li*¶, A. Shibata‡, C. Li§, P.E.Braun†, L. McKerracher, J. Roder§, S.B. Kater‡ and S. David¶. ¶Centre for Research in Neuroscience, and †Dept. of Biochemistry, McGill University, Montreal, Quebec, Canada, H3G 1A4; Dept. Anat. & Cell Biol., Colorado State Univ. Colorado, USA; §Samuel Lunenfeld Res. Inst., Mount Sinai Hosp. Univ. Toronto, Ontario, Canada.

We have shown previously that MAG inhibits neurite growth from NG108-15 cells. In this study we show that 60% of axonal growth cones of postnatal day 1 rat hippocampal neurons collapsed when they encountered polystyrene beads coated with rMAG. Neurite growth from these and neonatal rat cerebellar neurons was also inhibited about 80% on tissue culture substrates coated with rMAG. To investigate further the inhibitory effects of MAG in myelin, we purified myelin from MAG-deficient mice and separated octylglucoside extracts by DEAE ion-exchange chromatography. Although there was no significant difference in neurite growth on whole myelin purified from MAG-/- and MAG+/+ mice, differences were observed in the fractionated material. The first major peak of inhibitory activity associated with MAG in normal mice was significantly reduced in MAG-deficient mice. Axon regeneration in these mice was also examined after thoracic corticospinal tract lesions. A very small number of axons anterogradely labeled with WGA-HRP (maximum of 12 axons) extended 13.2 mm past the lesion in MAG-/- mice. The poor growth on whole myelin in vitro, and after spinal cord injury in MAG-/- mice may be due to compensatory changes. However, MAG is an important modulator of growth cone behaviour, since it can inhibit axon growth by arresting growth cone motility. To assess the contribution of various myelin-derived inhibitors to axon growth will require the identification and cloning of the non-MAG inhibitors.

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130.9

REGENERATIVE FAILURE: A POTENTIAL MECHANISM FOR NEURITIC DYSTROPHY IN ALZHEIMER'S DISEASE. D.A. DeWitt*, C. Doller, and J. Silver. Department of Neurosciences, Case Western Reserve University, Cleveland, OH 44106.

Neuronal pathology and synaptic loss are salient features of Alzheimer's disease, although the underlying mechanisms involved are unknown. Using double-immunolabeled preparations, we found that both the density and total lengths of axons are decreased within the Aβ containing area of senile plaques (SP) in comparison with the adjacent neuropil. these observations, along with the demonstration of synaptic loss in the SP, are consistent with axotomy. Since Aβ has been shown to be neurotoxic *in vitro*, SP cores isolated from AD brain were presented to retinal ganglion neurons. SPs did not appear to be repulsive to neurons since they adhered well and elaborated axons which wrapped around the SP core. Rat cortical astrocytes growing on isolated SPs accumulated chondroitin sulfate proteoglycan (CSPG). Neurons appeared to avoid astrocyte conditioned SP cores consistent with the axon outgrowth inhibitory nature of CSPGs. These results suggest that astrocytic reaction to SPs, including increased CSPG, may facilitate the decreased axon density and synaptic loss in AD brain. Moreover, the presence of CSPG and the similarity between both swollen axon endings in trauma and the dystrophic neurites of the SP suggest that dystrophic neurites may be exhibiting regenerative failure rather than aberrant sprouting.

Supported by NIH grant: NS 25713.

130.10

RECOMBINANT ADENO-ASSOCIATED VIRUS (AAV) VECTORS FOR BRAIN-DERIVED NEUROTROPHIC FACTOR (BDNF) AND GROWTH-ASSOCIATED PROTEIN (GAP-43) GENE DELIVERY.

R.L. Klein*, S. Zolutukhin, E.M. Meyer, N. Muzyczka. Dept. of Pharmacology and Therapeutics and Dept. of Molecular Genetics and Microbiology, Univ. of Florida, Gainesville, FL 32610

The design and use of adeno-associated virus (AAV) vectors can produce prolonged expression of genes of interest in brain. The degeneration of populations of neurons that are associated with specific diseases may be attenuated or prevented by expressing genes involved in growth and regeneration. AAV plasmids were designed for cytomegalovirus promoter-driven expression of BDNF or GAP-43 as well as green fluorescent protein (GFP). We prepared high-titer stocks of recombinant AAV virions that are free of adenovirus. Infection of primary cortical neurons with AAV virions resulted in detectable expression of BDNF mRNA at 2.2 kb that was not observed in controls. Pronounced expression of recombinant BDNF mRNA was observed after infection of neurons with adenovirus in addition to recombinant AAV. Although adenovirus is toxic to cultured neurons it may be useful to study short-term effects of AAV-mediated gene transfer, e.g. to observe the function of GAP-43 or to produce biologically active BDNF. Currently, we are testing the BDNF-AAV vector for induction of choline acetyltransferase activity in primary septal neurons and we are testing the GAP-43-AAV for expression of GAP-43 mRNA and protein. The long-term goals of these studies are injection of virions *in vivo* for models of neurodegeneration. Supported by NIH 2910378-11.

130.11

PROLONGED GENE EXPRESSION IN GLIAL CELLS BUT NOT NEURONS WITH RECOMBINANT ADENOVIRUSES IN THE FACIAL NUCLEUS OF ATHYMIC NU/NU RATS. W.T.J.M.C. Hermens[1,2], W.H. Gispen[2], J. Verhaagen[1,2]*. [1]Neth. Inst. Brain Res., A'dam, [2]Rudolf Magnus Inst., Utrecht, The Netherlands.

The aim of our research is to promote growth of injured neurons by using viral vectors encoding neurotrophic and growth-promoting proteins. This requires an efficient, non-toxic vector system. Recombinant adenovirus has been shown to induce inflammation in many tissues. We compared the performance of two replication deficient first generation adenoviral vectors, encoding the reporter gene lacZ (AdCMVLacZ) or the neural growth-associated protein B-50/GAP-43, following infusion in the facial nucleus of immunocompetent Wistar rats and thymus-deficient (Nu/Nu) rats. Titers of both adenoviral vectors as determined on 911-producer cells were $1x10^{11}$pfu/ml. Contamination with replication competent adenovirus (RCA) was determined on A549 cells and was less than 1 RCA per 10^8 recombinants. Injection of 2 x 10^7pfu of AdCMVLacZ or AdCMVB-50 in adult Wistar rats resulted in abundant transgene expression in facial motoneurons and glial cells during the first week following application. In the second week a substantial decline in transgene expression became apparent. Infiltrating macrophages were observed in the facial nucleus as early as 4 days following adenoviral vector infusion and reached peak levels at the end of the second week. In the facial nerve the macrophage response started at 8 days after vector application. T-cell infiltration was not observed during the first week but occurred during the second week. The decrement in transgene expression in Wistar rats correlated closely with the appearance of T-cells. In T-cell deficient Nu/Nu rats transgene expression persisted for up to 20 days in glial cells, however expression declined in motoneurons according to a time course that was similar to that observed in Wistar rats. In conclusion, recombinant first generation adenoviral vectors evoke a T-cell mediated immune response in the brain that appears not to be the direct cause of the extinction of transgene expression in neurons. However, transgene expression in glial cells is prolonged in rats that can not evoke a T-cell response. (Support: grant from NWO/GB-MW 90352121).

130.12

AAV VECTORS CAPABLE OF CELL TYPE-SPECIFIC TRANSGENE EXPRESSION IN THE CNS AND PNS. A.L. Peel,* S. Zolotukhin, G.W. Schrimsher, K. Chesnut, M. Potter, N. Muzyczka, and P.J. Reier. Depts. of Neuroscience, Neurological Surgery, Molecular Genetics and Microbiology, University of Florida, Gainesville, FL 32610.

A combination of different therapeutic approaches may be required to promote recovery from CNS trauma or disease, and a role for gene delivery techniques can be readily envisioned. Of the current viral vector systems available, adeno-associated virus (AAV) appears to be an optimal system for introducing foreign genes into the CNS by virtue of several inherent advantages. The objective of these experiments was to determine whether transgene expression can be optimized by capitalizing on cellular responses to CNS injury in combination with cell type-specific promoters. Accordingly, we have developed AAV vectors capable of transducing and eliciting expression of foreign genes from major cell types of both the CNS and PNS. We have placed the gene for a "humanized" form of green fluorescent protein (GFP) under the control of two neuron-specific promoters: the neuron-specific enolase (NSE) and the platelet-derived growth factor (PDGF) beta chain promoters. For potentially maximizing gene expression in reactive astrocytes, we have also developed a GFP vector driven by the glial fibrillary acidic protein (GFAP) promoter. Additionally, we have developed a similar GFP construct under the control of the myelin basic protein (MBP) promoter which is active in myelinating oligodendrocytes and Schwann cells. For *in vivo* analysis, the neuron-specific constructs packaged in AAV virions were injected into the intact spinal cords (C_4 spinal level) of six adult rats. Two weeks later, green fluorescing cells with neuronal morphology were seen in all animals. Expression from the PDGF construct was evident for as long as 15 weeks post-injection. Fluorescent fibers were observed extending rostrally to the base of the pons and caudally through the extent of the thoracic cord. Transduction of astrocytes and CNS stem/progenitor cells *in vitro* yielded positive expression of GFP driven by the GFAP promoter only in differentiated astrocytes. Finally, transduction of Schwann cells by the MBP promoter construct was positive for protein expression, whereas transduction of astrocytes and CNS stem cells did not yield evidence of GFP expression. *In vivo* experiments with the GFAP and MBP vectors are currently being undertaken. These initial data support the use of AAV vectors to drive foreign gene expression in a cell type-specific manner. (Supported by University of Florida Brain Institute, the Center for Neurobiological Sciences, and the Mark F. Overstreet Chair for Spinal Cord Regeneration Research)

130.13

ENHANCEMENT OF NERVE REGENERATION USING A LAMININ OLIGOPEPTIDE DERIVATIZED ARTIFICIAL EXTRACELLULAR MATRIX.

S. Makohliso*, M. Borkenhagen and P. Aebischer. Gene Therapy Center, CHUV, Lausanne University Medical School, Lausanne , Switzerland.

Growing neurites are guided through their environment during development and regeneration via different extracellular matrix (ECM) molecular cues. A three-dimensional (3-D) hydrogel based ECM equivalent containing different immobilized laminin oligopeptides has been designed. The specificity of the neurite outgrowth promoting activity was tested in a competitive binding assay, showing that neurite outgrowth from DRGs in a CDPGYIGSR derivatized gel was significantly inhibited after incubation with soluble CDPGYIGSR peptide. Scrambled peptides such as CDPGYIGSK and CDPGRGSYI had a signficantly smaller effect on neurite outgrowth compared to CDPGYIGSR.

The in vivo impact of derivatized gels was evaluated, studying the regeneration of transected spinal roots in a rat model. Six mm long polymeric nerve guidance channels were used to bridge a 4 mm gap. Cohorts of 6 animals were implanted with guidance channels filled either with saline, plain agarose, scrambled peptide or CDPGYIGSR derivatized agarose. Four weeks post-implantation, nerve guidance channels filled with a CDPGYIGSR derivatized gel, contained a regenerated nerve cable with a higher number of myelinated axons (384+/-196) compared to nerve guidance channels filled with saline solution (202+/38), plain (198+/-119) or scrambled peptide (83+/-70) derivatized agarose gel. The present work shows that 3-D bioartificial matrices containing ECM molecules or laminin oligopeptides can be constructed to promote neurite outgrowth both in vitro and in vivo.

Supported by the Swiss National Science Foundation.

130.14

A HYDROGEL BASED, 3D BIOPOLYMERIC MATRIX FOR NERVE REGENERATION. R. Bellamkonda* and X.J. Yu. Biomaterials, Cell and Tissue Engineering Laboratory, Department of Biomedical Engineering, Case Western Reserve University, OH 44106-7207.

The extracellular environment influences the degree of nerve regeneration in the PNS and CNS. Our laboratory focuses on using biomaterials to modify the extracellular environment at the site of nerve lesion to facilitate regeneration. We have developed a bioactive, 3D hydrogel matrix that has the following attributes: A) it has a 3D physical structure that is amenable to neurite extension B) it has the neurite outgrowth promoting extracellular matrix (ECM) protein laminin, covalently coupled to its backbone and C) it carries lipid microcylinders that release nerve growth factor (NGF) to attract nerve ingrowth into the polymer matrix.

Laminin was covalently immobilized onto agarose gels using solution and photochemical methods. Infrared spectroscopy confirmed covalent immobilization of laminin. E9 Chick dorsal root ganglia (DRGs) were embedded in modified and unmodified gels and cultured *in vitro* for 5 days. Significantly greater neurite extension was observed in laminin coupled agarose gels compared to gels carrying a control pentaglycine peptide or in unmodified agarose gels. This growth was contingent upon the addition of exogenous NGF to the culture medium.

Phosphatidylcholine based lipid microcylinders (1μ x 100μ) were loaded with NGF and embedded in unmodified agarose gels. DRGs were cultured in these gels without adding exogenous NGF. DRGs in NGF-microcylinder embedded gels had long neuronal process (>1000μ) while gels with saline filled microcylinders had limited neuronal processes. *In vitro* DRG bioassays and spectrophotometry show that lipid microcylinders release NGF at physiologically relevant concentrations for at least five days.

ECM protein and growth factor laden polymer matrices that are conducive for neurite growth in 3D can induce controlled nerve growth from neural cells *in vitro* and can potentially enhance nerve regeneration *in vivo*. (Supported by the Lindseth Endowment, BME, CWRU)

130.15

Free-radical scavengers support retinal ganglion cell survival after traumatic optic nerve lesions. G.W. Eschweiler and M. Bähr*. Neurologische Universitätsklinik, Hoppe-Seyler-Str.3, 72076 Tübingen, F.R.G.
After traumatic CNS lesions, a secondary neuronal loss can be observed which occurs rather late and might be prevented by interference with pathophysiological cascades that are triggered by posttraumatic loss of trophic factors, release of neurotoxic substances or activation of pro-apoptotic genes. We have used a CNS trauma model to examine the influences of potentially neuroprotective drugs like glutamate-receptor-antagonists, MAO-B-inhibitors, calcium-antagonists or free-radical scavengers. In adult female rats the optic nerve was transected close to the the optic disc. Retinal ganglion cells (RGCs) were retrogradely labeled by Di-I and Fast blue. At two weeks after optic nerve lesion the numbers of viable RGCs were determined in different retinal regions. To examine the effects of the various treatment strategies on physiological functions, electroretinogramms (ERGs) and pattern visual evoked potentials (pVEP) were recorded. Systemic application of a free radical scavenger (S-PBN) lead to a significant rescue of traumatized RGCs after optic nerve lesion. Calcium-antagonists were less effective and the NMDA-antagonist memantine and the MAO-B inhibitor deprenyl did not support survival of axotomized RGCs at all. Electrophysiological measurements were not influenced by treatment with SPBN. We conclude from these findings that free radical damage plays an important role in delayed posttraumatic neuronal death. Delayed administration of SPBN rescues a significant proportion of lesioned CNS neurons without influencing physiological parameters. In the future, free radical scavengers might contribute to a therapeutic strategy designed to prevent early axonal and neuononal damage after CNS lesions.
Supported by the Kuratorium ZNS, BMBF Neurotraumatologie and the Herrmann-und-Lilly-Schilling Stiftung

130.16

TUMOR FORMATION AND HYPERPIGMENTATION FOLLOWING WOUNDING OF NF1/nf1 MOUSE NERVE: **Tilat Rizvi* and Nancy Ratner.** Dept Cell Biology, Neurobiology & Anatomy, Univ. Cincinnati. Coll. Med., Cincinnati, OH-45267
Patients with type 1 neurofibromatosis (NF1) are predisposed to the formation of neurofibromas (benign peripheral nerve tumors) and cafe-au-lait macules (hyperpigmented spots on the skin-CALM). Mice heterozygous at NF1, unlike their human counterparts, fail to develop CALM or neurofibromas. It was suggested that wounding might precipitate neurofibroma formation. To test this hypothesis, we crushed or cut the sciatic nerve in NF1/nf1 mice. First a developmental study was carried out to test if any abnormalities are present in NF1/nf1 mouse nerves before injury. Histologic and EM analysis of sciatic nerve (from E14 through adult) in wild type and NF1/nf1 mice showed no significant abnormalities in Schwann cell differentiation (myelination) or fibroblast differentiation (perineurium formation). We next tested if crushing the sciatic nerve by mechanical pressure caused abnormalities. Mice sacrificed 6 and 10 weeks post surgery (PS) showed no significant abnormalities. We then transected the sciatic nerve and rejoined cut ends of the nerve with sutures. Eight NF1/nf1 and eight wild type mice have been evaluated. Four animals from each group were sacrificed at 1 month, 2 animals each at 2 months and 2 animals each at three months PS. Wild type mice did not show abnormality in nerve histology while nerves from all NF1/nf1 mice showed dramatic tissue changes including (1) intermingling of axons with surrounding muscles at 1 month PS and with the overlying muscles and skin at 2 and 3 month PS, (2) transient muscle atrophy, (3) patchy hyperpigmentation of muscles overlying the nerve and around and on the sciatic nerve itself at 1 month PS, and in the dermis at 2 and 3 month PS. Hyperpigmented cells were identified as melanocytes by EM, (4) formation of encapsulated tumors in the lesioned area at 3 month PS. Thus, wounding of the sciatic nerve in mice with a single mutant NF1 allele is sufficient to cause disruption of tissue organization with features resembling human NF1. Supported by NIH NS-28840

130.17

DEGENERATION OF EPIDERMAL SENSORY FIBERS IN MICE. S.-T. Hsieh*, W.-M. Lin, W.-P. Chen, and Y.-C. Chang. Departments of Anatomy and Neurology, National Taiwan Univ. College of Medicine, Taipei, 10018, TAIWAN
Innervation of the skin is important for nociception, which is required for normal protection. Neurons with axons belonging to the Aδ and C categories terminating in the skin have been characterized extensively by physiological means. However, the diameter of these fibers are usually less than one μm because of their non-myelination character. Morphological evaluation of the "free nerve endings" have been difficult. We recently took the advantage of sensitive immunocytochemistry to demonstrate these fibers unequivocally by immunolabelling the epidermal fibers with protein gene product 9.5 (PGP), a ubiquitin hydrase. Combining with sciatic nerve transection, we further addressed the following issues: (1) whether the cutaneous nerve fibers reach the epidermis, the outermost layer of the skin, and that (2) whether these fibers disappear after nerve degeneration. Adult ICR mice were rendered to sciatic nerve transection on one side with the other side sham-operated as control. The skin of the feet was sampled at different time points after transection, from one day-post surgery to four weeks. Our results indicated that the PGP-immunoreactive epidermal fibers were far more rich than previously expected. These fibers quickly degenerated one day after transection while many of the dermal fibers, usually myelinated, remained intact. The results suggest that unmyelinated fibers degenerate faster than myelinated ones. Thus this approach provides a new way to study degeneration and regeneration of unmyelinated nerve fibers, which has not been explored for years because of technical difficulties.
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130.18

NEURONAL SUB-POPULATION OF THE MAJOR PELVIC GANGLION INNERVATING THE CORPUS CAVERNOSUM: A FLUORESCENT TRACER STUDY OF THE CAVERNOSAL NERVE. S. J. Archibald*[1], K. Jovanovic[1], S. Batra[4], and R. D. Madison[1,2,3]. Division of Neurosurgery [1], Department of Neurobiology [2], Duke University Medical Center, Research Service of the VA Medical Center [3], Durham, NC., and Integra LifeSciences, Inc., Plainsboro, N.J.[4]
With the continued development of prosthetic nerve guides as replacements or supplements for nerve grafts, there has been increased interest in their use for cavernosal nerve repair (Ball et al., 1992 , J. Urology 148: 211-215). One of the main advantages of nerve guide or entubulation repair techniques is that the lumen environment of the tube can be modified to promote neuronal survival and axon regeneration. This study represents a baseline assessment of the population of autonomic (parasympathetic) neurons in the major pelvic ganglion (MPG) providing the cavernosal innervation and their response to nerve section and entubulation repair.
Ten young adult Sprague Dawley rats were divided into 2 groups, 1) unlesioned controls, 2) entubulation repair of the right cavernosal nerve, 4 mm polyethylene tube (Clay Adams PE-20, 0.58 mm I.D.) with a 2.5 mm nerve gap. Regeneration was allowed to proceed for one month at which time the MPG was unilaterally retrogradely labeled with Fluorogold from the cavernosal nerve at a point proximal to the entubulation repair and from the same location in the unlesioned normal animals. Histological analysis demonstrated significant drop out of cells from the major pelvic ganglion after entubulation repair (two tailed t-test, $p < 0.05$). The normal population of neurons in the MPG innervating the right cavernosal nerve was 2447±198 (n=4), and following entubulation repair was 1702±209 (n=6).
SBIR 1R43NS34224-01 (SJA & SB)

130.19

AXONAL DIE-BACK INDUCED BY CORTICOSPINAL AXOTOMY IN THE MIDTHORACIC SPINAL CORD OF ADULT RATS. H. Li[1], C.Y. Hsu[2], A.L. Pearlman*[2], J.E. Brunstrom[2], J. Perschbacher[1] and X.M. Xu[1]. [1]Dept. Anat. & Neurobiol. Saint Louis Univ. Sch. of Med., St. Louis, MO 63104; [2]Dept. Neurol. & CSNSI, Washington Univ. Sch. of Med., St. Louis, MO 63110.
When axons are severed, their entire distal segments undergo Wallerian degeneration. It is not clear whether the proximal segments of long projecting axons in the spinal cord die back from the site of injury and, if so, how far they retract. In the present study, the die-back phenomenon of the corticospinal tract was investigated using a phaseolus vulgaris leucoagglutinin (PHA-L) anterograde tracing method. Adult female Fischer rats received spinal cord overhemisection at T-8 to transect the corticospinal tract (CST) bilaterally. The injection of PHA-L was made iontophoretically into the hindlimb area of the left sensorimotor cortex either prior to or after corticospinal axotomy and transport allowed for 14 days. The animals were perfused 3, 7, 14, 21 and 28 days after CST axotomy. Tissues were sectioned and reacted with goat anti-PHA-L, processed with biotynylated rabbit anti-goat IgG and Vector Avidin-biotin-Peroxidase complex, and with H_2O_2 in the presence of diaminobenzidine. Our results demonstrate that: 1) axotomy of the CST causes axons proximal to the lesion to swell and to form bulb-like profiles near the lesion site 3 and 7 days postinjury, 2) the die-back of lesioned CST axons progresses over time and by day 14, labeled axons were found approximately 1.0 mm away from the rostral border of the remaining tissue, 3) regenerative sprouting was observed in later stages, i.e., 21 and 28 days postinjury, where a few fine labeled CST fibers had sprouted to the rostral tissue border. (Supported by American Paralysis Association to CYH and the Daniel Heumann Fund for Spinal Cord Research to XMX).

131.1

CAUDOTOMY DOES NOT PROVOKE THE SUSTAINED IMPROVEMENT OBSERVED IN IMPLANTED PARKINSON'S PATIENTS. J.J. López-Lozano*, G. Bravo, R. Martínez, J. Burzaco, P. Sánchez, C. de la Torre and the CPH Neural Transplantation Group. Clínica Puerta de Hierro, and GammaKnife Neurosurgical Unit, Clínica Ruber Internacional, 28035 Madrid, Spain.

The results obtained by our group to date show that the implantation of neural tissue into caudate nucleus by open surgery can improve the clinical symptomatology of severely impaired parkinsonian patients, and that the duration of the clinical improvement observed and the moment of onset differ depending on the tissue implanted, with that resulting from implantation of AM grafts presenting earlier and that observed with FVM occurring later and lasting longer. However, we have yet to determine whether the improvement detected in the early months may also be caused by trauma at the implantation site. To test this possibility, we have carried out a double-blind study in 8 parkinsonian patients (4 controls and 4 study subjects) who underwent stereotactical radiosurgical caudotomy- 4 mm ø (GammaKnife Leksell Unit). All the patients were installed in the GKLU but only 4 patients received radiation. The selection criteria for the patients were those used in our previous series of graft recipients (J.Neurosurg '91; Lancet '93; Transplantation proc '95). Their status was determined on the basis of internationally accepted rating scales (CAPIT'P, UPDS, NWDS). The results show that there were no significant changes in the control patients. After 6 months, the 4 study patients presented an area of radionecrosis measuring 4-5 mm in diameter. Two patients presented a transient moderate improvement in rigidity and akinesia with a moderate reduction in the amount of time spent in Off and fluctuations in dyskinesias. There was no substantial reduction in the L-dopa intake. In comparison with these graft recipients, the overall improvement in the patients in this study was less marked, was of shorter duration was barely appreciable and the clinical course and reduction of medication did not parallel those observed after transplantation of AM, FVM or AM+PN. The results indicate that the improvement observed in parkinsonian graft recipients can not be attributed to caudotomy. (Supported by FIS 96/428, CAM 95).

131.2

STRATEGIES TO ALLOW CONTINUED LEVODOPA THERAPY IN PARKINSONIAN GRAFT RECIPIENTS: IDENTIFICATION OF FACTORS INVOLVED IN LEVODOPA-INDUCED TOXICITY IN EMBRYONIC DOPAMINE NEURONS. K. Steece-Collier[1]*, T. Alexander[1], C.E. Sortwell[1], T.J. Collier[2], C.D. Sladek[3]. Dept of Neuroscience[1], and Dept of Physiology[3], Finch Univ. of the Health Sci/ The Chgo Med Sch, N. Chgo, IL 60064; Dept of Neurological Sciences, Rush Presb-St. Lukes Med Ctr, Chgo, IL 60612.

One potential caveat in parkinsonian graft recipients is that they require continued treatment with the antiparkinsonian medication levodopa (L-dopa). There is increasing evidence that levodopa is toxic to developing dopamine (DA) neurons. We have observed that chronic L-dopa adminstered to rats with embryonic DA neuron grafts is detrimental to morphology and function of grafted DA neurons. Further, we and others have observed that L-dopa and DA are toxic to catecholamine neurons in culture. In order to develop potential adjunct treatments which could decrease or alleviate the potential deleterious influence of L-dopa on survival of grafted embryonic neurons while allowing benefical symptomatic treatment in the patient we have examined the possible mechanisms of L-dopa toxicity. It is generally assumed that L-dopa produces toxicity due to oxidative stress derived from catabolism of DA. To verify this assumption, we compared the neurotoxic profile of L-dopa, the less active stereoisomer D-dopa, and DA. In cultures derived from embryonic day 14 (E14) F344 and Sprague Dawley rats, D-dopa was toxic to DA neurons only at high concentrations (>50uM). In contrast, L-dopa and DA produced significant toxicity at concentrations >1nM. In addition, statistical analysis indicated that DA produced more neuronal death at most concentrations ($p<0.001$) and that cultures from Sprague Dawley rats were significantly more susceptible to DA neurotoxicity than F344. Ongoing studies are examining downstream pathways that are important in producing this toxicity. In parallel studies, we have observed a dramatic decrease in neurotoxicity of L-dopa at earlier gestational ages. Whereas 4 applications of 100uM L-dopa caused complete death of DA neurons in E14 (CRL 10.5-11mm), there was only a 20% reduction in number DA neurons derived from E13 (CRL 9-9.5mm) donors. In contrast to this differential effect of L-dopa, there was no significant difference in degree of DA cell death following DA treatment. Again, ongoing studies are exploring potential factors responsible for the differential toxicity of L-dopa with earlier gestation age in hopes of identifying neuroprotective strategies which might enhance graft cell viability in parkinsonian patients requiring drug therapy.

131.3

POST-MORTEM HISTOLOGICAL CHARACTERIZATION OF SURVIVING PORCINE MESENCEPHALIC CELL SUSPENSION XENOGRAFTS IN A PARKINSONIAN PATIENT. T. Deacon[1,4]* C. Thomas[2] J. Dinsmore[3] E. Prather Palmer[2] D. Penney[2] S. Kott[2] P. Dempsey[2] O. Isacson[1] and J. Schumacher[2]. McLean Hospital, Belmont, MA 02178[1] Lahey Hitchcock Clinic, Burlington, MA 01805[2] Diacrin, Inc., Charlestown, MA 02129[3] Boston University, Boston, MA 02115[4]

In an ongoing FDA approved safety study of porcine fetal mesencephalic cell transplantation in Parkinson's disease (Schumacher, et al., in prep.) one patient died of unrelated causes 7.5 months after transplantation. The patient received multiple injections of a cell suspesion derived from E27 fetal pig ventral mesencephalon into the right putamen and caudate nucleus and was immune suppressed with Cyclosporin (5mg/kg/day). The brain was removed at autopsy and immersion fixed in 10% formalin. Histological analyses were performed using hematoxylin and eosin stain (H&E), Cresyl violet stain; antibodies to tyrosine hydroxylase (TH), pig-specific 70kD neurofilament (NF70) and pig-specific glial antigen (CD44); and in situ hybridization to a pig-specific DNA repeat element (PRE). H&E analysis showed no signs of inflammatory response around implantation sites. Nissl, TH and NF70 demonstrated TH and non-TH immunoreactive neurons in all grafts, with no sign of neuronal migration into the host. Porcine axons were identified with NF70 and TH extending from the graft into the host striatum. CD44 immunoreactive porcine glia were densely present in each graft and were also identified in small numbers in the surrounding host putamen. PRE labelled cell nuclei were concentrated within each graft site and significant numbers were also identified in the surrounding host striatum. This histologic study demonstrates survival of xenogeneic neurons and glia in a Parkinsonian patient for over 7 months. (Supported by Lahey Hitchcock Clinic and Diacrin, Inc.)

131.4

FUNCTIONAL FETAL NIGRAL GRAFTS IN TWO PATIENTS WITH PARKINSON'S DISEASE: IMMUNOLOGICAL STUDIES: R Hanbury[1]*, S.D. Styren[2], P. R. Sanberg[3], T.B. Freeman[3], and C.W. Olanow[4], J.H. Kordower[4]. [1]Dept. Neurological Sciences, Rush Presbyterian Med. Ctr.,Chicago Ill. 60612, [2]Dept. of Psychiatry, Univ. Pittsburgh, Med. Ctr. Pittsburgh PA, 15213, [3]Division of Neurosurgery, Univ. S. Florida, Tampa Fl, 33606, [4]Dept. of Neurology, Mt. Sinai Med. Ctr. N.Y. N.Y. 10029.

Little information is available regarding the status of the immune system following human fetal nigral transplantation. We are studying two patients with Parkinson's disease which have come to autopsy following fetal nigral transplantation and died 17-18 month following transplantation from evens unrelated to the grafting procedure. Both patients were immunosuppressed with cyclosporin 3 weeks prior to grafting and for 6 months post-transplantation. In both patients, healthy appearing grafts were observed in all implant sites within the post-commissural putamen. Most graft sites displayed few macrophages on Nissl stains. GFAP immunostaining revealed relatively few astrocytes within the grafts. The grafted host putamen displayed dense collections of GFAP-ir cells. However, the nongrafted caudate nucleus displayed a similar widespread expression of GFAP-ir astrocytes suggesting that the underlying disease and not the grafting procedure was responsible for this finding. Immunohistochemical studies were performed using an antibody directed against HLA-DR, a marker which is ubiquitously expressed in immune cells including microglia, T cells, B-cells, and cells expressing Class II antigens. Dense HLA-DR-ir was observed within graft sites and the perigraft region in both cases. HLA-DR-ir was especially dense around blood vessels. Further studies are underway defining which population(s) of immune cell(s) are expressing this marker within the grafts. (Supported in part by the United Parkinson's Foundation).

131.5

FUNCTIONAL FETAL NIGRAL GRAFTS IN A SECOND PATIENT WITH PARKINSON'S DISEASE: A POST-MORTEM ANALYSIS J.H. Kordower*[1]; J.M. Rosenstein[2]; T.J. Collier[1]; E.-Y Chen[1]; Jing Min Li[1]; E.J. Mufson[1]; P. Sanberg[4], T.B. Freeman[3]; and C.W. Olanow[6]. [1]Dept. Neurological Sciences, Rush Presbyterian Med. Ctr., [2]Dept. Anatomy, George Washington Medical Ctr., Dept. Neurology, Emory Univ., Dept. Neurology and [5]Div. Neurosurgery, Univ. South Florida at Tampa; [6]Dept. of Neurology, Mt. Sinai Medical Center, N.Y., N.Y. 10029

We recently reported post-mortem evidence for long-term survival of fetal nigral grafts survival in a patient with Parkinson's disease (Kordower et al., NEJM, 332: 1118-1124, 1995). This year, a second patient from our clinical transplantation program has come to autopsy. This patient received sterotaxic bilateral fetal ventral mesencephalic transplants into the post-commissural putamen employing seven embryos between 6 1/2-9 weeks post- conception. This patient displayed sustained improvement in motor function and a progressive increase of putamenal fluorodopa (FD) uptake on PET scan. This patients died 18 months following transplantation from asphyxiation. The autolysis time was 5h. One hemisphere was immersion fixed for histological evaluation and transplants were analyzed using tyrosine-hydroxylase immunohistochemistry (TH-ir).. Large organotypic grafts were observed at each graft site containing numerous TH-ir neurons. A quantitative evaluation revealed the presence of 135,673 TH-ir neurons in the right hemisphere. TH-ir grafted neurons displayed a round and triangular shape, were 25-30µm in diameter and exhibited long multipolar processes. These grafted extensively reinnervated the parkinsonian striatum in a patch-matrix pattern. No host dopaminergic sprouting was observed. These data support the concept that fetal nigral grafts can survive in the human brain and functionally reinnervate the parkinsonian striatum. (Supported by the United Parkinson's Foundation).

131.6

FUNCTIONAL FETAL NIGRAL GRAFTS IN A PATIENT WITH PARKINSON'S DISEASE: EFFECTS ON DOPAMINE SYNAPTIC MARKERS. J.K. Staley*[1], D.C. Mash[1], M. Basile[1], W. Weiner[1], A.I. Levey[2] and J.H. Kordower[3]. [1]Dept. Neurology, University of Miami Sch. Med., Miami, FL., [2]Dept. Neurology, Emory University, Atlanta, GA and [3]Dept. Neurological Sciences, Rush Presbyterian Med. Ctr. Chicago, IL.

The cardinal feature of Parkinson's disease (PD) is the loss of nigrostriatal dopamine (DA) neurons. Fetal nigral transplantation has been considered as a treatment for replacement of the DA deficit in PD. The present study used quantitative *in vitro* autoradiography and immunocytochemistry to assess the status of the DA transporter (DAT), D_1, D_2 and D_3 receptor subtypes in a patient who received a fetal ventral mesencephalic transplant into the post-commissural putamen and died 18 months following the transplantation from events unrelated to the grafting procedure. DAT densities were significantly lower in the anterior and posterior striatum in endstage PD patients as compared to aged control subjects. Labeling of the DAT was enriched in the posterior striatum of the PD/transplant case as compared to the PD cases. DAT-IR was extensive in the posterior putamen in a pattern similar to that seen with tyrosine hydroxylase. D_1 receptor densities were elevated in the anterior and posterior striatum of the PD and PD/transplant case as compared to control cases. D_2 receptor densities were elevated in the anterior putamen, but were not significantly different in the anterior caudate or posterior striatum of the PD and the PD/transplant cases. D_3 receptor densities were decreased in the posterior striatum and increased in the anterior striatum of the PD cases. D_3 receptor number normalized to the density observed in the control subjects in the posterior striatum of the PD/transplant case. These studies suggest that fetal nigral grafts may foster the recovery of some DA synaptic markers and may contribute to the beneficial behavioral effects observed after transplant. Supported by the National Parkinson's Foundation.

131.7

EX VIVO AND *IN VIVO* GENE TRANSFER IN THE RAT STRIATUM USING AN AAV VECTOR EXPRESSING GFP. L. Tenenbaum[1], E. Hooghe-Peters[1], B. Avalosse[2], A. Lubansu[3], M. Levivier*[3]. [1]Dept of Pharmacology, AZ-Vrije Univ. Brussel; [2]Laboratory of Molecular Biology, Univ. Libre Bruxelles-Institut Bordet, [3]Dept of Neurosurgery, Univ. Libre Bruxelles-Hôpital Erasme; Brussels, Belgium.

The green fluorescent protein (GFP) of *Aeqorea victoria* absorbs blue light and emits green fluorescence without exogenous substrates and can, therefore, be used as a reporter gene. We transfected the human glial cell line U373-MG with an AAV vector (kindly provided by Drs. N. Muzyczka and S. Zolotukhin) carrying two expression cassettes: [i] the GFP gene under the control of the CMV promoter and [ii] the neomycin-resistance gene under the control of the HSV-TK promoter. The clones constitutively expressing the GFP protein were still fluorescent after 3 months in culture. When these cells were stereotactically injected into the striatum of adult rats (10^5 cells in 2 µL), GFP+ cells were observed at the implantation site 6 hours after surgery. We then tested the intrastriatal injection of a concentrated AAV-GFP recombinant virus stock (2µL: 2.10^8 infectious units per ml). Starting 2 days after injection, we found a strong yellow-green fluorescence at the level of the cortex underlying the burr hole and along the needle tract in both AAV-GFP- and mock-injected (DMEM) rats, as well as in rats implanted with GFP+ cells. This artefactual fluorescence corresponded to endogenous proliferating cells, most presumably glial cells, and increased in intensity from 2 days to 2 weeks after surgery. We could not clearly distinguish GFP green fluorescence from this high fluorescent background, neither after implantation of GFP+ cells nor after direct AAV-GFP injection *in vivo*. This artefactual fluorescence was not linked to paraformaldehyde fixation since it was observed with the same intensity in unfixed fresh-frozen tissue and in both paraformaldehyde-post-fixed tissue and paraformaldehyde-perfused animals. These results suggest that technical aspects of the fluorescence detection may limit the use of GFP as a reporter gene *in vivo*. Long-term expression of the vector will be monitored to further evaluate interactions between GFP- and surgery-induced fluorescence. (Supported by A.S.L.K. and F.N.R.S., Belgium).

131.8

IMPLANTATION PARAMETERS FOR RELIABLE DELIVERY OF CELLS INTO THE STRIATUM OF MONKEYS USING AN AUTOMATED DELIVERY SYSTEM. J.R. Bringas, E.M. Kutzscher, B.Y. Yang, R.S. Hundal, D. Nagy*, W.W. McLaughlin, S. Leff, M.E. Emborg, S. Jungles and K.S. Bankiewicz. Somatix Therapy Corporation, Alameda, CA 94501.

Intracerebral grafting combined with gene transfer may provide a powerful technique for local delivery of therapeutic agents into the CNS. The present study was undertaken to: (i) determine upper safe limits of cellular implantation into the neostriatum of monkeys; (ii) determine optimal implantation parameters of cells into the striatum; and (iii) estimate cell survival in the grafts using BRDU-labeling methods. Autologous fibroblasts were infused into six sites of the striatum in non-human primates (Macaca Mulatta, n=12). Twenty-seven gauge cannulae were inserted vertically through cortical entry sites into the striatum (2 sites in the caudate nucleus and 4 sites in the putamen) at pre-defined coordinates based on magnetic resonance imaging (MRI). The cannulae were guided by an electronically-operated hydraulic micropositioner and withdrawn at controlled rates (0.375 or 0.75 mm/min.) while fibroblast cells (5, 10, 20, 40 or 80 µl/site) were infused simultaneously. Varying infusion rates (0.8, 1.6, 3.2 or 6.4 µl/min.) and fibroblast concentrations (1.0 or 2.0 x 10^5 cells/µl) were also evaluated. Visualization and evaluation of graft placement were performed using contrast MRI at 3-5 days post-surgery. Animals were monitored for signs of clinical complications and sacrificed two weeks following surgery.

Post-implantation MRI revealed mass effect of the implant with shifting of midline, edema and infiltration of the white tracts at 40 and 80 µl/site. In addition, these animals developed transient hemiparesis contralateral to the implant site. MRI of animals grafted with 20 µl/site exhibited columnar-shaped implants and evidence of infiltration into white matter tracts possibly due to a volume effect. No clinical side effects were seen in this group. At 14 days post-surgery, MRI scans showed consistent columnar grafts (measuring 5mm in height) throughout the striatum in animals implanted with 5 or 10 µl/site. There were no signs of clinical side effects associated with these volumes and post-mortem histological examination confirmed MRI observations. Fibroblast survival in the grafts was estimated at 30-50%. Optimal surgical parameters for delivery of fibroblasts into the striatum consist of a graft volume of 10 µl/site, a 1.6 µl/min. infusion rate, a cell concentration of 2.0 x 10^5 cells/µl and a cannula withdrawal rate of 0.75 mm/minute. These results show that infusion of genetically-altered primary fibroblasts into the striatum can be done in a safe and routine manner.

131.9

TRANSPLANTATION OF EGF/FGF RESPONSIVE HUMAN PRECURSOR CELLS INTO THE 6-OHDA LESIONED STRIATUM. M.A. Caldwell[1]*, S.B. Dunnett[1], J. Shen[2], Karmiol[2] and C.N. Svendsen[1]. [1]MRC Cambridge Centre for Brain Repair, University of Cambridge, UK ; [2]Clonetics Inc, San Diego, CA 92123

The whole brain from a single human embryo (20 weeks post conception) was acquired from a tissue bank in accordance with the Uniform Anatomical Gift Act, dissociated and grown in EGF supplemented serum free medium for 4 days before being shipped in hibernation medium to Cambridge where the cells were transferred to serum free medium containing both EGF (20ng/ml) and FGF (20ng/ml). At 7 days *in vitro* some of the cells were pulsed with 1µM BrdU for 3 days and then transplanted as intact spheres into the striatum of 15 rats previously given unilateral 6-OHDA lesions and showing an amphetamine induced rotation of greater than 10 turns/min. or plated onto substrate coated slides and allowed to differentiate. The remainder of the cells were passaged and the label, graft, plate and passage process repeated at 14d and 21d of growth. 5 grafted animals from each group were sacrificed at 2 weeks, 6 weeks and 20 weeks post transplantation. Over 80% of the cells plated were positive for BrdU, many were βtubulin III positive, and a sub population stained for tyrosine hydroxylase (TH) at each passage. Animals grafted with 10, 17 and 24 day old precursors had large, dense transplants at 2 weeks with a core of BrdU positive cells and penumbra of BrdU negative cells, with a few BrdU positive cells migrating out into the host tissue. In stark contrast, by 6 weeks post transplantation no BrdU positive cells could be seen in any animal although many had large tissue masses, strongly suggesting that BrdU had been lost due to cell division. Many TH positive neurons were also seen within the transplants at both time points but there were no effects on rotation in any group. Data from 20 week old transplants will also be presented. These results show that human precursor cells can be expanded, stored in hibernation medium, transported, and survive transplantation into the lesioned adult CNS. Supported by the Wellcome Trust and MRC.

131.10

AROMATIC L-AMINO ACID DECARBOXYLASE IN GENE THERAPY FOR PARKINSON'S DISEASE. S. R. Wachtel*, C. Bencsics and U. J. Kang. Dept. of Neurology, University of Chicago, Chicago, IL 60637.

Investigations of gene therapy for Parkinson's disease (PD) have focused on strategies that restore tyrosine hydroxylase (TH) activity. These strategies presume that aromatic L-amino acid decarboxylase (AADC) is present in sufficient quantities in the denervated brain, such that L-DOPA synthesized by TH can be efficiently decaboxylated to provide dopamine (DA). This hypothesis is supported by the clinical, therapeutic benefit of exogenous L-DOPA, and by its effects in animal models of PD. However, the source of AADC in the DA-denervated brain is unclear. Therefore, it is necessary to determine the role of AADC in gene therapy for PD. In the present study, we examined the potential benefit of adding the gene for AADC to TH gene therapy by cotransplanting primary fibroblasts (PF) transduced with AADC (PFAADC) with PF transduced with both TH and GTP cyclohydrolase I (GC), an enzyme necessary for the synthesis of tetrahydrobiopterin cofactor. To determine the optimal ratio at which to mix PFTHGC and PFAADC cells for transplantation, these cells were cocultured at ratios from 1:1 to 3:1, and the media from those cultures analyzed for L-DOPA and DA content. Although L-DOPA concentrations in the media increased as the ratio of PFTHGC to PFAADC cells increased, DA levels were highest at a ratio of 2:1. Thus, to determine the role of AADC *in vivo*, PFTHGC/PFAADC or PFTHGC/PF cells were grafted, both at a 2:1 ratio, into the 6-hydroxydopamine-denervated striatum of Fisher 344 rats, and microdialysate levels of L-DOPA and DA compared. Interestingly, PFTHGC/PFAADC grafts produced less L-DOPA than PFTHGC/PF grafts. Nevertheless, PFTHGC/PFAADC and PFTHGC/PF grafts did not produce significantly different levels of DA. These findings indicate that 1) there is sufficient AADC near striatal grafts for L-DOPA decarboxylation and that 2) TH gene therapy strategies may not benefit from additional modification with AADC. (Supported by Natl Park Fdn, Park Dis Fdn, United Park Fdn, and USPHS grants DA07255, NS07113, and NS32080.)

131.11

HUMAN FETAL RETINAL EPITHELIAL TRANSPLANTATION EFFECTS VISUALLY-GUIDED BEHAVIOR IN THE RCS RAT. C.W. Little1, C. Cox2, J. Wyatt3, C. del Cerro*1, and M. del Cerro1,4,5. Departments of Neurobiology1, Statistics2, Animal Medicine3, Neurology4, and Ophthalmology5, U. of Rochester Medical School, Rochester, N.Y., 14642.

The RCS rat is affected by a selective loss of retinal photoreceptor cells. This study evaluates the duration and functional effect of human fetal RPE grafts in the RCS rat.

Human fetal RPE cells were transplanted bilaterally into the subretinal space within the superior hemisphere of dystrophic RCS rats. Vehicle was similarly injected bilaterally into control rats. All animals were immunosuppressed. On the day of sacrifice, each animal was given ten consecutive trials in a water escape paradigm. The time was recorded for the rat to find a submerged platform, which was adjacent to a light bulb, the location of which was randomly assigned. The eyes were analyzed histologically, and the photoreceptor nuclear profiles within specified regions of the retina were counted.

Eight weeks after transplantation, a rescue effect was apparent, as was an effect on the visually-guided water escape behavior. An outer nuclear layer 2 to 4 profiles thick was present in the region of transplant; whereas, few photoreceptors remained in all other regions. The mean number of photoreceptors in the graft region was significantly greater than in the same region of sham-injected eyes ($p = 0.004$). The water escape times showed statistically different linear trends between the two groups ($p = 0.027$).

Photoreceptor cells are rescued by transplantation of human fetal RPE; they provide the dystrophic RCS rat with a functional advantage for visually-guided behavior.

Supported by Dr. Sam Williams and Research to Prevent Blindness.

131.12

FETAL NEURAL RETINAL GRAFTS INTO HUMAN RETINITIS PIGMENTOSA (RP). M. del Cerro*1,2; T.P. Das 3; E. Lazar 1,2; S. Jalali 3; D. DiLoreto 2; C. Little 2; A. Sreedharan 3; C. del Cerro 1,2; and G. N. Rao 3. L.V. Prasad Eye Institute, Hyderabad, India 1; Dept. of, Ophthalmology 2, and Neurobiology 3; U. of Rochester Medical School, Rochester, N. Y, 14642; USA

Human fetal neural retinal cells have been grafted into the subretinal space of 8 patients with advanced RP for possible treatment of the disease.

A suspension of dissociated human fetal neural retinal cells (14 to 19 weeks gestational age) was injected into the subretinal space in the paramacular area of adult patients with only light perception (LP) bilaterally. The recipient and donor mother blood was pre-screened for transmissible diseases. Pre- and post-operatively a full clinical exam including visual acuity, electroretinogram, visual evoked potential and fluorescein angiogram was performed at frequent intervals.

In the follow-up period (average 8.66 months; range 3-15 months), repeat examinations showed no evidence of inflammation, infection, retinal detachment, or clinically observable overt rejection of the subretinal graft. Furthermore, there have been indications of visual improvement in two patients; one advancing from LP to hand motion, and the other patient from LP to 20/200. No change was seen in other eyes or the fellow eyes of these or any other operated patients.

In sum, a procedure has been developed that allows the safe and efficient delivery of neural retinal cells into the subretinal space of RP patients. This series shows lack of overt rejection of the grafted cells, with no compromise to the host eyes. The study suggests that visual gain via retinal transplantation is possible in advanced RP.

Supported by a gift from Dr. Sam Williams; Research to Prevent Blindness; The Rochester Eye Bank; Hyderabad Eye Research Foundation.

131.13

EFFICACY OF NON-FETAL HUMAN RPE FOR PHOTORECEPTOR RESCUE: A STUDY IN DYSTROPHIC RCS RATS, B.V. Castillo,Jr.* 1, M. del Cerro 1,2, R.M.White 1, C. Cox 3, J. Wyatt 4, G. Nadiga 1, and C. del Cerro 1. 1Neurobiology & Anatomy, 2Ophthalmology, 3Biostatistics, & 4Lab Animal Med., University of Rochester Med. Ctr., Rochester, NY.

The purpose of this study is to determine the efficacy of non-fetal human retinal pigment epithelium for photoreceptor rescue utilizing the dystrophic RCS rat.

Eyes from 10 and 49 year old donors were obtained through the Rochester Eye and Human Parts Bank. The RPE was isolated by enzymatic treatment of the choroid-RPE with 2% dispase for 30 min. at 37°C . Mechanically dissociated RPE cells were injected at the superior hemisphere into the subretinal space of dystrophic RCS rats during the fourth postnatal week. Rats receiving vehicle injection served as sham controls. The animals were immunosuppressed with daily cyclosporine injections (10 mg/kg) and sacrificed 30 days post-transplantation for evaluation of the RPE graft and its effect on photoreceptor survival.

Transplantation of adult human RPE promoted the survival of photoreceptors in the dystrophic RCS rat. Morphometric analysis of the grafted superior hemisphere (149.2 + 50 SD) demonstrated a 3 fold increase in photoreceptor cell density compared to sham controls (39.7 + 31 SD) and the untouched inferior hemisphere (52.8 + 28 SD). RPE from the 49 y.o. donor was as effective as RPE from the 10 y.o. donor in promoting photoreceptor survival.

The results of this study in RCS rats suggests that RPE from adult human donors is suitable for transplantation and retains the capability to promote survival of photoreceptor cells. This finding opens the possibility of using non-fetal RPE cells in human retinal transplantation.

Rochester Eye Bank, Research to Prevent Blindness, & Private Gifts.

131.14

Effect of Schwann Cells and Macrophage Inhibitory Factor(MIF) on the Survival of Rat Retinal Ganglion Cells(RGCs) after Axotomy and Peripheral Nerve(PN) Grafting.

J.M. Lawrence, D.D.A. Lawson, S.J.O. Whiteley, R.D. Lund* and Y. Sauvé
Institute of Ophthalmology, London, UK.

Peripheral nerve grafts to the transected optic nerve support axonal regeneration from a small proportion of RGCs. This fraction might be increased by co-transplanting Schwann cells, a potential source of growth factors known to sustain RGCs. However, both axotomy and Schwann cells induce a microglial response, which may be inimical to neuron survival. The addition of intravitreal MIF at the time of PN grafting appears to suppress this response and so assist in RGC survival and axonal regeneration.

Therefore, following survival times of 6 to 8 weeks and using DiAsp as a retrograde tracer, we present results comparing the effects of combined MIF and neonatal homologous Schwann cells introduced into the eye at the time of PN grafting.

In comparison to untreated grafted retinae, MIF appears to promote axonal regeneration from more RGCs; these cells are mainly type II-like (with small soma and restricted dendritic arbours). After Schwann cell grafting, RGCs with much larger soma and dendritic fields are present in a significantly greater proportion. Furthermore, the radiating axon bundles appear more prominent in the Schwann cell-grafted retinae.

Supported by MRC, UK.

131.15

TARGET DENERVATION PROMOTES RETINAL TRANSPLANT PROJECTIONS IN ADULT RATS

D.D.A.Lawson* and R.D.Lund, Neural Transplant Program, Institute of Ophthalmology, Bath Street, London EC1V 9EL, UK.

Two important factors relevant to adult neural transplantation strategies are the extent of graft axon elongation and the influence of existing host circuitry. Here we investigated the tectal innervation patterns of intracerebral retinal transplants, employing a novel graft arrangement to examine the impact of the above parameters.

Embryonic(E10-E12) mouse retinae were transplanted unilaterally over the midbrain of adult immunosuppressed rats, and the projections studied using M4/M6 antibodies and the neuronal tracer DiI after a minimum of 4 weeks. A second set of adult rats received bilateral embryonic(E12-E14) rat retinal transplants with concomitant single eye enucleation at the time of grafting. Three months later a secondary degeneration protocol allowed a comparison of transplant-derived optic terminal numbers in the intact and denervated superior colliculi(SC) using EM.

Immunohistochemistry/DiI revealed sparse fibre outgrowth from the retina to the upper regions of the SC, the axons coursing a maximum of 200µm from the graft margin. Bilateral transplants demonstrated both a heavier retinal projection and a greater number of graft terminals in the denervated vs. intact SC.

These results sustain the idea that a significant component limiting graft incorporation in the mature CNS is the non-permissive environment for axon extension. However, once suitable targets have been attained the principal determinant of successful integration would appear to be the availability of synaptic sites.

Supported by grants from Action Research and MRC, UK.

131.16

CONTRIBUTION OF SYMPATHETIC TONE TO THE PUPILLARY LIGHT REFLEX MEDIATED THROUGH NORMAL AND TRANSPLANTED RETINAE. J.D. Radel *, Depts. of Occupational Therapy Ed. and Physiology, Univ. Kansas Medical Center, Kansas City, KS 64113-7602.

The pupillary light reflex (PRL) in mammals is a motor function which occurs in a graded fashion with changes in light intensity. Characterization of the PRL in individual rats has been utilized to monitor functional status of the visual system during development (Radel et al., Eur. J. Neurosci. 1992), and at maturity after intracranial transplantation of retinae (Radel et al., Neurosci., 1995) and the directed immunologic lesioning of such transplants (Bannerjee et al., Neurosci. 1993). The sympathetic nervous system also plays a role in governing pupillary responsiveness. The present studies are directed at clarifying that role, particularly in responses mediated through retinae transplanted to an ectopic location in the brain.

Pigmented (Long-Evans) rats were used. Pupillary responses of the left eye were recorded as the right eye was illuminated in 4 normal rats. Once the response characteristics were defined for each animal, the left superior cervical ganglion (SCG) was removed surgically. Pupillary responses again were characterized 4-6 weeks after the surgery. Parameters describing the pupillary response were compared across pre- and post-surgery sessions. Although there were no changes in the pre-stimulus (baseline) diameter, removal of the ipsilateral SCG produced a 10% reduction in constriction amplitude, a 50% longer response latency, a 40% increase in constriction velocity, and a 10% slowing in the rate of post-stimulus dilation rate. Sympathetic tone appears to influence component features of the PRL differentially.

In the second phase of the study, an E13 retina was transplanted to the dorsal midbrain surface in each of five newborn host rats. The right eye of each host was removed at that time to encourage a robust transplant projection to denervated visual nuclei. At maturity, the tip of a fiber optic was inserted through the host's skull and fixed in place to allow direct stimulation of the transplanted retina with light. Transplants were illuminated with stimuli of graded intensities, and pupillary responses recorded from the host's left eye. The procedure outlined above for normal rats was followed. The findings show that pupillary light responses mediated through transplanted retinae 1) are preserved following ipsilateral lesioninmg of the SCG, and 2) such responses may be more strongly influenced by sympathetic tone than normal.

Supported by NEI

STAINING, TRACING, AND IMAGING TECHNIQUES I

132.1

USE MANGANESE AS A CALCIUM TRANSPORT ANALOG FOR MRI OF NEURONAL ACTIVATION WHICH IS INDEPENDENT OF BLOOD FLOW.

Y. J. Lin *, and A. P. Koretsky Dept. Biological Sciences and NMR Center for Biomedical Research, and Center for Neural Basis of Cognition, Carnegie Mellon University, Pittsburgh, PA15213

Current functional imaging methods are all based on detecting hemodynamic changes resulting from neuronal activation. The goal of this work is to develop an alternative method that allows directly imaging neuronal activation independent of hemodynamic changes. Calcium (Ca^{2+}) is a major second messenger for neuronal signal transduction. The work presented here is to indirectly image Ca^{2+} influx during neuronal activation with MRI. The strategy is to use a Ca^{2+} analog which is also a paramagnetic MRI contrast agent. Manganese ion (Mn^{2+}) enters cells through calcium pathways such as ligand-gated and voltage-gated calcium channels. Mn^{2+} is also a known MRI contrast agent. Activated neurons will have elevated Ca^{2+} influx as well as Mn^{2+} influx if Mn^{2+} is supplied. Therefore, activated neurons will have higher intracellular Mn^{2+} concentration which will shorten the NMR relaxation time constant T_1. A shorter T_1 leads to enhanced contrast on T_1 weighted MRI.

Anesthetized rats were chemically challenged with glutamate and bicuculline. Mn^{2+} was constantly infused through a femoral vein at a rate of 3.6µmole/min. This dose of Mn^{2+} had little effect on heart rate and blood pressure. Both glutamate and bicuculline caused Mn^{2+} dependent increases in MRI signal intensity of 250%. This change in signal intensity was dependent on breaking the blood-brain-barrier to increase delivery of Mn^{2+} . This large increase in signal intensity was independent of blood flow, because high CO_2, which is known to increase cerebral blood flow, did not cause significant signal intensity changes. These data indicate that Mn^{2+} is a candidate for indicating Ca^{2+} influx for functional MRI. (The work is supported by the Center for Neural Basis of Cognition and NIH P41-RR03631-08 Multidisciplinary NMR Center for Biomed. Res,)

132.2

QUANTITATION OF LARGE MOLECULAR WEIGHT PROTEIN DISTRIBUTION IN THE CNS AFTER BULK FLOW INFUSION AND ITS NEUROSURGICAL IMPLICATION.

Alexander C. Cummins*, Paul F. Morrison[1], Marc-Etienne Corthésy, Russell R. Lonser, Yash Pannu, Nitin Gogate, Robert L. Dedrick[1] and Edward H. Oldfield, Surgical Neurology Branch, National Institute of Neurological Disorders and Stroke and [1]Biomedical Engineering and Instrumentation Program, National Center for Research Resources, National Institutes of Health, Bethesda, Md. 20892

High flow infusion has been demonstrated to be a viable means of uniform delivery of compounds into the CNS. The distribution of infusate remains to be quantified for accurate dose assessment. To quantify the infusion in grey matter, rats were infused with 2µl of C^{14} labeled albumin into the striatum. Delivery rate is one possible factor affecting accurate delivery. We examined recovery at fixed rates ranging from 0.02µl/min to 1µl/min. At a rate of 0.02µl/ the recovery was 87.8% (+3.5%) of the radioactive albumin infused into the striatum. There was a small decline in recovery at rates between 0.02µl/min and 0.1µl/min (80%). Above this rate, however, the recovery rate fell rapidly. A possible explanation for this decrease may be leakage along the needle tract which would be consistent with a mathematical model. Autoradiographs of the brain sections showed significant amounts of radioactivity along the needle tract only in rats infused at rates above 0.1µl/min. Additionally autoradiography showed that the infusate filled most of the striatum and the concentration was uniform throughout the infused area.

132.3

MAPPING OF PHOSPHOCHOLINE SECONDARY IONS IN THE RODENT BRAIN BY SECONDARY ION MASS SPECTROMETRY.
C.A. McCandlish*, J.M. McMahon, and P.J. Todd. Chemical and Analytical Sciences Division, Oak Ridge National Laboratory, Oak Ridge, TN 37831-6365.

Phosphocholine ions m/z 184 and m/z 86 are almost always abundant in secondary ion mass spectra (SIMS) of animal tissue, and have been previously identified by tandem mass spectrometry (MS/MS). The parent compounds of these ions, mainly phosphatidylcholine and sphingomyelin, are major constituents of animal membrane, particularly in the brain. We report that the distribution of phosphcholine ion emission corresponds to the density and composition of the membrane. For example, the distribution of neurons and associated cell axons detected in Nissl-stained tissue corresponds to the observed distribution of m/z 184 emission.

Rats and mice were anesthetized with metofane and perfused intracardially with buffered saline (pH. 7.3) followed by a 2% formalin solution (pH. 7.3). Following perfusion, the brain was removed from the skull and sectioned (50 microns) coronally using a Vibratome. Sections were mounted onto copper slides for SIMS imaging. Alternate (reference) sections were mounted onto glass slides to be stained with cresyl violet.

Analysis of m/z 184 ion images indicates that areas of greatest ion intensity are brain nuclei which are cell-rich, whereas areas which have little or no emission are acellular fiber tract sites. These results indicate that the heterogeneous emission of secondary ion yields of m/z 184 observed with SIMS imaging delineates brain structures in a way consistent with known histochemistry. Furthermore, the correspondence between alternate optical and secondary ion images suggests that secessive ion images may be used to produce a 3-D image of the brain.

132.4

METABOLIC POLARIZATION IN NEURONS AS RELATED TO THEIR STRUCTURAL AND FUNCTIONAL POLARIZATION. Glenn H. Kageyama*, Dept. Biol. Sci., Calif. State Polytechnic Univ., Pomona, CA 91768; Dept. Anat. & Neurobiol., College of Medicine, University of California, Irvine, CA 92717.

Major sites of energy utilization, as determined by the histochemical localization of Na^+,K^+-ATPase (p-nitrophenyl phosphatase, p-NPP), was compared to major sites of energy production by glycolytic vs. oxidative metabolic pathways, as determined by lactate dehydrogenase (LDH) and cytochrome oxidase (CO) histochemistry, respectively in various neuronal structures of knifefish (Apteronotus), rat and cat. Animals were perfused with aldehydes and 60 μm vibratome sections were processed for either p-NPP, LDH or CO histochemistry and compared. Cellular analysis of the rat hippocampus revealed that the highest levels of p-NPP and CO were found within the distal dendritic segments of granule and pyramidal cells, while LDH was highest in proximal dendrites, soma, axons and terminals, indicating an inverse intracellular distribution and partial segregation of oxidative and glycolytic enzymes in these cells. Similar distribution patterns were observed in the hypothalamo-hypophyseal magnocellular paraventricular neurosecretory neurons. In cat photoreceptors CO is localized to the inner segments and axon terminals (of cones, but not rods) along with Na^+,K^+-ATPase while the remainder of the cell is devoid of mitochondria, but rich in LDH. Neurogenic electrocytes of the weak electric fish *Apteronotus albifrons* exhibit extremely high CO levels in the cell body and proximal axons but are devoid of mitochondria in the electrogenic axon terminals. Since these neurons have a very high firng frequency, axonal discharges would have to be supported by elevated levels of glycolysis. These observations support the hypothesis that distinct neuron types have a particular pattern of metabolic polarity whereby oxidative and glycolytic metabolic pathways can be partially segregated within functionally different parts of the same cell. Support: Cal Poly Pomona RSCA Grant #1-11496 (Kageyama), & NIH Grants: DC 00450 & NS 30109 (RT Robertson & GH Kageyama).

132.5

SYNAPTIC ACTIVATION AND PROPAGATION OF ACTION POTENTIALS IN THE PROCESSES OF INDIVIDUAL NEURONS.
D. Zečević.* Yale University School of Medicine, New Haven, CT.

The aim of the experiments was to provide direct evidence for the early postulate that individual nerve cells might be functionally subdivided. If true, this postulate would have important implications for the functional complexity of individual neurons. The proposed functional subdivision will depend critically on two factors: (1) the existence of multiple spike trigger zones that can be independently activated, and (2) the ability of the neuron to initiate partial responses (action potentials which do not invade all regions of the cell). Both of these questions could be addressed directly using fast, multi-site, voltage-sensitive dye recordings from processes of an identified snail *(Helix)* neuron which has a complex geometry and multiple spike trigger zones. The first goal was to investigate whether different synaptic inputs might activate different trigger zones. The results showed that the site of spike initiation was different and specific for different modes of synaptic activation. Different trigger zones were associated with different sets of synaptic inputs. The second goal was to establish, by direct measurements, whether action potentials evoked selectively in one axonal branch spread into all other processes, or whether spikes might fail at certain branch points. Voltage-sensitive dye recordings allowed direct detection of action potential propagation across axonal branch points. While for some branches peripherally evoked action potentials invariably propagated into the whole neuron, failure of spike propagation was found at specific sites.

Together, these results provide direct evidence for the hypotheses that individual nerve cells can be functionally subdivided with some of the processes functioning as independent units. Supported by NIH Grants NS28443 and NS08437.

132.6

OPTICAL MONITORING OF ACTIVITY FROM INDIVIDUAL AND IDENTIFIED POPULATIONS OF NEURONS RETROGRADELY LABELED WITH VOLTAGE-SENSITIVE DYES. C. Hickie*, P. Wenner, M O'Donovan, Y. Tsau, J. Fang, and L.B. Cohen. Dept. of Physiology, Yale University, New Haven, CT 06520, and NINDS/NIH, Bethesda, MD 20892

Optical signals obtained from neural tissue labelled with voltage-sensitive dyes are often derived from the activity of many types of neurons as well as glia. To obtain specific signals from identified neuronal populations we developed a method for retrogradely labeling neurons. We report two advances. First, in the chick embryo spinal cord preparation, we compared the optical signals obtained from a population of retrogradely labelled motoneurons with simultaneous whole cell recordings of individual motoneurons antidromically identified from the labelled root. The optical and electrical signals were similar except that whole cell recordings of individual motoneurons revealed spiking activity. This is direct evidence that the optical signals are reliably following transmembrane potentials and suggests that labelled motoneurons are physiologically unaffected by the dyes. Second, we have preliminary evidence that the voltage-sensitive dye signal from motoneurons in a lamprey spinal cord is large enough to allow detection of individual action potentials in individual neurons. A concentrated solution of the styryl dye, di-8-ANEPPQ, provided by J. Wuskell and L. Loew, was injected into a cut ventral root of a larval lamprey (*Lampetra*) cord. After waiting 10 hours for the dye to be transported back to the cell bodies, we sometimes found that well separated individual neurons were stained. The dye fluorescence was monitored using a microscope with a 464 element photodiode array in the objective image plane. Motoneurons were activated by shocks to the rostral cord. Optical signals (df/f ~0.3%) from individual cell bodies could be recorded in single trials. These signals had a latency and a duration appropriate for individual action potentials in motoneurons. Supported by NIH grant NS08437.

132.7

COMPARISON OF OPTICAL SIGNALS IN THE TURTLE CNS GENERATED BY VOLTAGE- AND ION-SENSITIVE DYES, C. X. Falk*, Larry B. Cohen, and David M. Senseman. Dept of Physiology, Yale Medical School, New Haven, CT 06520, Div. of Life Sciences, Univ. of Texas at San Antonio, San Antonio, TX 78249

Extrinsic voltage-sensitive dye signals and intrinsic optical signals arising from changes in light-scattering, blood flow and/or oxygenation levels have proven useful in mapping the functional architecture of the visual cortex and other regions within the vertebrate CNS. There are, however, at least two ways in which the utility of this approach might be improved. Both intrinsic and extrinsic optical signals are relatively small compared to the optical noise generated, *in vivo*, by the movements associated with respiration and the pulsatile blood flow. Another problem is phototoxicity which is especially problematic when bright light sources are used to excite fluorescent dyes during long recording sessions. We asked whether pH-sensitive and/or ion-sensitive dyes might be more benign and generate more robust signals for optical mapping of neuronal activity. We used the hemi-sected turtle olfactory bulb because olfactory nerve stimulation evokes large and well-characterized voltage-sensitive dye signals. We tested 11 ion-sensitive dyes that would be expected to respond to changes in either the extracellular or the intracellular concentration of Na^+, H^+, Cl^-, or Ca^{2+}. The largest signals were obtained with RHOD-2-AM (presumably measuring intracellular Ca^{2+}) and phenol red (presumably measuring changes in the extracellular pH). Several of the ion-sensitive dyes had signals that were more localized than the voltage-sensitive dye signals measured on the same preparation. In each preparation we finished by adding a voltage-sensitive dye (either RH795 or RH155). In every case, the signal-to-noise ratio from the voltage-sensitive dye was larger than the signals found with ion-sensitive dyes. Supported by NIH grant NS08437.

132.8

LABELING BLASTOMERES WITH A CALCIUM INDICATOR: A NON-INVASIVE METHOD OF VISUALIZING NEURONAL ACTIVITY IN ZEBRAFISH. K. J. A. Cox* and J. R. Fetcho. Department of Neurobiology and Behavior, State University of New York at Stony Brook, Stony Brook, New York 11794-5230

The relationship between neuronal activity and behavior is clearly an important issue in neuroscience and has been studied using a variety of methods, including electrophysiology and more recently the use of voltage and calcium sensitive dyes. A problem with these recording approaches is that they are invasive, either inherently as with electrophysiology, or in the preparation, as with the introduction of dyes into cells. In order to circumvent this, we have developed an approach in which injections of the calcium indicator calcium green dextran (CGD) into zebrafish embryos at the 1-4 cell stages were used to monitor the activity of neurons in larval fish. Dye was pressure injected into a single cell and the fish allowed to develop until post-hatching, when they were embedded in agar and viewed under a confocal microscope. Labeled larval cells, including identifiable neuronal classes such as Rohon-Beard cells and olfactory neurons, were clearly visible with extensive labeling of the whole fish following injections at the one cell embryonic stage, and a mosaic labeling pattern following injections at the 2 or 4 cell stages. Activity of neurons in the spinal cord, as indicated by intracellular calcium concentration changes, was observed directly by monitoring fluorescence changes of individual spinal neurons and groups of spinal neurons on a confocal microscope. Fluorescence increases of between 9% and 55% in spinal neurons were seen during escape responses produced when the fish was tapped on the tail. This technique can potentially be used to monitor the activity of any neuron or group of neurons with respect to behaviour non-invasively in intact living zebrafish.

This work was supported by the Howard Hughes Medical Institute and National Institute of Neurological Disorders and Stroke Grant #NS26539 to J.R. Fetcho.

132.9

A HIGH PERFORMANCE MICROSCOPE FOR *IN-VITRO* SLICE IMAGING WITH VOLTAGE SENSITIVE DYES. M.C. Crair*, and M.P. Stryker. Keck Center for Integrative Neuroscience and Department of Physiology, University of California, San Francisco, CA 94143-0444

We have adapted the "macroscope", originally introduced by Grinvald et. al (*J. Neurosci. Methods*, 1991) for *in vivo* imaging, to an *in vitro* environment to achieve exceptional light gathering performance at relatively low magnification. This system is ideally suited for high spatial and temporal imaging using transmitance signals and voltage sensitive dyes with cameras like the Fuji HR Deltaron 1700.-

With Koehler illumination from below and a pair of conventional camera lenses attached front-to-front as our transmitted light gathering system, we can achieve a numerical aperature (NA) up to 0.5 for a range of magnifications from 2X to 8X. In comparison to stock microscopes whose NA is typically 0.14-0.16 at 4X, our microscope collects light approximately 10 times more efficiently than standard systems, thereby relieving the most stringent bottle neck for high signal to noise imaging and reducing the effects of photodynamic damage to our biological sample. Mounted on a free standing XY stage independent of the sample and sample chamber, this system is also stable enough to allow whole cell patch recording from cortical slices.

In a typical arrangement, we use a 50mm f/1.2 objective (with the back end closest to the sample) and a 70-210mm f/2.8 zoom lens as our telescope (attached to the camera). With this lens system, in addition to a 2X tele-extender, we can image at any magnification up to 8X by a continuous adjustment of the zoom lens. This "macroscope" is a convenient and efficient system for wide field imaging applications with the potential for combining the benefits of high resolution imaging with the microelectrode and pharmacological access available in an *in-vitro* system

Supported by NIH EY09760, NIH EY02874 and an NIMH fellowship.

132.10

'ULTRATHIN' CNS SLICES: ENHANCED VISUALIZATION OF NEURON STRUCTURE FOR ELECTROPHYSIOLOGY AND IMAGING. L. Song*, M. Sawchuk, & S. Hochman, Dept. Physiology, Univ. of Manitoba. Winnipeg, MB, Canada R3E 0W3.

Electrophysiological studies using patch clamp recordings in CNS slice preparations involve either 'blind' or visual targeting strategies. Recordings from visually-identified neurons generally require specialized upright microscopes equipped with Nomarski optics (DIC) (e.g. Konnerth et al *Pflugers Arch.* 414:600, 1989). Further image optimization to observe neuronal processes employ video-enhanced infrared wavelength illumination (e.g. Stuart et al *Pflugers Arch.* 423:511, 1993). Though powerful, these approaches require specialized, expensive equipment.

Since the ability to resolve detailed cellular features is generally limited to 40-50 μm from the slice surface, we have developed an alternate strategy to visualize neurons using a semi-transparent 'ultrathin' slice preparation (20-50 μm) visualized on an inverted microscope equipped with Hoffman modulation optics. Isolated cerebellum, hippocampus or spinal cord from neonatal rats (P1- P14) were embedded in AGAR (2.5% w/v) then sectioned with a Leica VT 1000 vibratome. Following incubation at 32°C for 1 hour slices were fixed to the bottom of the recording chamber and maintained at room temperature. The enhanced transparency due to reduced slice thickness permitted superior optical resolution of dendritic and axonal processes and cells were visible throughout the slice thickness. Live/dead cell staining revealed that many cells remained viable for imaging and electrophysiological experimentation. In all CNS regions examined, neurons were easily identified for successful patch recordings. While not yet tested, use of an inverted microscope should permit high numerical aperture oil-immersion objectives to be used for simultaneous imaging experiments and the reduced slice thickness would hasten drug equilibration and washout times. *Supported by the Canadian Neuroscience Network.*

132.11

PLASTIC EMBEDMENT OF CNS TISSUE: PRESERVING ULTRASTRUCTURE WITHOUT OSMIUM. K.D. Phend* and R.J. Weinberg. Department of Cell Biology & Anatomy, University of North Carolina, Chapel Hill, NC 27599.

Following glutaraldehyde fixation, tissue for electron microscopy is routinely postfixed in osmium tetroxide. This step is thought to be especially important for preservation of ultrastructure in lipid-rich tissues, such as brain. However, osmium compounds are expensive, toxic, and frequently impair antigenicity. We previously reported an osmium-free technique that enhances subsequent immunoreactivity while providing satisfactory ultrastructure (*J. Histochem. Cytochem.* **43**:283-292). We here report further progress in optimizing tissue preparation. For subsequent postembedding immunocytochemistry for glutamate receptors and associated proteins, we include calcium and iridium salts; antigenicity is further enhanced by briefly etching the grids in metaperiodate and borohydride. For cases where antigenic preservation is not needed, we report a simple postfixation technique, using tannic acid followed by zinc and uranyl salts, that provides excellent structural preservation without osmium. Resin infiltration may be improved by incubating tissue in digitonin before the tannic acid step. This research was supported by NIH award # NS29879.

132.12

IMAGING OF INTRINSIC OPTICAL SIGNALS SHOWS THAT ACUTE OSMOTIC AND ACUTE EXCITOTOXIC NEURONAL DEATH ARE DIFFERENT. *Michael E. Lobinowich, Trevor M. Polischuk and R. David Andrew**. Department of Anatomy & Cell Biology, Queen's University, Kingston, Ontario, Canada, K7L 3N6.

Irreversible swelling of the neuronal cell body is observed histologically in the cerebrel cortex hours after an excitotoxic/ischemic event. The assumption has been that osmotic stress swells the cell body, thereby contributing to neuronal death. Previous studies of acute osmotic or excitotoxic stress have focused on changes in cell body volume. In the hippocampal slice preparation, we have shown dynamic volume changes in both the cell body *and* dendritic regions evoked by excitotoxins.[1] Elevated light transmittance (LT) through the brain slice is an indirect measure of cell swelling, whereas reduced LT indicates cell shrinkage.[2] At 35°C, a 20 min exposure to saline diluted with distilled water to 175 mOsm (-120) led to an initial LT increase of 49 ± 11% (n=8 slices) in the CA1 stratum radiatum (RAD). This severe unphysiological osmotic stress caused no apparent volume regulation. Remarkably in 4 slices, upon return to control saline, the evoked CA1 field potential returned to control levels while the change in LT returned to -17 ± 4%. However in 4 other slices, it was reduced or eliminated (neuronal death) while the LT irreversibly decreased to -37 ± 10%. Similar exposure to 55 mOsm (-240) saline annihilated the evoked field and caused an irreversible LT decrease in CA1 RAD (-47 ± 21%) in all 3 slices tested. In each case, only a small *reversible* swelling was measured in the CA1 cell body region (PYR). In contrast, 4-10 min exposure to a glutamate agonist (100 μM NMDA, 10 μM domoic acid or 10 μM AMPA) at 37°C *irreversibly* increased LT in CA1 PYR within 10 min (n=25) coupled to an irretrievable loss of the evoked field (neuronal death). We conclude: **1)** Dendritic regions in CA1 exhibit dramatic volume changes in response to acute osmotic and acute excitotoxic stress but **2)** CA1 cell bodies can resist swelling during acute osmotic but not acute excitotoxic stress.

1. Polischuk TM and Andrew RD, this meeting.
2. Andrew RD and MacVicar BA (1994) *Neurosci.* **62**:371-383.

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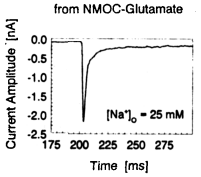
132.13

NEW PHOTORELEASABLE CAGED GLUTAMATE WITH VERY LOW PRE-PHOTOLYSIS ACTIVITY, J. P. Y. Kao*, C.-M. Tang and F. M. Rossi. Medical Biotechnology Center and Depts. of Physiology and Neurology, Univ. of Maryland School of Medicine, Baltimore, MD 21201

Because desensitization of non-NMDA GluR channels occurs at low (μM) concentrations of glutamate while full activation during synaptic transmission requires millimolar concentrations, an ideal caged glutamate should give high yield of glutamate on photolysis but show low pre-photolysis residual activity. (Residual activity is the sum of the intrinsic activity of the caged compound and the activity of any free glutamate arising from spontaneous, non-photolytic breakdown of the caged compound). Ideally, the ratio of yield to residual activity should be >1000. Such a caged glutamate has remained elusive. In an attempt to to maximize the yield/residual activity ratio while maintaining reasonable photorelease kinetics, we have prepared a new reagent: NMOC-glutamate. Photolysis of NMOC-glutamate with UV light liberates glutamate with quantum yield Ω = 0.11. The photochemical reaction is fast, while the dark reaction yielding glutamate is pH-dependent and is expected to proceed with a pseudo-unimolecular rate constant of $\sim 3\times10^{8}[H^{+}]$. The rate at which activating concentrations of glutamate are attained can thus be manipulated through both reagent concentration and medium pH. The figure illustrates the effect of glutamate photorelease from NMOC-glutamate on the whole-cell current recorded from a dissociated rat hippocampal neuron. Addition of 10 mM NMOC-glutamate at pH 6.3 caused no measurable change in the baseline current. Photorelease was achieved at the cell soma by exposure to UV light from an argon ion laser focused to a 30-μm spot. Photoreleased glutamate caused a rapid inward current, with time-to-peak <1 ms (shutter opening, ~400 μs), which then desensitized rapidly. (GM46956)



132.14

MONITORING CHANGES IN $[Ca^{2+}]_i$ AND CELL VOLUME USING FURA-2, W.E. Crowe , J. Altamirano and F.J. Alvarez-Leefmans*. Depto. Neurobiología, Inst. Mex. de Psiquiatría, Av. México-Xochimilco 101, México 14370 D.F.; Dept. Physiology & Biophysics, UTMB, Galveston, TX 77555; Depto. Farmacol. CINVESTAV-IPN, Ap. Postal 14740, México 07000, D.F.; & Dept. Physiol. MCPHU, Philadelphia, PA 19129.

The role of Ca^{2+} as a second messenger in cell water volume (CWV) control is controversial. This is partly due to the lack of techniques with enough sensitivity and time resolution to measure simultaneous changes (Δ) in CWV and $[Ca^{2+}]_i$. Fura-2 was used to monitor these variables in N1E-115 and NG108 cells. $[Ca^{2+}]_i$ was measured ratiometrically (350/358 or 358/380), whereas volume was measured at the intracellular isosbestic point (IP) that we determined *in vivo* (358 nm). Changes in fluorescence (ΔF) were measured from a region of each cell that was delimited by a pinhole placed at the image plane. ΔF at 358 nm reflect changes in intracellular furà-2 concentration that can be transformed to ΔCWV (Alvarez-Leefmans et al. Methods in Neurosciences 27:361-391,1995). For CWV measurements it is crucial to minimize the effect of changes in effective path length resulting from changes in cell shape. By altering the pinhole size and location using a digital imaging system we found that the noise (N) level in individual traces decreased by increasing the pinhole size. However, as more of the peripheral regions of the cell were included in the pinhole, the magnitude and time course of the volume signals (S) were distorted. Optimal S/N was obtained with a pinhole located well within the cell boundaries and comprising 5-8 % of the total area of the cell body. (Supported by NINDS grant NS29227 and CONACyT Mexico, grant F-285-N9209).

132.15

SUBCELLULAR LOCALIZATION OF THE PRION PROTEIN USING FUSION TO GREEN FLUORESCENCE PROTEIN. Z. Meiner, M. Vey, E.L. Clausnitzer, D.M. McDonald, P. Dazin, S.B. Prusiner*. University of California, San Francisco, CA 94143-0518.

While the function of PrP^C remains uncertain, PrP^C is the substrate from which PrP^{Sc} is formed in prion diseases. Although PrP^C is bound to cellular membranes by a glycosylphosphatidyl inositol anchor, it is controversial whether it is clustered within distinct domains of the cellular membranes or diffusely distributed. To localize PrP^C on the plasma membrane and in other cellular compartments, we transiently expressed fusion proteins consisting of PrP and the fluorescent marker, Green Fluorescent Protein, in normal (N2a) and scrapie-infected (ScN2a) neuroblastoma cells. The fluorescence was examined in living and fixed cells using fluorescence microscopy, confocal microscopy, and FACS analysis. The fluorescent fusion proteins were detected in patchy clusters on the plasma membrane of the cell soma and neurites. In addition, the fluorescence was detected in a perinuclear compartment identified as the trans-Golgi network. After treatment with phosphatidylinositol-specific phospholipase-C or proteinase K, most of the fusion protein disappeared from the cell membrane without any difference between N2a and ScN2a cells. We conclude that PrP^C is localized in clusters on the plasma membrane of the cell soma and neurites. Our results support the hypothesis that PrP^C is localized on the plasma membrane in distinct domains. This localization may be important for the function of PrP^C and the conversion of PrP^C into PrP^{Sc}.

132.16

LOCALIZATION OF ION TRANSPORTERS INVOLVED IN CALCIUM CONTROL IN PRESYNAPTIC NERVE TERMINALS. M. Juhaszova, M.P. Blaustein*, P.J. Church⁺ and E.F. Stanley⁺, Physiol. Dept., U. Md. Med. Sch., Baltimore, MD 21201 and ⁺Synaptic Mechanisms Sect., NINDS, NIH, Bethesda, MD, 20892

Isolated calyces from chick ciliary ganglion synapses are useful as model vertebrate presynaptic terminals because of their large size and accessibility (J. Neurosci. 11:985, 1991). We have applied immunofluorescence to the calyx to determine the spatial distribution of Na/Ca exchanger (NCX) and plasmalemmal (PM) Ca^{2+} pump in a presynaptic nerve terminal. Cross reactivity of antibodies (AB) generated against mammalian proteins was confirmed by immunoblots of ganglion membrane proteins. Chick ciliary ganglia were dissociated, fixed, and observed with high resolution imaging using the Cellscan (Scanalytics) system. Calyces were co-labeled with an anti-transporter AB (NCX from G.E. Lindenmayer; PM Ca^{2+} pump from E. Carafoli) and an AB against synaptotagmin (from R. Jahn) as a marker for secretory vesicle clusters at the release sites. The staining for all three AB was patchy, indicating that the proteins were highly clustered. NCX staining exhibited no obvious association with synaptotagmin whereas many Ca^{2+} pump clusters were co-localized with synaptotagmin. Our results suggest that the NCX, although present in the calyces, is not concentrated near transmitter release sites. In contrast, the Ca^{2+} pump in calyces is preferentially located near release sites. Further studies are required to determine whether all of the pump clusters are located on the surface membrane or whether some are co-localized with synaptotagmin on the secretory vesicles themselves.

Support: NIH/NS-16106; NINDS intramural program.

132.17

SELECTIVE SORTING OF EMBRYONIC DOPAMINERGIC CELLS BY FLOW CYTOMETRY USING A FLUORESCENT NEUROTENSIN DERIVATIVE. M.P. Faure*, K. McDonald, A. Gagner, A.F. Sadikot and A. Beaudet, Montreal Neurol. Inst., Univ. McGill, 3801 University St., Montreal, QC, Canada, H3A 2B4.

We have previously shown that 80-95% of rat ventral mesencephalic dopaminergic (DA) neurons were selectively endowed with high affinity neurotensin (NT) receptors (Szigethy et al., 1989). Taking advantage of this property, we devised a method for selectively sorting embryonic DA cells by flow cytometry (FACS) using a fluorescent NT derivative (fluo-NT, Faure et al., 1994). Mesencephalic cells from E15 rat embryos were rapidly dissociated and analyzed by flow cytometry either immediately (D0) or after 7 days in culture (D7). Both D0 and D7 cells were labeled by incubation for 20 min at 4°C with 10 nM fluo-NT in the presence or absence of 1 μM non fluorescent NT for determination of non specific binding. FACS analysis revealed an almost tenfold difference in mean fluorescence intensity between total and non specific binding at D7, but none at D0, in keeping with the late expression of NT receptors by DA cells. Control COS-7 cells transfected with a cDNA encoding the high affinity NT receptor exhibited the same shift to the right of fluorescence profiles as D7 neurons. Such a shift was not apparent in COS-7 cells either non transfected or transfected with an irrelevant receptor. Sorting through a window encompassing high specific binding/high survival events allowed for a sixfold enrichment in DA cells (from 14 to 85% of total neuronal population), as estimated by pre- and post-sorting tyrosine hydroxylase immunocytochemistry. This proportion remained constant up to 4 days after plating the sorted cells back in culture, attesting to their viability. These results demonstrate the feasibility of selectively enriching embryonic DA cell populations by FACS and of keeping these cells alive after sorting. Supported by The Parkinson Foundation.

132.18

THE APPLICATION OF NEAR-FIELD OPTICAL MICROSCOPY TO NEURONS AND MAST CELLS IN CULTURE. P.G. Haydon*, M. McCloskey, T. A. Basarsky and S. Marchese-Ragona. Laboratory of Cellular Signaling, Iowa State University, Ames, IA, USA.

Optical resolution is limited by diffraction. Thus, the theoretical limit of resolution is about 250-300 nm. Sub-diffraction optical resolution can be achieved however, if a fine aperture is used so that a "nano-spot" of illumination is provided. Using pulled optical fibers, with tip diameters of 20-50 nm, an illumination source regulated by this aperture diameter has been developed. In the physical sciences, such near-field fibers have been used to study the properties of single fluorescent molecules. In principle, this technique could be used in biology to provide a new level of optical resolution to study the dynamic behavior of individual molecules in or adjacent to the cell membrane.

Using near-field optical fibers we have determined that sub-diffraction optical resolution (20-50 nm) can be achieved when using immobilized, dehydrated biological samples. Such resolution should permit new opportunities for studying molecular associations in biological systems when using fluorescence labeling methods. Additionally, we demonstrate that near-field probes can be readily placed in feedback on living cell membranes of neurons, astrocytes and mast cells. When cells are loaded with calcium indicators, such as fluo-3, we demonstrate the ability to reliably detect local changes in calcium level in the cell cortex. Furthermore, as an initial test of the ability to monitor fluorescently labelled secretory organelles, we demonstrate the ability to monitor exocytosis of individual mast cell granules labelled with quinacrine.

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GENE STRUCTURE AND FUNCTION I

133.1

DEFECTIVE HERPES SIMPLEX VIRUS VECTORS EXPRESSING THE RAT BRAIN STRESS-INDUCIBLE HEAT SHOCK PROTEIN 72 PROTECT CULTURED NEURONS FROM SEVERE HEAT SHOCK BUT NOT 3-NITROPROPIONIC ACID TOXICITY. S. L. Fink, L. K. Chang, D. Y. Ho* and R. M. Sapolsky. Program in Neurosciences and Dept. of Biological Sciences, Stanford University, Stanford, CA 94305-5020.

Recently, pre-induction of the heat shock response has been shown to protect neurons undergoing a variety of stressful insults (e.g. heat, ischemia, exposure to excitotoxins). However, it is not known which of the proteins induced by the heat shock response mediate the protective effects. Previous correlative evidence points to a role for the highly stress-induced heat shock protein 72 (hsp72). The massive induction of hsp72 following neuronal perturbation, and the existence of induced tolerance phenomena in the brain both suggest a role for hsp72 in neuroprotection. However, studies to directly assess the ability of hsp72 to protect central nervous system neurons from heat shock and other, more common, neural stressors have not been performed.

We constructed a herpes simplex virus-1 vector carrying the rat brain stress-inducible *hsp72* and the *lacZ* (marker) genes. Infection with the vector caused neurons to co-express hsp 72 and ß-galactosidase. Infection with a control vector lead to marker gene expression only, and thus did not induce endogenous hsp72 expression. Overexpression of hsp72 protected cultured hippocampal neurons against a heat shock, but not against the metabolic toxin, 3-nitropropionic acid.

Supported by a grant from the Adler Foundation (R.M.S.), an MSTP grant from the National Institute of General Medical Sciences (S.L.F.) and a Summer Research Fellowship from the Howard Hughes Foundation (L.K.C.).

133.2

EFFICIENT AND LONG-TERM GENE DELIVERY INTO RAT BRAIN USING ADENO-ASSOCIATED VIRAL VECTORS. **P. Wu*[1], B. Du[1], M.I. Phillips[2], E.F. Terwilliger[1]. [1] Dept. Medicine, Deaconess Hospital, Harvard Medical School, Boston, MA 02215; [2] Dept. Physiology, Univ. of Florida College of Medicine, Gainesville, FL 32610.**

Adeno-associated virus (AAV) vectors were previously shown in this lab to efficiently transfer a ß-gal marker gene into several types of nondividing cells *in vitro*, including neurons. Further optimization of this system for gene delivery into the CNS was carried out by comparing the expression pattern of the ß-gal gene under the control of either the CMV promoter or a neuron specific cellular promoter, both *in vitro* and *in vivo*. The rat arginine vasopressin (AVP) promoter was chosen for this purpose. Initially, AVP positive (AtT20/D16v-F2) or negative (293) cell lines were transduced with the pCMVßgal or the pAVPßgal vector. The CMV promoter appeared more potent than the AVP promoter in terms of the level of ß-gal expression in both cell types *in vitro*. Treatment with the cyclic AMP activators dramatically enhanced the level of ß-gal expression directed by the AVP promoter, but not from the CMV promoter. To determine whether these AAV-ßgal vectors also induce expression of ß-gal effectively *in vivo*, either pAVPßgal or pCMVßgal packaged in AAV virions (4 µl each) was stereotaxically injected into the left lateral ventricle of normal male Sprague Dawley rats. ß-gal expression was subsequently detected in sites far from the injection, demonstrating dispersal of the vector via the CSF, and was primarily confined to neurons, particularly in the case of the pAVPßgal. In contrast to the *in vitro* results, the AVP promoter was stronger than the CMV promoter in its ability to drive the expression of ß-gal in neurons *in vivo*. ß-gal expression driven by the CMV promoter was more diffuse, while the AVP promoter-induced expression was more concentrated to the PVN and SON of the hypothalamus, and this expression was stable for at least 1 month. Supported by grants to EFT from AmFar.

133.3

STABLE TRANSDUCTION OF NEURONS IN THE ADULT BRAIN BY AN HIV-DERIVED LENTIVIRAL VECTOR.

U. Blömer, L. Naldini, I.M. Verma, D. Trono, F.H. Gage* The Salk Institute, 10010 North Torrey Pines Road, La Jolla, CA 92037, USA

Application of gene therapy for the central nervous system depends not only on the identification of genes responsible for neurological disorders and gene products ameliorating diseases or genetic defects, but requires new methods for gene delivery. Significant problems remain with the existing viral vector systems. In the present study a new retroviral based HIV vector system, using the unique ability of lentiviruses to stably infect quiescent cells, was investigated *in vitro* and *in vivo*. This viral vector contains karyophilic determinants in two virion proteins, the matrix and the Vpr, interacting with the nuclear import mechanism of the host cell, mediating the active transport of the HIV preintegration complex through the nucleopore. In order to test this HIV derived vector *in vivo* we injected highly concentrated viral vector stock with the LacZ reporter gene bilaterally into the striatum and hippocampus of adult female Fischer rats. Seven days, 4 and 6 weeks after injection the animals were sacrificed and immunofluorescent triple labeling for ßgal (reporter gene), NeuN (neuronal specific marker) and GFAP (glial fibrillary acidic protein) was performed on microtomed tissue sections. ßgal immunoreactive cells were detectable at all time points with a large number cells also NeuN positive. These results indicate that the HIV derived viral vector was able to tranduce terminally differentiated neurons very efficiently and persistently for 6 weeks without causing local damage or immune response. Control brains injected with a MLV based retroviral vector revealed no transduced neurons in the early time points and no ßgal immuno reactive cells after 6 weeks. Our results strongly suggest, that the HIV vector system is able to stably and efficiently infect terminally differentiated neurons *in vivo*. This replication defective lentiviral vector system also shows significant advantages compared to available vector systems and provides a new perspective on gene delivery to the brain. *Supported by the Deutsche Forschungsgemeinschaft BL* 389/2

133.4

IN VIVO TRANSFER OF LEPTIN (*OBESE*) GENE INTO VENTROMEDIAL HYPOTHALAMUS WITH A DEFECTIVE AAV-VECTOR DECREASES BODY WEIGHT AND FOOD INTAKE.

T. M. Mizuno*, A. Sugiyama, F. Isoda, M. Rosenfeld, M. Kaplitt, S. Tanaka, and C. V. Mobbs Fishberg Center for Neurobiology, Mt. Sinai School of Medicine, and Bronx VAMC, New York, NY 10029.

Leptin, the normal product of the *obese* gene, is hypothesized to control body weight by acting through leptin receptors in the ventromedial hypothalamus (VMH). To address this hypothesis, we examined the effects of transferring the leptin gene into VMH using a defective adeno-associated virus (AAV) vector. cDNA containing the coding region of leptin was produced from mouse adipose tissue by RT-PCR, then cloned into a plasmid containing a CMV promoter and AAV packaging sequences. Plasmid was packaged in the presence of helper adenovirus to produce defective vector dvACLep. PBS, dvACLacZ (a LacZ construct), or dvACLep, were infused into the VMH of male Sprague-Dawley rats. Body weight and food intake were determined daily before and up to 7 days after infusion. Compared to PBS or dvACLacZ, dvACLep significantly inhibited body weight gain and food intake after infusion ($p<0.05$, ANOVA). These results suggest that expression of leptin in the VMH, possibly acting locally on hypothalamic leptin receptors, can reduce body weight and food intake.

Supported by NIH (DK 50110-01).

133.5

TETRACYCLINE-CONTROLLED GENE EXPRESSION SYSTEM ACHIEVES BOTH HIGH LEVEL AND QUANTITATIVE CONTROL OF GENE EXPRESSION,

Patricia Whaley*, Dong X. Yin, Sara Cunningham, Li Zhu. CLONTECH Labortatories, Inc., Palo Alto, CA 94303-4230.

The tetracycline-controlled gene expression system utilizes the control elements of the tetracycline-resistance operon encoded in Tn10 of E. coli to control gene expression in eukaryotic cells. Here we demonstrate the quantitative control of the expression of luciferase gene, dihydrofolate reductase gene, and bcl-2 gene in HeLa S3 or Chinese hamster ovary AA8 cells using the tetracycline-controlled gene expression system. Regardless of the host cell lines or the genes being expressed, there is a common range of tetracycline concentration within which the expression of genes is most sensitively regulated. In addition, the maximal gene expression level of the tetracycline-controlled gene expression system is higher than that of the wild-type CMV promotor/enhancer driven systems. Nonetheless, careful selection of stably transfected clones is necessary to achieve the optimally regulated gene expression using this system. Furthermore, addition of the nuclear localization signal onto tTA or rtTA causes an increase of both background and maximal gene expression in this system. Therefore, both the experimental strategy and the system components should be carefully selected to achieve the expected results

133.6

ISOLATION AND CHARACTERIZATION OF THE RAT GTP CYCLOHYDROLASE I GENE PROMOTER.

S.L. Stegenga and G. Kapatos. Cellular and Clinical Neurobiology Program, Dept. of Psychiatry and Behavioral Neurosciences, Wayne State Univ. Sch. of Med., Detroit, MI 48201.

Tetrahydrobiopterin (BH4) is the essential cofactor for tyrosine, tryptophan and phenylalanine hydroxylases and nitric oxide synthases. The first and rate-limiting enzyme in the biosynthetic pathway for BH4 is GTP cyclohydrolase I (GTPCH). Various stimuli, including cAMP, glucocorticoids, NGF, tumor necrosis factor-α (TNF-α), γ-interferon (γ-IFN) and LIF/CNTF have been shown to alter GTPCH mRNA levels in a tissue specific fashion. The manner in which these diverse stimuli exert their effects on GTPCH mRNA levels is not understood but presumably involves alterations in GTPCH gene transcription. We have, therefore, cloned and begun to characterize the rat gene for GTPCH. A rat testis genomic library in the λ-DASH vector was screened using a 852 bp cDNA probe spanning the entire coding region of rat GTPCH. A 15 kb GTPCH insert was isolated. Restriction and Southern analyses indicated that this included 6 kb of sequence 5' to the translation initiation site. The 6 kb 5' flanking sequence was subcloned into pGEM5Z for sequencing and further manipulation. The transcription start site was localized 141 bp upstream from the translation initiation site by primer extension using RNA isolated from PC12 cells. 1.2 kb of the 3' end of the 6 kb clone has been sequenced. Analysis reveals the absence of a TATA box within the core promoter although an AT-rich motif (ATAAAAA) and a CCAAT box are found adjacent to or 50 bp upstream of the cap site, respectively. CRE or AP-1 response elements were not detected despite the regulation of GTPCH by cAMP and NGF. Consensus sites for SP1 and NF-κB are present, the later being concordant with the GTPCH response to TNF-α and γ-IFN. The 5' flanking sequence for rat GTPCH has a high degree of homology to that of mouse and human, especially within the first 550 bp. (supported by NS26081)

133.7

MOLECULAR CLONING OF THE RAT INDUCIBLE NITRIC OXIDE SYNTHASE GENE.

R. Keinänen*, N. Vartiainen, K. Kurkinen and J. Koistinaho. A.I.Virtanen Institute, Univ. Kuopio, P.O.B. 1627, FIN-70211 Kuopio, Finland.

Nitric oxide (NO) formation from L-arginine is catalyzed by nitric oxide synthase (NOS). Two of the NOS enzymes (NOS1 and NOS3) are expressed constitutively in a calcium-dependent manner, while the third (NOS2) is inducible and calcium-independent. Although NO is known to act in versatile ways, its precise role in brain diseases, such as ischemia, is not completely understood.

A rat genomic cosmid library was screened using the 3' end of the rat NOS2-cDNA as a probe. A positive hybridization signal was obtained from six clones, which were isolated and purified for further characterization. Based on hybridization with the 5' end of the mouse NOS2-cDNA, one of the clones (SC1A1) was selected for sequencing analysis. The length of the SC1A1 genomic insert is approximately 40 kb, and thus a strategy was designed to sequence the trapped exons first. Subsequently, SC1A1 was subcloned as shorter fragments, in both orientations, into a splicing vector and used to transfect COS 7 cells. Purified mRNA from the transfected cells was used as a template for cDNA synthesis, which in turn served as a template in PCR. The PCR products were further subcloned into a plasmid vector. Subclones for nucleotide sequencing were selected based on their specific hybridization with the original genomic fragment used for transfection. The current sequencing data obtained agrees with the cloned rat NOS2-cDNA.

When the 5' regulatory region of the NOS2 gene becomes available, detailed studies to understand the regulation of its transcriptional initiation will be started at the molecular level. Regardless of the eventual role NO plays in brain diseases, the control of the NOS2 gene transcription from the NOS family offers the most attractive target for pharmaceutical intervention.

Supported by the Maud Kuistila Foundation (Finland) and the Academy of Finland.

133.8

STRUCTURE OF THE HUMAN α2 GLYCINE RECEPTOR GENE AND IDENTIFICATION OF MOTOR NEURON SPECIFIC ELEMENTS.

U. Monani[1], A. D. Epstein*[2] and A. H. M. Burghes[1,2]. Departments of Molecular Genetics[1] and Neurology[2], Ohio State University, Columbus, OH 43210.

The α subunit of the glycine receptor (GlyR) is encoded by multiple genes which display developmental and tissue specific expression. The α1 subunit is expressed in adults, while the α2 subunit constitutes the fetal form of the glycine receptor and is expressed in the brain and spinal cord. Here, we describe the intron-exon structure of the human α2 subunit gene. In addition, we have defined the 5' end of this gene as well as the α1 subunit gene by RACE-PCR. A clone containing the α2 gene was isolated from the CEPH mega YAC library and used to define five of the nine exons by vectorette PCR. Alu-exon PCR on genomic DNA was used to identify boundaries of exons 5 and 6. A lambda phage library was screened with an α2 cDNA probe to isolate clones containing the remaining exons. This resulted in the identification of a previously unreported exon between exons 3 and 4 which probably gives rise to an alternative isoform similar to that seen in the rat. RACE-PCR on fetal human brain and adult spinal cord RNA was used to define the 5' ends of the α2 and α1 transcripts respectively. Our study shows that the human α2 subunit gene consists of nine exons. Its structure has been compared with and found to be very similar to other GlyR α subunit genes in humans as well as mice.

We have also isolated upstream genomic fragments of the α1 gene. These have been cloned into reporter constructs and will be delivered to rat spinal motor neurons via retrograde axonal transport to assay for motor neuron specific expression. Our lab has worked extensively on cloning the gene(s) causing the motor neuron disorder, spinal muscular atrophy. SMN^{tel}, the most likely candidate and its centromeric copy SMN^{cen} can be delivered to motor neurons in a similar manner. Genomic clones each containing one of these genes will be injected intraneurally and their expression or lack of it determined. PCR assays for unique isoforms from each of the two genes will be simultaneously carried out. This will aid in precisely defining the molecular events causing spinal muscular atrophy. (Supported by Families of SMA and MDA.)

133.9

GENE STRUCTURE OF THE RAT KAINATE RECEPTOR SUBUNIT KA2 AND TRANSCRIPTIONAL ANALYSIS OF AN INTRAGENIC NEGATIVE REGULATORY REGION, F. Huang, S.E. Scherer* and V. Gallo. Lab. of Cell. and Mol. Neurophysiol., NICHD, NIH, Bethesda, MD 20892, USA.

We have isolated and analyzed the structure of the gene *GRIK5*, encoding the rat kainate receptor subunit KA2. Six overlapping clones containing the entire *GRIK5* gene were identified in a bacteriophage λ rat genomic library. *GRIK5* is a unique gene composed of 19 translated and one 5'-untranslated exons (sizes between 54 and 944 bp) that span over 70 kilobases of genomic sequence. The intron sizes ranged between 0.1 and 12 kb. Previous analysis demonstrated that 2kb of *GRIK5* 5'-flanking genomic sequence confers tissue-specific expression of the chloramphenicol acetyltransferase (CAT) reporter gene *in vitro* and *in vivo* (Molne, Scherer, Huang and Gallo, Soc. Neurosci. Abstr. 1995, vol. 21, Abstr. 29.9). We now show that: i) the first intron of *GRIK5* (3.4 kb) inhibited (3-fold decrease) transcription of the CAT gene driven by the 2kb *GRIK5* 5'-flanking region in the oligodendrocyte cell line CG-4 and in PC12 cells, but did not modify its tissue-specific expression *in vitro*, as assessed by transient transfection assays of HeLa or 3T3 cells; ii) this effect was not due to aberrant splicing of the CAT coding region; iii) the negative regulatory element(s) resided within 600 bp of the 3'-end of intron 1, as demonstrated by deletion analysis; iv) these elements also inhibited CAT transcription driven by the heterologous β-actin promoter in neural and non-neural cells; and v) the negative effects of these elements on the *GRIK5* promoter in CG-4 cells were orientation- and distance-independent. Multiple bandshift assays demonstrated that this 600 bp region of intron 1 selectively bound nuclear proteins isolated from CG-4 or HeLa cells. We conclude that regulation of *GRIK5* transcription involves an interplay between positive and negative elements located in the promoter region and within the first intron. Supported by NIH.

133.10

CHARACTERIZATION OF THE NEURON-SPECIFIC REGULATORY CONTROL OF THE RAT CLASS III BETA -TUBULIN GENE. K. E. Dennis * and S. A. Moody. The Neuroscience Program and the Dept of Anatomy and Cell Biology at the George Washington University, Wahington D.C., 20037

The molecular events involved in the determination of neuron cell fate remain an enigma. The analysis of genetic mutations in several developmental models has identified a variety of signaling factors which are active in this process. However, the events surrounding the adoption of the neuronal cell fate at the time of terminal differentiation have yet to be determined. To address this question more directly, we have undertaken the analysis of the neuron-specific gene expression of the rat class III beta-tubulin gene. The appearance of the class III beta-tubulin protein is coincident with the time of terminal mitosis of several neuronal populations and is specific to neurons (Moody et al. 1989; Lee et al. 1990; Easter et al.1993). We are investigating the molecular mechanisms involved in this event through the analysis of the 5' flanking region of the rat class III beta-tubulin gene. Using a rat cDNA clone obtained from Anthony Frankfurter (Univ. of Virginia), a portion of the class III 5' untranslated region (UTR) was isolated using the rapid amplification of cDNA ends (RACE). This material was cloned and sequenced and used to identify two clones in a P1 rat genomic library that contain the gene encoding the rat class III beta tubulin protein (Genome Systems). The genomic clones were subjected to Southern blot hybridization analysis using the 5' UTR region as a probe. Two *Bam*H1 bands approximately 6.1 and 6.5 kb in size as well as two *Pst*1 bands approximately 0.5 and 1.0 kb in size hybridized to the 5' UTR probe. This material has been subcloned and will be subjected to S1 nuclease protection mapping and RNAse protection studies to identify the promoter of the rat class III beta tubulin gene. Future studies with this material will identify regions in the promoter which may have an important role in the neuron-specific expression of this gene. Supported by NIH grant NS23158. (SAM).

133.11

CLONING OF A PUTATIVE CRUSTACEAN SYNAPSIN HOMOLOG. R.E. Dearborn Jr., K.F. Arcaro*, B.G. Szaro and G.A. Lnenicka. Department of Biological Sciences, University at Albany, SUNY, Albany, New York 12222.

The synapsins represent an abundant group of neuron-specific phosphoproteins localized to the presynaptic terminal where they appear to regulate the availability of synaptic vesicles for release. Currently, molecular knowledge of the synapsins is limited to mammalian species. In an effort to identify invertebrate synapsins, a λgt10 cDNA library was constructed from poly-A selected RNA isolated from 60 adult crayfish ganglia. The library was screened with a 1700 bp fragment of the coding region of rat synapsin I (Kilimann and DeGennaro EMBO J. 4: 1997-2002, 1986) and yielded a 1.3 kb clone with stretches of nucleotide sequence similarity to bovine synapsins Ia and Ib. Northern blots probed with this clone showed it to be an abundant message specific to the crayfish nervous system (it was not found in muscle, liver or green gland tissues). More detailed analysis of the distribution of this message was conducted through *in situ* hybridization. Cross-sections of the abdominal nerve cord and ganglia probed with digoxigenin labeled cRNA from the crayfish clone fragments demonstrated that the message was expressed neuronally.

Subsequent library screens generated additional clone fragments, extending the sequence to a total of 3.3 kb. Further sequence analysis is ongoing at this time. It appears likely that this message represents an invertebrate homolog of the mammalian synapsins.

This work supported by NSF grant IBN-9511558 and NIH grant NS30682.

133.12

MOLECULAR CLONING OF THE 5'-FLANKING REGION OF THE GOLDFISH BRAIN AROMATASE GENE. A.Tchoudakova and G.V.Callard*. Department of Biology, Boston University, Boston, MA 02215

It is well-established that estrogen (E) synthesized in the central nervous system mediates androgen (A) actions on the growth, survival, differentiation, and functions of neural tissues. The rate limiting step in E biosynthesis is catalyzed by aromatase (P450arom), a product of the CYP19 gene. Expression of the mammalian CYP19 gene is under control of multiple tissue-specific promoters and regulators but gives rise to a single enzyme protein. Exceptionally high levels of P450arom are expressed in the brain of teleost fish, when compared to the ovaries of the same fish, or to the brain of other vertebrates. The presence of two CYP19 genes and mRNA species was established in goldfish (Carassius auratus). One aromatase mRNA type was predominantly neural and found in high abundance. The other, lower abundance mRNA, was detectable in both neural and gonadal tissues. Seasonal studies of the predominant brain CYP19 mRNA and in vivo manipulation of plasma steroids demonstrated upregulation by aromatizable A or E. To initiate characterization of the promoter of the CYP19 gene expressed primarily in brain the 5'-flanking region was cloned by ligation-mediated PCR of genomic DNA. DataBase analysis of a 372 bp genomic fragment revealed a TATA-box (-42 nt), an Ad4/SF1 site (-293 nt), and an estrogen response element (-360 nt). Future experiments are required to test the functional significance of these putative regulatory elements. Supported by NSF IBN89-16809.

133.13

CLONING AND CHARACTERIZATION OF A WORTMANNIN-SENSITIVE AND SOLUBLE PHOSPHATIDYLINOSITOL 4-KINASE OF 100kDa. T. Nakagawa, K. Goto & H. Kondo* Dept. of Anatomy, Tohoku Univ. Sch. of Med., Sendai 980, Japan

A phosphatidylinositol (PI) 4-kinase cDNA cloned from a rat brain cDNA library encoded a protein of 816 amino acids with a calculated molecular mass of 91,654Da. This molecule contained a lipid kinase unique domain and a presumed lipid/protein kinase homology domain that are found in other PI 4-kinases and PI 3-kinases. Furthermore, this molecule and a yeast PI 4-kinase, PIK1 had a region of similarity to each other that is not conserved in other lipid kinases. By examining PI kinase activity in transfected COS-7 cells, the product phosphatidylinositol phosphate (PIP) was identified as PI-4-P, but not PI-3-P. The PI 4-kinase activity was recovered predominantly from the soluble fraction. This PI 4-kinase activity was markedly enhanced in the presence of Triton X-100 and relatively insensitive to inhibition by adenosine. In addition, this PI 4-kinase activity was completely inhibited in the presence of 10μM wortmannin. By epitope tag immunohistochemistry, the immunoreactivity for this PI 4-kinase molecule was dominantly aggregated in a cytoplasmic region jaxtaposed to the nuclei and faintly dispersed widely in the cytoplasm. However, a deletion mutant molecule, which lacked the N-terminal region contained commonly and solely in both this PI 4-kinase and PIK1, localized to the nucleus. By *in situ* hybridization analysis, the mRNA for this PI 4-kinase was expressed ubiquitously and detected in most neurons throughout the gray matter of entire brain with higher expression intensity in fetus than adult.

133.14

GENE ORGANIZATION OF NEURONAL-SPECIFIC ACTIVATORS FOR CYCLIN-DEPENDENT KINASE 5. F. Nildén, D. Lindholm, A. Bäckström, P. Marin and I. C. Bark*. Department of Developmental Neuroscience, Uppsala University, S-751 23 Uppsala, Sweden.

Cyclin-dependent kinases (cdk's) are a family of proteins involved in cell cycle control. However, recent evidence shows the existence of cdk5 activity in post-mitotic neurons in the CNS. Neuronal cdk5 activation is mediated by complex formation to a new group of proteins, including p35/p25^{nck5a}, capable of phosphorylating neurofilaments and the tau protein (Tsai et al., 1994, Nature 371, 419-423; Lew et al., 1994, Nature 37, 423-426). In addition, p35/p25^{nck5a}, promotes neurite extension of cultured cortical neurons (Nikolic et al., Soc. Neuroscience Meeting, Abstract 785.13, 1995), and the cdk5/p35 complex interacts with proteins participating in regulated exocytosis of neurotransmitters (Grant et al., 35th Annual Meeting of the American Society for Cell Biology, Washington D.C, Abstract 2183, 1995; Shetty et al., 1995, J. of Neurochemistry, 1988-1995).

We have characterized the organization of p35/p25^{nck5a} related genes by isolating recombinant phages from a mouse genomic library using a p35/p25 probe. Exon/intron boundaries were identified by DNA analysis and to investigate the neuronal expression pattern of p35/p25^{nck5a} like genes, we are analysing potential regulatory signals in the promoter region. The regulation and subcellular localization of p35/p25^{nck5a} in the rat pheochromocytoma (PC12) cells after NGF (nerve growth factor) and dBcAMP treatment are also studied and compared with proteins known to participate in regulated exocytosis.

Supported by The Swedish Natural Science Research Council and Magn. Bergwalls Foundation to ICB.

133.15

ENC-1: A NOVEL MURINE GENE SPECIFICALLY EXPRESSED IN THE NERVOUS SYSTEM ENCODES AN ACTIN-BINDING PROTEIN. M. C. Hernandez*, P. J. Andres-Barquin, S. Martinez, A. Bulfone, J. L. R. Rubenstein and M. A. Israel, The Preuss Laboratory for Molecular Neuro-Oncology, The Brain Tumor Research Center, University of California San Francisco, CA 94143-0520.

We have isolated and characterized a novel murine gene whose expression is restricted to the nervous system. *Ectoderm-neural-cortex-1 (Enc-1)*, which is named based on its expression pattern, appears to be one of the earliest markers of neural induction, since its mRNA is detected very early during embryogenesis. Early in the gastrulation stage *Enc-1* is expressed in the prospective neuroectodermal region of the epiblast and from gastrulation to later stages of development its expression remains restricted to the nervous system. *Enc-1* expression is highly dynamic and after neurulation prominently marks prospective cortical areas. We have constructed a tagged ENC-1 to determine the subcellular localization of the protein in transfected cells and found that ENC-1 distribution is compatible with that of cytoskeletal associated proteins. Double fluorescent staining showed that ENC-1 co-localizes with F-actin and this co-localization is maintained after treatment with cytochalasin D. Moreover, ENC-1 was co-precipitated with actin in immunoprecipitation experiments using anti-actin antibodies demonstrating that ENC-1 associates *in vivo* with actin. National Cancer Institute Grant 5 U01 CA64898.

133.16

NERVANA 2 IS THE NEURONAL FORM OF β SUBUNIT OF Na^+, K^+-ATPASE IN *DROSOPHILA MELANOGASTER*. B. Sun and P. M. Salvaterra*, Division of Neurosciences, Beckman Research Institute of the City of Hope, 1450 E. Duarte Rd., Duarte, CA 91010

We have purified and characterized a nervous system specific glycoprotein antigen from adult *Drosophila* heads, designated Nervana (NRV), on the basis of immunoreactivity of its carbohydrate epitope(s) with anti-horseradish peroxidase (HRP) antibodies. Anti-HRP antibodies specifically stain *Drosophila* neurons. Three cDNA clones (designated Nrv1, Nrv2.1 and Nrv2.2) were isolated using a mixture of anti-NRV protein monoclonal antibodies. The NRV proteins deduced from these clones exhibit significant homology in both primary sequence and predicted topology to the β-subunit of Na^+,K^+-ATPase. Immunoaffinity-purified NRV is also associated with a protein (M_r=100,000) recognized on Western blots by an anti-ATPase α-subunit monoclonal antibody. In this study we have expressed *Drosophila* Na^+, K^+-ATPase α-subunit, along with NRV1 and/or NRV2 using a baculovirus expression system or in Xenopus oocytes by microinjection of cRNAs. In baculovirus-infected Sf-9 cells, we see no increase in sodium pump activity although both recombinant proteins (α-subunit, and NRV) are expressed at high levels in the same cells. In oocytes, injection of Nrv2.2 cRNA alone slightly increases the sodium pump activity. Co-injection of α-subunit and Nrv2.2 cRNAs leads to expression of more functional pumps in the plasma membrane as assessed by ouabain inhibititable Rb^+flux. Injection of Nrv1 or Nrv2.1 cRNA, with or without α-subunit cRNA, had no effect on the pump activity in oocytes. Furthermore, using isoform specific antibodies (anti-NRV1, and anti-NRV2) we have shown that NRV2 is the major nervous system form. Our results suggest that NRV2 is the neuronal form of the β-subunit of Na^+,K^+-ATPase in *Drosophila melanogaster*. (supported by the NIH-NINDS)

133.17

IDENTIFICATION AND CHARACTERIZATION OF TWO PROTEINS INTERACTING WITH THE NEURONAL PROTEIN SCG10. R. Lütjens*, G. Di Paolo, S. Catsicas and G. Grenningloh, GLAXO IMB, 14 ch. des Aulx, 1228 Plan-les-Ouates / Geneva, Switzerland.

SCG10 is a neuron-specific protein related to the ubiquitous phosphoprotein stathmin. This latter has been implicated in signal transduction and recently it has been shown to act directly on the dynamics of microtubules. Given the high degree of homology between stathmin and SCG10 and their differential subcellular localization, they may play similar roles in their respective compartments. However, the exact function of SCG10 is still unknown. Therefore, to further define it, we used the yeast two-hybrid system to identify interacting proteins. We generated a construct by fusing the rat SCG10 cDNA to the binding domain of the GAL-4 transcription factor which was used for screening an adult mouse brain cDNA library cloned into a plasmid containing the activation domain of GAL-4. Two million independent colonies were screened and 13 positives were purified and transformed into control yeast strains. 12 of these clones were specifically interacting with SCG10, but not with stathmin or other control proteins. They encoded two different cDNAs which we called SIGA1 and SIGA2. We analysed their pattern of expression by hybridization to multiple tissues and developing brain Northern blots. SIGA1 produces two forms of transcripts (~6.0Kb and 3.5Kb) and is expressed in different tissues but mainly in the brain. SIGA2 has a single transcript of ~12Kb most abundantly expressed in the brain. Both genes seem to have their expression upregulated during development and are maximal in adult. Polyclonal antibodies against SIGA1 and SIGA2 were raised and we are currently characterizing their subcellular localization in neurons and in transfected COS7 cells.

133.18

BOVINE NEUROFILAMENT L AND M SUBUNIT DNA SEQUENCES ARE HIGHLY CONSERVED. L.Zhang, M.M. Tompkins*, B.J. Balin*, T.J. Sprinkle† and W.D. Hill. Dept. of Cellular Biology and Anatomy, Dept. of Neurology† Medical College of Georgia, Augusta, GA 30912. Dept. of Pathology, MCP and Hahnemann Univ. Philadelphia, PA 19102§

Three full length cDNA clones coding for the bovine neurofilament L (NFL) subunit were identified from a bovine brain library and partially sequenced. One, L19, was fully sequenced. A second set of 3 clones for the bovine neurofilament M (NFM) subunit were identified from the same library and partially sequenced. The longest one, M3, was fully sequenced, it lacks approximately 400bp from the 5' end. Both NFL and NFM sequences demonstrate strong conservation of deduced amino acid sequences when compared to human and other mammalian species.

The bovine NFL head domain differs by only two amino acids from the human, while the tail domain, like that of the other known vertebrates, has about 80% homology with the human sequence. The amino acids in the most amino 2/3 of the tail domain show the most homology. The most COOH 13 amino acids are also well conserved and interestingly show homology with a portion of an acid stable protease inhibitor. This may suggest that the COOH end sequence of NFL may be involved in regulating the turnover of the subunit or the assembled filament.

The Bovine NFM subunit sequence is also highly homologous to the human sequence. The conservation of regions of the tail domain suggests that it has important functions which have been preserved. These subdomains may represent regulatory or binding domains. The NFM tail domain has at least three conserved subdomains in addition to the KSP repeats, KE regions and the final heptad repeat.

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133.19

GENE SEQUENCE FOR THE SERPIN, PROTEASE NEXIN I (PNI) IS IDENTICAL IN MOUSE BRAIN AND SEMINAL VESICLE. B. A. Citron*, K. T. Ratzlaff, I.V. Smirnova, and B. W. Festoff. Neurobiology Research Lab, VA Medical Center, Kansas City, MO 64128 and University of Kansas Medical Center, Kansas City, 66160.

PNI is a member of the serpin multigene family that includes α_1-protease inhibitor (α_1-antitrypsin), α_1-antichymotrypsin, and plasminogen activator inhibitors. Serpins are approximately 400 amino acids in length, with active sites near the C-terminus, and act as suicide substrates for their target serine proteases. PNI is secreted as a 378 amino acid protein (41.8 kDa), binds to and functions in the extracellular matrix to inhibit and form complexes with thrombin. Although prothrombin is chiefly synthesized in the liver and is present in the circulatory system, it is also synthesized locally in the brain (Dihanich,1991,Neuron,6:575), muscle and spinal cord (Smirnova *et al.*, in prep.). PNI is not expressed in the liver but is in brain, and is concentrated in seminal vesicles. The gene for PNI maps to human chromosome 2q33-35, and to mouse chromosome 1, between acetylcholine receptor γ subunit and villin. PNI is concentrated at synapses to balance thrombin and play an important role in development and plasticity. In this study, we confirmed that the gene expressed in brain is identical in sequence to that in seminal vesicle. PNI mRNA represented 0.0024% of mouse brain poly(A)+ RNA, and 0.04 % in seminal vesicle. Additionally, by Western immunoblot , a single ~47 kDa protein was visible in the mouse brain, seminal vesicle, and seminal fluid at approximately 0.008, 0.06, and 0.2 % of total protein, respectively. Analysis of PNI regulation will facilitate the understanding of serine protease signaling pathways in the central nervous system. Supported by the Medical Research Service, Department of Veterans Affairs.

134.1

ACTIVITY DEPENDENT POTENTIATION OF SYNAPTIC TRANSMISSION FROM L30 INHIBITORY INTERNEURONS OF *APLYSIA* DEPENDS ON RESIDUAL CALCIUM

T.M. Fischer [1], R.S. Zucker*[2], and T.J. Carew[1]. [1]Yale Univ., Dept. Psych., New Haven, CT 06520; [2] Dept. Molec. & Cell. Biol., Univ. California,, Berkeley, CA 94720

We have previously shown that activity-dependent potentiation (ADP) of inhibitory synaptic transmission in L30 interneurons provides a mechanism for dynamic gain control of the siphon withdrawal reflex of *Aplysia* (Fischer & Carew 1993; 1995). To explore the mechanisms of L30 synaptic plasticity, here we examine the role of residual free Ca^{++} in ADP through the use of the photo-activated calcium chelator diazo-4.

Diazo-4 was iontophoretically injected into L30 neurons, and was photolyzed by a UV flash (flash alone has no effect on ADP). L30 synaptic transmission was measured as inhibitory postsynaptic currents (IPSCs) in L29 excitatory interneurons. Four components of ADP were analyzed: **(1) Baseline** (non-potentiated) transmission was examined by eliciting a single spike in L30 at a 30 sec ISI. Photolysis of diazo-4 had no effect on IPSC amplitude (N = 6). **(2) Frequency facilitation** was examined during a 5 sec activation of L30 (≈8 Hz). Photolysis 2 sec following activation onset resulted in an immediate and significant reduction in the amplitude of the potentiated IPSC ($p<0.01$; N=7). **(3) Augmentation** was induced by a 5 sec activation of L30 at ≈ 8 Hz; this produces enhancement normally lasting for ≈ 60 sec. Photolysis 20 sec following activation immediately and significantly reduced the potentiated IPSC to pre-activation levels ($p<0.01$; N=7). **(4) Posttetanic potentiation** was induced by a one min activation of L30 at 5 Hz, which produces enhancement normally lasting ≈ 6 min. Photolysis 3 min following activation significantly reduced the potentiated IPSC to pre-activation levels ($p<0.05$; N=6). These data show that three different forms of ADP in L30 require presynaptic elevation of free calcium for their maintenance.

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134.2

FREQUENCY DEPENDENT ADAPTATION AT TWO DISTINCT EXCITATORY INPUTS IN THE HIPPOCAMPUS

M. Scanziani*, P.A. Salin, R.C. Malenka & R.A. Nicoll.
Depts. of Cellular & Molecular Pharmacology, Physiology & Psychiatry; UCSF, San Francisco, CA 94143-0450

The mossy fiber synaptic input on CA3 pyramidal neurons has been referred to as the detonator or teacher synapse in autoassociational network models of the hippocampus. In these models, the mossy fiber system is particularly important during storage of information because it determines which CA3 neurons fire based on the pattern of granule cell activity. However, the mechanism by which changes in the relatively low firing rates of granule cells is translated into changes in the efficacy of the mossy fiber input is not known. We addressed this issue by comparing associational/commissural and mossy fiber synaptic responses on the same CA3 neuron in guinea pig hippocampal slices. In striking contrast to commissural synapses, stimulation of the mossy fiber gave rise to an augmenting response, over a very wide range of low frequencies (0.0125 - 1 Hz). This augmenting response decayed with a time course of about 40- 60s and was reduced by bath perfusion of either the membrane permeant calcium chelator EGTA-AM or the CaMKII antagonist KN62, suggesting that it is triggered by presynaptic Ca^{2+} influx acting via CaMKII. Furthermore, the dynamic range of the augmenting response was markedly reduced at synapses expressing LTP. Thus, the mossy fiber synapse, is able to integrate granule cell spiking activity over a broad range of frequencies, and provides an interesting mechanisms by which spike frequency modulation is translated into changes in synaptic strength.

134.3

CYCLIC-ADP RIBOSE MODULATES ACETYLCHOLINE RELEASE AT AN IDENTIFIED SYNAPSE OF *APLYSIA*.

J. P. Mothet, P. Fossier, J. Stinnakre* and G. Baux. Laboratoire de Neurobiologie cellullaire et moléculaire, CNRS, 91198 Gif-sur-Yvette cedex, FRANCE.

Ca^{2+}-induced calcium release (CICR) *via* ryanodine receptors occurs in neurons. In this study, we investigated the hypothesis that CICR is involved in transmitter release. For this purpose, we injected into the presynaptic neuron of an identified neuro-neuronal cholinergic synapse of *Aplysia*, a metabolite of NAD^+, cyclic-ADP-ribose (cADPr), which has been identified as a potent endogenous modulator of CICR in a variety of cells. Below 10 µM, cADPr induced a sustained increase in acetylcholine (ACh) release whereas higher concentrations led to a transient facilitation of ACh release followed by a decrease. These effects were not due to changes in Ca^{2+} influx through N- and P-type channels triggering ACh release but to modifications of the number of evoked released quanta. Three different approaches showed that both increase and decrease in ACh release originated in an elevation of the intracellular Ca^{2+} concentration: *i)* injection of cADPr induced a fluorescence rise using Rhod2 as a probe, *ii)* the decreasing effect of high concentrations of cADPr on ACh release was partially reversed by presynaptic injection of EGTA and *iii)* the Ca^{2+}-activated K^+ current was potentiated with cADPr.

These observations show that *i)* in *Aplysia* neurons cADPr is able to release Ca^{2+} from internal stores, *ii)* this release facilitates ACh release but can result in a secondary desensitization of the release mechanism when the Ca^{2+} concentration is too high, *iii)* CICR can be involved in the building up of the intracellular Ca^{2+} concentration triggering neurotransmitter release.

134.4

PROPERTIES OF SYNAPTIC DEPRESSION DURING HIGH-FREQUENCY ACTIVATION OF MEDIAL PERFORANT PATH SYNAPSES IN THE RAT DENTATE GYRUS.

A.E. Talpalar*, Y. Grossman and J.F. Storm. Dept. Physiol. Fac. Health Sciences Ben Gurion Univ. Israel. Inst. of Neurophysiol. University of Oslo, Norway.

Central neurons often produce high - frequency bursts of action potentials, whose effects are modulated by various form of short-term synaptic plasticity. In particular, many synapses show reduced transmission (depression) during high-frequency activation, a property often attributed to reduction of releasable synaptic vesicles. We have tested this hypothesis in medial perforant path synapses in the rat dentate gyrus. Whole-cell voltage-clamp and field potential recordings were obtained from dentate granule cells in hippocampal slices from young rats (17-23 d). Presynaptic fibers of the medial perforant path were stimulated by an electrode placed in the middle third of the molecular layer. Trains of 5-10 stimuli at 1-100 Hz elicited excitatory synaptic currents (EPSCs) whose amplitudes declined to a plateau in a frequency-dependent manner (e.g. $\tau \approx 75$ ms at 50 Hz). Using K gluconate or CsCl in the whole-cell pipette, or shifting the holding potential of the postsynaptic cell had also no significant effect on the depression. Similar results were observed during blockade of NMDA-receptors by APV, and GABA-A receptors by bicuculline or picrotoxin. To test whether desensitization of postsynaptic AMPA-type glutamate receptors contributes, cyclothiazide (CT) was applied. CT (100 µM) slowed the decay of individual EPSCs and reduced the depression at certain frequencies, but most of the depression persisted. Increasing the extracellular calcium concentration, $[Ca^{2+}]_o$, enhanced the depression, while low $[Ca^{2+}]_o$ reduced and delayed the depression during each train, often inducing an initial facilitation. These results are compatible with depression being mostly due to presynaptic factors, such as reduction of releasable quanta, while desensitization of postsynaptic glutamate receptors seems to play a minor role. [Supported by NFR]

134.5

DIFFERENT FREQUENCY MODULATION AND $GABA_B$ INVOLVEMENT AT THALAMOCORTICAL AND INTRACORTICAL SYNAPSES

Z. Gil[1]*, Y. Amitai[1], M.A. Castro-Alamancos[2] and B.W. Connors[2]. [1]Dept. of Physiology, Ben-Gurion University, Israel, 84105, and [2]Dept. of Neuroscience, Brown University, RI, 02912.

Synaptic efficacy is sensitive to repetitive activation, but the polarity strength and neurotransmitter involvement of such modulation may vary between pathways. We compared thalamocortical (TC) and intracortical (IC) EPSPs evoked on single layer 3 neurons in slices of rat barrel cortex prepared with the TC input intact. Paired stimuli of either the thalamus or horizontal layer 3 IC axons evoked small (~2mV) EPSPs. $GABA_A$ receptors were blocked by local application of bicuculline (10 µM), and postsynaptic $GABA_B$-inhibition was blocked with intracellular QX-314 and Cs^{2+}. Both pathways exhibited paired-pulse depression at intervals of 10-2000 ms. Depression of TC-EPSPs was significantly stronger than that of IC-EPSPs. The $GABA_B$-agonist baclofen attenuated the first IC-EPSP and reduced paired-pulse depression. The $GABA_B$ antagonist CGP-35348 reduced IC paired-pulse depression by enhancing the second EPSP. Neither baclofen nor CGP-35348 had any effect on TC-evoked paired-pulse responses.

The results show that frequency modulation of convergent pathways onto neocortical neurons can have distinctly different strengths and mechanisms.

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134.6

INTERACTION BETWEEN DOPAMINE AND GLUTAMATE IN THE PARS RETICULATA OF THE RAT SUBTANTIA NIGRA,

D. Martínez-Fong*, M. G. Rosales, D. Limón, B. Florán, G. Flores, A. Nuñez and J. Aceves. Depto. de Fisiología, Biofísica y Neurociencias, CINVESTAV-IPN, A.P.14-740, 0700 México, D.F., Centro de Investigación Biomédica, U.A. de C., Torreón, Coah., México.

We have studied the interactions between glutamate (Glu), dopamine (DA) and GABA in the pars reticulata of the substantia nigra (SNr) by using microdialysis in unanesthetized male Wistar rats. Increased levels of Glu in the SNr were obtained by microinjecting carbachol (1 µg/0.25 µl) in the ipsilateral subthalamic nucleus (STN). The increase in Glu was followed by increases in extracellular levels of DA and GABA. Increased levels of the three transmitters were also observed during the administration of N-methyl-D-aspartate (NMDA, 1 mM) through the microdialysis probe. The NMDA-induced increase in glutamate and GABA was blocked by the selective D_1 agonist SCH 23390 (100 µM), yet it did not prevent the increase in DA levels. The addition to the dialysis solution of the selective D_1 agonist SKF 38393 (200 µM) also increased the levels of the three neurotransmitters. However, after the kainic-induced lesion of the STN, SKF 38393 only produced increase in the level of GABA but not in those of Glu or DA. The lesion of the STN produced, in addition, a drastic (80%) fall in the extracellular level of Glu.

The data suggest that Glu stimulates the release of DA from dopaminergic dendrites present in substantia nigra pars reticulata, and that DA in turn stimulates the release of Glu and GABA. Both effects are mediated by D_1 receptors present on sutbthalamonigral and striatonigral axon terminals respectively.

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134.7

ACTIVITY-DEPENDENT POTENTIATION OF [^{3}H]-D-ASPARTATE RELEASE IN HIPPOCAMPAL SLICES. S.A. Queen, L.L. Paxton and D.D. Savage. Dept. Pharmacology, Univ. New Mexico H.S.C., Albuquerque, NM, 87131-5316.

Studies on the mechanisms that regulate activity-dependent glutamate (GLU) release in brain slices has been limited by the methods available to evoke GLU release. Neither high K^+ or 4-aminopyridine allow one to study how GLU release is altered by changes in neural activity. Further, *in vitro* electrical stimulation of slices in standard perfusion mediums evokes immeasurably low quantities of GLU release due to neural and glial transporter reuptake of GLU.

Using preloaded [^{3}H]-D-aspartate (D-ASP) as a marker for GLU release, we have determined that the addition of 300 μM L-cysteinesulfinic acid to perfusion medium blocks D-ASP reuptake sufficiently to measure calcium-dependent D-ASP release. Subsequently, we have optimized the basic parameters for studying electrically-evoked D-ASP release in superfused dorsal hippocampal slices including: 1) D-ASP preloading conditions, 2) slice thickness, 3) Ca^{++} concentration and 4) test stimulus (TS) parameters (1 msec 20 mA biphasic square wave pulses at 4 Hz for three minutes). Operationally, we have established the stimulation parameters so that the TS produces a fractional release of 1% to 1.5% and D-ASP release evoked from a second TS is equivalent to D-ASP release evoked from the first TS, when the second TS is delivered at least 30 min. after the first TS. Applying the TS at 100 Hz (2 sec. trains at 30 sec. inter-train intervals) potentiated TS-induced D-ASP release. Potentiation was not apparent until 30 min. after tetanus and remained fairly stable for at least 2 hours. The degree of potentiation was a function of the number of tetanus trains applied. One tetanus train produced a 14% increase in D-ASP release, three trains produced a half-maximal chamge and 7 to 10 trains produced a maximum potentiation of about 55% above current control. Using the parameters described above, we have initiated studies on the intrinsic and extrinsic mechanisms that regulate activity-dependent changes in GLU release. Supported by AI25555 (SAQ) and AA06548 (LLP and DDS).

134.8

EFFECT OF PRENATAL ETHANOL EXPOSURE ON PRESYNAPTIC MECHANISMS OF LONG-TERM POTENTIATION IN HIPPOCAMPUS OF ADULT RAT OFFSPRING. DD Savage*, LL Cruz, LM Duran and LL Paxton. Dept. of Pharmacology, Univ. of New Mexico H.S.C., Albuquerque, NM, 87131.

Prenatal ethanol exposure has been associated with long-lasting intellectual impairments in children. Previous studies from our lab suggest that these impairments are, in part, linked to neurochemical deficits affecting long-term potentiation (LTP) in the hippocampal formation. Whether this LTP deficit is principally presynaptic or postsynaptic in origin is unclear at present. Using a recently developed method to study tetanus stimulus-induced potentiation of electrically evoked [^{3}H]-D-aspartate (D-ASP) release from hippocampal slices, we explored whether FAE affected activity-dependent potentiation of excitatory amino acid neurotransmitter release, a presynaptic component of LTP.

Rat dams consumed one of three diets throughout gestation: 1) a BioServ liquid diet containing 5% ethanol, which produces a mean nightly maternal peak blood ethanol concentration of 83 mg/dL, 2) pair-fed an isocalorically equivalent amount of 0% ethanol (EtOH) liquid diet or 3) lab chow *ad libitum*. D-ASP release was studied in 400 μm thick hippocampal slices prepared from adult offspring from each of the three diet groups. The incorporation of D-ASP into hippocampal slices, spontaneous release of D-ASP and evoked release produced by a test stimulus (TS) were not different among the three diet groups. Potentiation of TS-evoked D-ASP release was elevated by 42 to 48% at 30, 60 and 90 minutes after a tetanizing stimulus in both *ad lib* and pair-fed control offspring. In contrast, virtually no potentiation of TS-evoked release was observed in the 5% EtOH diet group. These results suggest that prenatal exposure to moderate quantities of ethanol produces a long-lasting deficit in neurochemical mechanisms that sustain potentiation of synaptic communication. Whether this deficit is a function of intrinsic deficits in synaptic vesicle mobilization or extrinsic mechanisms activating synaptic vesicle mobilization is under investigation. Supported by AA06548 (DDS and LLP) and RR08139 (LLC and LMD).

134.9

EFFECT OF PRENATAL ETHANOL EXPOSURE ON GAP-43 PHOSPHORYLATION AND PKC ACTIVITY IN RAT HIPPOCAMPUS. N. Perrone-Bizzozero, G.Keidan, C.Erigat, D.D.Savage A.M. Allan*. Depts Biochem. and Pharmacology, Univ. of New Mexico Sch. Med., Albuq., NM, 87131.

Previous studies using a rat model of fetal alcohol exposure (FAE) suggest that observed behavioral deficits are, in part, linked to neurochemical and electrophysiological deficits in long-term potentiation (LTP) in the perforant path projection to the hippocampus (HC). PKC-mediated phosphorylation of GAP-43 may be critical to the induction and maintenance of LTP. Thus, alterations within this phosphorylation pathway may play a significant role in LTP deficits seen in FAE animals. Rat dams consumed one of three diets throughout gestation: 1) a Bio Serve liquid diet containing 5% ethanol (v/v), 2) an isocalorically equivalent amount of 0% ethanol liquid diet (pair-fed) or 3) lab chow ad libitum. PKC activity was measured by monitoring ^{32}P-phosphorylation of a peptide substrate in a combined HC soluble and particulate membrane fraction from adult rat offspring of these dams. GAP-43 phosphorylation levels in HC homogenates were determined *in vitro* by immunoprecipitation. GAP-43 levels in the same samples were measured by quantitative Western blots. PKC activity was lower by approximately 50 % in HC of FAE rats compared to ad lib or pair-fed control rats. GAP-43 levels were 35 % greater in FAE rats compared to controls, while GAP-43 phosphorylation was reduced in these rats by 50%. The measurably lower level of PKC activity in the FAE rats is consistent with the decrease in phosphorylated form of GAP-43. Since GAP-43 is one of the main substrates for PKC in HC synapses, alterations in these proteins are likely to result in significant changes in synaptic function and signaling mechanisms associated with LTP. Thus, these findings may underlie the behavioral deficits seen in FAE. Supported by AA06548 (DDS) AA0821 (AMA), NS30255 (NPB) and GM08139 (DDS, AMA, NPB).

134.10

DIRECT MODULATION OF THE SECRETORY MACHINERY UNDERLIES PKA-DEPENDENT SYNAPTIC FACILITATION IN HIPPOCAMPAL NEURONS. L.-E. Trudeau*, D.G. Emery and P.G. Haydon. Lab. of Cellular Signaling, Iowa State University, Ames, IA 50011.

Activation of the cAMP-PKA pathway has been previously reported to produce facilitation of synaptic transmission in a number of preparations. This facilitation may be of presynaptic origin. Using dual whole-cell patch clamp recordings in cultured hippocampal neurons we confirmed that monosynaptic IPSCs are reversibly facilitated (+126%, n=8) by the adenylate cyclase activator forskolin (20 μM). Inclusion of the protein kinase inhibitor H-7 in the presynaptic, but not the postsynaptic pipette, blocked the synaptic facilitation. Similarly, inclusion of the PKA antagonist Rp-cAMPS or a peptide inhibitor in the presynaptic pipette blocked the facilitation, supporting a presynaptic mechanism. Using Fura-2 imaging we found no evidence of any action of forskolin on either resting or depolarization-evoked elevations of intracellular calcium. This suggests that changes in presynaptic calcium dynamics are unlikely to be necessary for the synaptic facilitation. Ruthenium red (RR) was used to probe the state of the presynaptic machinery for exocytosis. This agent elevates the frequency of miniature synaptic currents independently of calcium (Trudeau et al., 1996; J.Neurosci. 16:46-54). RR-evoked release was markedly facilitated by forskolin (+197%, n=8). This effect was also blocked by Rp-cAMPS and was not accompanied by any change in the number of functional synaptic terminals as assessed by uptake of the fluorescent indicator of synaptic vesicle cycling FM1-43. These results suggest that PKA directly acts on the release machinery in previously functional synaptic terminals to facilitate neurotransmitter release. Funded in part by the HFSP (L.-E.T.) and the NIH (P.G.H.).

134.11

THE PRESYNAPTIC MODULATION OF GLUTAMATE RELEASE AFFECTS DIFFERENT CALCIUM CHANNELS. J. Sánchez-Prieto* and E. Vázquez Dpto. Bioquímica, Fac. Veterinaria, Univ. Complutense. Madrid 28040, Spain.

We have studied which type/s of Ca^{2+}-channel/s support glutamate exocytosis and its modulation by presynaptic receptors in cerebrocortical nerve terminals. Depolarization of nerve terminals with 30 mM KCl induced a Ca^{2+}-dependent release of 3.46±0.25 nmol/mg of protein. The addition of either 2μM ω-conotoxin GVIA or 200 nM ω-agatoxin-IVA reduced the KCl-evoked release by 47.7±3.5% and 70.4±8.9%, respectively, and by 85.7±4.1% when both toxins were co-applied, indicating the involvement of N and P/Q type Ca^{2+}-channels in glutamate exocytosis. The activation of adenosine A_1 receptors with N^6-cyclohexyladenosine or the activation of metabotropic glutamate receptors with L(+)-2-amino-4-phosphonobutyrate inhibited the KCl-evoked release by 41.0±5.9% and 54.3±10%, respectively. The extent of these inhibitions was not altered by the prior addition of 2μM ω-conotoxin-GVIA but were significantly enhanced when ω-agatoxin-IVA was added together with the adenosine A_1 receptor agonist or the metabotropic glutamate receptor agonist, suggesting that ω-conotoxin-GVIA-sensitive but not ω-agatoxin-IVA-senstive Ca^{2+}-channels are involved in the action of these inhibitory receptors. In contrast, the facilitation of glutamate release that follows the activation of the protein kinase C either with phorbol esters or with the stimulation of phospholipase C-linked metabotropic receptors was expressed by both ω-conotoxin-GVIA-sensitive and ω-agatoxin-sensitive Ca^{2+}-channels. It is concluded that N-type Ca^{2+} channels are the target of both adenosine A_1 receptors and L-AP4-sensitive metabotropic glutamate receptors, while N and P/Q type Ca^{2+} channels are involved in the facilitation of glutamate release.

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134.12

MODULATION OF EXOCYTOSIS BY INTRACELLULAR Ca^{2+} STORES IN BOVINE CHROMAFFIN CELLS. A.F. Fomina & M.C. Nowycky*. Dept. Neurobiol. & Anat., Med. Coll. Penn., Phila., PA 19129.

Elevation of intracellular Ca^{2+} ($[Ca^{2+}]i$) resulting directly from store mobilization can trigger catecholamine release in bovine chromaffin cells. However, Ca^{2+} influx via voltage-gated Ca^{2+} channels (VGCC) is more effective in causing secretion. Here we investigate the possible role of intracellular stores in modulation of the secretory response. We employed the capacitance tracking technique in perforated patch voltage-clamp mode to assay single cell exocytosis from bovine chromaffin cells. Simultaneously, $[Ca^{2+}]i$ was measured as changes in the fluorescence of Fluo-3 (loaded as the AM form) and monitored on a confocal laser microscope. Application of thapsigargin (Tg; 1-5 μM) frequently induced slow exocytosis which was correlated with a $[Ca^{2+}]i$ transient. Lower concentrations (Tg = 0.5 - 1 μM) facilitated depolarization-stimulated exocytosis even though VGCC were partially inhibited. The development of this facilitation was gradual, prolonged (up to 20 min) and not correlated with $[Ca^{2+}]i$ elevation. In contrast, caffeine (10-20 mM) significantly inhibited both VGCC and depolarization-induced exocytosis. Caffeine caused only a small rise in $[Ca^{2+}]i$ and did not induce exocytosis. Thus, in bovine chromaffin cells recorded in perforated patch mode: 1) Ca^{2+} release from Tg sensitive stores can induce depolarization independent exocytosis; 2) depletion of Tg sensitive stores facilitates depolarization-induced exocytosis; 3) caffeine sensitive stores play a minor role in the maintanence and simulation of exocytosis. Supported by NS22281.

134.13

ASSOCIATION OF B-50/GAP-43 AND CALMODULIN IN SITU, AT THE PLASMA MEMBRANE OF UNMYELINATED AXONS IN THE REGENERATING RAT SCIATIC NERVE. A.B. Oestreicher [1], P.Verkade[1], A.J.Verklei[2] and W.H.Gispen.[1] Rudolf Magnus Institute for Neurosciences[1] Institute of Biomembranes[2], University of Utrecht, Universiteitweg 100, 3584 CH Utrecht, NL. (Spon: ENA)

The ultrastructural distribution of B-50 and calmodulin in unmyelinated axons of regenerating rat sciatic nerve was investigated by means of single and double immuno gold labelling and quantification, to test whether the association of B-50 and calmodulin, previously shown to occur **in vitro**, can be demonstrated **in situ**. Electron microscopical analysis of nerve pieces dissected proximal to the site crushed 7 days earlier revealed that the B-50 immunoreactivity (BIR) at the plasma membrane (pm) was significantly increased (270%) with respect to that of the intact unmyelinated axon and to that of the axoplasma (450%) of the umyelinated axon of the regenerating nerve. Similarly to BIR, the calmodulin immunoreactivity (CAMIR) was preferentially accumulated (68.8%) at the pm, detected in association with vesicles in the axoplasma, but unlike BIR, also found to be located at microtubules and more distributed in the axoplasm. Double immuno labelling demonstrated that over 60% of the pm-associated CAMIR and the pm-associated BIR was colocalised. Statistical analysis provided proof that this pattern of colocalisation was not random. Thus, using immuno electron microscopy, we were able to visualise in unmyelinated axons sites of association of B-50 and calmodulin at the pm **in situ**, indicating that these proteins may be present as a molecular complex. The accumulation of B-50 and calmodulin bound in a complex at the pm in proximal axon segments which are not active in regeneration is in agreement with a "reciprocal regulator" hypothesis, namely that the complex serves a storage function in which the proteins are inactive. Moreover, on increase in intracellular local calcium level, the complex dissociates and both proteins become available to participate in various processes occuring during axon outgrowth and remodelling of neurons.

134.14

INVOLVEMENT OF ADENYLATE CYCLASE IN THE DEVELOPMENT OF TRANSMITTER RELEASE IN AREA CA1 OF THE RAT HIPPOCAMPUS. T.C. Dumas* and T.C. Foster, Department. of Psychology, University of Virginia, Charlottesville, VA 22903.

We have previously reported an increase in hippocampal CA3-CA1 presynaptic function *in vitro* from the third to the fifth postnatal week (wk) of development. Related studies show that transmitter release is modulated by the adenylate cyclase (AC) activator, forskolin, in slices from neonate and adult rats. We hypothesize that increased presynaptic function beyond 3 wk postnatal is due to increased activity of AC. The hypothesis predicts that direct activation of AC by forskolin will increase transmitter release more in slices from 3 wk than from 5 wk rats. This idea was tested by bath application of forskolin to hippocampal slices and measurement of changes in paired-pulse facilitation (50 ms ISI) of the field EPSP slope in the stratum radiatum of area CA1. Forskolin (50 μM, 25 min) significantly increased the EPSP slope at both ages (3 wk, n=8 slices: 125.78±3.76%; 5 wk, n=7 slices: 113.31±4.76%). Facilitation was decreased more in the 3 wk (85.52±0.95%) than the 5 wk pups (97.07±1.33%). A forskolin analog that does not activate AC, 1,9-dideoxyforskolin (50 μM, 25 min), increased the EPSP slope (3 wk, n=7 slices: 134.6±6.11%; 5 wk, n=9 slices: 124.77±4.17%) and decreased facilitation at both ages (3 wk: 90.84±1.37%; 5 wk: 89.63±2.52%). These results suggest that part of the effect of forskolin on facilitation is due to modulation of K^+ channel function; however, the age difference in the presynaptic effect of forskolin appears to be specific to activation of AC. We conclude that the development of transmitter release at CA3-CA1 synapses from the third to the fifth postnatal wk is regulated, in part, by an increase in AC activity. This study was supported by NIH grants NS31830 to TCF and 1F31MH11005 to TCD.

134.15

SYNAPTIC DEPRESSION INDUCED BY HYPOXIA BUT NOT BY METABOTROPIC GLUTAMATE RECEPTOR AGONIST 1S, 3R-ACPD IS ENHANCED BY FORSKOLIN. M. Windisch*, A. Baskys and J. M. Wojtowicz. Ctr. of Animal Biology, Univ. of Graz Med. School, Graz 8036, Austria and Dept. of Physiology, Univ. of Toronto, Toronto, Ont. M5S 1A8, Canada.

To understand pathophysiology of the early stages of hypoxic/ischemic brain damage and the role of presynaptic inhibition of glutamate release, we studied synaptic depression in in vitro hippocampal slices prepared from 4-8 week old CD1 mice. Field potentials (fEPSP) elicited by paired pulse stimulation (interstimulus interval = 50 ms) of lateral perforant pathway-dentate gyrus neurons were recorded in the upper dendritic layer of the dentate gyrus and served as a measure of synaptic transmission. Hypoxia was induced by switching the perfusion of the slice from artificial cerebrospinal fluid (ACSF) saturated with 95% O_2/5% CO_2 to ACSF saturated with 95% N_2 - 5% CO_2 for brief (10 min.) periods of time. The hypoxic episodes induced a rapid and reversible suppression of fEPSPs and an increase in paired pulse facilitation. Addition of adenylate cyclase activator forskolin (10 μM) increased fEPSPs in the dentate gyrus and significantly enhanced the suppression of synaptic transmission due to hypoxia. 1S,3R-ACPD (50 μM), which acts as an agonist for several metabotropic glutamate receptors and was shown to reduce forskolin-induced increases in cAMP, depressed fEPSPs and increased paired pulse facilitation in the dentate gyrus. In contrast to the hypoxic depression, the metabotropic agonist-induced decrease of the fEPSP showed no consistent changes in the presence of forskolin, suggesting that metabotropic glutamate receptors are not involved in the hypoxic depression of fEPSPs. The experiments also suggest that adenylate cyclase-cAMP regulation may be a factor influencing the degree of synaptic depression following hypoxic nerve tissue challenge.

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LONG-TERM POTENTIATION: PHARMACOLOGY I

135.1

A POSSIBLE ROLE FOR HEPATOCYTE GROWTH FACTOR IN SYNAPTIC PLASTICITY THROUGH ACTIVATION BY TISSUE PLASMINOGEN ACTIVATOR. D. Lederfein, Y.-Y. Huang, H. Golan, and D. Baranes*. HHMI, Ctr. Neurobiol. & Behav., Columbia Univ., HHMI, NY, NY 10032.

Hepatocyte growth factor (HGF), also known as Scatter factor, induces morphogenesis and invasive growth of epithelial and endothelial cells. This factor is a plasminogen-like protein that is secreted as a single chain percursor devoid of biological activity and can be converted to its active dimer form by tissue plasminogen activator (tPA) (Naldini et al., *EMBO J.*,**11**:4825,1992; Naldini et al., *J. Biol. Chem.*, **270**:603, 1995)

Since the expression of tPA increases during long-term potentiation (LTP), and ablation of tPA in mutant mice impairs late phase LTP, we examined whether HGF also has a role in synaptic plasticity. We have found that when paired with a subthreshold tetanus, HGF produced late phase LTP (more than 5 h.), whereas this tetanus by itself produced only early phase LTP (less than 90 min.). The late phase LTP produced by HGF was blocked by a tPA inhibitor, suggesting that it might be mediated through tPA activation. We also found that in dissociated hippocampus cells in culture tPA induced autophosphorylation of the HGF receptor, the *c-met* proto-oncogene product. In addition, HGF immunoreactivity was colocalized with a presynaptic marker, synaptophysin, whereas HGF receptor was found on dendrites. These results suggest that HGF may play a paracrine role in the maintenance of LTP: it is produced in the presynaptic terminal and acts on its receptor in the postsynaptic site. This role is apparently mediated by tPA. Supported by HHMI and NYSPI.

135.2

ORPHANIN INHIBITS LONG-TERM POTENTIATION IN HIPPOCAMPAL CA1 REGION. T.-P. Yu*, J. Fein, C. J. Evans and C.W. Xie Dept. of Psychiatry and Biobehavioral Sciences, UCLA-NPI, Los Angeles, CA 90024.

A novel member of the opioid receptor family, ORL_1 receptor, has been cloned from a variety of vertebrates. Its endogenous agonist, orphanin, has also been identified in rat brain. Our recent immunocytochemical study showed the presence of a high density of ORL_1 receptors in the rat hippocampus. The present study further examined if activation of ORL_1 receptor by orphanin would affect synaptic transmission and long-term potentiation (LTP) in the hippocampal CA1 region.

Extracellular field potentials evoked by stimulation of Schaffer collaterals were recorded in both pyramidal cell body and dendritic layers of CA1 region in transverse rat hippocampal slices (500μm). Our results showed that bath application of synthetic orphanin (0.1-10 μM) reduced population spike and EPSP slope in a dose-dependent manner. Paired-pulse facilitation of EPSP was significantly increased in the presence of 1μM orphanin. When high frequency stimuli (4 pulses at 100Hz, repeated every 200ms for a total of 40 pulses) were delivered, 1μM orphanin attenuated LTP. In orphanin-treated slices, the increase in EPSP slope and population spike after high frequency stimulation was 22 ±5%, 12 ± 4%, respectively (n = 5-10), which were significantly smaller than those in control slices (43 ± 7% and 47 ± 4%, n = 6-12). These data suggest that hippocampal function including LTP can be modulated by ORL_1 receptors (Supported by NIH-NIDA grants, DA05010 and DA08571).

135.3

CHOLINERGIC INDUCTION OF LONG-TERM TRANSFORMATION OF GABAergic SYNAPSES IN HIPPOCAMPAL FIELD CA1. D. Dahl*, J. Arrowood, and D.L. Alkon. School of Human Development, The University of Texas @ Dallas and the Laboratory of Adaptive Systems, NINDS, Bethesda, MD.

A distinctive modification of hippocampal field CA1 pyramidal cells was reported by Collin *et al.* (*PNAS*, Oct. 1995). Activation of an inhibitory afferent (10 trains, 10 pulses each, 100 Hz) paired with post-synaptic depolarizing current injection transformed γ-aminobutyric acid (GABA)-mediated inhibition into a depolarizing synaptic response. This modification is termed long-term synaptic transformation (LTT) and persisted for at least 60 min. Maintenance of LTT did not require further pre- or post-synaptic activation. This LTT may be significant in the induction of associative long-term potentiation (LTP).

In the present experiments, we have modified the LTT paradigm by pairing application of carbachol (a cholinergic agonist [20 μM]) with activation of an inhibitory afferent (10 trains, 10 pulses each, 100 Hz). These conditions produced LTT that also persisted for at least 60 min without further activation.

Carbachol- and pairing-induced LTT appear pharmacologically similar, in that they are both prevented by anandamide (an endogenous cannibinoid ligand, 1 μM) and eliminated by application of bicuculline ($GABA_A$ antagonist, 1 μM). Carbachol-induced LTT is prevented by concurrent application of the glutamatergic antagonists CNQX (100 μM) and APV (50 μM), but CNQX and APV do not eliminate LTT once it has been induced. Carbachol-induced LTT is also prevented by Dantrolene (preventing intracellular release of Ca^{2+}, 20 μM) or acetazolamide (blocking synthesis of HCO_3^-, 10 μM).

These results suggest an interaction of acetylcholine and GABA activation in the induction of associative LTP in hippocampal field CA1.

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135.4

LONG-LASTING ENHANCEMENT OF SYNAPTIC TRANSMISSION IN DISSOCIATED CHICK CEREBRAL NEURONS. S.Kudoh*, K.Kiyosue, M.Kasai, T.Taguchi†, Lab of Mol. Biol., Fac. of Eng. Sci., Osaka Univ., Toyonaka 560, Japan, and †Osaka Nat'l. Res. Inst. AIST, Ikeda 563, Japan.

Long-term enhancement of synaptic transmission were demonstrated in a dissociated cell culture containing embryonic chick cerebral neurons . Using whole-cell patch clamp technique, long-lasting increase in amplitude of spontaneous excitatory post synaptic currents (SEPSCs) was confirmed to be induced by Mg^{2+}-free medium. This enhancement was depends on neural activity and the activation of NMDA type glutamate receptor. Analysis of miniature EPSCs in the presence of TTX revealed that the enhancement is due to the increase of the number of transmitter release site and/or synaptic sites. Furthermore the similar potentiation was found to be able to be caused by exposing neurons to conditioned Mg^{2+}-free medium which was collected after induction of the enhancement and adjusted the Mg^{2+} concentration at the same in normal extra cellular recording solution. This result suggests that a factor which released to the medium from neurons or glia are involved in the mechanism of the potentiation. Using glia-free culture which we developed by the addition of peptides containing cell adhesion-related sequences, we examined whether the presence of glia is essential for the long-lasting increase in amplitude of SEPSCs, or not. In the neuron-selective culture, synapse formation proceeded in a similar way to that in the control one and the amplitude of SEPSCs increased by Mg^{2+}-free medium, suggesting that the potentiation was caused by the intrinsic mechanisms. This work was Supported in part by JSPS Research Fellowships (072875).

135.5

DIFFERENTIAL MECHANISMS UNDERLYING LONG-TERM POTENTIATION IN THE MEDIAL AND LATERAL AMYGDALA. K. Abe, S. Okuda*, Y. Watanabe and H. Saito. Dept. of Chem. Pharmacol., Fac. of Pharmaceut. Sci., Univ. of Tokyo, Tokyo 113, Japan.

The amygdala plays an important role in learning and memory associated with emotion. Although it has been reported that the synapses of the amygdala display long-term potentiation (LTP), the cellular mechanisms of amygdala LTP have not been well understood. In the present study, we investigated the mechanisms underlying LTP in the medial and lateral amygdala synapses in vitro. Amygdala slices (400-500 μm) were prepared from male Wistar rats (7-9 weeks old) and maintained in a submersion chamber. A bipolar tungsten electrode was placed on the stria terminalis or the external capsule to stimulate the afferent fibers, and the evoked potential was extracellularly recorded from the medial amygdaloid nucleus or the lateral amygdaloid nucleus, respectively. LTP was induced by applying tetanic stimulation (100 pulses at 100 Hz) in the presence of a GABAA blocker picrotoxin (10 μM). LTP in the medial amygdala was blocked by 2-amino-5-phosphonovalerate (APV), an NMDA receptor antagonist, and was significantly reduced by scopolamine, a muscarinic receptor antagonist. On the other hand, LTP in the lateral amygdala was not affected by APV, but was significantly reduced by scopolamine. These results suggest that both NMDA receptors and muscarinic receptors are involved in medial amygdala LTP, while muscarinic receptors, but not NMDA receptors, are involved in lateral amygdala LTP. Furthermore, isoproterenol, a β-adrenoceptor agonist, facilitated the induction of LTP in the medial amygdala, but suppressed the induction of LTP in the lateral amygdala. The effects of isoproterenol were mimicked by forskolin, an adenylate cyclase activator, in both medial and lateral amygdala. These results suggest that the β-adrenoceptor-cyclic AMP system plays a role in facilitating LTP in the medial amygdala, but suppresses synaptic plasticity in the lateral amygdala.

135.6

LIPOXYGENASE METABOLITES OF ARACHIDONIC ACID AS MODULATORS OF SYNAPTIC TRANSMISSION IN RAT HIPPOCAMPUS. **B. Liu, T. Tieman, S.J. Feinmark and J.H. Schwartz*. Center for Neurobiology & Behavior and Department of Pharmacology, Columbia University, NY, NY 10032.**

Arachidonic acid (AA) metabolites produced by lipoxygenase have been proposed as second messengers for presynaptic inhibition of *Aplysia* sensory neurons and as retrograde messengers in long-term potentiation (LTP) in hippocampus. In the present study, we examined the effect of a selective 12-lipoxygenase inhibitor, CDC (cinnamyl-3,4-dihydroxy-α-cyanocinnamate), on synaptic activity in the CA1 region of transverse hippocampal slices. CDC (0.1-1 μM) reversibly reduced the basal slope of EPSPs. When CDC-treated slices had reached a stable but reduced EPSP slope, the stimulus intensity was increased to return the response to control levels. At that point a train of tetanic stimuli were delivered to the Schaffer-commissural pathway. LTP in slices treated with CDC was impaired compared to those in control (DMSO, 0.1%) slices. Further, we confirmed the presence of lipoxygenase in hippocampus by Western blotting using an antibody which cross-reacts with several lipoxygenase isoforms. Hippocampal tissue metabolized radiolabeled AA into at least four products. The formation of these products was blocked by NDGA (nordihyroguaiaretic acid, a nonspecific inhibitor), as well as by CDC, indicating that they are probably derived from the action of a 12-lipoxygenase. Our data suggest that AA metabolites of the 12-lipoxygenase pathway may play an important role as modulators of synaptic activity in rat hippocampus. **(Supported by NIH grants NS29832, MH00921 [JHS] and NS10035 [BL])**

135.7

β-ADRENERGIC AND MUSCARINIC RECEPTORS PARTICIPATE IN THE LTP INDUCTION IN RAT HIPPACAMPAL CA1. M. Kobayashi, M. Ohno, S. Shibata#, T. Yamamoto and S. Watanabe*. Dept. Pharmacol. Fac. Pharmaceut. Sci., Kyushu Univ. Fukuoka 812-82, # Dept. Pharmacol. Waseda Univ. Tokorozawa 359, Japan.

Neurotransmitters acting through G-protein-coupled receptors regulate the electrical excitability of neurons by modulating various ion channels. There are good evidences that the metabotropic GluR activation is critical for the induction of LTP and the muscarinic ACh receptor activation can facilitate the LTP formation. However, there is no evidence indicating the participation of norepinephrine in the tetanus-induced LTP formation in area CA1 of the hippocampus. To clarify an involvement of NE system in LTP in area CA1, we examined the effect of concurrent blockade of adrenergic and muscarinic receptors on population spike and the induction of LTP in rat hippocampal CA1 neurons. Consistent with previous findings, separate application of the α-adrenergic antagonist phentolamine (50 μM), the β-adrenergic antagonist propranolol (10 μM) and the β1-adrenergic antagonist atenolol (10 μM) had no effect on the induction of LTP by tetanic stimulation (100 Hz, 1 sec). The muscarinic receptor antagonist scopolamine (10 μM) suppressed the increase of population spike amplitude after tetanus (60 min, 136±17%), but low dose (1 μM) did not. Co-application of propranolol, but not phentolamine, with ineffective dose of scopolamine (1 μM), completely prevented the induction of LTP (103±8%), suggesting the induction of LTP requires co-activation of β-adrenoceptor and muscarinic receptor. Furthermore, atenolol, which has no potential membrane stabilizing action, also prevented the induction of LTP when combined with scopolamine (92±9%). In the experiment of extracellular EPSP recordings, concomitant application of atenolol and scopolamine showed the similar effects.

We conclude that β1-adrenoceptor plays some role in the induction of LTP in area CA1 of the hippocampus.

135.8

RAPID TOLERANCE TO BENZODIAZEPINE MODIFIES HIPPOCAMPAL SYNAPTIC PLASTICITY. O. A.Ramirez†, Raúl H. Marín‡, Nancy A. Salvatierra‡, D. Masco*‡ †Departamento de Farmacología, Fac. Ciencias Químicas, ‡Catedra de Química Biológica, Fac. Ciencias Exactas Físicas y Naturales, Universidad Nacional de Córdoba, Argentina.

Glutamate antagonists to N-methyl-D-aspartate (NMDA) receptors blocks the development of "rapid" tolerance to the sedative action of benzodiazepines (BZDs). This kind of glutamate receptors is closely related to synaptic plasticity in different areas of the brain such as hippocampus. In the present investigation, we studied the synaptic plasticity in dentate gyrus (GD) of the hippocampus during the development of tolerance to the hypomotility action induced by diazepam (DZ). The results show an increased hippocampal synaptic plasticity in slices from rats treated with diazepam (5mg/kg/day) during four days, assesed as a decrease of the threshold in the stimulation frequency for Long-Term Potentiation (LTP) elicitation. These results reveal a positive correlation between the synaptic plasticity and development of BZD tolerance to locomotor activity. Supported by grants from CONICET and CONICOR Argentina to O.A.R

135.9

ROLE OF PROTEIN PHOSPHATASES IN HIPPOCAMPAL LONG-TERM POTENTIATION.

K. Fukunaga[1]*, D. Muller[2] and E. Miyamoto[1]. [1]Dept. of Pharmacol., Kumamoto Univ. Sch. of Med., Kumamoto 860, Japan and [2]Dept. of Pharmacol., Centre Médical Univ., 1211 Geneva 4, Switzerland

The activation of CaM kinase II plays a crucial role in the molecular mechanisms that have been proposed to contribute to long-term potentiation (LTP) in the hippocampus. Induction of LTP in CA1 regions of hippocampal slices was associated with long-lasting increases in Ca^{2+}-independent and total activities of CaM kinase II (J. Biol. Chem., 270: 6119, 1995). In the experiments with cell extracts from CA1 regions, calyculin A-sensitive protein phosphatases and Mg^{2+}-dependent protein phosphatase were involved in the dephosphorylation of autophosphorylated CaM kinase II. Among calyculin A-sensitive protein phosphatases, phosphatase 2A was mainly contributed to dephosphorylation of CaM kinase II, because inhibitor 2 had little effects. In the LTP-induced slices, a significant decrease in calyculin A-sensitive protein phosphatase activity was observed without change in Mg^{2+}-dependent protein phosphatase activity using autophosphorylated CaM kinase II as substrate. The decrease was prevented when LTP induction was inhibited by treatment with D-AP5. We further demonstrated with ^{32}P-labeled hippocampal slices that application of high frequency stimulation resulted in an increase in phosphorylation of inhibitor 1 1 hr after LTP induction. These results support the idea that the reduced changes in protein phosphatase activities during LTP induction may be implicated in the maintenance of constitutively active CaM kinase II and phosphorylation of its substrates

135.10

ACTIVATION OF PROTEIN PHOSPHATASES 1 AND 2A IN ASSOCIATION WITH LONG-TERM DEPRESSION IN HIPPOCAMPUS *IN VIVO*.

E.D. Norman*, E. Thiels, G. Barrionuevo, E. Klann. *Department of Neuroscience, University of Pittsburgh, Pittsburgh, PA 15260*

We previously have shown that the maintenance of NMDA receptor-dependent paired-pulse stimulation-induced long-term depression (PPS-LTD) of the commissural input to CA1 pyramidal cells in the hippocampus of anesthetized adult rats is associated with increase protein phosphatase (PPase) activity (*Soc. Neurosci. Abstr.* 21:1097). In order to determine the time course of activation of PPases after induction of PPS-LTD, we measured PPase activity in homogenates of dorsal area CA1 (recording site) excised either 5, 35, or 65 min after the final of three trains of 200 paired pulses (25-ms interpulse interval; 2-sec interpair interval; 15-min intertrain interval) delivered to the contralateral dorsal area CA3.

LTD (dorsal) CA1 and control (ventral) CA1 homogenates were added to PPase assays that included ^{32}P-labeled phosphorylase kinase (PK), a PPase substrate consisting of an α subunit, which is dephosphorylated by PPases 2A, 2B, and 2C, and a β subunit, which is dephosphorylated by PPase 1. Dephosphorylation of the α and the β subunit was quantified using autoradiograpy and densitometry.

In PPase assays of the 5-min samples, we observed a significant increase in dephosphorylation of both the α subunit (control=94±8%; LTD=75±5%) and the β subunit (control=88±5%; LTD=75±4%) of PK. In contrast, a significant increase in dephosphorylation of only the α subunit was detected in the 35-min samples (control=89±4%; LTD=72±3%). No significant increase in dephosphorylation of either subunit was detected in the 65-min samples. In PPase assays of the 35-min samples, addition of okadaic acid (1μM), a potent inhibitor of PPase 2A, blocked the LTD-associated increase in dephosphorylation of the α subunit (control=100±6%; LTD=100±9%). These results indicate that PPS-LTD is associated with an increase in activity of PPases 1 and 2A, and that the increase of PPase 2A activity lasts longer than that of PPase 1 activity. [Supported by NIMH (E.T.) and NINDS (E.K.)].

135.11

SEROTONIN INHIBITS THE INDUCTION OF LONG-TERM POTENTIATION IN RAT PRIMARY VISUAL CORTEX.

Y. Edagawa*, H. Saito and K. Abe. Dept. of Chem. Pharmacol., Fac. of Pharmaceut. Sci., The Univ. of Tokyo, Bunkyo-ku, Tokyo 113, Japan.

The synapses of the mammalian primary visual cortex display long-term potentiation (LTP), which may underlie experience-dependent plasticity. It has been reported that serotonergic fibers are observed in the early development of primary visual cortex in rats. To determine the role of serotonin in synaptic plasticity in this brain region, we investigated the effect of serotonin on the induction of LTP by using slice preparations in vitro. Acute slices (400 μm thickness) were prepared from primary visual cortex region of male Wistar rats (3-5 weeks old). The slices were maintained in a chamber at 34 ℃, where they were continuously perfused with physiological salt solution. Bipolar stimulating electrode was placed in layer IV, and the evoked potentials were extracellularly recorded from layer II/III. Single-pulse test stimulation of layer IV evoked two distinct components of sharp negative-going potentials, i.e. antidromic and orthodromic responses. When tetanic stimulation (100 Hz for 1 sec, twice at an interval of 30 sec) was applied, only synaptic component was potentiated. The potentiation lasted longer than 60 min and was regarded as LTP (response amplitude 30-60 min after tetanus, 140.18±7.52 % of baseline, n=8). Serotonin (10 μM) did not affect the baseline synaptic potentials evoked by single-pulse test stimulation, but inhibited the induction of LTP (105.23±3.99 %, n=5). The inhibitory effect of serotonin was concentration dependent (0.1-10 μM), and was blocked by the concomitant presence of 10 μM pindolol, 5-HT1A,1B receptor antagonist, or 10 μM ritanserin, 5-HT2 receptor antagonist. These results suggest that serotonin plays a role in suppressing the induction of LTP in the primary visual cortex of rats.

135.12

SEROTONIN-INDUCED PLASTICITY IN THE KITTEN VISUAL CORTEX IS ASSOCIATED WITH AN ACTIVATION OF NMDA RECEPTORS AND VOLTAGE-GATED CALCIUM CHANNELS.

L. Kojic*, Q. Gu, R.M. Douglas, J. Matsubara and M. Cynader. Department of Ophthalmology, University of British Columbia, 2550 Willow St., Vancouver, B.C., Canada V5Z 3N9

Recently, we suggested an involvement of a particular family of serotonin receptors (5-HT_2) in the regulation of visual cortex plasticity, a hypothesis supported by both anatomical and electrophysiological data. To further study the mechanisms of serotonin-induced plasticity we here define an interaction with the two previously reported induction pathways of visual cortex plasticity, namely the activation of NMDA receptors and of voltage-gated Ca^{2+} channels. To directly test the role of serotonin receptors in cortical plasticity, we recorded field potentials in visual cortex slices from 40-65 and >120 day old kittens. Field potentials were recorded from layer IV after white matter stimulation, before and after a period of low frequency stimulation (LFS; 1Hz, 15 min). In visual cortical slices, from 40-65 day old kittens, LTD or LTP was induced by LFS only if serotonin (1 or 10μM) was applied before LFS. In the presence of the NMDA receptor antagonist dAPV or the L-type Ca^{2+} channel blocker nifedipine no long-term plasticity was observed after coapplication of LFS and serotonin. Furthermore, no such serotonin-induced long-term plasticity was ever detected in >120 day old animals. The present results suggest that the serotonin-induced long-term plasticity in the kitten visual cortex involves an increase of intracellular calcium, which occurs through an activation of NMDA receptors and/or L-type Ca^{2+} channels. One possibility is that the calcium release, consequent to an induced PI turnover by the activation of 5-HT_2 receptors, might be additive with the calcium influx through NMDA receptors and L-type voltage-gated Ca^{2+} channels. Furthermore, our finding that there was no serotonin-induced long-term plasticity detected in visual cortical slices from adult animals (>120 days) is consistent with this hypothesis, since a substantial reduction of NMDA receptors, L-type Ca^{2+} channel binding sites, and 5HT_2 receptors was observed in layer IV of the adult cat.

135.13

ADENOSINE A_{2b} RECEPTORS MODULATE HIPPOCAMPAL SYNAPTIC TRANSMISSION VIA A cAMP DEPENDENT PATHWAY.

K. Kessey* and D.J. Mogul. Depts.of Biomedical Engineering and Neurobiology& Physiology, Northwestern Univ., Evanston, IL 60208 USA.

The effects of A_2 receptor activation on normal synaptic transmission and tetanus-induced LTP were examined at the Schaffer collateral/CA1 synapse in rat transverse hippocampal slices. Receptor activation with the A_2 agonist DPMA reversibly enhanced the field EPSP slope during low frequency test pulses (0.033Hz). In the presence of A_1 receptor blockade with CPT, DPMA further enhanced the EPSP over that observed with CPT. A_2 receptor blockade using DMPX in the presence of CPT led to a reversible decrease of the EPSP. These effects appear to be a result of A_{2b} receptor manipulation because the A_{2a} receptor agonist CGS21680 had no effect on the EPSP. During A_1 receptor blockade, A_2 receptors significantly modulated the level of tetanus-induced LTP (100Hz,1s). In the presence of CPT alone, the level of LTP attained was 163.5±9.1% (mean±SEM). In comparison, when A_2 and A_1 receptors were blocked with DMPX and CPT respectively, the level of LTP was 121.3± 1.9%. In contrast, A_2 receptor activation with DPMA in the presence of CPT resulted in LTP of 190.2±10.5%. Using forskolin, the direct adenylate cyclase activator, we were able to restore 53% of the LTP blocked by DMPX suggesting that the effects of A_2 receptors on synaptic transmission may be mediated through the regulation of intracellular cAMP. These results suggest that A_2 receptors may play a significant modulatory role in normal synaptic transmission and contribute to LTP through their stimulatory influence on cAMP. Supported by the NIH (NINDS NS31764) and the Whitaker Foundation.

135.14

D1 DOPAMINE RECEPTOR ACTIVATION INCREASES EARLY LTP IN SCHAFFER - CA1 HIPPOCAMPAL SYNAPSES

N. Otmakhova*, J. Lisman. *Department of Biology and Volen Center for Complex Systems, Brandeis University, Waltham, MA 02254.*

The role of the mesolimbic dopaminergic system in the reinforcement of learning suggests that dopamine should be able to modulate activity-dependent synaptic plasticity in a synapse-specific manner. We have examined the effect of D1 agonists and antagonist on early LTP (40 min. after a single tetanus). Field EPSPs were monitored in the CA1 region of the hippocampal slices using 2 independent pathways. The drugs were applied in 5-10 µM concentrations into the bath for 5 min. before the tetanus. D1 agonists (Bromo-APB, Chloro-PB, Dihydrexidine) increased the magnitude of LTP in a synapse-specific manner by 5-20%. The effect of D1 agonists could be mimicked by the activator of adenylyl cyclase, forskolin (10 µM). The D1 antagonist, SCH 23390 (5 µM), reduced early LTP by 5-10%, suggesting that under normal conditions dopamine released during the tetanus (Frey et al., 1990) facilitates the induction of LTP.

In slices depleted of catecholamines LTP was substantially (by 10-25%) decreased. D1 antagonist became ineffective. On the other hand, both D1 agonist and forskolin produced larger enhancements of LTP in depleted slices, restoring it to the normal level.

These results suggest that dopamine can participate in the mechanisms of LTP induction and produce a synapse-specific enhancement of early LTP through D1 receptors and cAMP.

This work was supported by a NIH grant (5 R01 NS27337-7), W. M. Keck Foundation, and generous donations of dopaminergic drugs from NIMH Synthesis Program at RBI (Natick, MA), Abbot Laboratories (Abbot Park, IL), and Interneuron Pharmaceuticals Inc. (Lexington, MA).

135.15

DOPAMINERGIC MODULATION OF LONG-TERM POTENTIATION IN THE HIPPOCAMPAL-PREFRONTAL CORTEX PATHWAY. T.M. Jay*, F. Burette and S. Laroche. NAM, CNRS URA 1491, Université Paris-Sud, 91405 Orsay, France.

High-frequency stimulation of the hippocampus (CA1-subicular region) induces long-term potentiation (LTP) in the prelimbic area of the prefrontal cortex (PFC). Previous studies have shown that the hippocampal input to the PFC is glutamatergic and reaches the deep layers of the prelimbic cortex. As DA innervation is particularly dense in this area and in close proximity to excitatory axonal terminals, we examined the effects of DA on synaptic transmission and LTP in the hippocampal-PFC pathway. Intracerebral microdialysis combined with recording of PFC extracellular fields potentials evoked by stimulation of the hippocampus was carried out in anaesthetized rats. After one hour baseline stimulation in the presence of aCSF (2µl/min), DA was infused in the PFC for one hour (30 min prior to tetanic stimulation). In the first series of experiments, DA was infused at 1mM. PFC potentials evoked by test stimuli were significantly increased (9.86 %) by DA. Tetanus-induced LTP in the presence of DA was greater in amplitude (71.7 %) when compared to the controls (47.9 %) and, one hour after the tetanus, LTP was maintained and significantly greater than in controls (74.5% compared to 28.9 % in the controls). In a second series of experiments, DA was infused at 5 mM in rats receiving a perfusion of nomifensine (5 µM in aCSF; uptake inhibitor) one hour prior to DA infusion. Nomifensine increased significantly (15.6 %) the amplitude of the evoked response and this increase was enhanced to 22.6 % with the infusion of 5mM DA. During infusion of DA, tetanic stimulation of the hippocampus induced reliable LTP of the evoked response in the PFC and the amplitude of LTP was higher (68.2 %) than that of the controls. These data indicate that DA enhances excitatory synaptic transmission in the hippocampal-PFC pathway and suggest that, in vivo, DA may exert a facilitatory effect on the expression of LTP in this pathway. Studies to further investigate this modulation are currently underway.

135.16

D1/D5 RECEPTORS MEDIATE A PROTEIN SYNTHESIS-DEPENDENT LATE PHASE OF LTP IN THE AMYGDALA. Y.-Y. Huang* and E.R. Kandel. Ctr. Neurobiol. & Behav., Columbia Univ., HHMI, NY, NY 10032.

There is now good evidence that the major synaptic pathways of the hippocampus, a structure thought to be important for explicit memory, show a late phase of LTP that require cAMP, PKA, and protein synthesis. What about implicit forms of storage in the mammalian brain? Does LTP in the amygdala, thought to be critical for emotionally charged implicit storage, have a protein synthesis-dependent late phase? If so, which second messenger mediates this phase? We found that multiple tetani applied to external capsule of rat brain slices induced a protein synthesis-dependent late phase LTP (> 3 hrs) in the basal lateral amygdala. A similar late phase could be induced 1 hr after application of an agonist of the D1/D5 receptor. This late agonist-induced potentiation is mediated by PKA and blocked by anisomycin. Moreover, antagonists of the D1/D5 receptors significantly depress the late phase of LTP in the amygdala. These results suggest that the LTP in the amygdala has a late as well as an early phase and that D1/D5 receptors and cAMP/PKA play a role in the late phase.

(Supported by HHMI and Grant GM32099 from NIH, as well as Columbia component of the Dana Consortium on age-related memory loss.)

LONG-TERM POTENTIATION: PHARMACOLOGY II

136.1

STREPTOZOCIN-INDUCED DIABETES MELLITUS IMPAIRS WATER-MAZE LEARNING AND EXPRESSION OF LONG-TERM POTENTIATION IN RAT HIPPOCAMPUS IN VITRO

A. Kamal , G.J. Biessels, I.J.A. Urban and W.H. Gispen
Rudolf Magnus Institute for Neuroscience, University of Utrecht, The Netherlands. (Spon: ENA)

Peripheral neuropathies and cognitive impairments have been among recognized complications of diabetes mellitus (DM). We have studied the influence of a severe form of diabetes mellitus(DM), (induced by i.v. injections of streptozocin(STZ; 40mgSTZ/kg body weight) on conduction velocity of the sciatic nerves, water-maze (WM) learning and the expression of long-term potentiation (LTP) in CA1 field of the hippocampus slice from rats with a severe DM, mild DM (a partial insulin substitution) and age-matched controls. The sciatic nerve conduction velocity and WM learning in rats with a severe form of DM (25.6±1 mMol/l blood glucose) was significantly slower than thhose in rats with the mild form of DM (18.9±1.8 mMol/l blood glucose) or age-matched controls. In addition, the expression of LTP in the CA1 field of the hippocampus in slices from severely DM rats was significantly below that in slices from moderately DM and control rats. Neither WM learning nor expression of the hippocampus LTP in rats with mild form of DM differed significantly from these measures in controls. However, the conduction velocity of the sciatic nerve was slower than that in control rats. It is demonstrated that the severe form of DM, in addition to the sciatic nerve neuropathy, also impairs the hippocampus synaptic plasticity and water-maze learning. The mild DM affects the peripheral nerves only.

136.2

DIFFERENCE IN EXPRESSION OF LONG-TERM DEPRESSION IN THE CA1 FIELD OF THE HIPPOCAMPUS IN SLICES FROM DIABETES MELLITUS AND CONTROL RATS. I.J.A. Urban, A. Kamal, G.J. Biessels and W.H.Gispen. Rudolf Magnus Institute for Neuroscience, P.O. Box 80040, 3508 AT Utrecht, The Netherlands. (Spon: ENA)

Long term depression (LTD) is a decrease in synaptic strength produced by a low frequency stimulation of afferent fibers. LTD is considered to be an opposite to long-term potentiation (LTP). Elsewhere we shown (see Kamal et al., this Meeting) that streptozocin(STZ)-induced diabetes mellitus (DM) impairs the expression of LTP in the CA1 field of the hippocampus slices. Here we studied the expression of LTD in the CA1 field of the hippocampus in slices from rats suffering 10 or 20 weeks from DM, from 20 weeks DM rats that the 10 last weeks received insulin, in slices from rats with 10 weeks of DM with or without insulin substitution and from age-matched control rats. LTD was produced by stimulating the radiate layer either two times for 15 min at 1 Hz in a 15 min interval (type 1) or 5 min at 1 Hz, followed by 5 min test stimulation, 5 min at 1 Hz followed by 5 min at 5 Hz (type 2). The slices from 20 months DM rats showed at 60 min after the stimulation on average a 30±% LTD in the slope of the field excitatory postsynaptic potentials (fEPSPs), regardless the stimulation type used. However, control slices stimulated with the type 1 showed only 11.4-±2.3% LTD in the slope of the fEPSPs. In slices stimulated wirth type 2 was LTD 14.1±5.0.2%. In insulin substituted slices LTD to stimulation 1 was on average 13.7%. LTD induced by type 2 stimulation was on average 5.5%. The slices from 10 weeks DM rats showed a similar pattern in response to both stimulation types, however, the difference in the amount of LTD between amongst groups was less pronounced than that in the 20 weeks DM rats. Thus, severe DM significantly enhances the LTD expression in the CA1 field of the rat hippocampus. Insuline failed to abolish the LTD enhancing effect of this DM.

136.3

ROLE OF CADHERIN MOLECULES IN SYNAPTIC PLASTICITY IN THE ADULT RAT HIPPOCAMPUS. L. Tang*, C. P. Hung and E. M. Schuman, Division of Biology 216-76, Caltech, Pasadena, CA 91125

It has been suggested that certain cell adhesion molecules (CAM) can modulate synaptic transmission and hippocampal long-term potentiation (LTP). In the present study, we explored the role of classic cadherins, in particular N-cadherins, in synaptic transmission and LTP in CA1 region of young adult rat hippocampus. Immunostaining and detection with confocal microscopy suggest that cadherins and the associated cytoplasmic proteins, catenins, are present in young adult rat hippocampus. Additional studies in dissociated cultured hippocampal neurons indicate that much of the cadherin staining colocalizes with staining for the synaptic vesicle protein, synapsin I. There is evidence that synthetic peptides containing the consensus sequence "HAV", which is highly conserved among many different cadherins, can inhibit cadherin-mediated processes. In "two-pathway" experiments, bath application the synthetic peptide AHAVD (0.2 mM) significantly reduced LTP [mean % baseline @ 50-60 min post tetanus: control 130.5±7.4%, AHAVD 110.8±3.1% ($p<0.01$, n=10)]. The peptides were without effect on basal synaptic transmission. In contrast, bath application of 3 different control peptides (0.2 mM) were without effect on LTP ["AEAVD": control 139.3±7.6%, AEAVD 137.5±4.9% ($p>0.8$, n=6); "AHSVD": control 135.7±6.1%, AHSVD 130.6±4.3% ($p>0.1$, n=7); scrambled sequence "AADHV": control 138.1±7.3%, AADHV 137.0±6.7% ($p>0.8$, n=7)]. Further studies suggested that the observed reduction of LTP by "AHAVD" peptides is not due to blockade of NMDA receptor mediated responses. We also investigated whether treating slices with a function-blocking N-cadherin antibody can affect subsequently recorded LTP. We found that N-cadherin antibody preincubated slices showed less LTP than control antibody preincubated adjacent slices [control antibody 157.7±13.0%, N-cadherin antibody 110.0±7.0% ($p<0.001$, n=10)]. Our immunostaining data confirm that these antibodies can efficiently penetrate slices after ≥ 1 hr incubation. Taken together, these data suggest that cadherins, presumably N-cadherins, are involved in hippocampal synaptic plasticity.

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136.4

ACTIVITY SWITCHING OF CAMKII IN LONG-TERM POTENTIATION---THEORETICAL ANALYSIS, Hideyuki Câteau* and Shigeru Tanaka, Lab. for Neural Modeling, The Institute of Physical and Chemical Research (RIKEN), Hirosawa, Wako, Saitama 351-01, Japan.

Semipermanent switching of the kinase activity of CaMKII caused by its autophosphorylation has been suggested to play a central role in the induction of long-term potentiation (LTP). Cooperative activation among subunits constituting a CaMKII holoenzyme is speculated to maintain its kinase activity, resulting in a long-lasting conformational change and asociated change in syanaptic efficacy. To test this hypothesis, we carried out simulations of dynamical transitions of CaMKII states triggered by a transient rise in $[Ca^{2+}]_i$ and observed the following behavior: 1) If the CaMKII is initially in an off (catalytically inactive) state, it remains in the off state, and if it is initially in an on (catalytically active) state it remains in the on state. 2) A large transient rise in $[Ca^{2+}]_i$ induces a switching of the CaMKII state from off to on. In order to identify the critical determinant for this clear-cut switching, we reformulated the CaMKII dynamics based on statistical mechanics. We found that the on/off states are described by an energy function, which justifies the stability of these states. The energy function derived is mathematically equivalent to that of magnetic material, so that we can understand the clear-cut nature of on/off switching of CaMKII activity in terms of magnetism.

136.5

SIGNALING TARGETS OF REACTIVE OXYGEN SPECIES IN THE HIPPOCAMPUS. L.T. Knapp*, B.I. Kanterewicz and E. Klann. *Department of Neuroscience, University of Pittsburgh, Pittsburgh, PA 15260.*

We previously have shown that superoxide ($\bullet O_2^-$), a reactive oxygen species (ROS), modulates the induction of hippocampal long-term potentiation (LTP), possibly via interactions with protein kinase C (PKC) (*Soc. Neurosci. Abstr.* **21**:109). Consistent with this idea, hippocampal glutamate receptor activation, including NMDA receptor activation, results in increased production of ROS, including $\bullet O_2^-$ (*J. Neurosci.* **16**:1324). Furthermore, oxidation with hydrogen peroxide (H_2O_2) of either purified PKC or hippocampal homogenates results in an increase in PKC activity (*Biochem. Biophys. Res. Comm.* **187**:1439). Therefore, we determined if $\bullet O_2^-$ activates PKC directly by incubating either hippocampal homogenates or purified PKC with xanthine/xanthine oxidase (X/XO), an $\bullet O_2^-$-generating system. Treatment of either purified PKC or homogenates with X/XO resulted in an increase of both autonomous and cofactor-dependent PKC activity. The X/XO-induced increase in PKC activity was attenuated by superoxide dismutase which suggests that $\bullet O_2^-$ can activate PKC directly. To determine if ROS such as H_2O_2 and $\bullet O_2^-$ can activate biochemical signaling cascades in hippocampal slices, we incubated slices with H_2O_2. Treatment of slices with H_2O_2 also resulted in an increase of both autonomous and cofactor-dependent PKC activity. Because activation of PKC has been shown to increase protein tyrosine phosphorylation in hippocampal slices, we used a phosphotyrosine antibody to determine changes in protein tyrosine phosphorylation in slices exposed to H_2O_2. Incubation of slices with H_2O_2 resulted in increased tyrosine phosphorylation of a number of proteins. Bath application of NMDA to activate NMDA receptors resulted in increased tyrosine phosphorylation of a similar subset of proteins. The NMDA-induced increase in phosphorylation was blocked by the antioxidant N-acetylcysteine. Taken together these results suggest that ROS are capable of activating signaling cascades in the hippocampus, and that similar mechanisms may occur during induction of hippocampal LTP. Supported by NINDS grant NS34007, the Winters Foundation, and a University of Pittsburgh CRDF grant.

136.6

PRENATAL COCAINE EXPOSURE RESULTS IN INCREASED SENSITIVITY OF LTP TO A SEROTONIN ANTAGONIST J.Z. LITTLE* and T.J. TEYLER Dept. of Neurobiology, Northeastern Ohio Univ. Coll. of Med., Rootstown, Ohio 22472

Cocaine exposure during development is known to decrease the number of serotonin staining projections to the hippocampus. Serotonin neurotransmission modulates the magnitude of LTP in hippocampal area CA1. In a rabbit model of in utero cocaine exposure we examined the effects of 1μM methiothrepin maleate, a serotonin autoreceptor antagonist, on the induction and maintenance of LTP in area CA1. Induction of LTP by a 100Hz/1sec tetanus was not altered in control animals by the presence of methiothrepin maleate. LTP in offspring exposed to cocaine during gestation was significantly reduced compared to LTP in the absence of methiothrepin maleate. The magnitude of LTP in cocaine exposed animals following methiothrepin maleate was similar to LTP induced in control animals regardless of whether methiothrepin maleate was present or absent during tetanus.

This project was supported by NIDA Grant #DA06871

136.7

ISOLATION OF "MINI"-SYNAPTONEUROSOMES FOR SYNAPTIC PLASTICITY STUDIES IN HIPPOCAMPAL SLICES. [1]M. W. Johnson, [1]J. J. Rubino, [2]T. J. O'Dell, and [1]J. B. Watson*. [1]Department of Psychiatry and Biobehavioral Sciences, [2]Department of Physiology, UCLA School of Medicine. 90024-1759.

The availability of a reliable method for subcellular fractionation of synapses in individual hippocampal slices would greatly enhance our ability to reconstruct the neurochemical signalling pathways that underlie LTP/LTD forms of plasticity. We have scaled down the original synaptoneurosome preparation from whole brain (Hollingsworth et al, 1985, J Neurosci 5, 2240) to a "mini" synaptoneurosome fraction isolated from mouse hippocampal slices. Slices were maintained in interface chambers under conditions normally used for electrophysiological studies of LTP, homogenized on ice in an Eppendorf tube with a teflon hand pestle, and sequentially filtered [nylon mesh, Millipore filter (5μm)] in a filter-holder attached to a 1 cc Tuberculin syringe. The filtrate was centrifuged at low speed to generate a pellet ("mini" synaptoneurosomes) and supernatant (cytosol). Western blotting studies with antisera to known pre- and post-synaptic markers (α-CaM Kinase II subunit, GAP-43, NMDAR1 receptor subunit) show that "mini" preps exhibit expression profiles comparable to controls. The "mini" synaptoneurosome preparation has the potential to identify a variety of subcellular neurochemical changes including novel kinase activities and substrates after the induction of plasticity. Supported by NIH grant NS32521 and a UCLA Stein-Oppenheimer Award.

136.8

DANTROLENE (BUT NOT THAPSIGARGIN) PREVENTS THE LONG-TERM POTENTIATION INDUCED IN CA1 REGION OF RAT HIPPOCAMPAL SLICES BY 2-DEOXYGLUCOSE (2DG).

A.T. Tan*, S. Tekkök and K. Krnjević Anaesthesia Research & Physiology Depts., McGill University, Montréal, PQ, H3G 1Y6 Canada.

As reported elsewhere (Tekkök and Krnjević, this meeting), Ca^{2+} released from an internal store appears to be essential for the induction of 2DG LTP in CA1. Dantrolene, which blocks internal Ca^{2+} release in hippocampal cells, at 10 μM completely blocked induction of 2DG LTP -- afferent volley-EPSP (V-E) relation up by 2 ±1.2 %, n=7. Tetanic LTP was unaffected by dantrolene (≤ 50 μM).

A curious finding was that a preceding tetanic LTP prevented the suppression of 2DG LTP: in 10 μM dantrolene, V-E slope increased by 41 ± 13.6 % after 2-DG application, n=5). Also, even 20 μM dantrolene did not suppress 2DG LTP of NMDA receptor-mediated EPSPs (53 ± 14.6 % increase in V-E slope, n=6), indicating a significant dissociation of the components of EPSPs in 2DG LTP.

In agreement with previous studies, depotentiation by 1 Hz stimulation (for 15 min) was abolished by 20 μM dantrolene. Moreover, 10 μM thapsigargin, which blocks tetanic LTP, did not prevent 2DG LTP (V-E slope increased by 75 ± 15.6 %, n=5).

These results suggest that the induction of 2DG LTP requires Ca^{+2} release from a dantrolene-sensitive store; whereas tetanic LTP depends on Ca^{+2} release from a thapsigargin-sensitive store. This is further evidence that different mechanisms are involved in 2DG- and tetanic-LTP.

(Supported by the Medical Research Council of Canada and Hacettepe University, Ankara).

136.9

THE INVOLVEMENT OF METABOTROPIC GLUTAMATE CLASS I RECEPTORS IN LTP DEPENDS ON TETANIZATION STRENGTH.

V. Wilsch, T. Behnisch*, T. Jäger, D. Balschun, R. Pellicciari[1] and K.G. Reymann[2] Federal Inst. for Neurobiology, Dept. Neurophysiol.; [2]Inst. of Applied Neuroscience GmbH, Brenneckestr. 6, D-39118 Magdeburg; [1]Istituto di Chimica e tecnologia del Farmaco Univ. degli studi de Perugia, Via del Liceo, 06100 Perugia

The eight subtypes of the metabotropic glutamate receptors (mGluRs) which are known so far can be pharmacologically divided in three classes. Beyond other functions mGluRs have been found to play a role in synaptic plasticity. ACPD an mGluR class 1 and 2 agonist enhances LTP whereas MCPG an unspecific mGluR class 1 and 2 antagonist either to an inhibits LTP or has no effect. To further resolve the role of class 1 mGluR in LTP, we employed MCPG and the specific class 1 antagonists 4-CPG and (RS)-1-Aminoindan-1,5-dicarboxylic acid (UPF 523) as well as the specific class 1 agonist DHPG in two different types of LTP. Field excitatory postsynaptic potentials (fEPSPs) were recorded in the hippocampal CA1 area and a 'weak' or a 'strong' LTP were induced by two different tetanization paradigms (tree trains of 100 Hz for 500 ms every 2 minutes or a single train of 100 Hz for 400 ms, pulsewidth 200 μs).

In the 'weak' LTP, MCPG (400 μM) and 4-CPG (100 μM) led to clear-cut reduction of potentiation in comparison to controls. DHPG (15 μM) resulted in a decline of baseline values immediately after application which apparently masked a facilitation of LTP induction. However, 50 min after tetanization when this baseline depression had ceased, the potentiation was significantly enhanced compared to controls. 4-CPG acted in a concentration dependent manner with an IC_{50} of 9.9 ± 0.7 μM and a Hill coefficient of 1.03. UPF 523 had no effect. This can be attributed to its supposed selectivity towards mGluR 1 which seems to be limited to glial cells and CA1 interneurons. All drugs did not exert any influence on 'strong' LTP. We conclude that activation of class 1 mGluRs plays a role in a 'weak' LTP but in the 'strong' LTP other mechanisms compensate mGluR activation.

136.10

REMOVAL OF EXTRACELLULAR CALCIUM AFTER CONDITIONING STIMULATION DISRUPTS CA1 HIPPOCAMPAL LONG-TERM POTENTIATION.

H. Katsuki*, Y. Izumi and C.F. Zorumski. Departments of Psychiatry and Anatomy / Neurobiology, Washington Univ. Sch. Med., St. Louis, MO 63110.

Ca^{2+} influx from extracellular milieu into cytoplasm is generally believed to be crucial for the induction of long-term potentiation (LTP) in hippocampal synapses. In this study we demonstrate that extracellular Ca^{2+} is required for full production of LTP for a much longer period than previously thought. Field excitatory postsynaptic potentials evoked by Schaffer collateral stimulation were recorded from the stratum radiatum in the CA1 region of rat hippocampal slices. An extracellular solution containing no added Ca^{2+} and 10 mM Mg^{2+} was perfused for 15 min after the induction of LTP by theta burst stimulation (TBS) of Schaffer collateral input. Although synaptic responses in the control (non-potentiated) pathway recovered to baseline level after washout of 0 mM Ca^{2+} / 10 mM Mg^{2+} solution, those in the test pathway did not regain their potentiated level. The effect of Ca^{2+} removal was most prominent when the solution was applied beginning 15 min after TBS, while either earlier or later application (beginning 5 min and 30 min after TBS, respectively) was less effective. The effect of the 0 mM Ca^{2+} / 10 mM Mg^{2+} solution was observed even when afferent stimulation was not delivered during the application of the solution, suggesting that evoked synaptic activity is not required for this effect.

Perfusion with an extracellular solution containing Cd^{2+} (40 μM), a broad spectrum blocker of voltage-dependent Ca^{2+} channels, resulted in similar reduction in the magnitude of LTP. A low concentration (50 μM) of Ni^{2+}, which preferentially blocks low-voltage-activated T-type Ca^{2+} channels, also induced a significant decrease in the magnitude of LTP, whereas an L-type Ca^{2+} channel blocker nifedipine (20 μM) had no effect. These results suggest that the continuous presence of extracellular Ca^{2+} during a critical period after high-frequency synaptic activity is necessary for the production of LTP, and that basal activity of voltage-dependent Ca^{2+} channels plays an important role in the stabilization of plastic changes in hippocampal synaptic transmission.

Supported by Human Frontier Science Program.

136.11

PROLINE-INDUCED POTENTIATION AND TETANUS-INDUCED LONG-TERM POTENTIATION ARE PRODUCED BY DISTINCT, BUT OVERLAPPING, MECHANISMS. S.M. Cohen* and J.V. Nadler. Depts. Pharmacology and Neurobiology, Duke Univ. Med. Ctr., Durham, NC 27710.

We previously reported that either transient or continuous exposure of rat hippocampal slices to proline enhances transmission at Schaffer collateral-commissural synapses. Proline-induced potentiation occurs with proline concentrations that are present in human CSF, is long-lasting and involves activation of the NMDA receptor. We have now investigated the relationship between this form of synaptic plasticity and tetanus-induced long-term potentiation (LTP). Two electrical stimulation protocols were used to induce LTP: a 60-train protocol that evoked saturating LTP and a submaximal protocol (100 Hz for 0.5 s) that evoked LTP of a magnitude somewhat greater than potentiation evoked by 30 μM proline (which averaged 36%). Proline-induced potentiation resembled the LTP produced by the submaximal protocol in not altering paired-pulse facilitation of the fEPSP. However, the saturating LTP protocol reduced paired-pulse facilitation by ~15%. When either saturating or submaximal tetani were delivered after proline-induced potentiation had been established, the effects of the two processes appeared to be additive. Either tetanus, when presented before proline application, prevented the induction of proline-induced potentiation. This was an effect of strong afferent stimulation, not of prior LTP, because the tetani also prevented proline-induced potentiation when they were applied in the presence of 50 μM D-AP5. We suggest that proline-induced potentiation and tetanus-induced LTP are produced by distinct mechanisms, even though both processes depend on activation of the NMDA receptor.
(Supported by NIH grant NS 16064.)

136.12

NITRIC OXIDE SYNTHETASE AND NADH-DIAPHORASE: COMAPARTIVE HISTOCHEMISTRY AND PHARMACOLOGY, K. Talbot *[1], Eric J. Buenz[1], and L.L. Butcher[2]. [1]Dept. of Psychology, St. Olaf College, Northfield, MN 55057 and [2]Dept. of Psychology and Brain Research Institute, UCLA, Los Angeles, CA 90095

The comparative distribution and pharmacological properties of NADPH-diaphorase (a form of nitric oxide synthetase) and of NADH-diaphorase were studied in the brains of female albino rats. Sections were reacted for NADH-d according to the technique of Friede et al. (J. Histochem. Cytochem. 11:232-245, 1963) and for NADPH-d using the method of Hope and Vincent (J. Histochem. Cytochem. 37: 653-661, 1989). The two enzymes were found to differ markedly in their distributionand susceptibility to pharmacological inhibition. NADPH-d was markedly inhibited by 10^{-3} M dichloropheno-lindophenol (DPIP) but not by 10^{-4}M dicumarol, whereas NADH-d was moderately inhibited by 10^{-4}M dicumarol (a classic suppressant of DT-diaphorase) but not by 10^{-3}M DPIP. In the olfactory bulbs, both enzymes were concentrated in the glomeruli, but only NADH-d was prominent in the underlying plexiform layer, and only NADPH-d was prominent in the granule layer. In the hippocampal formation, there were also striking contrasts. NADH-d was concentrated in the terminal field of the perforant path, wheras NADPH-d was restricted to blood vessels and to neuropil deep in CA1 (especially CA1a) and in the adjoining subiculum. Both enzymes were seen, however, in layer IV of the entorhinal cortex (ERC) , as well as in neurons of the diagonal band projecting to the ERC. In fact, both enzymes were similarly distributed throughout parts of the magnocellular basal forebrain projecting to limbic cortex. The distribution of NADH-d neurons in the basal forebrain of macaques proved very similar to the distribution of choline acetyltransferase (ChAT) neurons in alternate sections, suggesting colocalization of the two enzymes. These findings suggest that separate enzymes account for NADPH-d and NADH-d activity , that both enzymes may nevertheless be found in certain cholinergic neurons of the basal forebrain, and that NADH-d may be related to DT-diaphorase (i.e., NADH: quinone-acceptor) oxidoreductase (EC 1.6.99.2]. Supported by St. Olaf intramural grant 011290.

136.13

PRENATAL CO EXPOSURE IMPAIRS LTP IN HIPPOCAMPAL CA1 NEURONS IN SLICES G. Mereu*, W. Francesconi, C. Piccirilli, M.Cammalleri, R. Cagiano, L. Trabace, M. Brunelli, and V. Cuomo. Universities of Cagliari, Pisa, and Bari, Italy.

In rats, prenatal exposure to carbon monoxide (CO, 150 ppm) reduces their performance in an active avoidance task[1]. We have initiated a study on neuronal excitability and long term potentiation (LTP) of CA1 hippocampal neurons in slices taken from male rats (age 20-30 days) whose mothers were exposed, during the gestational period, to normal or CO enriched atmosphere. Slices (350 µm thick) were maintained at 30 °C in an interface chamber and perfused with an artificial CSF. Evoked field potentials were recorded extracellularly in the regions of apical dendrites of the CA1 pyramidal cells following the electrical stimulation (square waves: 20 µs, 1-100 µA, at 0.1 Hz,) of Shaffer collateral/commissural pathway. The responses were evaluated as the slope (mV/ms) of the 80 % of the rising phase of the evoked field potential. Input-output (I/O) curves were used to assess for the neuronal excitability and to establish the stimulus strength for producing half maximal response. This intensity was used for both single and tetanic (1 s, 100 Hz) stimulation.

In the CO treated rats, the I/O curves and the magnitude of the post tetanus potentiation (PTP) responses did not differ with those observed in control animals (183 ± 32 % vs 198 ± 44 %). However, the LTP was altered in the CO group. While in control animals the LTP lasted for about 45 min, in the slices from treated animals the potentiation subsided toward control values in about 25 min. The results indicate that although neither CA1 neuronal excitability nor presynaptic mechanism appear to be modified, and expression of LTP was similar in both groups, the maintenance of LTP in the CO group was reduced, suggesting an impairment of a post-synaptic mechanism, likely involving glutamate receptors[2].

It is widely accepted that LTP represents a reliable model for learning and memory[2]. Thus, our results are in line with the deficit in learning and memory previously observed in offspring of rats exposed to 150 ppm of CO[1]. Since this CO concentrations are present in the cigarette smoke and in the exhausts gases of motor vehicles, this and previous[1] results may have relevance in the neurotoxicity of CO.

(1) De Salvia M.A. et al., (1995) *Psychopharmacology* **122**: 66-71
(2) Bliss T.V.P. and Collingridge G.L. (1993) *Nature* **361**: 31-39

LIGAND-GATED ION CHANNELS: NICOTINIC ACETYLCHOLINE AND P2X RECEPTORS

137.1

THE NICOTINIC ACETYLCHOLINE RECEPTOR CHANNEL IN THE OPEN STATE : A MOLECULAR MODELLING STUDY. M.O. Ortells[1], G.E. Barrantes[1], C. Wood[2], G. G. Lunt[2]* and F.J. Barrantes[1] ,[1], Instituto de Investigaciones Bioquímicas de Bahía Blanca, CONICET, CC 857, 8000, Bahía Blanca, Argentina, [2] School of Biology and Biochemistry, University of Bath, Bath, BA1 7AY, UK

Based on a previous closed-state model (Ortells & Lunt, Prot.Eng.9,51-59,1996), of the transmebrane region (TM) of the nAChr, we propose a model of the open-state by replacing the M2 helices with kinked helices to better match the M2's observed in the e.m. images of Unwin (Nature, 373:37, 1995). In both models (open and closed), M1 is a three-strand β-sheet, M3 is half β-sheet and half helical, and M4 is an α-helix. The new model accounts for those residues known to affect ion permeation and assumes that such residues are accessible from the lumen of the channel. Following the *Torpedo* α-subunit nomenclature, residues E241, T244, L245, S248, L250, L251, S252, T254, V255, L258, V259, I260, and E262, face the ion channel in one or more of the three ion-channel states (open, closed and desensitized). All, with the exception of L250, are visible from the lumen in our model. In the upper half of the channel M2's are widely separated and their side chains are readily accesible by probes from the channel lumen. The lower half is more closely packed, and because of the kinked M2's, the side chains of three residues (T244, S248 and L251), labeled by channel blockers, are at almost the same level, in a plane parallel to the membrane. The M1-M2 loop region partially shapes the ion-channel proper, giving partial explanation to mutational studies in this region that change the ion selectivity from cationic to anionic.
Supported by CONICET and Fundación Antorchas from Argentina, and European Union grant No. CI1-CT94-0127.

137.2

IDENTIFICATION OF A NOVEL CURRENT MEDIATED BY α7 NICOTINIC RECEPTORS IN RAT HIPPOCAMPAL STRATUM RADIATUM INTERNEURONS, BUT NOT IN CA1 PYRAMIDAL CELLS. C.J. Frazier*, W.R. Proctor, G.M. Rose, and T.V. Dunwiddie. Neuroscience Training Program, UCHSC, and Medical Research, VAMC, Denver, CO 80220

We have identified a novel nicotinic current in the interneurons of stratum radiatum of rat hippocampus. Hippocampal slices (300 microns thick) were prepared from 2-4 week old Sprague Dawley rats and maintained under in vitro conditions at room temperature in artificial CSF. Interneurons in stratum radiatum of the CA1 region were readily identified using DIC (Nomarski) optics. Recordings were made in voltage clamp mode using whole cell patch clamp techniques. Pressure application (2-20 p.s.i., 5-15 msec) of acetylcholine on the soma induced a fast, rapidly desensitizing, inward current, very similar to the type IA current previously identified by Alkondon and Albuquerque *(J. Pharmacol. Exp. Ther. 265:1455-1473)* in cultured hippocampal cells. This current was insensitive to atropine (5 µM), but was completely antagonized by low concentrations of either methyllycaconitine (50 nM), or α-bungarotoxin (100-500 nM). These results strongly suggest that the current was mediated by nicotinic receptors containing the α7 subunit. Complete desensitization of the current was apparent when the interval between applications of ACh was 5 seconds or less. Similarly, near complete desensitization could be induced by superfusion of 1-5 µM nicotine. Identical applications of acetylcholine to CA1 pyramidal cells did not produce any detectable nicotinic response. These results suggest that the stratum radiatum interneurons in CA1 are the primary targets of nicotinic cholinergic input to this hippocampal subregion.
Supported by AG10755, MH44212, and the Veterans Administration Medical Research Service.

137.3

NICOTINIC CHOLINERGIC RECEPTORS IN CULTURED MYENTERIC NEURONS OF GUINEA-PIG ILEUM. X. Zhou* and J. J. Galligan, Department of Pharmacology and Toxicology, Michigan State University, E. Lansing, MI 48824

Acetylcholine (ACh) is an excitatory transmitter in the enteric nervous system but little is known about enteric neuronal nicotinic cholinergic receptors (nNAChRs). Fast excitatory postsynaptic currents (fEPSCs) and the pharmacological and electrophysiological properties of nNAChRs were studied using whole-cell and outside-out configurations of the patch clamp method. Whole-cell spontaneous and evoked fEPSCs were inhibited by the nicotinic antagonist, hexamethonium (C6, 100 μM, n=20). ACh and nicotinic receptor agonists (10-1000 μM) caused inward currents (rise time < 100 ms) which desensitized in the presence of agonist. The agonist rank order potency was: ACh>nicotine>DMPP>cytisine. Currents evoked by ACh were reversibly blocked by C6 (n=12). Preincubation with α-bungarotoxin (α-BgTX, 1-3 hours, 100-300 nM, n=27) did not alter agonist dose-response curves. Acute treatment of individual neurons with α-BgTX (100 nM) reduced ACh-evoked currents by a maximum of 20% (n=4). The current-voltage relationship for whole-cell ACh-evoked currents rectified inwardly (conductances at -50 and +50 mV were 15.1±3.12 and 3.3±1.7 nS, respectively). The ACh current had a reversal potential of +10 mV (n=11) which was shifted by -20 mV when $[Na^+]_o$ was reduced by 50% (n=3). The amplitude of ACh-evoked currents increased as $[Ca^{2+}]_o$ was raised from 0 to 2.5 mM but decreased as $[Ca^{2+}]_o$ was raised from 2.5 to 60 mM. In outside-out patches, ACh caused single channel currents with a amplitude of -1.6 ± 0.6 pA at -70 mV. Single channel conductance at -70 mV was 20 pS (n=5). These data demonstrate that nNAChRs mediate fEPSCs in cultured myenteric neurons. Although myenteric nNAChRs are calcium permeable they are not α-BgTX-sensitive, α7-containing receptors. Agonist rank order potency suggests that myenteric neurons express α4β2-containing nNAChRs. (Supported by DK40210, NS01738 and NS33289)

137.4

N-TERMINAL DOMAIN MEDIATES THE VOLATILE GENERAL ANESTHETIC SENSITIVITY OF NICOTINIC ACETYLCHOLINE TYPE α7 RECEPTORS. **L. Zhang, M. Oz, R.R. Stewart and F.F. Weight*. Lab. Mol. & Cellular Neurobiology, NIAAA/NIH, Bethesda, MD 20892**

Neurotransmitter-gated ion channels have been proposed to be one of the primary action sites for volatile general anesthetics in the central nervous system. However, the molecular action sites of volatile anesthetics on the receptor protein remain unclear. In the present study, we examined the effects of halothane and isoflurane on the function of recombinant nicotinic acetylcholine$_{\alpha 7}$ ($ACh_{\alpha 7}$) receptors, serotonin type 3 (5-HT_3) receptors, and a chimeric receptor constructed with the extracellular N-terminal domain from the $ACh_{\alpha 7}$ receptor and the C-terminal and transmembrane domains from the 5-HT_3 receptor expressed in the *Xenopus* oocytes. 0.3-10 mM halothane and isoflurane inhibited the $ACh_{\alpha 7}$ receptor-activated currents and potentiated the 5-HT_3 receptor-activated currents in a concentration-dependent manner. The chimeric receptors exhibited an inhibition with a sensitivity similar to that of $ACh_{\alpha 7}$ receptors. Since the N-terminal domain of the chimera is from the $ACh_{\alpha 7}$ receptor, our observations suggest that the sensitivity of the $ACh_{\alpha 7}$ receptors to volatile general anesthetics may be mediated by the extracellular N-terminal domain of the receptor protein.

This work was supported by NIAAA, NIH.

137.5

POTENT MODULATION OF NEURONAL NICOTINIC ACETYLCHOLINE RECEPTOR-CHANNEL BY ETHANOL K. Nagata*, C.-S. Huang, G.L. Aistrup, W. Marszalec, J.-H. Song, J.Z. Yeh, and T. Narahashi. Dept. of Mol. Pharmacol. & Biol. Chem., Northwestern Univ. Med. Sch., 303 E. Chicago Ave., Chicago, IL 60611.

A number of studies have been performed to elucidate the mechanism of action of ethanol on various ion channels including the $GABA_A$-, NMDA-, and serotonin-activated channels, and voltage-gated Ca^{2+} channels. However, controversies still remain over which channel is the most important target site. We studied the effect of ethanol on the neuronal nicotinic acetylcholine (ACh) receptor-channel in rat pheochromocytoma (PC12) cells using whole-cell and single-channel patch clamp techniques. Ethanol potently modulated the ACh channel at micromolar concentrations. The lowest effective concentration of ethanol was 30 μM which was several hundred to one thousand times lower than those previously reported for other ion channels. Ethanol accelerated the decay phase of current in a dose-dependent manner, and shortened single-channel mean open time with channels opening in bursts. These observations suggest that ethanol accelerates channel desensitization. The dissociation of ACh from its receptor, measured as the decay rate of current following a brief (10 ms) application of ACh, was slowed by ethanol. Thus, the affinity of ACh for its receptor is increased by ethanol. It is concluded that neuronal nicotinic ACh receptors are one of the most important target sites of ethanol. Supported by NIH grant AA07836.

137.6

MODULATION OF RECOMBINANT ACETYLCHOLINE RECEPTOR CHANNELS BY ETHANOL IS DEPENDENT ON THE RECEPTOR SUBUNIT COMPOSITION. A. Ravindran*, K. Masood, A. Ghazanfari and F. F. Weight. Lab. of Molecular & Cellular Neurobiology, NIAAA, NIH, Bethesda, MD 20892.

The nicotinic acetylcholine receptor (nAChR) of vertebrate skeletal muscle is a ligand-gated ion channel composed of four homologous subunits assembled into a $\alpha_2\beta\gamma\delta$ pentamer. In mammalian muscle there are two forms of the nAChR, one that appears in fetal muscle as well as in denervated adult muscle composed of $\alpha_2\beta\gamma\delta$ and another that is seen in adult endplates in which an ε subunit replaces the γ subunit. Ethanol has been shown to potentiate the adult form of the nAChR (Ravindran et al., Soc. Neurosci. Abstr., 20: 1611, 1994). In an attempt to further elucidate the molecular mechanism of ethanol action we have compared the effects of ethanol on different combinations of nAChR subunits. The modulation of acetylcholine (ACh) induced current by ethanol was studied in HEK-293 cells transfected with mouse $\alpha\beta\gamma\delta$ and $\alpha\beta\delta$ nAChR subunit cDNAs. Forty hr after transfection whole-cell and outside-out patch clamp techniques were used to record ACh induced inward current from cells expressing nAChR. ACh alone and in combination with ethanol (EtOH) were applied to the recorded cell and outside-out patch by a theta-tube rapid perfusion system that enabled complete exchange of solutions in 0.6-3 ms. ACh activated rapidly desensitizing inward currents with EC_{50} of 7 μM in $\alpha\beta\gamma\delta$ nAChR and 10 μM in $\alpha\beta\delta$ subunit combination of nAChR. Co-application of EtOH (10-150 mM) potentiated currents activated by 1-5 μM ACh in $\alpha\beta\gamma\delta$ combination. The potentiation was between 15-45% of the control response. With higher concentrations of ACh >5 μM, EtOH significantly reduced the peak current amplitude. By contrast, ethanol (10-100 mM) failed to produce any significant effect on peak amplitude of currents in cells transfected with $\alpha\beta\delta$ nAChR subunits. Further studies are underway to elucidate the molecular mechanism of receptor subunit dependence of ethanol modulation of nAChR channels. This work was supported by the intramural research program of NIAAA/NIH.

137.7

DISTRIBUTION AND LOCALIZATION OF P2X7 RECEPTORS IN RAT TISSUES G. Collo, S. Neidhart, F. Rassendren, E. Kawashima, and G. Buell.* Geneva Biomedical Research Institute, CH1228 Geneva, Switzerland.

We have recently identified a novel P2X receptor by molecular cloning from adult rat brain. Transiently expressed in HEK293 cells it forms an ATP gated channel with properties known for P2Z receptors (Surprenant A. et al., Science, in press). P2Z function has been primarily studied in the immune system, and has been shown in macrophage cell lines such J774, but not in the brain. Digoxigenin-labelled riboprobes were used to localize P2X7 RNA by Northern blotting and in situ hybridization in the E19 rat embryo and in adult rat tissues. In E19 embryos P2X7 mRNA was weakly detected in the brain, but a strong signal was seen in bone marrow. In the adult bone marrow, spleen and testis P2X7 mRNA was also observed. In situ hybrdization with normal adult brain did not detect P2X7 RNA but brain-derived microglial cells do contain this mRNA, as judged by Northern blotting. When the rat brain was studied following a procedure of cerebral ischemia (24 hours post-occlusion of the meddle cerebral artery) strong P2X7 hybridization signal was detected in cells scattered in the area of penumbra that surrounded the necrotic core of the lesion. Preliminary studies using a specific antibody raised against the C-terminus of P2X7 confirmed the results obtained by in situ hybridization for bone marrow and localized the signal to monocytes and polymorphonucleated cells. Overall, these findings suggest that P2X7 receptor is transiently expressed in the brain during development but may be induced in the adult brain by ischemia or inflammation. Funding was private.

137.8

CELLULAR DISTRIBUTION OF P2x4 ATP-GATED CHANNELS IN RAT BRAIN AND SPINAL CORD. **K.-T. Lê, P. Villeneuve, A. Ramjaun, J. Mukerji, P. McPherson, A. Beaudet and P. Séguéla*. Montreal Neurological Institute, Dept. Neurology & Neurosurgery, McGill University, Montreal, Quebec, Canada H3A 2B4.**

Central ionotropic effects of extracellular ATP are exerted through a family of P2x receptor-channels expressed in brain and spinal cord. We report here the immunocytochemical localization of a major central receptor subunit, P2x4, using affinity-purified polyclonal antibodies directed against the C-terminal domain of the predicted protein. Subunit-specific anti-P2x4 antibodies detected a single band of Mr 56 ± 3 kDa corresponding to glycosylated P2x4 subunits in homogenates from brain and transfected HEK-293 cells. In rat brain sections, the strongest expression of P2x4 was observed in olfactory bulb, septum, cerebellum and spinal cord. Moderate to strong levels of P2x4 receptors were also evident in widespread areas including cerebral cortex, hippocampus, thalamus and brainstem. In most brain regions examined, P2x4 immunoreactivity was associated with dendrites and neuronal perikarya, suggesting a post-synaptic involvement of ATP-gated channels in fast excitatory transmission. Nevertheless, high densities of P2x4 purinoceptors were found in association with axon terminals in the olfactory bulb and spinal cord, suggesting an important presynaptic role for ATP-gated channels in the modulation of neurotransmitter release in sensory systems. Finally, non-neuronal cells from vascular endothelial layer and pia matter also expressed high levels of P2x4, indicating an unexpected role for extracellular ATP in central epithelial tissues.

(Supported by MRC-Canada)

137.9

Identification of residues lining the ionic pore of P2X receptors by SCAM mutagenesis.

Rassendren, FA, North RA, Buell, G. and Surprenant, AM*
Glaxo Institute for Molecular Biology. 1228 Plan-les-Ouates, Geneva, Switzerland.

P2X receptors are ATP gated-channels expressed in various tissues including central and peripheric nervous systems. Cloning of P2X receptor cDNAs revealed that these proteins have two putative hydrophobic transmembrane domains separated by a large extracellular loop. This structure differs from other ligand gated channels where four hydrophobic regions are found. No structural data on P2X receptors are yet available and the localisation of the pore forming region remains unknown. The second transmembrane domain of the seven P2X subunits cloned so far could probably form an amphipathic a-helice; every third residue is either polar or has a small side chain and therefore susceptible to interaction with an aqueous environment. Using the systematic cysteine accessibility method (SCAM), we have tested this hypothesis. In a first set of experiments we have mutated the polar residues to cysteine in P2X2, P2X4 and P2X7 subunits. All mutated proteins expressed when transiently transfected in HEK293 cells. MTSET blocked P2X channels when two of thoses residues were replaced with cysteines in all three subunits tested. However cysteines substitution of the always conserved glycine (position 342 in P2X2) did not react with MTSET in any of the subunits tested. These results indicate that the second transmembrane domain of P2X receptors contribute to pore formation and may be compatible with an α-helical pore structure.

137.10

STRUCTURAL DOMAINS AFFECTING PHARMACOLOGICAL DIFFERENCES BETWEEN THE HUMAN ATP-GATED CHANNEL $P2X_4$ AND ITS RAT HOMOLOGUE

F.Soto*, M. Garcia-Guzman and W. Stühmer. Max-Planck Institute for Experimental Medicine, Hermann-Rein Str. 3, 37075-Göttingen, Germany.

Rat and human $P2X_4$ receptors (rP2X4 and hP2X) cloned from brain display a very similar profile of agonist sensitivity. However, despite the high sequence identity (87 %), antagonists such as pyridoxalphosphate-6-axophenyl-2', 4'-disulphonic acid (PPADS) and suramin were at least twice more effective for the human clone than for the rat homologue. For instance, 100 μM PPADS blocks 65% of the human $P2X_4$ current elicited by 5 μM ATP, while for the rat clone, 70 % of the current remains under the same conditions (N>6). To check which domains where involved in conferring the antagonist sensitivity, we constructed chimeric receptors and expressed them in *Xenopus* oocytes. For all the measurements, we used 5 μM ATP and 100 μM of the antagonist. To ensure maximal effect of block, the oocytes were preincubated with the antagonist for 4 min before every agonist application. This approach allowed us to identify a region in the putative extracellular loop of $hP2X_4$ (from aa 82 to 183) that confers antagonist sensitivity when introduced into $rP2X_4$ without affecting agonist sensitivity. The reverse chimera was as sensitive to antagonists as $rP2X_4$. In conclusion, we demonstrated that there is some interspecies differences between human and rat $P2X_4$ receptors concerning antagonist block and identified the domain responsible for that. Work actually in progress aims to identify which amino acid(s) may account for the interspecies differences.

137.11

EXPRESSION OF ATP-GATED $P2X_2$ AND $P2X_3$ RECEPTOR SUBUNITS IN BACULOVIRUS

E. Kawashima, K. Radford, A.M. Surprenant, M. Ward, A. Michel and R.A. North*, Geneva Biomedical Research Institute, CH-1228, Switz. and Glaxo-Wellcome Medicines Research Center, Stevenage, SG1 2NY, U.K.

The family of P2x receptor genes predict a new structural subclass of ligand-gated cation channels, which contain two membrane-spanning domains and a large extracellular loop. We have used the baculovirus system to express the $P2X_2$ and $P2X_3$ receptors. Infection of Sf9 cells with baculovirus-containing the $P2X_2$ cDNA generates a fully functional receptor when assayed electrophysiologically. Expression of the $P2X_2$ receptor at high volumes (24 l.) yields significant quantities of protein, which we have identified as $P2X_2$ by ^{35}S-ATP binding (22 pmol/mg-protein) and by micro-sequence analysis using electrospray mass spectrometry. Further micro-sequence analysis has revealed three sites of glycosylation in the extracellular loop. We are currently characterising Sf9 cells co-infected with epitope-tagged $P2X_2$ and $P2X_3$ for expression of functional heteromeric receptors by electrophysiological and immunochemical techiques. Funding was private.

137.12

PHYSIOLOGICAL AND PHARMACOLOGICAL CHARACTERIZATION OF P_{2x}- AND P_{2Y}-PURINOCEPTORS OF THE GUINEA-PIG SUBMUCOSAL NEURONS.

C. Barajas-López*[1], and F.L. Christofi[2]. [1]Biomed. Sci. Depart., McMaster Univ., Hamilton, ON. [2]Anesthesiol. Depart., The Ohio State Univ., Columbus, OH.

In S-type submucosal neurons, extracellular application of ATP induces a fast and a slow depolarization by activation of cationic channels and inhibition of the membrane potassium conductance, respectively. In this study, we characterized the receptors and intracellular events associated with such ATP effects. In whole-cell recordings, ATP application evoked a rapid inward current which was antagonized by pyridoxalphosphate-6-azophenyl-2',4'-disulfonic acid (PPADS; IC_{50}=4 μM). Suramin potentiated these currents; whereas, reactive blue 2 was a very weak antagonist (IC_{50}=300 μM). In intracellular recordings, none of these three antagonists, neither pertussis toxin nor KT5720 (protein kinase (PK) inhibitor), affected the slow depolarization. Whereas, N-ethylmaleimide (uncouples receptors from G-proteins), staurosporine (non-specific PK inhibitor) and calphostin (PKC inhibitor) inhibited this response. In Fura-2/AM Ca^{2+} imaging experiments, ATP induced a rapid increase in the intracellular free Ca^{2+} concentration, which was completely prevented by PPADS, unaffected by staurosporine, and partially inhibited by blocking the voltage-activated Ca^{2+} channels with Cd^{2+}. In conclusion, the fast and slow depolarization are mediated by activation of P_{2x}- and P_{2y}-purinoceptors, respectively. Activation of P_{2x}-purinoceptors leads to an increase in intracellular Ca^{2+}. P_{2y}-purinoceptors linked to G-proteins and PKC activation are likely mediating inhibition of the membrane potassium conductance. Supported by the grants MRC-MT-13491 (CBL), OMH-04500 (CBL), and NIHR29DK44179-04 (FLC).

137.13

INWARD RECTIFICATION OF $P2X_2$ RECEPTORS IS CAUSED BY VOLTAGE-DEPENDENT GATING

Z. Zhou and R. I. Hume*, Dept. of Biology, Univ. of Michigan, Ann Arbor, MI 48109

Many different cation channels show profound inward rectification when expressed in Xenopus oocytes. For AMPA class glutamate receptors and some K^+ channels, the cause of inward rectification is a voltage dependent block of intracellular polyamines. For other K^+ channels, the cause is a voltage dependent block of intracellular Mg^{++}.

We have tested whether inward rectification of $P2X_2$ receptors is also mediated by intracellular polyamines or Mg^{++}. $P2X_2$ receptors were expressed in Xenopus oocytes and then outside-out patches were excised. ATP (50uM) was used as the agonist in all experiments. Inward rectification was not alleviated in the absence of internal Mg^{++} and/or polyamines. Furthermore, the degree of rectification did not increase when a high concentration of polyamines (e.g. 100uM spermine) was included in the internal solution. It seems that blocking by internal Mg^{++} and polyamines is not the cause of inward rectification in these channels. Voltage jump experiments were done to study the voltage-dependence of gating. After rapid voltage jumps, we observed current relaxations which could be fitted by single exponential functions. These results indicate that voltage-dependent gating is the underlying mechanism of inward rectification of $P2X_2$ receptors. (Supported by NS 25782)

137.14

INTERACTION BETWEEN ATP-GATED AND VOLTAGE-GATED CALCIUM CHANNELS IN THE PC12 CELL,

J.R. Keath*, G. Balan, and E.W. Westhead. Program in Neuroscience and Behavior and Dept. of Biochemistry, Univ. of Mass at Amherst, Amherst, MA, 01003-4505.

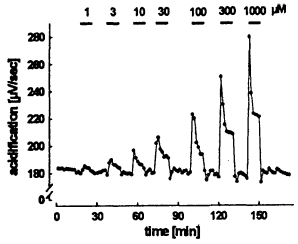
We have investigated the possible contributions of voltage-operated calcium channels (VOCCs) to PC12 cell secretion in response to ATP stimulation. Although ATP stimulation does depolarize the cell, VOCC blockers, Nicardapine and Cadmium ion, do not inhibit the secretion of PC12 cells at either saturating or half-maximal concentrations. This suggests that the presence or absence of VOCC activity does not influence calcium concentration rise in response to ATP stimulation. Both VOCC blockers do, however, increase the rate at which the PC12 cells desensitize to ATP stimulation. This indicates that, when VOCC blockers are not present, VOCCs are opening to allow calcium into the cell. This presents us with a paradox: VOCCs open during ATP stimulation to let calcium into the cell, but this calcium does not modify the increase in internal calcium concentration that occurs in response to the ATP stimulation, To explain this quandary, we propose that both the ATP-gated calcium channels and the VOCCs are activated during ATP stimulation, but that the calcium entry is self-limiting within domains of the plasma membrane. Support for this model is provided by desensitizing PC12 cells in a solution in which the calcium is replaced with barium, which is known to induce secretion without inhibiting calcium channels. When stimulated with ATP under these circumstances, the PC12 cells desensitize even faster thatnthey would if they were treated with VOCC blockers.

137.15

EXAMINATION OF ATP-INDUCED RESPONSES IN PC12 CELLS. INVESTIGATIONS OF P_{2x}-RECEPTORS USING MICROPHYSIOMETER AND ELECTROPHYSIOLOGICAL TECHNIQUES

A. Rohlfs, P. Salmeso, F. Fery and R. Netzer. Department of Electrophysiology, Battelle, CH-1227 Carouge/Geneva, Switzerland.*

The response of NGF-treated PC12 cells to extracellular application of ATP was investigated using a microphysiometer and compared to electrophysiological experiments. ATP induced a rapid concentration-dependent rise of extracellular acidification with an EC_{50} of 105 ± 9.5 μM. Preincubation with the PLC-inhibitor U-73122 did not affect the response indicating that only P_{2x}-receptors are involved. The response was reduced by the P_2-receptor antagonist suramin. The agonist αβ-methylene-ATP interacting with specific subtypes of P_{2x}-receptors induced only marginal responses. During the electrophysiological investigations ATP induced an inward current with an EC_{50} of 14.1 ± 1.8 μM. The application of glutamate, NMDA, AMPA and kainate induced in these cells a marginal peak decrease of relative acidification and no current in electrophysiological experiments. It is to conclude that the described microphysiometer assay serves as a non-invasive and functional system to examine effects of compounds on P_{2x}-receptors. *Support: Battelle-IR&D-fund*



LIGAND-GATED ION CHANNELS: GLUTAMATE, GABA, AND GLYCINE RECEPTORS

138.1

SINGLE-CHANNEL PROPERTIES OF GLUTAMATE-GATED CHLORIDE CHANNEL RECEPTOR SUBUNITS. M. Amar[1], A. Etter[2], K. Liu[2], D.J. Beadle[1], J. Arena[2]* and I. Bermudez[1]. [1]School of Biological & Molecular Sciences, Oxford Brookes University, Oxford, U. K. and [2]Merck Research Labs., Rahway, NJ 07065, USA.

Two subunits (GluClα and GluClβ) of a glutamate-gated chloride channel have been cloned from the free living nematode *Caenorhabditis elegans* (Cully *et al.*, Nature 371, 707-710). Homomeric GluClβ channels are sensitive to glutamate, while homomeric GluClα are not. Co-expression of GluClα with GluClβ channels shifts the glutamate dose response curve to the left, and changes the rectification properties of the whole cell currents. To gain a molecular insight into the mechanism underlying these changes we have recorded glutamate-gated channel currents from outside-out patches excised from *Xenopus* oocytes injected with GluClα and GluClβ or GluClβ specific-RNAs. Both GluClα-β and GluClβ channels exhibited a single conductance of 12 ± 0.2 pS and 15 ± 0.3 pS, respectively. GluClαβ channel open time histograms are best fitted with two exponential components, one of which is strongly voltage-dependent. The open time kinetics of the GluClβ channel is dominated by a time constant which is comparable to the voltage-dependent component of the GluClα-β channels. These results suggest that the open time kinetics of the GluClα-β channel are defined predominantly by the β subunit. Supported by the Wellcome Trust, UK.

138.2

INHIBITORY GLUTAMATE RECEPTORS IN A MOTOR PATTERN GENERATOR: CHANNEL PROPERTIES AND PHYLOGENETIC ANALYSIS T.A. Cleland* and A.I. Selverston. Dept. of Biology 0357, University of California San Diego, La Jolla, CA 92093-0357.

Inhibitory ionotropic glutamate receptors (iGluHRs) are a family of ion channel proteins closely related to ionotropic glycine and GABA receptors. They are gated rapidly by glutamate; the open channel is permeable to chloride and/or potassium. They are expressed extrajunctionally on striated muscle fibers and postsynaptically in neurons across many taxa. Generally, iGluHR orthologues exhibit consistent pharmacological profiles; however, some pharmacological characters are uniquely derived within discrete clades. For example, while iGluHRs within other taxa are insensitive to quisqualic acid, it is a potent agonist of all known iGluHRs within the pulmonate molluscs. The iGluHR is a target of the insecticidal avermectins, which can modulate, activate, and block receptor gating.

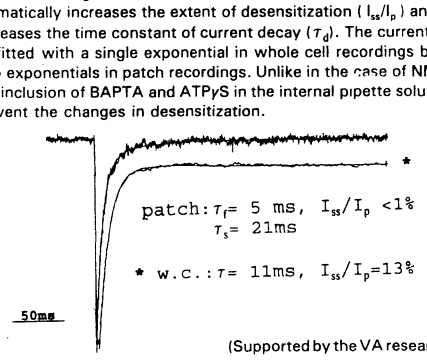
Neuronal iGluHRs mediate rhythmic synaptic inhibition within the central pattern generator circuits of the crustacean stomatogastric ganglion (STG). Superfusion of glutamate over voltage-clamped isolated STG neurons gated an outwardly rectifying conductance permeable primarily to chloride and pharmacologically consistent with iGluHRs in related taxa. In outside-out excised patches from STG neurons, 10 μM glutamate evoked at least two unitary conductance states, the larger of which (~50 pS) was the more prevalent under experimental conditions. As glutamatergic synaptic inhibition is fundamental to the bursting properties of this oscillatory motor network, any alterations in receptor properties directly affect rhythmogenesis and consequently behavioral output.

Supported by NIGMS training grant GM08107 to TAC and NIH grant PO1NS25916 to AIS.

138.3

THE DESENSITIZATION PROPERTIES OF NON-NMDA GLUR CHANNELS ARE ALTERED BY MEMBRANE EXCISION. M. Margulis, and C.-M. Tang. Dept of Neurology, Univ. of Maryland Sch. of Medicine.

Excised patch recordings have provided the bulk of data on the fast kinetic properties of non-NMDA GluR channels. Unfortunately, whether membrane excision alters the gating properties of these channels is not known. We addressed this issue with the use of laser induced rapid photolysis of "caged" glutamate. This approach result in identical kinetics of glutamate/receptor interaction for whole-cell and excised patch recordings. We found that membrane excision consistently and dramatically increases the extent of desensitization (I_{ss}/I_p) and modestly increases the time constant of current decay (τ_d). The current decay can be fitted with a single exponential in whole cell recordings but requires two exponentials in patch recordings. Unlike in the case of NMDA GluRs the inclusion of BAPTA and ATPγS in the internal pipette solution did not prevent the changes in desensitization.



(Supported by the VA research service)

138.4

17β-ESTRADIOL POTENTIATION OF KAINATE CURRENTS: SITE OF ACTION Qin Gu* and Robert L. Moss, Department of Physiology, The University of Texas Southwestern Medical Center, Dallas, Texas, 75235.

Previously we reported that 17β-estradiol (E_2) potentiates kainate-induced currents in acutely dissociated hippocampal CA1 neurons. This potentiation was not due to direct interaction of E_2 with kainate receptors but rather the activation of a second messenger system. A specific PKA inhibitor blocks the E_2 potentiation (Gu & Moss, 1996).

The present study was designed to investigate whether E_2 acts extracellularly or intracellularly to potentiate the kainate current. Experiments using standard whole-cell voltage-clamp techniques were performed on dissociated hippocampal CA1 neurons from Sprague-Dawley rats. The kainate current was elicited by applying kainate pulses (100 μM, 0.033 Hz at 0.1-1 psi for 20 ms) via a puffer electrode to the dendrite of a CA1 neuron. The effects of E_2, an impermeable BSA-conjugated E_2 (E_2-BSA), and GTP-γ-S on kainate-induced currents were tested on either side of the neuronal membrane.

Extracellular application of E_2 (100 nM, 3 min) significantly increased the amplitude of kainate-induced currents within 3 min, whereas extracellularly applied E_2-BSA had no effect. Similarly intracellular E_2 also potentiated kainate currents within 12 min, but intracellular E_2-BSA did not. The simultaneous application of E_2-BSA on both sides of the membrane could restore the estrogenic potentiation within 3 min. Intracellular GDT-β-S supressed the effect of E_2. Extracellular E_2-BSA potentiated the kainate-induced currents in the presence of intracellular GTP-γ-S, although either alone had no effect.

The results indicate that potentiation of kainate currents in rat hippocampus requires the presence of E_2 on both sides of the membrane. We suggest that E_2 acts at an extracellular site to trigger G-protein-coupled modulation, whereas E_2 intracellularly acts to maintain phosphorylation of kainate receptor channels. Supported by NIH grant MH 47418.

138.5

AGMATINE BLOCKS NMDA RECEPTOR CHANNELS IN RAT HIPPOCAMPAL NEURONS. X.-C. Yang* and D.J. Reis. Dept. of Neurol. and Neurosci., Cornell Univ. Med. Col., New York, N.Y 10021.

Agmatine (decarboxylated arginine) is an endogenous polyamine and organic cation with two positive charges at physiological pH. Agmatine is synthesized, stored and released in mammalian CNS and may be a novel neurotransmitter/modulator (Li et al., *Science*, 1994; Regunathan et al., *Soc. Neurosci. Abstr.*, 1996). Because agmatine may be taken up into neurons via some Ca^{2+} channels (Sastre et al., *Soc. Neurosci. Abstr.*, 1996), we investigated whether the amine may interact with the Ca^{2+}-permeable NMDA receptor channel. Hippocampal neurons were obtained on prenatal day 19 rats and maintained in primary culture. Ion currents through the NMDA channel were recorded using the whole-cell patch clamp technique. The Mg^{2+}-free extracellular solution contained (in mM) NaCl 140, KCl 5, $CaCl_2$ 0.2, HEPES-NaOH 10, pH 7.4, supplemented with 50 μM NMDA and 10 μM glycine. The electrode-filling solution contained (in mM) CsCl 145, $MgCl_2$ 2, EGTA 5, HEPES-CsOH 10, plus ATP 3, GTP 0.1, and leupeptin 0.1, pH 7.2. When applied extracellularly agmatine, at concentrations as low as 1 μM, reversibly reduced the NMDA current at a holding potential of -60 mV. The half-block concentration of agmatine was about 100 μM, which reduced the NMDA current by 50%. External agmatine blocked preferentially the inward NMDA current with a small blocking effect at positive voltages. As a result the linear I-V curve of the NMDA current became inwardly rectifying in the presence of external agmatine. Agmatine, possibly released presynaptically, may act to modulate ligand-gated cation channels either at the polyamine site and/or in the channel pore. (NHLBI-HL18974)

138.6

SENSITIVITY OF NATIVE AND RECOMBINANT GLUTAMATE RECEPTORS TO ALIPHATIC *n*-ALCOHOL INHIBITION. B.E. Akinshola*, R.W. Peoples, R. Stewart and F. F. Weight. Lab. Molecular and Cellular Neurobiology, NIAAA, NIH, Bethesda, MD 20892-8205.

In order to compare recombinant glutamate (GluR) receptor sensitivity to alcohol with that of neurons, we used two-electrode voltage clamp and whole-cell patch clamp techniques, respectively, to study recombinant receptors expressed in *Xenopus* oocytes, and native receptors in cultured hippocampal neurons. Receptor currents in both oocytes and hippocampal neurons were measured in response to kainate as an agonist. For short-chain aliphatic alcohols from methanol to hexanol, potency for inhibition of native and recombinant receptor currents increased in proportion to chain-length or hydrophobicity, whereas inhibitory activity could not be observed with octanol for GluR1, nonanol for GluR3 (*Soc. Neurosci. Abstr.,21;*1815,1995), and decanol for neurons. Our studies indicate that the characteristics of *n*-alcohol inhibition of neuronal currents show similarities to the inhibition of recombinant receptor subunits for alcohols from methanol to hexanol. The differences in sensitivity found with higher alcohols may be an indication of differences in the alcohol binding sites on these receptor-channels. In addition, the difference seen with the hippocampal neurons may also reflect the involvement of additional subunits. This work was supported by NIAAA, NIH.

138.7

Investigation of the Ethanol Site of Action on the NMDA Receptor Using Site Directed Mutagenesis. Tooraj Mirshahi* and John J. Woodward. Dept. of Pharmacology and Toxicology, Box 980524 Medical College of Virginia, Virginia Commonwealth University, Richmond, VA 23298.

Ethanol has been shown to inhibit both native and recombinant NMDA receptors although no specific site of action has been defined. Ethanol's inhibition does not appear to be competitive with any of the known modulatory sites on the NMDA receptor. Due to its hydrophobic nature, ethanol could alter receptor function by interaction with a hydrophobic pocket on the NMDA receptor. Amino acids with large hydrophobic side chains are candidates for formation of such an ethanol binding pocket. There are several Phe (F), Leu (L) and Ile (I) residues in the NMDA receptors which could participate in formation of this pocket. We have begun to investigate this possibility using the oocyte expression system to test the ethanol sensitivity of NMDAR1 receptors with mutations at these hydrophobic sites.

Two mutant NMDAR1 receptors, F554A and F558A were expressed in combination with the NR2A subunit. Application of 100 µM NMDA and 10 µM glycine induced robust currents in oocytes expressing the mutant receptor combinations. NMDA-induced currents in these mutant receptors were significantly inhibited by ethanol. However the extent of ethanol inhibition of the mutant receptors was similar to that observed for wild-type (NR1/NR2A) receptors expressed in oocytes. This approach should provide valuable insights into defining a possible site of action for ethanol on the NMDA receptor. Supported by AA09986 and DA07027.

138.8

EFFECTS OF ACUTE AND CHRONIC ETHANOL EXPOSURE ON NMDA RECEPTOR EXPRESSION AND FUNCTION IN TRANSFECTED HEK 293 CELLS T.L. Blevins and J.J. Woodward*, Dept. of Pharmacology/Toxicology, Virginia Commonwealth Univ., Richmond, VA 23298

The N-methyl-D-aspartate (NMDA) subtype of neuronal glutamate receptors is a multi-subunit, calcium-permeable ion channel that helps regulate synaptic activity. Previous studies have demonstrated that both native and recombinant NMDA receptors are inhibited by ethanol at behaviorally relevant concentrations (10-100 mM). In a previous study (Neurosci. Lett. 200:214, 1995), we observed that chronic ethanol exposure enhanced the agonist sensitivity of NMDA receptors expressed in cultured neurons. The sensitivity of these receptors to ifenprodil was also enhanced suggesting that changes in the expression of the NR2B subunit may have been involved.

To investigate the possibility that these changes occur at a post-transcriptional level, we have used a transient transfection system and monitored changes in NMDA receptor expression and function following exposure to ethanol. HEK 293 cells transfected with the NR1 subunit alone were not functionally active although significant amounts of NR1 protein were present as measured by immunoblotting and immunofluorescence. Cells transfected with NR1/NR2A, NR1/NR2B, or NR1/NR2A/NR2B subunits showed NMDA and glycine-dependent increases in intracellular calcium as measured by fura-2 imaging. In the double subunit transfected cells, both subunits appeared to be co-localized as revealed by antibody labeling and confocal imaging. This was also observed in cells transfected with an NR2 subunit and a fusion protein consisting of NR1 and the green fluorescent protein (NR1/GFP). Ethanol inhibited NMDA-induced changes in intracellular calcium with equal potency in all combinations. Chronic ethanol exposure of NR1/2A/2B transfected cells enhanced the ifenprodil sensitivity of these cells. These results suggest that some of the effects of chronic ethanol observed in neurons may result from changes in the post-transcriptional and post-translational processing of NMDA receptor subunits. Supported by AA09986.

138.9

BIPHASIC EFFECT OF ETHANOL ON GLYCINE CURRENTS OF CORTICAL AND HYPOTHALAMIC NEURONS. D. Schiller*, J.-H. Ye and J.J. McArdle. Depts Pharmacology & Physiology and Anesthesiology, NJ Medical School (UMDNJ), Newark, NJ 07103.

Glycine-activated current (I_{Gly}) was recorded from freshly isolated murine cortical and hypothalamic neurons with the nystatin perforated patch technique. Approximately 40% of the cortical and 90% of the hypothalamic neurons examined responded to glycine; this current was resistant to the $GABA_A$ receptor blocking agent picrotoxin. Co-application of ethanol at concentrations of 11, 22, 43, and 65 mM reversibly potentiated peak I_{Gly} in 86% and 82% of the cortical and hypothalamic neurons, respectively. In contrast, 130 mM ethanol suppressed peak I_{Gly} of both types of neurons. At the lower concentrations ethanol potentiated I_{Gly} whether or not this current was sensitive to strychnine. The acute potentiating effect of ethanol was observed when 50, 100, 200, 300 or 400 μM glycine induced I_{Gly}. Furthermore, pre-exposure to 65 mM ethanol for 2 min prior to the co-application of glycine and 65 mM ethanol did not alter the potentiating effect. Thus, desensitization or acute tolerance to ethanol does not occur in glycine responsive cortical neurons. Evaluation of voltage ramps revealed that the potentiation of I_{Gly} occurred in association with a rightward shift in the current voltage relation. This suggests that ethanol may alter the conductile properties of the glycine-gated chloride channel. Finally, in 68 of the 94 neurons examined, the facilitatory effect of ethanol was associated with a decrease in the rise time of I_{Gly}. This kinetic effect suggests that ethanol may increase open probability of the glycine-gated ion channel.

138.10

ZINC MODULATORY SITES AT THE INHIBITORY GLYCINE RECEPTOR. B. Laube*, J. Kuhse and H. Betz. Department of Neurochemistry, Max-Planck-Institute for Brain Research, Deutschordenstraße 46, D-60528 Frankfurt, Germany

Zinc is a divalent cation endogenously present in the synaptic cleft and released in an activity-dependent manner. The biphasic modulatory effect of extracellularly applied zinc on glycine-gated chloride currents in embryonal spinal cord neurons implicates zinc in the regulation of synaptic efficacy within glycinergic pathways (Laube et al., *J. of Physiol.* 483.3: 613-9). Glycine responses of heterologously expressed glycine receptors (GlyRs) are 2-3 fold potentiated in the presence of 0.5-10 µM zinc, whereas >10 µM zinc decreases the glycine response by changing the apparent agonist affinity. Single channel recording revealed that low concentrations of zinc enhance the open probability of the GlyR channel, whereas higher concentrations decrease the latter without affecting single-channel conductances. Taurine, a partial agonist of the GlyR, also showed an increased agonist potency in the presence of zinc, whereas its antagonist activity was not affected by the metal ion. Chimeric GlyR subunit constructs showed that the positive and negative regulatory effects of zinc are mediated by different regions of the GlyR protein, suggesting the existence of at least two distinct zinc binding sites. Site-directed mutagenesis of the human α1 subunit identified negatively charged residues in the extracellular N-terminal region as essential determinants of high-affinity zinc binding. A model of the modulatory zinc binding sites of the GlyR will be presented.

138.11

DICHLOROKYNURENIC ACID SELECTIVELY SUPPRESSES A SLOW INHIBITORY GLYCINE CURRENT. Yi Han and Malcolm M. Slaughter*. **Departments of Biophysical Sciences, Physiology, and Ophthalmology. School of Medicine, State University of New York, Buffalo, NY 14214, USA.**

The inhibitory glycine receptor (GlyR) is a pentamer of α(48 KD) and β(58 KD) subunits. It is known that multiple isoforms exist and the distribution of GlyR subunits is inhomogeneous in the retina. This differential expression may indicate a functional diversity. We recently reported that glycine mediated chloride currents observed in retinal ganglion cells can be separated into two components which have different kinetics of activation and desensitization. These two components could be distinguished by strychnine antagonism. The fast component is blocked by strychnine with an IC_{50} of 34 nM, while the efficacy of this antagonist for the slow component is 2.2 μM. The purpose of this study was to investigate the properties that distinguish the slow glycine receptor. Whole-cell voltage clamp techniques and a rapid perfusion system were used to study isolated ganglion cells from the amphibian retina, *Ambystoma tigrinum*. Both components were carried by chloride and had similar affinities for glycine. While the fast component was selectively suppressed by low concentrations of strychnine (<100nM), 5,7-dichlorokynurenic acid (500μM) manifested a selective reduction in the slow glycine current. There was little overlap between the effects of these antagonists. Several glycine analogs (β-alanine, L-alanine, L-serine) activated both components but had different efficacies on each component The results suggest that there are two inhibitory glycine receptors which have distinct kinetics and pharmacologies. The slow glycine receptor has a pharmacology that is similar to that of the glycine recognition site at the NMDA receptor.

Supported by NEI EY-05725

138.12

CONVULSANT SITE PROPERTIES IN GLYCINE RECEPTORS FROM RAT SPINAL CORD AND HIPPOCAMPAL NEURONAL CULTURES. V. E. Wotring* and K.-W. Yoon. Dept. of Pharmacological and Physiological Sciences and Division of Neurosurgery and Surgical Research Institute, Saint Louis University Health Sciences Center, St. Louis MO 63104.

Actions of picrotoxinin and butyrolactones were investigated in dissociated cultures from hippocampi of 1 day old or spinal cords from 12-14 embryonic day rats and kept in culture for 8-16 days and 15- 35 days, respectively. Recordings were made in voltage clamp mode using whole-cell and excised outside-out patch clamp techniques. In spinal cord, the glycine EC_{50} was 32 μM, with a Hill slope of 1.7, while in hippocampal cells, the EC_{50} was 185 μM and the Hill slope 2.3. Despite the difference in glycine EC_{50}, preliminary data show that cells from both hippocampus and spinal cord required similar, and relatively high, concentrations of picrotoxinin to block control glycine-elicited currents (100 μM glycine; IC_{50}s were ~300 μM). Unlike what has been previously reported for $GABA_A$ receptors, the picrotoxinin block of glycine showed no signs of use-dependence or voltage dependence and recoveries were rapid and complete. To further explore the possibility of a convulsant site at the glycine receptor, we used αIMGBL, a substituted γ-butyrolactone that has been previously shown to block the convulsant site of the $GABA_A$ receptor and to reverse the picrotoxinin block of the $GABA_A$ receptor. (Yoon et al., 1993) With a relatively high concentration of αIMGBL (5mM) we saw no significant effect on control glycine currents in either spinal cord or hippocampal cells (n=6, p> 0.2) or on the picrotoxinin block seen (n=6, p>0.2). We have attempted to further explore the mechanism of the picrotoxinin block of glycine receptors and to determine if the glycine receptor has activities that are similar to those associated with the convulsant site of the $GABA_A$ receptor. Potential functional roles of such a glycinergic convulsant site will be considered.

This work was supported by K08NS01547 (K.-W.Y.) and NS07254-08 (V.E.W.)

138.13

STRUCTURAL ELEMENTS DETERMINING THE GATING OF GLYCINE RECEPTOR ISOFORMS. J. Bormann*, N. Rundström, H. Betz and D. Langosch. Max-Planck-Institut für Hirnforschung, D-60528 Frankfurt, Germany.

The glycine receptor (GlyR) is a ligand-gated ion channel protein that mediates synaptic inhibition in spinal cord and other CNS regions. We have analyzed the kinetic properties of human GlyR isoforms using patch-clamp recording from transfected HEK-293 cells. Elementary currents through α1 or α2 homooligomeric GlyRs had a burstlike appearence. Distributions of shut time and burst duration could be fitted with four and three exponential functions, respectively. The average length of long bursts was 32 ms for α1-channels, interrupted by 9.4 closing gaps. In contrast, α2-channels showed much longer bursts of 392 ms duration, containing only 2.3 closures. To identify the structural elements responsible for the different gating properties, chimera were constructed between α1 and α2. The gating properties of mutant α1 containing both the extracellular N-terminus and transmembrane domain M2 of α2 closely resembled that of α2 GlyRs. This indicates that receptor and channel both contribute to the gating properties of human GlyR isoforms. A kinetic scheme is proposed with five sequential binding steps for glycine and three open states of the channel.

Supported by the Deutsche Forschungsgemeinschaft

138.14

MULTIPLE RESPONSES AND UNDERLYING SUBUNIT COMPOSITION OF GABA AND GLYCINE RECEPTORS IN HIPPOCAMPAL NEURONS

K.Fatima-Shad, K. Pierce, P. H. Barry & P. R. Schofield[†*], School of Physiology and Pharmacology, The University of New South Wales, Sydney 2052, Australia and [†]Neurobiology Division, The Garvan Institute of Medical Research, Darlinghurst, NSW 2010, Australia.

Whole cell studies of inhibitory synapses between neurons of the early post-natal rat hippocampus in dissociated culture have indicated the presence of IPSPs and IPSCs. The coincident reversal potentials for IPSCs and for GABA and glycine-evoked currents, and the sensitivity of the IPSCs to bicuculline and strychnine, indicated that the IPSCs described here were Cl^- dependent and mediated by either $GABA_A$ or glycine receptors. Heterogenous desensitization responses were observed in all cells with GABA- and glycine-gated channels. For GABA and glycine-gated receptor channels the estimated values of the receptor channel density were approximately 2 and 3 / μm^2 respectively. This functional heterogeneity of GABA and glycine receptor channels, observed in cultured hippocampal neurons suggested the possible presence of different subunit compositions for the receptor channels. To investigate this possibility, we are coupling patch-clamp recording with Reverse Transcription followed by Polymerase Chain Reaction amplification (RT-PCR) in order to correlate the properties of GABA and glycine-gated whole cell currents with the expression of mRNAs for each subunit in individual neurons. To determine which of the GABA and glycine receptor subunit mRNAs were present, the cellular content of each neuron was aspirated into the recording pipette after the cell had been classified as "fast" or "slow". RT-PCR was performed with primers common to all known GABA and glycine cDNAs. The presence of the different subunits of the receptor in the amplified product was then investigated by restriction analysis with enzymes specific for each subunit fragment and so far has indicated the presence of α1 and β1 subunits for these glycine receptors. Supported by the NH&MRC of Australia R.T. Hall Trust & Vincent Fairfax Family Foundation.

138.15

POSITIVE MODULATION OF GABA-INDUCED CURRENTS BY HISTAMINE AND RELATED COMPOUNDS IN RAT CEREBELLAR PURKINJE CELLS I.N.Sharonova, V.S.Vorobjev, G.D. Prell and H.L. Haas* Heinrich-Heine-Universität, Department of Physiology, Universitätsstr. 1, D-40225 Düsseldorf, Germany

We have studied the effects of histamine (HA) and some related compounds on GABA responses of voltage-clamped acutely isolated cerebellar Purkinje cells in whole-cell configuration. Conditioning application (2-10 s) of HA, histidine and the HA metabolites *tele*-methylhistamine (*tele*-MH), *tele*-methylimidazole acetic acid (*tele*-MIAA) and carnosine potentiated the current induced by 2 μM GABA 1 s after termination of the conditioning application. This effect was spontaneously reversible; 30 s or less were typically required for 50% recovery. Both the degree of potentiation and the time constant of recovery increased with the duration of conditioning application and the elevation of tested compound concentration. Preapplication of 10 μM histidine during 10 s doubled the GABA current, the EC_{50} was about 2 μM. Other HA related compounds potentiated GABA-mediated currents with the following order of potency: carnosine=HA=*tele*-MH<*tele*-MIAA<L-histidine=D-histidine. *Pros*-MH and *pros*-MIAA were without effect. The divalent ion chelators EGTA (0.5 mM), EDTA (0.1 mM), iminodiacetic acid and dithizon (10 μM) also potentiated GABA responses. The effects of histidine and divalent ion chelators were not additive at high doses. HA and related compounds may thus act as endogenous potentiating modulators of GABAA receptor mediated inhibition. The effect may depend on the ability of these componds to chelate metal ions.

138.16

MODULATION OF GABA-ACTIVATED CURRENT BY REDOX AGENTS IN CEREBELLAR PURKINJE CELLS MAY BE RELATED TO THEIR CHELATING PROPERTIES. H.L. Haas, H.J. Luhmann*, I.N. Sharonova and V.S. Vorobjev Department of Physiology, Heinrich-Heine University, POB 101007, D-40001 Düsseldorf, Germany

We have studied the influence of sulfhydryl redox reagents on GABA-gated chloride currents in acutely dissociated Purkinje cells with concentration jump techniques. Pre-exposure to the disulfide-reducing agent, dithiothreitol (DTT, 10 μM) during 10 s reversibly doubled the current, induced by 2 μM GABA 1 s after DTT withdrawal. The EC_{50} for DTT potentiation was 2.0±2 μM. Reduced and oxidized glutathione (GSH and GSSG) had the same effect on GABA-induced currents. The oxidizing agent 5,5'-dithiobis-2-nitrobenzoic acid (DTNB; 0.1-0.5 mM) and the thiol alkylating agent N-ethylmaleimide (0.1-1mM) had no such effect. Divalent ion chelators like EGTA (0.5 mM), EDTA (0.1 mM) and dithizone (10 μM) also augmented GABA-mediated responses. The potentiating effects of DTT and divalent ion chelators were not additive at high doses. Our observations suggest that these agents potentiate GABA-gated currents via common mechanisms. Thus, the widely used disulfide-reducing agent, DTT, can act through a mechanism different from breaking disulfide bonds. The data do not exclude a possible interaction of DTT with redox sites on the GABA receptor.

138.17

A NOVEL *DROSOPHILA* CHLORIDE CHANNEL GENE SHOWING HIGH HOMOLOGY WITH HUMAN *RHO* GENES. E.P. Semenov and W.L. Pak*, Department of Biological Sciences, Purdue University, W. Lafayette, IN 47907-1392

Using human *rho1* cDNA as hybridization probe, we have identified a novel gene encoding a GABA receptor-like protein in *D. melanogaster*. The protein sequence contains two cystein residues (Cys-loop) in its N-terminal extracellular portion that are conserved among chloride channels and conserved transmembrane domains within the C-terminal half. Sequence alignments show that the gene DNA sequence has highest homology with the human GABA *rho1* gene, which is expressed predominantly in the retina, rather than with other known *Drosophila* chloride channel genes. *In situ* hybridization to the fly polytene chromosomes maps the gene to a new site on chromosome 3.

Supported by NIMH grant MH 49727.

CALCIUM CHANNELS: PHYSIOLOGY, PHARMACOLOGY, AND MODULATION I

139.1

EXTRACELLULAR APPLICATION OF β-NAD^+ OR cADPR AFFECTS DEPOLARIZATION-INDUCED INTRACELLULAR Ca^{2+} RISE IN CEREBELLAR GRANULE. NEURONS C.Usai*, C.Marchetti, L.Moccagatta and A.Barberis, Istituto di Cibernetica e Biofisica, CNR, via De Marini, 6, 16149, Genova, Italy.

Experiments where cADPR was introduced in different cell types, suggested for this cyclic nucleotide the role of putative physiological ligand for the ryanodine-sensitive receptors of endoplasmic reticulum. However CD38, enzyme catalyzing the production of cADPR from βNAD, was localized on the external surface of the cell membrane. We studied the effect of external application of both βNAD and cADPR on $[Ca^{2+}]_i$ levels in Fura2-loaded cerebellar granule cells from 8-day old rats. Neurons were incubated with different doses (10μM÷1mM) of βNAD for ten minutes and then challenged with 25 mM external KCl, after a brief washout. The peak of $[Ca^{2+}]_i$ response was significantly increased in more than 80% of these experiments in a dose-dependent manner (12±2%÷47±15%). In addition, a small increase in the basal calcium level was observed during 1 mM βNAD treatment, but not with lower doses. The enzimatically inactive form αNAD failed to cause any increase in the KCl response. In a second group of experiments, cells were treated with 100μM cADPR for 6 minutes, then stimulated by 25 mM external KCl. This treatment did not modify the basal calcium concentration, but the peak of $[Ca^{2-}]_i$ response to KCl induced depolarization was significantly enhanced (44±18%, n=8). This effect was observed in approximately 50% of the experiments, suggesting some functional heterogeneity of the granule cells. Patch-clamp control experiments showed that βNAD reversibly inhibits the voltage-dependent calcium current, but potentiation of VDCC at later time after treatment was never observed. Therefore, since it has been reported that cADPR does not affect plasmalemmal calcium channel properties, both βNAD- and cADPR-induced increases of $[Ca^{2+}]_i$ following depolarization can be apparently view as a potentiation of calcium-induced calcium release. The nature of the intracellular calcium stores involved in this effect has not yet been characterized.

139.2

BODIPY® FL-X RYANODINE LABELING OF RYANODINE RECEPTOR CHANNELS IN RAT SYMPATHETIC NEURONS. González, C.E[1]., L. Granados [3], A. Cárabez [2] and A. Hernández-Cruz[1]*, [1] Instituto de Fisiología Celular, UNAM, [2] Dept. Biología Celular, Facultad de Medicina UNAM, Ciudad Universitaria, and [3] Inst. Nal. Pediatría, SS. México City, México.

The plant alkaloid ryanodine (Ry) is a specific and potent modulator of ryanodine receptor (RyR) Ca^{2+} release channels. The monosubstituted BODIPY FL-X analogue (B-Ry) was used to reveal the spatial distribution of RyRs present in cultured rat sympathetic neurons. Confocal microscopy showed cytoplasmic labeling of non-mitochondrial cisternae-like membranes. The nuclear membrane was also prominently stained. The time course of B-Ry binding was recorded with fluorescence digital microscopy. Labeling of individual neurons with 1 μM B-Ry reached steady-state within 15-20 min, and was reversible upon wash-out with a monoexponential time constant of decay of about 10 min. Pre-incubation with non-fluorescent Ry (20 μM, 5 min) reduced the rate of rise and peak amplitude of B-Ry binding by 60% and 50% respectively. However, non-fluorescent Ry did not increase significantly the rate of B-Ry unbinding. The basic pharmacology of B-Ry showed marked differences with normal Ry: a) it reduced, but never eliminated caffeine-induced Ca^{2+} release in a non-use dependent manner. b) it diminished voltage-gated Ca^{2+} influx by reversibly affecting voltage dependence and magnitude of plasmalemmal Ca^{2+} currents. These pharmacological differences between B-Ry and normal Ry must be considered in future studies using this compound. Supported by grant 400-346-5-2366PN from CONACyT (México).

139.3

STABLE TRANSFECTION OF PC12 CELLS WITH CALBINDIN-D_{28k} REDUCES STIMULATED $[Ca^{2+}]_i$ INCREASES. B. S. Wong*, M. C. Ng, A. M. Iacopino, A. McMahon and D. C. German, Dept. of Biomedical Sci., Baylor College of Dentistry, Dallas, TX 75246 and Dept. of Psychiatry, Univ. of Texas Southwestern Med. Center, Dallas, TX 75235.

The present study sought to determine whether calbindin-D_{28k} (CB) reduces intracellular calcium concentrations $[Ca^{2+}]_i$, in response to drugs that elevate $[Ca^{2+}]_i$. PC12 cells were stably transfected with CB and four clonal lines, with different expression levels, were selected. A vector control clonal line was also isolated. $[Ca^{2+}]_i$ was measured using the fluorescent dye fluo-3 and confocal microscopy. Bradykinin (0.5 μM) induced a rapid, transient ~80% increase in $[Ca^{2+}]_i$, followed by a 35% sustained increase (to 140 sec.) in control and vector control cells. The CB clonal lines exhibited the same transient increase in $[Ca^{2+}]_i$ as the control cells, however, all four lines exhibited a 70-85% reduction in the magnitude of the sustained increase. The extent of the reduction, however, was not dependent upon the amount of CB in the cells. ATP (100 μM) also caused a marked increase in $[Ca^{2+}]_i$, which was also significantly reduced ($p<0.01$) by the presence of CB. The rapid, transient elevation in $[Ca^{2+}]_i$ was decreased 20-40% of control, as was the sustained $[Ca^{2+}]_i$ elevation (decreased 45-64% of control). These data indicate that the presence of CB in PC12 cells reduces stimulated $[Ca^{2+}]_i$ increases. Studies are in progress to identify the specific sources of $[Ca^{2+}]_i$ that are altered by CB. Support by NS30406.

139.4

ALL-OR-NONE Ca^{2+} RELEASE FROM INTRACELLULAR STORES TRIGGERED BY Ca^{2+} INFLUX THROUGH VOLTAGE-GATED Ca^{2+} CHANNELS IN RAT DRG NEURONS. Y.M.Usachev* and S.A.Thayer, Dept. of Pharmacology, Univ. of Minnesota, Minneapolis, MN 55455.

Ca^{2+}-induced Ca^{2+} release from intracellular stores (CICR) serves to amplify the $[Ca^{2+}]_i$ signal that results from depolarization. In contrast to cardiac muscle, the amplification in neurons has previously been shown to occur in a graded manner. Now using combined indo-1-based microfluorimetry and whole-cell patch-clamp recording we show that in large DRG neurons, the stores act as a switch that when triggered by an elevation in $[Ca^{2+}]_i$ above threshold, elicits a regenerative response. The threshold was determined by stimulation with trains of action potentials (APs) of increasing number in the presence of caffeine (2-5 mM). For subthreshold stimulation, the amplitude of the $[Ca^{2+}]_i$ transient rose linearly with the number of APs. Suprathreshold stimulation triggered a process that was no longer proportional to the number of APs. The switch from a subthreshold to a maximal response increased dramatically both, the amplitude of the $[Ca^{2+}]_i$ transient (from 64±4 nM to 306±14 nM, n=26) and the time between termination of the stimulus and the peak $[Ca^{2+}]_i$ (from 1.2±0.1 s to 16.1±1.8 s, n=26). The maximal response was suppressed by ryanodine (10 μM) and inhibitors of voltage-gated Ca^{2+} channels (Cd^{2+} 200 μM and ω-conotoxin GVIA 1 μM) indicating that it results from CICR. This all-or-none reaction may serve as a gatekeeper that determines whether an electrical signal will be transduced into a local or widespread increase in $[Ca^{2+}]_i$.

Supported by the NIH (DA07304, T32 DA07234) and the NSF (IBN9412654).

139.5

EXTRACELLULAR SYNTHESIS OF CYCLIC ADENOSINE DIPHOSPHO-RIBOSE (CYCLIC ADP RIBOSE) FROM NAD BY RAT CORTICAL ASTROCYTES IN CULTURE. P.A. Rosenberg*, L. Pawlikowska, S. E. Cottrell, M. Harms, and Y. Li. Department of Neurology, Children's Hospital & Harvard Medical School, Boston MA 02115

Cyclic ADP ribose (cADPR) is an endogenous calcium-mobilizing agent synthesized from NAD by NADases with ADP-ribosyl cyclase and cADPR hydrolase activity. In the present study, selective culturing techniques were employed in order to localize ADP-ribosyl cyclase activity and cyclic ADPR hydrolase activity to astrocytes or neurons in cultures derived from rat embryonic cerebral cortex. ADP-ribosyl cyclase activity was determined by incubating cultures with 1 mM NAD in the extracellular medium for 60 minutes at 37°C, and measuring formation of cADPR by bioassay using sea urchin microsomes and by HPLC. Astrocyte cultures and mixed cultures of astrocytes and neurons had mean specific activities of 0.84 ± 0.06 and 0.9 ± 0.18 nmol cyclic ADPR produced/mg protein/hr, respectively. No detectable ADP-ribosyl cyclase activity was found in neuron-enriched astrocyte-poor cultures. The extracellular localization of the ADP ribosyl cyclase activity was demonstrated by showing that this activity did not increase when cultures were permeabilized with Triton X-100 or by acetone extraction. Cyclic ADPR hydrolase activity was detectable in astrocyte cultures and in mixed cultures by incubating cultures with 300 μM cADPR for 60 minutes at 37°C, and assaying loss of cADPR or accumulation of ADP ribose. The demonstration of extracellular ADP ribosyl cyclase and cADPR hydrolase activities associated with astrocytes may have important implications for the role of extracellular cyclic ADPR in signal transduction and in intercellular communication in the nervous system.

This work was funded by a grant from the NINDS (NS26830).

139.6

EFFECTS OF BETA-AMYLOID PROTEIN ON CALCIUM DYNAMICS IN PLATELETS AND C6 GLIOMA CELLS.
H. Ishikawa, H. Ozawa*, K. Imoto[1], H. Takemura[1], T. Saito, H. Ohshika[1], N. Takahata. Dept. of Neuropsychiatry and Pharmacology[1], School of Medicine, Sapporo Medical University, Sapporo, Japan.

Recent studies link amyloid beta-protein (Aβ) to disruption of neuronal Ca^{2+} dynamics and the neurotoxic action in terms of Alzheimer's disease (AD) pathology. Platelets are one of the major sources of Aβ in the circulation. The present study examined that the effect of Aβ on free cytosolic calcium ($[Ca^{2+}]i$) in human platelets compared to C6 glioma cells. Using the fluorescent calcium probe fura-2/AM, ($[Ca^{2+}]i$) levels in platelets and cells were measured during the resting state and when stimulated by Aβ. The addition of Aβ fragments 25-35 (Aβ 25-35) gradually increased $[Ca^{2+}]i$ and induced aggregation in platelets. After the maximum response, $[Ca^{2+}i]$ decreased and then reached a sustained, higher level. The maximum Ca^{2+} response over resting $[Ca^{2+}]i$ after Aβ 25-35 stimulation (5 mmol/l) was about 160 %. Similar effects were also observed with Aβ 1-40, whereas 1-28, 12-28 and 31-35 did not affect the Ca2+ response. In the absence of external Ca2+, Aβ 25-35 caused a transient increase in $[Ca^{2+}]i$, which returned to the resting level. Moreover, Aβ augments the formation of inositol 1,4,5-triphosphate(IP3) in platelets, which are similar effects to thrombin. In contrast to platelets, there were no enhancement of cytosolic calcium concentrations by Aβ 25-35 (up to 25 μM) in C6 glioma cells.

These findings suggest that Aβ directly induced cytosolic calcium enhancement is due to both extracellular influx and the release of intracellular Ca^{2+} stores involving IP3 signal cascades in platelets not but C6 glioma cells.

139.7

INTRACELLULAR CALCIUM DYNAMICS IN CNS MYELINATED AXONS. L. Steffensen* and P.K. Stys. Loeb Medical Research Institute, Ottawa Civic Hospital, University of Ottawa, Canada K1Y 4E9.

As in neurons, irreversible anoxic/ischemic injury in myelinated axons of central white matter has been associated with intracellular Ca overload. Central myelinated axons have been shown to contain high total (free and bound) levels of internal Ca and although the contribution of extracellular Ca to Ca overload has been well documented, the contribution of intracellular Ca has not been well examined. Pharmacological tools were used to examine Ca stores in myelinated axons of rat optic nerve and to determine what role internal Ca plays under physiological and pathophysiological conditions. We used an electrophysiological recording technique with paired stimulation at various interstimulus intervals, which we believe reflects changes in free [Ca]. Application of caffeine (10 mM), an activator of Ca-dependent Ca release stores, caused electrophysiological changes consistent with an increase in free [Ca] in the absence of changes in the area under the compound action potential (CAP). Caffeine (10 mM) plus bepridil (30μM), an inhibitor of the Na-Ca exchanger, irreversibly reduced CAP area to 69 ± 17% of control (n=7; $p<0.01$), whereas, application of bepridil alone had no effect. Ionomycin (2.5μM), a Ca ionophore, plus bepridil (30μM) caused an irreversible drop in CAP area to 51% ± 11% of control (n=8; $p<0.001$). Ryanodine (10 nM), a modulator of the internal Ca release channel, had no effect on resting nerves but increased free [Ca] in nerves stimulated by high frequency trains as judged by our paired stimulation protocol. These results suggest a relationship between electrical activity in axons and internal Ca dynamics, and may point to a physiological link between activity and cellular Ca-dependent metabolism. Under energy deprived conditions, release of Ca from stores may be contributing to Ca overload and irreversible injury. Supported by the Loeb Medical Research Institute.

139.8

CAFFEINE-INDUCED CALCIUM TRANSIENT IN MICE HIPPOCAMPAL NEURONS. T. Shimahara*, P. Chameau and R. Bournaud Laboratoire de Neurobiologie Cellulaire et Moléculaire, CNRS 91198 Gif-sur-Yvette, France.

Calcium transient induced by caffeine was studied in the cultured neurons from mice hippocampus. The calcium transient in the cell body was monitored by the fluorescent imaging analysis technique with the calcium indicator Fluo-3. In the 4-days cultured neurons, caffeine (20 mM) induced a calcium transient in 40 % of neurons, while 8 % of neurons showed a transient signal if caffeine application was preceded by KCl (70 mM) depolarization. The remaining neurons did not show the caffeine-induced calcium transient. In the 8-days cultured cells, the number of neurons which are sensitive to caffeine increased (70 % of neurons). In these neurons, the calcium store in the endoplasmic reticulum was depleted by repetitive application of caffeine but the endoplasmic reticulum refilled spontaneouly 80 % of the initial Ca^{2+} stock within 4 min. Ryanodine (50 μM), thapsigarin (2 μM) and dantrolene (10 μM) suppressed the caffeine-induced calcium transient. This study showed that at resting conditions the Ca^{2+} stores are continuously filled by releasable Ca^{2+}.

Supported by the grant from AFM.

139.9

CIRCADIAN VARIATIONS OF RYANODINE RECEPTOR IN THE SUPRACHIASMATIC NUCLEUS OF THE RAT. Myrna Dent#, Mauricio Diaz-Muñoz, José Luis Chávez and Raúl Aguilar-Roblero. SPON: Brain Research Association. #Facultad de Medicina, U. Autónoma Edo. de México, Toluca, Edo. de México 50180 and Dpto. Neurociencias, Instituto de Fisiología Celular, UNAM. México, D.F. 04510.

The hypothalamic suprachiasmatic nucleus (SCN) contains a circadian pacemaker responsible for most of the biological rhythms shown by mammals. Some reports have postulated that intracellular calcium ($Ca^{2+}i$) homeostasis might be playing an important role in the generation or maintaining of the SCN rhythmicity. To support this hypothsis, the properties of the ryanodine receptor (RyR) from the SCN were studied. RyR is a key element in the $Ca^{2+}i$ dynamics acting as a calcium release channel. Specific binding of [^{3}H]ryanodine to its receptor was performed in the SCN throughout a cycle of 12 h light/12 h darkness. The results show a significant peak in the [^{3}H]ryanodine specific binding to the SCN membranes at 12:00 and 15:00 h. This oscillatory pattern in the [^{3}H]ryanodine specific binding was detected exclusively in the SCN and not in other brain areas. IP_3 receptor, which is other calcium release channel, did not present any oscillatory pattern. The rhythm in the [^{3}H]ryanodine specific binding to SCN was still present during continuos darkness, suggesting its circadian nature. Scatchard analysis showed that the peak of [^{3}H]ryanodine specific binding was due to an increase in the number of RyR in the SCN, and not to an enhancement in the RyR affinity for its ligand. Immunohystochemical characterization demonstrated that the RYR present in the SCN is the type II, and that it is present mainly in neuronal cells. In conclusion, we are reporting the existence of a specific rhythm in one of the intracellular calcium release channels in the SCN which could have significant consequences in the $Ca^{2+}i$ homeostasis of this circadian pacemaker.

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139.10

RELEASE AND SEQUESTRATION OF CALCIUM BY RYANODINE-SENSITIVE STORES IN CA1 HIPPOCAMPAL NEURONS.
O. Garaschuk, Y. Yaari and A. Konnerth*. Universität des Saarlandes, 66421 Homburg, Germany.

The properties of ryanodine-sensitive Ca^{2+} stores in CA1 pyramidal cells were investigated in hippocampal slices by using fura-2 based Ca^{2+} imaging combined with whole-cell recordings. Local applications of caffeine induced Ca^{2+} transients which (1) persisted in Ca^{2+}-free saline, (2) were not associated with inward currents and (3) were blocked by ryanodine, indicating that they were caused by Ca^{2+} release from ryanodine-sensitive Ca^{2+} stores. The Ca^{2+}-transients evoked by closely spaced caffeine pulses rapidly decreased in amplitude, indicating progressive depletion of the stores. The amplitude of the Ca^{2+}-transients recovered 'spontaneously' with a time constant τ = 59 s. Depleted Ca^{2+} stores did not refill in Ca^{2+}-free saline. Thus, refilling of stores depends upon extracellular Ca^{2+} influx through 'capacitative' Ca^{2+} channels in the plasma membrane. Refilling was accelerated by depolarization-induced elevations in $[Ca^{2+}]_i$ and was blocked by bath-applied cyclopiazonic acid (CPA, 30 μM) or thapsigargin (1-3 μM). Even without prior store depletion, the caffeine-induced Ca^{2+}-transients disappeared after 6 min exposure to CPA, suggesting that Ca^{2+} level inside the stores is maintained by continuous Ca^{2+} sequestration. Elevations of basal $[Ca^{2+}]_i$ markedly potentiated (up to 6-fold) the caffeine-induced Ca^{2+}-transients. The degree of potentiation was positively related to the basal $[Ca^{2+}]_i$. The Ca^{2+}-transients remained potentiated up to 9 min. Thus, the stores can overcharge following a transient increase in $[Ca^{2+}]_i$ and slowly discharge excess Ca^{2+} after basal $[Ca^{2+}]_i$ returns to the resting level. We propose that neuronal ryanodine-sensitive calcium stores can act as either sinks or sources for Ca^{2+} when basal $[Ca^{2+}]_i$ increases or decreases, respectively.

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139.11

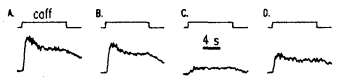
METHYLXANTHINES INHIBIT SPONTANEOUS $[Ca^{2+}]_i$ OSCILLATIONS IN THE ANTERIOR PITUITARY CELL LINE GH3. M. Cataldi, N. Santangelo, R. Caporaso, S. Amoroso, G.F. Di Renzo and L. Annunziato*
Section of Pharmacology, Dept. Neuroscience, School of Medicine, Federico II University of Naples, Via S. Pansini 5, 80131 Naples, ITALY

The clonal anterior pituitary GH3 cells exhibit wide spontaneous oscillations of $[Ca^{2+}]i$. Since methylxanthines have been reported to prevent the occurrence of $[Ca^{2+}]i$ oscillations in rat hepatocytes (Combettes et al. Biochem. J. 301:737,1994), in the present study the effect of these compounds on $[Ca^{2+}]i$ oscillations in GH3 cells was explored by fura-2 digital videoimaging. Both 400 μM 3-isobutyl-methylxanthine (IBMX) and 2 mM theophylline reversibly suppressed spontaneous $[Ca^{2+}]i$ oscillations. The effect of methylxanthines could be attributed either to the blockade of adenosine receptors or to the inhibition of phosphodiesterases (PDE). The hypothesis that the effect of IBMX and theophylline on $[Ca^{2+}]i$ oscillations depends on the blockade of adenosine receptors seems unlikely since 100 μM IBMX, a concentration reported to maximally block adenosine receptors, was ineffective. Furthermore, the adenosine receptor antagonist 8-cyclopentyl-1,3-dimethylxanthine (20 nM-1μM) did not induce any change in the pattern of spontaneous $[Ca^{2+}]i$ oscillations. By contrast, Vinpocetine (50 μM), a selective inhibitor of type-I PDE (Ca^{2+}/Calmodulin dependent), mimicked the effect of IBMX and theophylline on the pattern of spontaneous $[Ca^{2+}]i$ oscillations. These results suggest that methylxanthines interfere with the pattern of spontaneous $[Ca^{2+}]i$ oscillations by blocking type-I PDE activity. *(This study was supported by CNR and 40% and 60% MURST grants to L.A. and GF D.R.).*

139.12

WARMING INHIBITS CAFFEINE-STIMULATED Ca^{2+} TRANSIENTS IN CHICK SENSORY NEURONS. J. L. Kenyon* and H. R. Goff, Dept. of Physiol. & Cell Biol., University of Nevada School of Medicine, Reno, NV, 89557.

We used the amphotericin-perforated patch-clamp to measure I_{Ca} and $I_{Cl(Ca)}$, and indo-1 fluorometry to measure Ca_i^{2+} in neurons isolated from 10 day old chick embryos. Step depolarizations activated I_{Ca} and $I_{Cl(Ca)}$, the latter showing slow deactivation upon repolarization. Warming neurons (from 20 to 37°C) increased peak I_{Ca} (Q_{10}=2.3±0.15, mean ± SEM, n=5) and the rate of decline of $I_{Cl(Ca)}$ (Q_{10}=2.8±0.10, n=3). Below 21°C, 10 mM caffeine activated $I_{Cl(Ca)}$ by releasing Ca^{2+} from ryanodine-sensitive stores (J. Neurophysiol. 70:710, 1993). Warming reversibly abolished the activation of $I_{Cl(Ca)}$ by caffeine; the response disappeared and reappeared over a 3° range located between 21 and 30°C (n=5). Similarly, below 23 °C caffeine caused a rapid increase in the indo-1 fluorescence ratio. Warming to 30°C reduced the rate of rise and the amplitude of the signal (figure, n=4). Because the affinity of indo-1 for Ca^{2+} increases with warming, these data imply that warming reduces the increase in Ca_i^{2+} caused by caffeine. These observations suggest that the release of Ca^{2+} by ryanodine-sensitive intracellular Ca^{2+} stores is less effective at raising Ca_i^{2+} at physiological temperature compared with room temperature. Possibly the stores contain less Ca^{2+} or uptake mechanisms buffer release. The "break" in the temperature-dependence of caffeine-activated $I_{Cl(Ca)}$ suggests that a phase transition in the endoplasmic reticulum alters Ca^{2+} uptake, release, or both by intracellular stores.



Indo-1 fluorescence ratios in response to 10 mM caffeine recorded at 23° (A,B,D) or 29.5° (C).

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139.13

XESTOSPONGIN C: A POTENT CELL PERMEANT BLOCKER OF INOSITOL 1,4,5-TRISPHOSPHATE RECEPTOR CALCIUM PORE. J. Gafni*, J.A. Munsch#, T. Lam*, T.F. Molinski#, V. Kumari*, and I.N. Pessah*. Depts. of Vet. Molec. Biosci.*, Chemistry# and Med. Cell Biology and Human Anatomy*, University of California, Davis, CA 95616.

Components of the phosphoinositide signaling pathway abound in the brain, with particularly high levels of inositol 1,4,5-trisphosphate receptor (IP_3R) in the cerebellum. Stimulation of the IP_3R results in Ca^{2+} mobilization from intracellular stores, activating many processes. Known inibitors of the IP_3R have limited uses, due to problems such as low affinity, membrane impermeability and targeting of the IP_3-binding site. We show that Xestospongins A, C and D, a group of macrocyclic bis-1-oxaquinolizidines isolated from the Australian sponge, *Xestospongia sp.*, are potent blockers of IP_3-mediated Ca^{2+} release. IP_3-induced Ca^{2+} release from rabbit cerebellar endoplasmic reticulum (ER), reveals that Xestospongin C (Xest. C) is the most potent of these blockers (IC_{50}=300 nM). Competitive binding studies with [^{3}H] IP_3 show that Xest. C does not bind to the IP_3 binding site, suggesting that Xest. C is a pore blocker. [^{3}H] ryanodine binding studies and caffeine-induced Ca^{2+} transport assays also show that Xest. C blocks Ca^{2+} release via the ryanodine receptor with 5-30 fold lower potency. PC12 cells, a neuronal cell line which responds to bradykinin *via* an IP_3-mediated pathway, are used to study whole cell activity of Xest. C. Calcium imaging of fura-2 loaded PC12 cells reveal that 20 μM Xest. C decreases bradykinin-induced Ca^{2+} efflux from the ER store by 70%. Xestospongins represent a new class of potent, membrane permeable IP_3 blockers which exhibit a high selectivity over ryanodine receptors. Xest. C is a valuable tool for investigating the structure of the IP_3 pore and Ca^{2+} signaling in neuronal and non-neuronal cells.

This work was supported in part by NIH grant ES05002 and Training Grant ES07059.

139.14

TRI-ORTHO-SUBSTITUTED 2,2',3,5',6-PENTACHLORINATED BIPHENYL (PCB 95) ALTERS RAT BRAIN NEUROPLASTICITY IN VITRO THROUGH A RYANODINE RECEPTOR MEDIATED MECHANISM. P. W. Wong, T. E. Albertson, W. F. Walby, S. L. Schantz*, I. N. Pessah. Department of Molecular Biosciences, School of Veterinary Medicine, University of California Davis, Davis, CA 95616. *Institute for Environmental Studies, University of Illinois, Urbana, IL 61801.

Studies have revealed that non-coplanar polychlorinated biphenyls alter intracellular Ca^{2+} signaling in both cerebellar granular cells and PC12 cells *in vitro*. Recently we have shown that several *ortho*-substituted PCB congeners mobilize microsomal Ca^{2+} by activation of ryanodine sensitive microsomal Ca^{2+} channel/ryanodine receptor (RyR) through a FKBP12 (FK506 binding protein 12 KDa) mediated pathway. Study of several congeners have revealed a stringent structural requirement for the activity of PCBs towards RyRs. Measurements of electrical excitability of hippocampal slices were employed to futher elucidate the actions of non-coplanar PCBs in CNS. Single pulse orthodromic stimulation of Schaffer collateral/commissural (SC/C) fibers was performed by stimulating the striatum radiatum of CA1 with a bipolar electrode. Population spike (PS) and excitatory postsynaptic potential (EPSP) were recorded at striatum pyramidale. After perfusing the hippocampal slices with 25 μM PCB 95 (channel active congener) for 30 min, PS amplitude and EPSP slope were depressed, especially at high stimulus intensities. Significant reductions in both maximal PS amplitude and EPSP slope were observed, even after the induction of long term potentiation. However, these effects were not observed in 25 μM PCB 66 (channel inactive congener), suggesting that persistent activation of ryanodine receptor was responsible for the general depression of pyramidal cell excitability. Recently, preliminary studies on the effect of ryanodine on electrical excitability of hippocampal slice revealed that 300 μM ryanodine caused general depressions in both PS and EPSP of pyramidal cells. The similar effects of PCB 95 and ryanodine on the hippocampus, suggests that the action of PCB 95 on hippocampal excitability is through a ryanodine receptor mediated pathway.

139.15

LYSOPHOSPHATIDIC ACID ELEVATES INTRACELLULAR CALCIUM IN HIPPOCAMPAL NEURONS IN A NOVEL MANNER. F.W. Holtsberg*, M.R. Steiner, M.P. Mattson and S.M. Steiner, Univ. of Kentucky, Lexington, KY 40506.

The brain is a rich source of both lysophosphatidic acid (LPA) and the receptors for this potent lipid biomediator. Nonetheless, little is known about the intracellular signals generated by LPA in neurons. This study focuses on LPA-induced, calcium-related events in NGF differentiated PC12 cells and in primary neurons from embryonic rat hippocampi. LPA induced a rapid and transient increase in the concentration of intracellular free calcium ($[Ca^{2+}]_i$) in PC12 cells. This increase was inhibited by pretreatment of the cells with pertussis toxin, phorbol myristic acid or genistein, while it was not dependent upon extracellular calcium. These data are consistent with the increase in $[Ca^{2+}]_i$ in NGF differentiated PC12 cells resulting from signaling via a G protein coupled receptor, leading to activation of protein kinase C and a protein tyrosine kinase, which in turn result in the release of intracellular calcium stores. In contrast, in neurons, LPA stimulated a rapid and sustained increase in $[Ca^{2+}]_i$, which was not affected by pretreatment of the cells with any of the above inhibitors but was dependent upon extracellular calcium. This calcium response was also not affected by the addition of either cobalt or cadmium. However, treatment with the AMPA receptor antagonist, CNQX, or enzymatic depletion of extracellular glutamate partially inhibited the LPA-induced increase in $[Ca^{2+}]_i$. Thus, in hippocampal neurons, extracellular calcium is important in the LPA-induced increase in $[Ca^{2+}]_i$, which may be mediated, in part, via AMPA receptors.
(Supported by the NIH & Alzheimer's Assoc.)

139.16

CALCIUM-DEPENDENT PROPERTIES OF HIPPOCAMPAL PYRAMIDAL NEURONS IN TRANSGENIC MICE WITH REDUCED CALBINDIN D_{28K} EXPRESSION. P. Dutar*, A. Jouvenceau, J.M. Billard, Y. Lamour (1), and S. Ferrari (2). (1) INSERM U161, 75014 Paris France; (2) Univ. di Modena, 41100 Modena, Italia.

Calbindin D_{28K} (CaBP) is expressed in a subpopulation of CA1 pyramidal neurons of the hippocampus. We previously showed that the maintenance of long-term potentiation (LTP) is altered in the CA1 area in transgenic homozygous mice lacking CaBP expression. Using the *ex vivo* hippocampal slice preparation from wild-type and homozygous transgenic mice, we studied 1) the mechanisms of synaptic plasticity using extracellular recordings and, 2) the membrane properties of CA1 pyramidal neurons as a function of their CaBP content using intracellular recordings and immunofluorescence labelling.

1) Short-term potentiation (STP) induced by a weak tetanus (30 Hz; 0.5 sec), as well as paired-pulse facilitation of the field EPSP were not different in wild-type and homozygous mice. This contrasts with the lack of LTP maintenance and suggests the involvement of CaBP in long-term plasticity. The fEPSP slope measured as a function of stimulus intensity was significantly higher in homozygous while the afferent volley amplitude was unchanged. This suggests a modification of the synaptic efficacy of the CA1 afferent fibers in the homozygous mice.

2) Neurons recorded with sharp electrodes were filled with biocytin and the presence of CaBP in the neurons was checked using antiCaBP antibodies coupled to a fluorescent probe (FITC). In CaBP-depleted neurons from homozygous mice, a significant decrease in AHP duration as well as a reduction in spike discharge accommodation were observed. In contrast, membrane potential, membrane resistance, sodium spike amplitude and duration or EPSP and IPSP amplitude and duration were not affected. These results suggest a smaller activation of Ca-activated potassium conductance in the transgenic mice. This could be due to a stronger inactivation of Ca^{2+} currents or to an alteration in the intracellular transport of Ca^{2+}.

Supported by a grant from BAYER-Pharma France.

139.17

SPHINGOMYELIN AND ITS DERIVATIVES INCREASE VULNERABILITY TO OXIDATIVE STRESS IN PC-12 CELLS. N.A. Denisova*, I. Cantuti-Castelvetri and J.A. Joseph. USDA Human Nutrition Research Ctr. on Aging at Tufts University, Boston, MA 02111.

Previous research has shown that membrane sphingomyelin (SPM) incorporation may dramatically increase deficits in Ca^{2+} regulation induced by oxidative stress (OS) in PC-12 cells and increase conjugated diene levels. Since membrane SPM levels increase as a function of age, these findings suggest that interactions between OS and increased membrane SPM may be responsible for the age-related deficits seen in hippocampal Ca^{2+} homeostasis. Present experiments were carried out to determine whether derivatives of SPM (e.g., C2-ceremide (C2-cer), 100 μM; sphingosine, 20 μM; and sphingosine-1-phosphate, 1 μM; as well as SPMase, 100 mU/ml, pretreated 1-20 min.) would have similar effects on Ca^{2+} activity. Cells were then exposed to 0 or 300 μM H_2O_2 for 30 min., loaded with Fura-2 and Ca^{2+} flux imaged. No derivatives of SPM altered effects on pre-stimulation (baseline) Ca^{2+} levels, or peak Ca^{2+} depolarization in the absence of H_2O_2. However, each derivative of SPM, except C2-cer, acted similarly to H_2O_2 and significantly increased $Ca^{2+}RT$; C2-cer did not affect $Ca^{2+}RT$. These results suggest the both SPM and its derivatives could increase vulnerability to OS through initiation of Ca^{2+} disregulation. The nature of the C2-cer differences are being explored.

(Supported by USDA intramural)

CALCIUM CHANNELS: PHYSIOLOGY, PHARMACOLOGY, AND MODULATION II

140.1

ALTERNATIVE SPLICING IN THE DOMAIN I-II LINKER ALTERS α_{1A} Ca CHANNEL GATING AND MODULATION BY G PROTEINS. E. Bourinet*[1,2], T.W. Soong[1], G.W. Zamponi[1], A. Stea[1], J. Nargeot[2], and T.P. Snutch[1]. (1)Biotechnology Laboratory, University of British Columbia, Vancouver, B.C., V6T 1Z3 Canada ; (2)CRBM-CNRS UPR 9008, INSERM U249 1919 route de Mende, BP 5051, F34033 Montpellier France.

We have previously reported[1] that alternative splicing of the α_{1A} gene generates calcium channels with different physiological properties (α_{1A-a}, α_{1A-b}, α_{1A-c}). Of particular interest, small amino acid alterations in the domain I-II linker influence channel kinetics. This region has also been shown to be crucial in β subunit binding and for modulation of Ca channels by protein kinase C. To examine the potential role of the I-II linker on channel regulation by direct G-protein interaction, we reconstituted this second messenger cascade by coexpressing rat brain α_{1A} isoforms with either μ-opioid or α2-adrenergic receptors. Application of their respective agonists, DAMGO or phenylephrine, resulted in a voltage dependent inhibition of α_{1A} whole cell current. While all α_{1A} splice variants exhibited a similar magnitude of agonist-induced inhibition (~20%), they differed significantly in their rate of activation. Of particular note, agonist application resulted in α_{1A-b} currents that were much more slowly activating compared to α_{1A-a} or α_{1A-c} currents. Single channel current recordings in the absence of agonist revealed that the α_{1A-b} channel is characterized by an additional mode of gating with prolonged open times. Direct G-protein modulation has been suggested to alter Ca channel gating and we are currently investigating the single channel mechanism of the differential G-protein-dependent modulation of the α_{1A} splice variants. *Supported by grants from the MRC of Canada, the Howard Hughes Medical Institute and the NATO.*

[1] Soong et al (1994) Soc. Neurosci. Abstr. 34-20.

140.2

EXPRESSION OF RECOMBINANT ω-AGA-IVA AND MODIFICATION OF A PUTATIVE BINDING DOMAIN FOR P-TYPE CA CHANNELS. T. M. Norris*, V. Francis, M. Mehra, S. S. Gill and M. E. Adams. Depts. of Entomology and Neuroscience, University of California, Riverside, CA 92521.

The funnel web spider toxins ω-Aga-IVA and ω-Aga-IVB are high affinity antagonists (K_d ~ 1-3 nM) of P-type Ca channels in the mammalian brain. Structural analyses of Aga-IVA and Aga-IVB suggest identical disulfide-patterns and have led to a hypothesis that a cluster of conserved, highly basic residues (Arg21, 23, and 39) is important in channel binding. We have begun site-directed mutagenesis of recombinant Aga-IVA and evaluation of toxin mutants using whole-cell recording. A synthetic Aga-IVA gene was incorporated into a plasmid vector system and expressed in *E. coli* as a 6x His-Aga-IVA toxin fusion protein. The initial construct was designed to replace methionines at positions 30 and 42 with leucine residues, while a methionine was added between the His-tag and toxin gene for convenient cleavage of the fusion protein with cyanogen bromide. Replacement of methionine with leucine residues at positions 30 and 42 in the recombinant toxin had no effect on the kinetics of P-type channel block or recovery from block. The recombinant peptide blocked P-type channels with a K_d of 0.5 nM, a value almost identical to that of native Aga-IVA. Replacement of Arg39 with the less basic residue (lysine) reduced the rate of binding (k_{on}) ~ 8-fold and increased the unbinding rate (k_{off}) by ~ 2.5-fold, resulting in an approximate 18-fold decrease in K_d (~ 9 nM). These results suggest that Arg39 may be part of the Aga-IVA binding domain involved in P-type Ca channel antagonism.

140.3

CHARACTERIZATION OF ω-AGA-IIIA BINDING TO RAT BRAIN MEMBRANES. L. Z. Yan* and M. E. Adams. Environmental Toxicology Graduate Program and Depts. of Entomology and Neuroscience, Univ. of California, Riverside, CA 92521.

Electrophysiological studies have shown that ω-Aga-IIIA (IIIA), an 8.5 kDa peptide toxin from *Agelenopsis aperta* spider venom, is a high affinity (K_d ~0.5-1.0 nM) antagonist of high-threshold calcium channels, but spares low threshold T-type channels. The data suggest that IIIA identifies a conserved binding site unique to high threshold channels. To further evaluate IIIA binding to mammalian CNS sites, the toxin was iodinated directly with chloramine-T method and HPLC purified. Binding experiments were conducted at 25°C in 0.5-2 ml incubation volumes containing 50 mM HEPES/NaOH (pH 7.4) and 0.1% BSA for 3 hr. Saturation assays, performed in the concentration range of 0.1-15 μM [^{125}I]IIIA, showed that binding to rat brain membranes is saturable and involves high and low affinity sites. The K_d for the high affinity binding site was estimated to be ~1.3 pM from Scatchard analysis. Kinetic studies showed that the ligand-receptor interaction reaches equilibrium within 1.5 hr, with an association rate constant (k_{on}) of 0.0036 $min^{-1}pM^{-1}$, and dissociation rate constant (k_{off}) of 0.0055 min^{-1}, the latter corresponding to a τ_{off} of 181 min. The K_d value computed from the kinetic data is estimated to be ~1.5 pM, which is in agreement with Scatchard analysis. Ongoing experiments are aimed at the characterization of IIIA binding to uniform populations of calcium channels.

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140.4

DW13.3, A PEPTIDE TOXIN FROM THE SPIDER *FILISTATA*, DIFFERENTIALY BLOCKS TRANSIENTLY EXPRESSED NEURONAL CALCIUM CHANNELS. K.G. Sutton[1], A. Stea*[1], L.D. Artman[2], S. Heck[3], R.A. Volkmann[3], M.K. Ahlijanian[4] and T.P. Snutch[1]. [1]Biotech. Lab., Univ. of British Columbia, Vancouver, B.C. Canada, [2]NPS, Inc., Salt Lake City, UT, 84108, Depts. [3]Med. Chem. and [4]Neurosci., Pfizer, Inc., Groton, CT 06340.

The peptide toxin DW13.3 (Mr 8668, 74 amino acids), isolated from the venom of the spider *Filistata hibernalis* is a potent, relatively non-selective blocker of voltage-dependent calcium channels (CaCh). We have characterized the ability of DW13.3 to block rat brain α_{1A}, α_{1B}, α_{1C} and α_{1E} subunits coexpressed with α_2 and β_{1b} in *Xenopus* oocytes. DW13.3 produced a rapid onset of block for all classes of CaCh, however recovery from inhibition was significantly slower for α_{1A} (τ_{off} = 1738±341 s, n=5), when compared to α_{1B}, α_{1C} and α_{1E} (τ_{off} = 193±73 s, 59.8±4.9 s, 43.0±6.2 s respectively, n=4/6/5). IC_{50} values for DW13.3 block of α_{1B}, α_{1C} and α_{1E} were 28.4 nM, 27.3 nM and 86.7 nM. At the highest concentration used (1.15 μM) toxin block appeared to be nearly complete for these three isoforms. In contrast, the IC_{50} for DW13.3 inhibition of α_{1A} was 4.1 nM, yet 1.15 μM DW13.3 failed to produce total block (69.0±8.9 %, n=3). Kinetic analysis of τ_{on} and τ_{off} for α_{1A} produced an affinity ($k_d = k_{off}/k_{on}$ = 4.4±1.5 nM, n=5) comparable with the IC_{50} value determined from the dose response relation. The dose response data of the α_{1A} isoform is well described assuming a Hill coefficient of 1.0, although fit of the data required a model based on incomplete block. The reciprocal of the blocking time constant for α_{1A} ($1/\tau_{on}$) was linearly dependent upon DW13.3 concentration, consistent with 1:1 binding of the toxin molecule with the channel. This raises the possibility that DW13.3 acts on α_{1A} as a partial pore blocker. DW13.3 is a potent inhibitor of all CaCh studied to date, however its effect on α_{1A} contrasts with that observed for the other CaCh isoforms. *Research funded by:* Izaak Walton Killam Fellowship, MRC of Canada, Pfizer Central Research, NPS Pharmaceuticals.

140.5

EFFECT OF β_{1b}, β_{2e}, β_{3a}, OR β_{4a} SUBUNITS ON THE PHARMACOLOGICAL SENSITIVITY OF HEK293 CELLS STABLY TRANSFECTED WITH α_{1A} SUBUNITS OF HUMAN VOLTAGE-GATED Ca^{2+} CHANNELS. K.A. Stauderman, S. Simerson, P. Brust, M. Williams*, P. Prodanovich, C.-C. Lu, and M. Harpold. SIBIA Neurosciences, Inc., La Jolla, CA 92037.

We examined the pharmacological sensitivities of HEK293 cells stably transfected with cDNAs encoding full-length human α_{1A-2} and $\alpha_{2b}\delta$ subunits together with the β_{1b} (cell line A68-9o), β_{2e} (cell line E1H2), β_{3a} (cell line PB1-14), or β_{4a} (cell line 10-13) subunit. Western analysis confirmed the expression of α_{1A}, $\alpha_2\delta$, and β subunits in each cell line. The biophysical properties of the cell lines were also consistent with the expression of different β subunits in the functional Ca^{2+} channels (see Hans *et al.*, this meeting). Cells loaded with the Ca^{2+}-sensitive fluorescent dye fluo-3 responded to depolarization with 70 mM KCl with a transient increase in cytosolic $[Ca^{2+}]$ ($[Ca^{2+}]_i$) that was at least 200 nM at the peak. The KCl-induced $[Ca^{2+}]_i$ signals were not inhibited by 1 μM ω-CgTx-GVIA or 5 μM nimodipine. On the other hand, KCl-stimulated $[Ca^{2+}]_i$ responses were inhibited by ω-Aga-IVA in a concentration-dependent manner in all three cell lines with IC_{50} (nM) values as follows (mean ± SEM): 51 ± 9 (A68-9o), 93 ± 20 (E1H2), 54 ± 5 (PB1-14), and 80 ± 13 (10-13). Inhibition of KCl-evoked $[Ca^{2+}]_i$ signals was also produced by ω-CTx-MVIIC, with mean IC_{50} (nM) values (± SEM) of 48 ± 10 (A68-9o), 105 ± 12 (E1H2), 77 ± 11 (PB1-14), and 113 ± 10 (10-13). These data indicate that the β_{1b}, β_{2e}, β_{3a}, and β_{4a} subunits result in less than a 2.4-fold change in the potency of ω-Aga-IVA and ω-CTx-MVIIC to block human voltage-gated Ca^{2+} channels containing α_{1A-2} and $\alpha_{2b}\delta$ subunits. Although the β_{1b}, β_{2e}, β_{3a} and β_{4a} subunits do not appear to influence markedly the potency of peptide toxins, the effect of β subunits on the potency of small non-peptidergic organic antagonists is yet to be determined.

140.6

IDENTIFICATION OF BENZ(OTHI)AZEPINE BINDING REGIONS WITHIN L-TYPE CALCIUM CHANNEL ALPHA1 SUBUNITS. R. Kraus, B. Reichl, M. Grabner, S. D. Kimball, B. J. Murphy, W. A. Catterall, and J. Striessnig* Institut für Biochemische Pharmakologie, Universität Innsbruck, A-6020 Innsbruck, Austria; Bristol-Myers-Squibb, Princeton, NJ 08543-4000, USA; Department of Pharmacology, University of Washington, Seattle, WA 98115, USA

To identify the binding domain for diltiazem-like Ca^{2+} antagonists on L-type Ca^{2+} channel α1 subunits we synthesized the benzazepine [^{3}H]benziazem (35Ci/mmol) as a novel photoaffinity probe. [^{3}H]Benziazem reversibly labeled the benzothiazepine (BTZ) binding domain of partially purified skeletal muscle Ca^{2+} channels (Kd = 12 nM) with high yield (>66%). Antibody mapping of proteolytic labeled fragments revealed specific labeling of regions associated with segments S6 in repeats III and IV. More than 50% of the labeling was found in the tryptic fragment alanine-1023 - lysine 1077 containing IIIS6 together with extracellular and intracellular amino acid residues. The remaining labeling was identified in a second site comprising segment S6 in repeat IV and adjacent residues. Unlike for dihydropyridines, no labeling was observed in the connecting IIIS5-IIIS6 linker. The [^{3}H]benziazem photolabeled regions must be in close contact to the drug molecule when bound to the channel. Therefore the determinants for high affinity BTZ binding must be located within or in close proximity to segments IIIS6 and/or IVS6. The photolabeled regions may also contain residues that control BTZ access to its binding site.

We conclude that the binding domain for BTZs is located within pore-forming regions of the channel and in close proximity to the binding domains of the other main classes of Ca^{2+} antagonists (dihydropyridines and phenylalkylamines). Our findings provide the molecular basis for the non-competitive binding interactions observed between the major Ca^{2+}-antagonist binding domains of L-type Ca^{2+} channels.

This work was supported by grants from the Austrian Science Foundation (FWF S6602 to JS) and the NIH (HL44948 to WAC).

140.7

ANTIBODY CROSS-REACTIVITY AND PHARMACOLOGICAL SIMILARITIES BETWEEN CHICK AND RAT SYNAPTOSOMAL N-TYPE CALCIUM CHANNELS, J.J. Geer*, D.J. Dooley, and J. Offord. Parke-Davis Pharmaceutical Research Division, Warner-Lambert Co. Ann Arbor, MI 48105

N-type Ca^{2+} channels have been implicated in avian synaptosomal Ca^{2+} flux due to complete inhibition of flux by ω-conopeptide GVIA. It is well documented that rat synaptosomal Ca^{2+} flux, however, is not sensitive to these peptides despite the presence of ω-conopeptide binding sites. These functional discrepancies were addressed by comparing avian (chick) and mammalian (rat) synaptosomal N-type Ca^{2+} channels, using methods which targeted specific extracellular and intracellular domains. Extracellular domain similarity was assessed by comparing the dissociation constants (K_Ds) for binding of [^{125}I]-ω-conotoxin MVIIA to rat and chick synaptosomal membranes. ω-Conotoxin MVIIA, a large peptide which has a complex interaction with the channel, may bind to more than one extracellular site and might be expected to show differential affinity if critical binding sites vary between the two species. The K_Ds (rat, 1.6; chick, 2.0) were not statistically different. Additionally, the rank order of displacement of MVIIA binding by eight ω-conopeptides and two ω-agatoxins was found to be virtually identical in both preparations. Potencies of the peptides, as determined by the IC_{50} values, did not differ appreciably. The seven most potent inhibitors of binding were also the most potent inhibitors of $^{45}Ca^{2+}$ flux into chick synaptosomes, although rank order varied. Intracellular similarity was probed with an antibody directed against a GST fusion protein containing the internal loop between transmembrane regions II-III of the α_{1B} channel, the most highly variable region between Ca^{2+} channel subtypes. Protein from rat and chick membranes containing equal amounts of ω-conopeptide receptor was loaded onto SDS gels and probed with the antibody. Cross-reactivity of this antibody with chick as well as rat synaptosomal membranes indicated similarity between the N-type channel in the two species. A high degree of homology has been demonstrated between avian and mammalian N-type channels both at extracellular conopeptide binding domains and at the II-III intracellular loop. Failure of the conopeptides to block Ca^{2+} flux into mammalian synaptosomes remains unexplained from these comparisons. [Supported by Warner-Lambert Co.]

140.8

IDENTIFICATION OF BENZOTHIAZEPINE BINDING REGIONS WITHIN THE α1 SUBUNIT OF CALCIUM CHANNELS. A.Kuniyasu, N.Yoshida, H.Yabana, K.Naito, K.Itagaki, A.Schwartz, H.Nakayama*. Faculty of Pharm. Sci., Kumamoto Univ., Kumamoto, 862 Japan. Tanabe Pharm. Co., Toda, 335, Japan. LCMP, NIEHS, Research Triangles Park, NC 27709-704. Insititute of Mol. Pharm. Biophys., Univ. of Cincinnati Coll. of Med. OH 45267-0828.

To identify regions which are involved in the formation of the benzothiazepine (BTZ) receptor site of skeletal muscle L-type calcium channels, the α1 subunit of the channel complex was specifically labeled with a diltiazem-type photoaffinity probe, [^{3}H]azidobutyryl clentiazem (ABC). Two photolabeled regions were located by probing labeled proteolytic fragments with several anti-peptide antibodies recognizing different segments of the α1 sequence. A part of the α1 associated [^{3}H]ABC label was incorporated in a 13 kDa Lys-C fragment derived from the loop between S5 and S6 and the adjacent S6 segment in repeat IV (FEBS Lett., 334, 261-4, 1993). This region was partly shared with the photolabeled sites by dihydropyridine and phenylalkylamine probes. Another part of the labeling occured in a smaller Lys-C fragment which contained in a transmembrane segment of repeat IV. Our data suggest that the BTZ site is formed by close apposition of two discontinuous regions of the α1 subunit sequence in repeat IV. By identifying the BTZ binding site as the last of the three typical calcium antagonists, we can now obtain their overview and imply their allosteric interactions in the molecular model.

140.9

INVOLVEMENT OF PERTUSSIS TOXIN-SENSITIVE G PROTEINS IN EVOKED TRANSMITTER RELEASE AT THE FROG NEUROMUSCULAR JUNCTION. Y. Sugiura*, R. Tolentino and C.-P. Ko Department of Biological Science, University of Southern California, Los Angeles, CA90089-2520.

G proteins are thought to play an essential role in the signaling pathways of nervous system. To explore the G protein-linked modulation of neuromuscular transmission, we examined the effects of pertussis toxin (PTX), a Gi/Go inhibitor, on transmitter release at frog neuromuscular junctions (NMJs). Cutaneous pectoris muscles were incubated with, or without, PTX (2 μg/ml) for 22-24 hr at 4°C. Intracellular recording was performed in low Ca^{2+}/high Mg^{2+} saline and end-plate potentials (epps) and spontaneous miniature epps (mepps) were collected. PTX significantly increased epp amplitude (PTX; 6 95 ± 0.58 mV, n=53 vs. control; 3.78 ± 0.27 mV, n=52). PTX did not affect the amplitude and frequency of mepps, indicating that the effect was presynaptic. The result is consistent with the idea that G proteins inhibit voltage-sensitive calcium channels (VSCCs). Similar presynaptic potentiation of epps by PTX was seen at regenerating NMJs 2 weeks after nerve crush (PTX; 4.05 ± 0.43 mV, n=26 vs. control; 2.01 ± 0.26 mV, n=25). We have previously observed that L-type VSCC blockers *potentiate* evoked transmitter release at regenerating, but not intact, frog NMJs. This potentiation at regenerating NMJ was abolished by PTX pretreatment, indicating an involvement of G proteins. The present results suggest the existence of PTX-sensitive G protein-linked modulation in evoked transmitter release at normal and regenerating frog NMJs. At the regenerating NMJ, the PTX-sensitive G protein may be a link between N-type VSCCs that mediate transmitter release and L-type VSCCs that modulate transmitter release. (Supported by NIH grant NS 30051).

140.10

INHIBITION BY G PROTEINS OF N-TYPE Ca^{2+} CHANNELS EXPRESSED IN *XENOPUS* OOCYTES. AN ANALYSIS USING 'CUT-OPEN' METHOD. S. Kaneko*,[1] Y. Mitani[1], T. Hata[1], M. Kikuwaka[1], A. Akaike[1] and M. Satoh[2]. Depts. of [1]Pharmacol. and [2]Mol. Pharmacol., Facul. of Pharm. Sci., Kyoto Univ. Kyoto 606-01, Japan.

To investigate the intracellular modulation of N-type Ca^{2+} channels by G protein $\beta\gamma$ subunits, 'Cut-Open' recordings were made in *Xenopus* oocytes expressing cloned N-type channels and κ opioid receptors. Before intracellular perfusion, application of strong prepulse to oocytes (+100 mV, 100 ms duration, 20 ms before recording pulse) significantly increased the amplitude of Ca^{2+} channel current and fastened the activation kinetics of channel opening. Intracellular perfusion with a Cesium-EGTA-GTP solution for 30 min completely abolished the effects of prepulse without run-down of the channel current. However, κ agonist stimulation decreased the amplitude of Ca^{2+} channel current but not influenced the activation or inactivation kinetics. These results indicate that the intrinsic retardation of channel kinetics may be mediated by a mechanism which is independent of the G protein-mediated inhibition of current amplitude.

140.11

REGULATION OF HUMAN NEURONAL N- AND E- TYPE Ca^{2+} CHANNELS BY G-PROTEIN β/γ SUBUNITS IN HEK293 CELLS. L. R. Shekter*, P. T. Toth, R. Taussig+, and R. J. Miller. Dept. Pharm./Phys. Sci., U. Chicago, Chicago IL 60637 & +Dept. Biol. Chem., U. Michigan, Ann Arbor MI 48109

We previously compared receptor regulation of N (α_{1B}) and R (α_{1E}) currents in HEK293 cells (courtesy SIBIA Neurosciences). We observed strong inhibition of Ca currents by κ–opioid and SRIF agonists in α_{1B} expressing but not α_{1E} expressing cells containing κ–opioid and SRIF receptors. In the present study we examined the effects of G-protein β/γ subunit overexpression in both α_{1B} and α_{1E} containing cells. In α_{1B} containing cells the whole-cell current was facilitated an average of 339.7±86% while the time course of activation was reduced from τ=6.00±0.54 ms before the prepulse (p.p.) to τ=2.51±0.29 ms after the p.p. (n=6). The I-V relationship also shifted from an average peak of +30 mV before the p.p. to +10mV after. Interestingly, we also observed a population of cells in which there was no facilitation (-2.3±0.12% n=7) but in which time course of activation was distinctly inhibited (τ=9.38±0.92 ms before p.p., τ=6.69±0.50ms after p.p.). Varying the p.p. duration and magnitude had no effect on G-protein relief. In untransfected cells there was no effect on either facilitation (1.9±0.24%; n=7) nor on time course of activation (τ=2.35±0.40 ms before the p.p. and τ=2.51±0.50 ms after). In α_{1E} containing cells under the same conditions, a reduced but distinct facilitation was observed; the same paradigm was employed but V_{test} was 0mV instead of +10mV. The current was facilitated an average of 41.2±19.7% (n=5) while the time course of activation was reduced from τ=3.26±1.46 ms to τ=1.76±0.50 ms. In addition, the peak I-V shifted from 0mV to +30mV. In untransfected cells, the average peak current after the p.p. was *reduced* 24.04±5.83% (n=4) while the time course of activation remained essentially unchanged (τ=1.49±0.26 ms before p.p. compared with τ=1.70±0.36 ms after p.p.). Constituitively active forms of the G-protein subunits α_i and α_o were also tested for their effects on α_{1B} and α_{1E} expressing cells but none were observed. This work was supported by PHS Grants DA-02575, DA-02121, MH-40165 and NS-33502 to R.J.M.

140.12

CHARACTERISTICS OF G PROTEIN-MEDIATED INHIBITION OF α_{1A} and α_{1B} Ca^{2+} CHANNELS, J.P. Roche* and S.N. Treistman. Dept. of Pharmacology and Mol. Tox., Program in Neuroscience, Univ. of Mass. Medical Ctr., Worcester, MA 01655.

α_{1A} and α_{1B} calcium channels have been found to co-localize at synapses and are both thought to be capable of mediating synaptic transmission. Differential modulation of these channels by G proteins may provide neurons with mechanisms for precisely regulated exocytotic release. We have compared the characteristics of inhibition of α_{1A} and α_{1B} calcium currents by a tonically active G protein population in *Xenopus* oocytes, as well as after activation of a co-expressed M_2 or M_3 muscarinic receptor by 50 μM acetylcholine. We have found for both the α_{1A} and α_{1B} Ca^{2+} current, that upon removal of the initial application of acetylcholine there is a 2-3 fold rebound of current amplitude over the initial control levels. The rebound current then slowly returns towards the control current amplitude. This effect is seen with co-expression of either the M_2 or M_3 receptor. We conclude that the rebound is due to temporary loss of tonic inhibition based on 1) the loss of depolarizing prepulse facilitation at the peak of the rebound and 2) the correlation between the amplitude of rebound current and the amplitude of the current after treatment with either NEM or GDPβS. Additionally, there was a significant difference in the voltage-dependence of G protein-mediated inhibition between α_{1A} and α_{1B} calcium currents. The reversal of G protein inhibition by depolarizing prepulses was much greater for the α_{1B} channel. This may be due to a differential affinity of the G protein for the two α_1's at strongly depolarized potentials. The difference in the effects of depolarizing prepulses may indicate that that the α_{1B} channel plays a greater role in transmitter release in situations where high frequency trains of action potentials occur in the presynaptic neuron. In this situation the α_{1B} current would more readily overcome the G protein inhibition. (This work supported by NIH grant AA05542 to S.N.T.)

140.13

HUMAN TERATOCARCINOMA-DERIVED NEURONAL NT2-N CELLS CONTAIN ω–CGTX-GVIA AND NIFEDIPINE SENSITIVE Ca^{2+} CURRENTS. Torben R. Neelands*#, R. Scott Turner*& and Robert L. Macdonald*@, Departments of Neurology* and Physiology@ and the Graduate Program in the Neurosciences#, University of Michigan Health Sciences Center and Department of Veteran Affairs Medical Center&, Ann Arbor, MI.

Treatment of human NT2 teratocarcinoma cell line twice weekly for four weeks with 1 μM retinoic acid causes them to differentiate into neuron-like NT2-N cells. We investigated whether these neuron-like NT2-N cells expressed calcium currents using whole cell voltage-clamp electrophysiological techniques. NT2-N cells were replated onto 35 mm dishes 1-7 days prior to recording. Cells were held at -80 mV in an external solution using Ba^{2+} as the charge carrier and blockers of other voltage-gated ion channels. The pipette solution contained 5 mM ATP and 0.3 mM GTP. A series of hyperpolarizing and depolarizing voltage commands were applied. Inward Ba^{2+} currents were evoked starting at -35 mV, peaking at +5 mV, and reversing at +60 mV. Individual voltage step commands to +5 mV (the peak inward current) were then repeated until the peak current had stabilized (300-600 pA). Individual 300 msec voltage commands to +5 mV were then repeated in the presence of nifedipine, ω–CgTx-GVIA (conotoxin) or Cd^{2+}. All NT2-N cells were partially blocked by nifedipine and conotoxin and completely blocked by Cd^{2+}. The presence of N- and L-type Ca^{2+} currents in a dividing human neuronal-type cell line provides a novel model for the study of human derived calcium channel regulation. [*Supported by R01-NS33300 to RLM and the Dept. of Veterans Affairs*].

140.14

RATE OF RE-INHIBITION OF CA^{2+} CURRENT BY GTP-γ-S AND 5-HT DIFFERS BEFORE AND AFTER STIMULATION OF PKC. Y. Chen, and N.J. Penington*, Pharmacol. SUNY (H.S.C.) Brooklyn N.Y. 11203.

We studied the rate of association of the G-protein subunit responsible for 5-HT-induced inhibition of current carried by Ba^{2+} in serotonergic neurons, with or without the phorbol ester PMA. We inserted a gap between the prepulse to +80mV and the test pulse. At lower concentrations of 5-HT the re-inhibition of the Ba^{2+} current was slower (Elmslie and Jones 1994). The averaged data was fit by a single exponential of τ = 56.8 ms for 1μM and τ= 124.9 ms for 15nM 5-HT. PMA (1μM) reduced the percent inhibition of peak Ba^{2+} current by 5-HT (1μM) and the rate of re-inhibition from 56.8 to 93.5 ms (n=7 cells). Swartz (1993) found that reversal of GTP-γ-S effects by PMA was voltage dependent, and required a 200ms step to +80mV every 5 seconds. In dorsal raphe (DR) neurons using this protocol, PMA did not reverse the effect of GTP-γ-S. The average rate of re-inhibition by GTP-γ-S had a τ =58.1 ms (n=6) that did not change in PMA (1μM). However, when PMA was added to the bath before going whole cell, GTP-γ-S was significantly ($p<.001$) less effective at slowing Ba^{2+} current activation (average facilitation=71%, reduced to 39% in PMA), and the rate of re-inhibition of the Ba^{2+} current was slowed (τ =88.5 ms n=3). As PMA slowed the rate of re-inhibition by 5-HT, fewer activated G-protein subunits may be available to interact with the Ca^{2+} channel; appearing to rule out a direct action of PMA at that site. In DR cells, only prior PMA pretreatment can reduce the effects of GTP-γ-S. This result suggests an action of PMA at the G-protein, but an effect on the receptor cannot be ruled out.
Supported by an NIMH First award to N.J.P.

140.15

INHIBITION OF Ca^{2+}/CALMODULIN-DEPENDENT PROTEIN KINASE II REDUCES VOLTAGE-GATED Ca^{2+} CURRENT IN HIPPOCAMPAL NEURONS . E.M. Blalock and P.W. Landfield*. Dept. Pharmacology, Coll. Med., U. Ky, Lexington, KY 40536.

Although several groups have found that inhibition of Ca^{2+}/calmodulin-dependent kinase II (CaMKII) prevents voltage- and Ca^{2+}-dependent facilitation of Ca^{2+} current, the basic effects of CaMKII inhibition on non-facilitated Ca^{2+} channel activity are still not well understood. Some studies have reported inhibition of voltage-gated Ca^{2+} current in peripheral tissue with KN-62, a blocker of CaMKII. However, there is some evidence that this inhibition may be due to direct block of Ca^{2+} channels, rather than inhibition of CaMKII. Here we tested the hypothesis that CaMKII modulates the amplitude of basal, non-facilitated Ca^{2+} current in hippocampal neurons.

In cultured rat hippocampal cells, KN-62 reduced whole-cell Ca^{2+} current amplitude by greater than 40% regardless of whether it was applied internally or externally (consistent with its lipophilicity). When 60 mM Na^+ was the charge carrier through voltage-gated Ca^{2+} channels, KN-62 had no effect on current amplitude. Calmidazolium, a calmodulin inhibitor, paralleled the effect of KN-62. Single L-type Ca^{2+} channel activity was unchanged by the addition of KN-62 to the on-cell pipette solution. Together these results suggest that basal, non-facilitated Ca^{2+} current is modulated by Ca^{2+}- and calmodulin- dependent CaMPKII activity; and that KN-62 does not directly block voltage-gated Ca^{2+} channels (supported by AG04542 and Bayer, Inc.).

140.16

TYROSINE KINASES EXERT A TONIC CONTROL ON CALCIUM CHANNELS IN RAT HIPPOCAMPAL NEURONS. C. Rovira, B. Potier, Y. Lamour*, INSERM U161, 2, rue d'Alésia, 75014 Paris France.

Protein phosphorylation is a major regulatory process of synaptic plasticity and calcium channels activity. Tyrosine kinases, present in large amounts in the hippocampus, play an important role in synaptic plasticity. They are activated by neurokines and oncogene products which also cause a rise in intracellular calcium. High-Voltage-Activated calcium channels (HVA) of hippocampal pyramidal cells are substrate for serine/threonine protein kinases but it is not known wether they are modulated by tyrosine kinases. We studied the effects of genistein, a tyrosine kinase inhibitor, and orthovanadate, a tyrosine phosphatase inhibitor on HVA using whole-cell patch-clamp recordings of CA1 pyramidal neurons in hippocampal slices (300 μM thick) from 14-20 day-old rats. Barium currents through HVA were evoked by regular (0.03 Hz) voltage steps to 0 mV from a holding potential of -60 mV. Intrapipette solution contained either BAPTA or EGTA as calcium buffers. Genistein (100μM) and orthovanadate (300μM) were bath applied. In the presence of intracellular BAPTA, genistein reduced the HVA by 24%(n=3) in an irreversible manner.The inactive analogue, genistin (100μM), had no effect on the current amplitude. Orthovanadate reversibly increased the current amplitude by 21% (n=3). In the presence of intracellular EGTA the effect of orthovanadate was more pronounced and HVA were increased by 47% (n=2). These data show that tyrosine kinases tonically control the activity of HVA in rat hippocampal pyramidal cells.

140.17

THE INHIBITORY EFFECT OF GENISTEIN ON THE ACTIVITY OF L-TYPE Ca^{2+} CHANNELS IS NOT EXERTED AT THE LEVEL OF α_{1C} SUBUNIT. A. Bassi, M. Cataldi, F. Hofmann¶, GF Di Renzo, M. Taglialatela* and L. Annunziato. *Section of Pharmacology, Dept. Neuroscience, School of Medicine, Federico II University of Naples, Via S. Pansini, 80131 Naples, ITALY and ¶ Institut für Pharmakologie und Toxikologie, Technische Universität München, Munchen, GERMANY*

Recently, we reported that tyrosine-kinase (TK) inhibitors reduce the activity of L-type Ca^{2+} channels in pituitary GH_3 cells (Cataldi et al J. Biol. Chem. in press). In order to understand whether this modulation is exerted on the α_{1C} subunit of this channel, expressed in GH_3 cells, the effect of genistein on $[Ca^{2+}]_i$ response to K^+ depolarization was studied by fura-2 single cell videoimaging in a cell clone stably transfected with the α_{1C} subunit of L-type Ca^{2+} channels, the CHO α9 cells (Bosse et al. EMBO J. 11: 2033;1992). In these cells, basal $[Ca^{2+}]_i$ was stable (88.4±1 nM) and no spontaneous $[Ca^{2+}]_i$ oscillations were detected. When CHO α9 cells were perfused with a depolarizing 55 mM K^+ solution, $[Ca^{2+}]_i$ increased by 44% over basal values. This K^+-induced $[Ca^{2+}]_i$ increase was markedly lower than the ~400% increase observed in GH_3 cells. No increase in $[Ca^{2+}]_i$ was observed when untransfected control CHO cells were exposed to high K^+ concentrations. Interestingly, in CHO α9 cells genistein (100 μM) did not exert any inhibitory action on the $[Ca^{2+}]_i$ increase induced by 55 mM K^+ (54% increase over basal values) whereas in GH_3 cells it blocked the K^+-induced $[Ca^{2+}]_i$ increase by 50%. These data indicate that the effect of TK inhibitors is not exerted directly on the α_{1C} subunit and that additional Ca^{2+}-channel subunits and/or modulatory proteins migth be involved. *(This study was supported by CNR and 40% and 60% MURST grants to L.A. and GF.D.R.).*

140.18

ROLE OF TYROSINE KINASES IN $GABA_B$-MEDIATED INHIBITION OF N-TYPE CALCIUM CURRENT. M. Diversé-Pierluissi* and K. Dunlap. Departments of Physiology and Neuroscience, Tufts University School of Medicine, Boston, MA 02111.

In embryonic chick dorsal root ganglion neurons, N-type calcium current is inhibited by γ-aminobutyric acid (GABA) via $GABA_B$ receptors coupled to the GTP-binding protein G_O. The inhibition has two components, a voltage-dependent slowing of the activation kinetics and a voltage-independent steady-state inhibition. These inhibitory components do not appear to involve serine-threonine kinases. Here we present evidence that the steady-state inhibition induced by GABA requires the activation of tyrosine kinase. Genistein, a tyrosine kinase inhibitor, blocked the GABA-mediated steady-state inhibition. Its inactive analog, daidzein had no effect. A tyrosine kinase inhibitor containing amino acids 137-157 from the regulatory region of src kinase prevented the steady-state inhibition but not the kinetic slowing produced by GABA. This pathway is likely to be mediated by α_o as a G$\beta\gamma$-binding peptide (derived from the $\beta\gamma$ binding domain of βARK2) failed to alter the GABA response. Recombinant α_o will be used to directly test this idea. In contrast to other α_o-mediated tyrosine kinase pathways (van Biesen et al. 1996, *JBC* **271**, 1266-1269), this effect of GABA is unique in that it does not require protein kinase C.

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140.19

ACTION POTENTIAL WAVEFORM-DEPENDENT MODULATION OF N-TYPE CALCIUM CHANNELS. D. Park* and K. Dunlap. Dept. of Neuroscience, Tufts Univ. Sch. of Med., Boston, MA 02111.

We studied GABA-mediated inhibition of N-type Ca^{2+} current evoked by command potentials derived from chick sensory neuron action potentials (APs). Voltage-dependent forms of inhibition were identified by their sensitivity to a conditioning depolarization (to 80 mV) preceding the AP. The relative proportions of voltage-dependent and -independent inhibition were studied as a function of variations in AP amplitude and duration of repolarization. As the peak of AP was decreased from 44 to -5 mV, control N current decreased by 40 %. The total GABA-induced inhibition increased slightly from 57±7 to 67±9 % (mean±S.D.) with decrease in AP amplitude, but the proportion of voltage-dependent to -independent inhibition (~1.1) did not change significantly within the physiological range. With increases in the duration of repolarization (from 0.65 to 31.2 ms), peak N current decreased, but total charge entry through the N channel increased. GABA-induced inhibition also decreased from 59±10 to 37±6 %. This time-dependent relief of inhibition was associated entirely with the voltage-dependent component (33±6 to 16± 2 %); voltage-independent component was unchanged throughout the duration sequence. These results suggest that the extend and types of N current inhibition produced by GABA will vary under conditions of changing physiological stimuli and suggest a physiological relevance of multiple modulatory components.

(This work was supported by NS16483.)

140.20

UP-REGULATION OF L-TYPE AND NON-L-, NON-N-TYPE CALCIUM CHANNELS BY PROTEIN KINASE C IN INSULIN-SECRETING RINm5F CELLS. D. Platano, A. Pollo*, E. Carbone and G. Aicardi§. Dept. of Neuroscience, Univ. of Turin, Italy. (§)Dept. of Human and General Physiology, Univ. of Bologna, Italy.

We studied the effect of protein kinase C (PKC) inhibition and activation on voltage-dependent Ca^{2+} channels in rat insulinoma RINm5F cells. PKC down-regulation by chronic (24 h) treatment with the PKC activator phorbol 12-myristate 13-acetate (PMA, 1 μM) reduced by about 60% the Ba^{2+} currents through L-type and non-L-, non-N-type Ca^{2+} channels, indicating that PKC tonically up-regulates these channels under basal conditions. Consistently, PKC activation by acute PMA (30-300 nM) application caused only a modest increase (average 23%) of Ba^{2+} currents in a minority of cells (24%). L-type and non-L-, non-N-type channels were differently up-regulated by either basal or stimulated PKC activation. Acute cell exposure to PMA preferentially caused Ba^{2+} current increases in a voltage range in which L-type channels were mostly activated (-30 to -10 mV in 10 mM Ba^{2+}), and the action was greatly reduced or even abolished by 5 μM nifedipine. On the other hand, the non-L-, non-N-type channel was more effectively up-regulated under basal conditions since acute application of PKC activators caused only minor increases of the ω-conotoxin-GVIA- and nifedipine-resistant current in a small percentage of cells. Unexpectedly, the run-up of Ba^{2+} currents during acute PMA application was followed by a progressive current decrease, which was also observed in isolation in another 24% of the cells. This action was abolished when 15 mM phosphocreatine was added to the internal perfusate, and could thus be ascribed to PKC-induced ATP depletion, rather than to a direct effect of PKC on Ca^{2+} channels. We also found that PKC-mediated phosphorylation is not involved in the voltage-dependent and voltage-independent G-protein mediated α_2-noradrenergic modulation of Ca^{2+} channels in RINm5F cells (Aicardi et al., 1991. *FEBS Letters* **281**:201-204).

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POTASSIUM CHANNELS: PHYSIOLOGY

141.1

LESIONING OF THE DOPAMINERGIC NEURONS OF THE SUBSTANTIA NIGRA WITH 6-HYDROXY DOPAMINE RESULTS IN LOSS OF AN ATP-SENSITIVE K^+ CHANNEL ACTIVATION. A. McGroarty* and S. A. Greenfield. University Dept. of Pharmacology, Mansfield Rd, Oxford, OX1 3QT, U.K.

A sub-population of neurons within the guinea-pig substantia nigra pars compacta (SNpc), which fire in a phasic fashion, show a large K^+ conductance with the characteristics of an ATP sensitive K^+ channel (K_{ATP}). This channel can be activated by agents which decrease intracellular ATP levels, e.g. hypoxia and blocked by the K_{ATP} blocker tolbutamide (100-500μm). We have previously investigated the effect of dopamine (DA) depleting agents on this hypoxic response using *in vitro* current clamp techniques. Previous data has shown that reserpine 6mg/kg i.p. (depletes DA storage), but not α-methyl para tyrosine 200mg/kg i.p. (decreases DA synthesis) causes a loss of the response to a 5 min hypoxic insult. We now show that the DA neurotoxin 6-hydroxy dopamine (6-OHDA) has a comparable effect to reserpine. Unilateral lesions of the nigral DAergic neurons were produced by 6-OHDA injection into the medial forebrain bundle. Only the lesioned hemisphere showed loss of response. Cells recorded from this hemisphere showed a mean decrease in membrane resistance of merely 17.99±4.44MΩ, whereas the decrease seen in cells from the non-lesioned hemispheres was 54.01±14.46MΩ and indistinguishable from control (41.29± 3.69MΩ) and sham operated cells (51.28±4.66MΩ). Values from 6-OHDA lesioned SNpc (n=7) were significantly different from control ($p<0.005$, n=5), non-lesion ($p<0.05$, n=8) and sham values ($p<0.001$, n=4, unpaired Student t-test). Extent of lesion was assessed using HPLC, or immunocytochemistry for tyrosine hydroxylase. We conclude that 2 quite different DA toxins cause very similar effects, strongly suggesting a link between DA systems and K_{ATP} channels in the SNpc. The results suggest that loss of DA storage could render this brain region vulnerable to further degeneration, due to the loss of K_{ATP} activity and hence of protection against metabolic insult. A.M. is supported by a Sandoz Pharma Scholarship.

141.2

FUNCTIONAL ANALYSIS OF G PROTEIN $\beta\gamma$ STRUCTURE, B. Velimirovic,* Y. Li, D.E. Clapham and E.J. Neer Dept. of Pharmacology, Mayo Foundation, Rochester, MN 55905 and Cardiovascular Division, Brigham and Women's Hospital, Harvard Medical School, Boston, MA 02115.

G protein $\beta\gamma$ subunits (G$\beta\gamma$) transduce signals from neurotransmitter and hormone receptors to both the classical G protein-coupled and the mitogen activated protein kinase pathways. G$\beta\gamma$ acts directly to activate several effectors including inwardly rectifying potassium channels in cardiac (I_{KACh}) and neuronal cells and phospholipase C β (PLCβ). Crystallographic analysis of G$\beta\gamma$ reveal a propeller-like structure of Gβ with seven blades each made up of characteristic WD-40. In order to test what regions of the G$\beta\gamma$ dimer are responsible for effector activation we introduced various mutations into the Gβ subunit and tested for its ability to activate recombinant I_{KACh}. Mutants were designed by substituting amino acid residues positioned on the outer surface of the dimer. The regions that were probed include the N-terminal part of Gβ contributing to the coiled-coil structure and parts of each of the seven propeller blades. This work was supported by AHA-Minnesota (BV), AHA (YL) and NIH grants (DEC and EJN).

141.3

ABNORMAL CALCIUM HOMEOSTASIS IN CEREBELLAR GRANULE CELLS OF WEAVER MICE. M.D. Womack*, K. Thompson, E. Fanselow, G.J. Augustine and A.S. Peterson. Departments of Neurobiology and Genetics, Duke University, Durham, NC 27710.

Weaver mice have a genetic defect that causes a drastic and selective loss of cerebellar granule cells within the first two postnatal weeks. This defect is caused by a point mutation in GIRK2, an inwardly rectifying, G-protein coupled potassium channel. We have used fluorescence imaging methods to test the hypothesis that the channel mutation leads to granule cell defects by altering the concentration of calcium ($[Ca^{2+}]_i$) within granule cells. $[Ca^{2+}]_i$ was measured in individual neurons within cerebellar slices from mice (postnatal day 4-5) via the calcium indicator fura-2. In wild type mice, the average $[Ca^{2+}]_i$ in granule cells was about 60 nM. The average $[Ca^{2+}]_i$ in *weaver* granule cells was nearly twice that of wild type granule cells. Elevated $[Ca^{2+}]_i$ was observed only in *weaver* granule cells; $[Ca^{2+}]_i$ in Purkinje cells of *weaver* mice was only slightly elevated over that of wild type Purkinje cells (also about 60 nM). This is consistent with the observation that Purkinje cells and other cerebellar neurons of *weaver* mice do not undergo the drastic cell loss characteristic of granule cells. $[Ca^{2+}]_i$ in neurons of heterozygous mice was similar to that in neurons of homozygous *weaver* mice. Thus, at the time that cell death begins, *weaver* granule cells express both mutant GIRK2 channels and elevated $[Ca^{2+}]_i$. Because $[Ca^{2+}]_i$ can regulate cell migration, neurite extension, and cell survival, elevated $[Ca^{2+}]_i$ may comprise the link between mutant GIRK2 channels and granule cell defects in *weaver* mice. Supported by NS17771, NS34045 and the National Downs Syndrome Society. A.S.P. is a Sandoz Scholar.

141.4

EFFECTS OF SHAKER K CHANNEL (Kv1.1)-NULL MUTATION ON CEREBELLAR PHYSIOLOGY IN MICE. C. L. Zhang*, A. Messing, and S.Y. Chiu. Dept. of Neurophysiology and Vet. Medicine, Univ. of Wisconsin, Madison, WI 53706.

In the cerebellum, the output of the Purkinje cells is subjected to inhibitory control by basket cells that release GABA onto the Purkinje cell body. The mechanism that regulates this release is unknown, but likely involves voltage-gated K channels. Immunohistochemistry shows that Kv1.1 & Kv1.2, two Shaker K channels, are co-localized to the basket-cell-Purkinje-cell junction. In this study, we used Kv1.1-null mutant mice to assess the role of Kv1.1 (as well as the compensatory role of Kv1.2) in regulating the tonic inhibition of Purkinje cells. Whole-cell patch clamp recordings of spontaneous inhibitory postsynaptic currents (IPSCs) were made from Purkinje cells in thin cerebellar slices from P10-P13 Kv1.1-null mutants using wild type littermates as control. In normal saline solutions, mutation caused a specific increase in the frequency (~2 fold) but not the amplitude of IPSCs. Mutation had no effects on the IPSCs measured in TTX. The IPSCs in the mutant were blocked by bicuculine (10 uM) but not affected by CNQX (10 uM) and APV (100 uM). In wild type slices, Shaker channel blockers 4-AP (0.3 mM), TEA (0.3 mM) and DTX (0.8, 8 nM) mimicked null-mutations by causing a differential increase of frequency over amplitude of the IPSCs. The role of residual Shaker K channels (like Kv1.2) in modulating IPSCs in the mutant was investigated by applying these blockers to mutant slices. Interestingly, TEA (0.3 mM) and DTX (0.8 nM) were without effects on the mutant IPSCs. Granted that Kv1.2 is more sensitive than Kv1.1 to these blockers at these concentrations, a negligible role of Kv1.2 in the mutant is suggested. We conclude that Kv1.1 plays an important role in regulating spontaneous GABA release onto Purkinje cells, with limited ability from a closely related member Kv1.2 to compensate for the loss of function. Supported by NIH grant 23375.

141.5

POTASSIUM SPATIAL BUFFER EXPLORED BY DIFFUSION ANALYSIS AND BARIUM IN NEOCORTEX, W. Schwindt*, C.-K. Tong and C. Nicholson, Dept. of Physiology & Neuroscience, NYU Medical Center, New York, NY 10016.

Prominent among proposed mechanisms for brain potassium (K^+) homeostasis is spatial buffering (SB) by glial cells (Orkand et. al. 1966, *J. Neurophysiol.* **29**: 788; Gardner-Medwin 1983, *J. Physiol.* **335**: 393). The relative importance of SB for K^+ homeostasis was assessed using diffusion analysis and applying Ba^{2+} (0.5 to 5 mM) to block the inward rectifier K^+ channel (K_{IR}) in rat neocortical slices.

Slices were cut at 400 μm from Sprague-Dawley rats. K^+ and the extracellular probe tetramethylammonium (TMA^+) were iontophoresed from microelectrodes and monitored about 100 μm away with ion-selective microelectrodes. Diffusion analysis (Nicholson 1993, *J. Neurosci. Meth.* **48**: 199) in 0.3 % control agarose gel yielded the free diffusion coefficient $D = 1.86 \pm 0.03 \times 10^{-5}$ cm^2 s^{-1} (mean ± SEM, 34 °C). In slices it provided extracellular volume fraction α, apparent diffusion coefficient (D^*), tortuosity factor $\lambda = (D/D^*)^{0.5}$ and non-specific uptake k'. In normal conditions $\lambda_{K^+} = 1.77 \pm 0.04$. It was unaffected by Ba^{2+} and similar to that obtained with TMA^+ ($\lambda_{TMA^+} = 1.78 \pm 0.04$). In contrast, the apparent $\alpha_{K^+} = 0.62 \pm 0.02$ declined to 0.43 ± 0.01 with Ba^{2+} while $\alpha_{TMA^+} = 0.22 \pm 0.01$. Non-specific uptake was exceptionally low ($k'_{K^+} = 7.24 \pm 2.5 \times 10^{-4}$ s^{-1}) and Ba^{2+} induced a rise to $k'_{K^+} = 4.6 \pm 0.6 \times 10^{-3}$ s^{-1}, matching $k'_{TMA^+} = 4.9 \pm 0.9 \times 10^{-3}$ s^{-1}. Ba^{2+} did not affect parameters measured with TMA^+.

Surprisingly, despite the postulated presence of SB, K^+ diffusion curves could be fitted accurately by standard diffusion theory. However, α_{K^+} and k'_{K^+} deviated substantially from those obtained with TMA^+ as shown in the in vivo rat (Nicholson et al. 1979, *Brain Res.* **169**: 580-84). The threefold larger value of α_{K^+}, compared to α_{TMA^+}, suggests that α_{K^+} is not measuring true extracellular space. Furthermore, although the presumed blocking of the K_{IR} reduced α_{K^+}, it did *not* lead to normal values (i.e. α_{TMA^+}). Thus SB seems to contribute to K^+ homeostasis but is apparently not the only mechanism involved. Regarding k' it was striking again that SB, a mechanism based on K^+ entering glial cells, was *not* characterized by a high uptake. But k'_{K^+} did increase to values comparable to k'_{TMA^+} when the K_{IR} was presumably blocked. Finally we note that λ_{K^+} was similar to λ_{TMA^+} and unchanged by Ba^{2+}. These paradoxical findings emphasize the need for detailed modeling of these SB experiments. Supported by NIH Grant NS 28642.

141.6

ELECTROPHYSIOLOGICAL PROPERTIES OF RAT MEDIAN RAPHE NEURONS. Y. Nishimura*, Y. Yoshioka, K. Ito, H. Kitagawa, M. Lin, T. Asahara and T. Yamamoto. Dept. Physiol. Sch. Med. Mie Univ. Tsu, Mie 514 Japan

Electrophysiological properties of the median raphe neurons were investigated by intracellular recording and whole-cell patch clamp techniques. Slices of 400 μm for intracellular recording and of 150-200 μm for whole-cell patch clamp were made from the young rat (2 - 21 d. o.) The intracellular recording was performed using micro-pipet filled with Biocytin or Lucifer yellow. The whole-cell patch clamp was employed after cleaning the surface of the soma. TEA (20 mM) prolonged the action potential in the depolarized membrane potential, though it affected the spike duration little in the resting membrane potential. Action potential was prolonged by 4-AP (300 μM) in the resting membrane potential. Firing patterns induced by long outward current (500ms duration) were grouped into regular spiking and phasic spiking. Ca^{2+} channel blockers (replacement of Ca^{2+} with Co^{2+}, Ni^{2+}) increased the firing rate in the regular spiking cells but not in the phasic spiking cells. These agents did not alter the phasic spiking patterns. The phasic spiking patterns, however, were changed into the regular spiking patterns by 4-AP. The 4-AP sensitive outward current was transient and was easily inactivated by depolarizing prepulses. The TEA-sensitive outward current showed weak inactivation. The outward current blocked by Ca^{2+} channel blockers was persistent. In the median raphe neurons the spike repolarization was controlled by mainly 4-AP sensitive current. The firing rate in the regular spiking cells was controlled by Ca^{2+}-mediated K^+ current. This study was supported by Ministry of Education, Science and Culture of Japan.

141.7

CONTRIBUTIONS OF THREE POTASSIUM CURRENTS TO FREQUENCY-DEPENDENT SPIKE BROADENING OF *APLYSIA* R20 NEURONS. M. Ma* and J. Koester. Center for Neurobiology and Behavior, Columbia University, N.Y., NY 10032.

The R20 neurons of *Aplysia* exhibit frequency-dependent spike broadening. We had previously identified three K+ currents that mediate action potential repolarization: a transient A-type K+ current (I_{Adepol}), a delayed rectifier current ($I_{K\text{-}V}$), and a Ca^{2+}-sensitive K+ current ($I_{K\text{-}Ca}$). A major constraint in the earlier study was the lack of completely selective blockers for I_{Adepol} and $I_{K\text{-}V}$, resulting in an inability to assess directly the effects of their activation and inactivation on spike broadening. In the present study, the dynamic clamp technique, which employs computer simulation to inject biologically realistic currents into a cell under current clamp conditions, was used either to block I_{Adepol} or $I_{K\text{-}V}$ or to modify their inactivation properties.

The data lead to the following hypothesis for the mechanism of spike broadening in the R20 cells. As the spike train progresses, the primary responsibility for spike repolarization gradually shifts from I_{Adepol} to $I_{K\text{-}V}$, to $I_{K\text{-}Ca}$. This sequence can be explained on the basis of the relative rates of activation and inactivation of each current with respect to the constantly changing spike durations, the cumulative inactivation of I_{Adepol} and $I_{K\text{-}V}$, and the progressive potentiation of $I_{K\text{-}Ca}$. Positive feedback interactions between spike broadening and inactivation contribute to the cumulative inactivation of both I_{Adepol} and $I_{K\text{-}V}$. The data also illustrate that when two or more currents have similar driving forces and partially overlapping activation characteristics, selectively blocking one current under current clamp conditions can lead to a significant underestimate of its normal physiological importance, due to the interplay of different currents via their effects on spike shape. (This work was supported by NIH grant NS14385.)

141.8

OUTWARD POTASSIUM CURRENTS OF MAGNOCELLULAR NEUROSECRETORY CELLS ISOLATED FROM THE SUPRAOPTIC NUCLEUS OF THE ADULT GUINEA PIG. M. D. Hlubek* and P. Cobbett. Dept. of Pharmacology and Toxicology and The Neuroscience Program, Michigan State University. East Lansing, MI 48824.

A necessary prerequisite to understanding the mechanisms which underlie firing patterns of oxytocinergic and vasopressinergic magnocellular neurosecretory cells (MNCs) is a thorough characterization of the specific ionic conductances present in the membrane of these cells. Using whole-cell patch clamp techniques, we have identified four different outward K^+ currents in MNCs acutely isolated from the SON of the adult guinea pig. Depolarizing steps from a holding potential (V_H) of -40 mV produced a long-lasting, slowly-activating K^+ current which activated at test potentials more positive than -20 mV. This voltage-activated delayed K^+ current was preferentially blocked by extracellular tetraethylammonium (TEA). In addition, extracellular Cd^{2+} significantly reduced this sustained current, suggesting the presence of a long-lasting Ca^{2+}-dependent K^+ current. An additional, more rapidly activating and decaying outward K^+ current which activated at test potentials more positive than -60 mV was recorded during depolarizing steps from V_H -90 mV. This voltage-activated transient K^+ current was preferentially blocked by extracellular 4-aminopyridine (4-AP). In many recordings from MNCs, a transient, 4-AP-insensitive outward current was evoked by depolarizing voltage steps from V_H -40 mV. This transient K^+ current was abolished by extracellular Cd^{2+}, suggesting that it is a transient Ca^{2+}-dependent K^+ current. Supported by NINDS (NS28206) and Neuroscience Training Grant NINDS (NS07279).

141.9

GATING KINETICS OF D_2 DOPAMINE RECEPTOR-MODULATED K^+ CHANNELS ON RAT STRIATAL NEURONS. Gabriela J. Greif*[1] and Jonathan E. Freedman. Dept. Pharmaceutical Sciences, Northeastern Univ., (and [1]Dept. Neurobiology, Harvard Medical School), Boston, MA 02115.

We used cell-attached patch-clamp recording to characterize the kinetics of activation of an 85 pS inwardly rectifying K^+ channel by D_2-like dopamine receptors on freshly dissociated rat caudate-putamen neurons. Previous studies indicated that channel activation has an absolute requirement for the presence of D_2-like dopamine agonists, and occurs in a membrane-delimited fashion. At concentrations near the dissociation constant (1-10 nM), the D_2 agonist quinpirole elicited isolated brief openings and short bursts, while increasing the quinpirole concentration to 10 μM markedly prolonged burst durations. Within a burst, there was a single population of open dwell times, which increased in duration as a function of quinpirole concentration, and two populations of closed dwell times, which were insensitive to agonist concentration. Similar results were obtained with a different agonist, 7-OH-DPAT, and with the G-protein activating peptide mastoparan, but not when the channel was metabolically activated independently of the receptor by rotenone. These results suggest that dopamine receptor-dependent activation of this channel stabilizes a burst-opening state. (Supported by NIH grant MH-48545.)

141.10

POTASSIUM CURRENTS IN NEURONS FROM THE VENTRAL LATERAL MEDULLA (VLM) OF RATS. J.W. Peters*, J. S. Allard, and C.O. Trouth. Dept. of Physiology & Biophysics, Howard Univ., Wash., DC 20059.

Central respiratory CO_2/pH chemosensitivity has been described on the ventrolateral brainstem (VLM) surface. These studies were undertaken to identify K^+ currents present in developing VLM neurons. Cells were isolated for whole-cell patch-clamp recording, without the use of enzymes, by placing the surface of a tissue section in a petri dish coated with high molecular weight poly-lysine. The tissue slice was removed after 30-180 minutes and cells adhering to the coating were cultured overnight. The reversal potential was -33 ± 3.3 mV (mean ± SEM; N=16); increasing K_o by 35mM resulted in a depolarizing shift of 15.8 ± 1.7 mV(N=13). Preliminary results indicate a variety of K^+ channel types and combinations that are present in these neurons. Inwardly rectifying K^+ channels were found in 7 of 16 cells tested. A slowly inactivating potassium current (I_{Ka}) was found in 12 of 15 cells examined. JWP and JSA were supported by ONR Grant # N00014-94-1-0523.

141.11

ROLES OF VOLTAGE-DEPENDENT TRANSIENT POTASSIUM CURRENT IN THE EXCITABILITY OF SUPRACHIASMATIC NUCLEUS NEURONS. Rong-Chi Huang*Department of Physiology, Chang Gung College of Medicine and Technology, Taiwan, ROC

The purpose of this study is to investigate the physiological roles of a voltage-dependent transient potassium current (I_A) in electrical excitability of suprachiasmatic nucleus (SCN) neurons. Experiments were performed on isolated hypothalamic slices using the extracellular recording technique, and neuronal excitability of SCN neurons was examined by evoking population action potentials (PAP) with a bipolar electrode located at the optic chiasm near SCN.

Addition of TTX and total substitution of choline$^+$ for Na^+ completely eliminated the evoked PAP, indicating that the PAP is due to activation of the voltage-dependent Na^+ current in these neurons. Double pulses stimulation suppressed the amplitude of the second PAP as opposed to that of the first control PAP, and the degree of suppression decreased with longer interpulse intervals. Recovery from suppression was complete in about 1 second, similar to the time course of recovery from Na^+ current inactivation. Addition of 1 mM 4-aminopyridine (4-AP) decreased the amplitude and slope of PAP, and markedly prolonged the duration of PAP. Furthermore, 4-AP greatly enhanced the double pulse suppression at short interpulse intervals. The results indicate that the 4-AP-sensitive I_A current participates in setting the duration of action potential and more importantly in regulating firing rate of SCN neurons. This work was supported by Chang Gung Medical Research Foundation (CMRP506).

141.12

CARDIAC CELLS ALTER THE EXPRESSION OF POTASSIUM CURRENT IN CHICK SYMPATHETIC NEURONS. D.A. Przywara*and A.R. Wakade. Department of Pharmacology, Wayne State University, Detroit, MI 48201.

The pharmacologic behavior of sympathetic neurons (SN) in culture is different from their counterparts growing in the body. The K^+-channel antagonist tetraethylammonium (TEA) has little effect on evoked release of [^{3}H]norepinephrine ([^{3}H]NE) from cultured SN, but acts in the expected manner seen in mature neuroeffector organs when SN are co-cultured with heart cells (Wakade et al., J.Physiol. 488, 1995). We examined the effects of several K^+-channel antagonists in SN grown alone and with cardiac cells. Action potentials (AP) of SN and co-cultured SN had similar shape and duration (3.7 ± 1 and 3.0 ± 0.4 ms at 50% repolarization). TEA caused 8 to 10 fold increase in AP duration (APD) in co-cultured SN but had little effect on SN alone. 4-aminopyridine (4-AP, 1mM) which blocks I_A in SN had little effect on APD in mono or co-cultured SN, suggesting that differential expression of I_A was not involved. Charybdotoxin caused a small increase in evoked [^{3}H]NE release under both culture conditions and produced similar block of isolated, voltage-clamped K^+ current. Outward current in the presence or absence of Ca^{2+} also showed similar density in SN alone or with cardiac cells, suggesting that Ca^{2+}-dependent K^+ current was not differentially expressed. However, Ca^{2+} current density was significantly lower in SN plus cardiac cells compared to SN alone (0.19 ± 0.03 and 0.61 ± 0.13 pA μm^{-2}, respectively) and when $[Ca^{2+}]_i$ was buffered to ≤ 100nM in SN, TEA increased APD 8 to 10 fold. These data support the conclusion that cardiac cells control TEA sensitivity of SN K^+ channels via altered voltage-dependent Ca^{2+} entry.

141.13

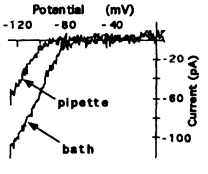
EVIDENCE THAT SMOCS RESULT FROM THE ACTIVATION OF K_{Ca} CHANNELS BY CALCIUM RELEASED FROM RYANODINE SENSITIVE CALCIUM STORES. L.A. Merriam*, J.C. Hardwick and R.L. Parsons. Dept. of Anatomy & Neurobiology, Univ. of VT., Burlington, VT 05405.

Spontaneous miniature outward currents (SMOCs) are thought to result from the release of Ca^{++} from intracellular stores, which then activates clusters of large conductance K_{Ca} channels. In vascular smooth muscle cells, the Ca^{++} is released from a ryanodine-sensitive store located close to the sarcolemma. Less is known about the Ca^{++} store responsible for these transient currents in neurons. We have extended the previous observations of Satin and Adams (1987) to test if the Ca^{++} which initates SMOCs is released from an intracellular ryanodine-sensitive Ca^{++} store. We recorded SMOCs from dissociated mudpuppy parasympathetic postganglionic neurons using the perforated patch technique in the presence of 200μM $CdCl_2$ to block voltage-dependent Ca^{++} influx. At -10mV, ryanodine (10 or 50μM) caused an increase in SMOC frequency followed by a concentration dependent elimination of SMOC activity as Ca^{++} stores became depleted. During exposure to ryanodine, SMOC configuration changed as would be expected from ryanodine locking the Ca^{++}-induced release channel in an open, subconductance state. Thapsigargin (100 nM), which inhibits endoplasmic reticulum Ca^{++}ATPase, also eliminated SMOCs. Exposure to low concentrations of caffeine (<3mM) increased SMOC frequency; following multiple challenges with 30 mM caffeine, SMOCs were eliminated. These results suggest that neuronal SMOCs result from Ca^{++} released from ryanodine-sensitive stores. Using immunofluorescence microscopy, we also demonstrated ryanodine receptor-immunoreactivity in these neurons. Supported by NS 23978.

141.14

LOW POTASSIUM SALT BRIDGES RECTIFY ANOMALOUS RESULTS. G.G. Schofield, J.T. Weber* and M.J. Mason. Depts of Physiology and Anatomy, Tulane University Medical School, New Orleans, LA 70112.

During initial patch-clamp investigations of possible stretch-induce activation of rat basophilic leukemia cells, we employed drug applicatio from a large diameter micropipette to single cells in a static bath. T reduce possible junction potential errors we grounded the preparation via 3 M KCl salt bridge. Under these conditions, application of control solution from either glass or polyethylene micropipettes induced a dramatic reduction of whole-cell inward rectifier (IK_{in}) currents (see figure). This decrease in the amplitude of IK_{in} resembled channel block by a contaminant released from the micropipette. However, a concomitant negative shift of the reversal potential of the ramp current suggested an alteration of the [K^+] bathing the cell. Flame photometry confirmed K^+ accumulation in the chamber over a 15 min period from 3.0 mM to 7.4 1.2 mM (n = 4). Raising extracellular [Ca^{2+}] from 1 to 5 mM also induce a dramatic decrease in the amplitude of IK_{in}, but without a shift of revers potential. These data demonstrate the extreme sensitivity of IK_{in} to change in external [K^+] and an unexpected sensitivity of IK_{in} to external [Ca^{2+} Thus, a small junction potential (< 5 mV) from a low K^+-containing sa bridge may be preferable to the effects of uncontrolled accumulation c external [K^+] which can occur when using a 3 M KCl salt bridge to obviat junction potential correction. Supported by LEQSF.



141.15

Ca^{2+}-ACTIVATED K^+ CHANNELS OF SYMPATHETIC NEURONS FROM ADULT HYPERTENSIVE RATS. Walter P. Robertson and Geoffrey G. Schofield.* Dept. of Physiology, Tulane University Medical Center, New Orleans, LA 70112.

Essential hypertension has been linked with hyperexcitability of sympathetic neurons. Since K_{Ca} channels of adult rat sympathetic neurons have not been characterized, we examined if altered K_{Ca} channel properties underlie neuronal hyperexcitability in hypertensive rats (SHR). In excised patches bathed in symmetrical 130 mM K^+, single K_{Ca} channel I-V relationships were linear between -50 and +50 mV and reversed near 0 mV. Mean slope conductances were 126±4 pS for SHR (n=6) and 124±16 pS for normotensive WKY neurons (n=4). With 0.1 μM cytoplasmic $[Ca^{2+}]$, channels activated positive to -50 mV and reached half maximal Po near -20 mV in both rat strains. Preliminary estimates of Ca^{2+} binding affinities of K_{Ca} channels were 630 nM and 203 nM at -50 and -30 mV, respectively, for SHR (see figure), and 643 nM and 233 nM in WKY neurons. Based on these data, altered steady-state properties of K_{Ca} channels cannot explain sympathetic neuron hyperexcitability in the SHR.

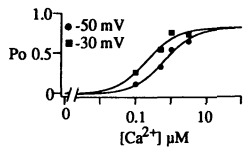


Figure. Calcium dependence of P_O for a K_{Ca} channel from an SHR. P_O calculated as the fraction of time spent in the open state at -50 mV (circles) and -30 mV (squares) is plotted against cytoplasmic $[Ca^{2+}]$. Solid lines are least-squares fits of the data to a single site binding isotherm. The apparent affinity of K_{Ca} channels for Ca^{2+} increases with depolarization.

Supported by NIH and LEQSF.

141.16

BK CHANNELS IN EMBRYONIC RAT NEUROEPITHELIUM ARE STRETCH SENSITIVE. GD Lange*[2], JL Barker[1] and J-M Mienville[1]. [1]Lab of Neurophysiology & [2]Instrumentation and Computer Section, NINDS, NIH, Bethesda, MD 20892.

The mechanosensitive properties of large-conductance Ca^{2+}-activated K^+ (BK) channels from embryonic rat neuroepithelium were investigated with the cell-attached and inside-out configurations of the patch-clamp technique. The channels were activated in both recording configurations by negative pressures applied to the patch electrode, but reversal of the effect was total and immediate in inside-out patches whereas it was incomplete and delayed in on-cell patches. This mechanosensitivity was not mediated by Ca^{2+} ions or fatty acids, suggesting that it is an intrinsic property of these channels. Cytochalasin B did not affect mechanosensitivity in on-cell patches but increased it in inside-out patches. Kinetic studies showed that stretch increased the mean open time of the channels and decreased the slowest time constant of their closed-time distributions. The present as well as previous results suggest complex interactions between embryonic BK channels and their membranous and submembranous environment.

141.17

EFFECTS OF INTRACELLULAR CALCIUM CHELATION ON THE TRANSIENT POTASSIUM CHANNEL (K_A) IN NEOCORTICAL PYRAMIDAL NEURONS L. Jiang[1] and J. Kang*[2]. [1]Department of Hepatitis Clinic, People Hospital of Liaoning, Shenyang, Liaoning 110015, P.R. China and [2]Department of Cell Biology and Anatomy, New York Medical College, Basic Science Building, Valhalla, NY 10595

To investigate influence of intracellular Ca^{2+} on the K^+ channel for A-current (K_A) during neuronal spiking, single large layer V pyramidal neurons in visualized neocortical slices were patched by two electrodes: a whole-cell current clamp and a cell-attached patch. Electrodes for whole-cell recordings were filled with K·MeSO4 plus a Ca^{2+} chelator, 1,2-bis (2-aminopheoxy) ethane-N,N,N',N'-tetraacetic acid (BAPTA, 10 mM). Before membranes in whole-cell electrodes were ruptured, spikes were induced by depolarization pulses delivered to whole-cell electrodes. Activities of K_A channels and a small conductance Ca^{2+}-activated K^+ channel (SK_{Ca}) were observed following spikes in cell-attached patch recordings. K_A channel openings immediately followed each spikes and contributed to repolarization and the fast afterhyperpolarization (AHP). After breaking the membrane in whole-cell patches, cell-attached patch recordings showed an inhibition of SK_{Ca} channel activity and an enhancement of K_A channel activity by intracellular BAPTA. Meanwhile, whole-cell current clamp recordings showed that spiking adaptation was inhibited and the fast AHP was increased by intracellular BAPTA. The results indicated that depolarization-induced increase in intracellular Ca^{2+} levels not only activated SK_{Ca} channels but also inhibited K_A channel activity. The inhibitoty effect of intracellular Ca^{2+} on K_A channels may play roles in neuronal bursting properteis.

141.18

PATCH-CLAMP RECORDINGS OF AN OUTWARD K^+ CURRENT IN RETINAL GLIAL CELLS. T. Pannicke and W. Reichelt*. Paul-Flechsig-Inst. Brain Res., University of Leipzig, 04109 Leipzig, Germany.

We performed cell-attached patch-clamp recordings from isolated, non-cultivated retinal (Müller) glial cells from guinea pigs. Bathing solution contained 3 mM K^+, whereas the pipette solution contained 0.5 to 20 mM K^+.

Recordings were performed from 330 patches, but only 23.6% of the patches displayed a current at depolarizing voltage steps,whereas hyperpolarizing voltage steps did never evoke any current. The current was exclusively macroscopic, with an amplitude of 19.1±12.8 pA at a 90 mV step, except once, when unitary current transients were found with a conductance of 4.8 pS. An average (51 trials) of the single channel traces, containing transients from 4 channels, resembled the macroscopic currents found in the other patches. Those had a time-to-peak of 15.2±6.7 ms during an 80 mV step and 6.3±2.6 ms during an 150 mV step, and were partly inactivating to about one third of their peak amplitude. The potentials for half-maximal steady-state activation and inactivation were +16.6 mV and -15.5 mV, respectively. Steady-state inactivation was incomplete over the tested voltage range of up to +30 mV. During the recording procedure the currents were running down and disappeared after a period of several minutes.

The current features are characteristic for a delayed rectifier channel, which is partly inactivating due to the activity of a β-subunit. Obviously, the channels are arranged in small clusters all along the cell membrane. At present the function of these channels remains elusive.

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141.19

CHARACTERIZATION OF FAST AND SLOW POTASSIUM CURRENTS IN THE CELL BODIES OF IDENTIFIED CUTANEOUS AFFERENT DORSAL ROOT GANGLION (DRG) NEURONS. B. Everill*, M. A. Rizzo and J. D. Kocsis. Dept. of Neurology, Yale Univ. Med. Sch., New Haven, CT. 06510, and Neuroscience Res. Ctr., VAMC, West Haven, CT. 06516.

The present study was undertaken to characterize K^+ currents on the cell bodies of DRG neurons as a precursive examination of their injury-induced plasticity. DRG neurons from adult female Wistar rats were dissociated and short-term cultured for examination 16-24 hrs. later. Whole cell patch clamp recordings were obtained from medium-size (39-49 um diameter) cells, retrogradely labelled with Fluorogold. K^+ currents were isolated by blocking Na^+ and Ca^{2+} currents with appropriate ion replacement and channel blockers. Stable recordings of up to 50 min were obtained at a holding potential of -80 mV. Depolarization-activated K^+ currents were routinely recorded during 300 ms voltage steps to potentials positive to -40 mV, from conditioning potentials ranging from -120 to -40 mV. The neurons displayed complex K^+ currents composed of distinct kinetic and pharmacological properties. Separation of these components was achieved on the basis of sensitivities to 4-aminopyridine (4-AP), dendrotoxin (DTx), and by the response to variation in conditioning voltage. Concentrations as low as 10 μM 4-AP could be seen to reduce peak current by as much as 70%, with a recovery up to 85% of the total peak after 15 min wash. With subtraction of traces, recorded before and after 10 μM 4-AP, a rapidly activating K^+-current, sensitive to low doses of 4-AP, was revealed. High concentrations of 4-AP (6mM) were seen to extinguish all inactivating current, thus isolating I_K. Perfusion with 1 uM DTx blocked slowly inactivating transient current (I_D) while sparing most of the initial fast transient current (I_A). A reversible effect of DTx was to significantly reduce input resistance. At least three components of K^+ current, in varying ratios, were defined in identified cutaneous afferent DRG neurons. Supported in part by the VA and the NIH.

142.1

POTENTIATION OF CLONED Ca^{2+}-ACTIVATED K^+ (mSLO) CHANNELS BY A SERIES OF N-ALKANOLS DEMONSTRATES CUTOFF. B. Chu* and S. N. Treistman, Department of Pharmacology and Molecular Toxicology and Program in Neuroscience, University of Massachusetts Medical Center, Worcester, MA. 01655.

Using electrophysiological methods, our laboratory has shown that EtOH (10-100mM) rapidly and reversibly potentiates the activity of both native neurohypophysial and cloned dSlo and mSlo Ca^{2+}-activated K^+ (BK) channels. Cutoff describes the phenomenon where in a homologous series of anesthetic agents, one observes a direct correlation between potency and hydrophobicity until a sudden loss of anesthetic efficacy among higher members occurs in spite of high lipid solubility. It has been argued that cutoff results from the inability of large hydrophobic alcohols to interact with hydrophobic pockets on channel proteins. To determine whether the potentiation of mSlo channels by alcohols displays a cutoff, we measured the degree of potentiation by a series of n-alkanols of mSlo BK channels expressed in Xenopus oocytes. Currents were elicited by stepping the voltage from -80mV to +50mV in the two electrode voltage clamp configuration. Ethanol, butanol, hexanol, and heptanol rapidly and reversibly enhanced the current. Nonanol (1-3mM) did not significantly potentiate the current. Interestingly, the degree of potentiation by ethanol was less than that seen in excised patches. Ethanol (8, 25, 50, 100, 200mM) enhanced the current by 9, 12, 17, 36, and 66% respectively; butanol (5, 10, 20, 30mM) by 17, 31, 59, and 71%; hexanol (0.5, 1, 2, 3mM) by 19, 29, 46, and 45%; and heptanol (0.25, 0.5, 1, 2, 3mM) by 16, 20, 27, 33, and 62%. 50mM ethanol was previously shown by our laboratory to potentiate mSlo channels expressed in Xenopus oocytes by ~100% in excised patches. Our results indicate that 1) cloned mSlo BK channels demonstrate a cutoff and 2) intracellular modulators such as kinases or G-proteins may modulate the sensitivity of mSlo BK channels to ethanol. Supported by NIH grant AA08003.

142.2

INTERNALLY APPLIED GLUCONATE REVERSIBLY INHIBITS I_K, I_{AHP}, I_h AND I_{Ca} IN RAT HIPPOCAMPAL PYRAMIDAL NEURONES A.A.Velumian*, L.Zhang, P.Pennefather, P.L.Carlen. Playfair Neuroscience Unit, Toronto Hospital and University of Toronto, Toronto, Ontario M5T 2S8, Canada

Earlier we reported that the spike frequency adaptation and slow afterhyperpolarization (sAHP) in hippocampal pyramidal neurons highly depend on the nature of anions used in the patch pipette solution. In particular, we showed that gluconate ($Gluc^-$), an anion widely used in whole-cell recording experiments, causes a fast rundown of the sAHP and of spike frequency adaptation, while methylsulphate was found to be a suitable anion to preserve these cellular features. Here we show, using internal perfusion of patch pipettes in whole-cell recording experiments on hippocampal CA1 neurons in brain slices, that inhibitory effects of $Gluc^-$ on spike frequency adaptation and on sAHP are reversible. Contrary to what might be expected based on $Gluc^-$ binding of Ca^{2+}, the sAHP and its underlying current could be temporarily *enhanced* by adding 1-3 mM of calcium chelator BAPTA to internal solution in the presence of $Gluc^-$. Moreover, the sAHP could be "revived" by BAPTA after its complete rundown in the presence of $Gluc^-$. Replacement of internal methylsulphate with $Gluc^-$ did not affect the membrane resting potential or the amplitude and duration of action potentials, but reversibly increased the cell input resistance and, accordingly, decreased the threshold current for spike generation. Internally applied $Gluc^-$ reversibly inhibited several cationic currents: the hyperpolarization-activated current (I_h), the depolarization-activated delayed rectifier K^+ current (I_K), the high voltage-activated Ca^{2+} current and the Ca^{2+}-activated K^+ current (I_{AHP}) that underlies the sAHP. While the mechanisms of these effects remain to be elucidated, our data indicate that $Gluc^-$ should be used with caution in experiments where the above membrane currents are involved.

Supported by MRC and NCE grants to P.L.Carlen.

142.3

COPPER INDUCES CALCIUM-DEPENDENT NEUROTRANSMITTER RELEASE FROM CATECHOLAMINERGIC NERVE TERMINALS. J.K.T. Wang*, Department of Neuroscience, Tufts University School of Medicine, Boston, MA 02111.

Rat hippocampal or striatal synaptosomes, preloaded with ^{3}H-norepinephrine (NE) or ^{3}H-dopamine, respectively, were exposed to 3-100 μM Cu^{2+} under superfusion. Cu^{2+} caused the release of labeled transmitters in a concentration- and time-dependent manner. At 100 μM Cu^{2+}, transmitter release rises at 1 min, peaks at 2 min, and is sustained over a period of 5 min. This time course is slower than that of K^+-depolarization or that of the K^+ channel blocker 4-aminopyridine (4AP), but the amount of release induced by Cu^{2+} over the 5 min period is similar to that induced by K^+ or 4AP. Zn^{2+} and Ni^{2+} (0.1-1mM), but not Cd^{2+} or Co^{2+}, also induced transmitter release, but their effects were much smaller that of Cu^{2+}. The effect of Cu^{2+} is dependent on extracellular Ca^{2+} and Na^+, since it was eliminated by omitting Ca^{2+} from the superfusion buffer, or by substituting Na^+ with choline Cl. Moreover, 3 μM TTX also inhibited the Cu^{2+}-induced NE release. The effect of 4AP is similarly dependent on extracellular Ca^{2+} and Na^+. These results suggest that Cu^{2+} and certain divalent heavy metals may block K^+ channels in catecholaminergic nerve terminals, leading to opening of voltage-gated Na^+ channels, membrane depolarization, opening of voltage-gated Ca^{2+} channels, and transmitter release. These events, and the possible resultant changes in neuronal functions, may occur *in vivo* when the levels of heavy metals are modified by physiological or pathological processes.

Supported by grant R01NS30590 from the NIH.

142.4

THE INACTIVATING INWARD-RECTIFYING POTASSIUM CURRENT IN GH_3B_6 CELLS IS HIGHLY SENSITIVE TO THE CLASS III ANTIARRHYTHMIC AGENT E-4031. F. Weinsberg, C.K. Bauer and J.R. Schwarz*, Inst. Physiol., Univ. Hamburg, D-20246 Hamburg, Germany

Clonal rat anterior pituitary cells (GH_3B_6 cells) exhibit an inward-rectifying potassium current (K_{IR}), characterized by a voltage-dependent and sodium-independent inactivation during hyperpolarizing voltage pulses (Bauer et al., J. Physiol: 429: 169, 1990). K_{IR} is relatively insensitive to TEA and barium.

We now report that K_{IR} is effectively blocked by the class III anti-arrhythmic agent E-4031. K_{IR} was activated by voltage pulses to -110 mV from a holding potential of -40 mV (bath solution: isotonic KCl). The maximum block of the transient inward current was 65%, achieved with 100 nM E-4031; increasing the concentration up to10 μM induced no further reduction. The IC_{50} value was 3 nM. The voltage-dependence of K_{IR} steady-state inactivation was not altered by 10 nM E-4031. The maximal reduction of the holding current in isotonic KCl at -40 mV was 26% (≥100 nM E-4031), suggesting the presence of additional ionic currents at this potential. Inward and outward currents of GH_3B_6 cells in normal Ringer's solution (holding potential: -80 mV, test pulse: 0 mV, prepulse:-120 mV) were not affected by 100 nM E-4031.

E-4031 is a selective blocker of K_{IR} in GH_3B_6 cells, with an IC_{50} value much lower than those reported for other potassium currents. Supported by the Deutsche Forschungsgemeinschaft (Schw 292; Ba 1436).

142.5

VOLTAGE AND CALCIUM DEPENDENCE OF PAXILLINE BLOCK IN NEURONS AND ENDOCRINE CELL LINES. D.E. Johnson[1], H. Wieland, S. Terhart-Krabbe, M. Neuberg, R.T. McCarthy*[1] and R. Wittka. [1]Institute for Dementia Res., Bayer Corp., West Haven, CT 06516 and Institute for Neurobiology., Troponwerke GmbH & Co. KG, D-51063 Koeln, F.R.G.

The indole alkaloid fungal mycotoxin paxilline was examined for effects on large conductance calcium activated potassium channels (BK) in a variety of cell types. In an in vitro slice preparation of rat cortical neurons, paxilline (100 nM) markedly broadened the action potential duration (APD). The mechanism of toxin block was studied in GH_3 and undifferentiated PC12 cell lines using whole cell, cell attached and excised patch recording techniques. Results demonstrated that paxilline was a potent blocker of BK channels in both cell lines. Furthermore, paxilline inhibition of BK current was both calcium and voltage dependent. Paxilline block displayed a unique voltage dependence such that current inhibition was pronounced at hyperpolarized holding potentials (V_H < -30 mV) but diminished at depolarized potentials (V_H > -20 mV). Examination of toxin block at hyperpolarized potentials revealed that paxilline inhibited BK current at concentrations as low as 6 nM in the presence of low intracellular calcium (Ca_i = 1μM). Increasing Ca_i to 50 μM reversed paxilline block. These results suggest that paxilline broadens the cortical action potential by inhibiting BK channels which play a critical role in cortical repolarization. (Bayer)

142.6

TETRAHYDROBERBERINE INHIBITS ACETYLCHOLINE-INDUCED K^+ CURRENT IN ACUTELY DISSOCIATED RAT HIPPOCAMPAL CA1 PYRAMIDAL NEURONS. P.X. Chen, J. Wu, and Chibing Xu* Dept. Physiol., Sun Yat-Sen Univ. Med. Sci., Guangzhou, 510089, P.R. China, and *Dept. Neurol. Univ. New Mexico Sch Med., Albuquerque, NM 87108

It is well established that tetrahydroprotoberberines (THPBs) is a novel class of dopamine (DA) receptor antagonists. In this study, effects of THPBs on acetylcholine (Ach)-induced current were investigated in freshly dissociated CA1 pyramidal neurons from rat hippocampus using nystatin perforated patch-clamp whole-cell recording technique under voltage-clamp model. At the holding potential (V_H) of -20 mV, Ach evoked an outward current (I_{Ach}) with the half-maximum effective concentrations (EC_{50}) of $2x10^{-6}$ M. I-V relationship curve showed that the reversal potential of I_{Ach} was -82 mV which was close to the K^+ equilibrium potential of -86 mV calculated from the Nernst equation, and K^+ channel blocker, TEA, clearly inhibited the I_{Ach}, indicating that Ach-induced outward current is mainly carried by K^+. THB, but not *l*-THP or *l*-SPD, clearly inhibited I_{Ach} in a concentration-dependent manner with the IC_{50} of $1.3x10^{-5}$ M. THB suppressed the maximum of concentration-response curve of I_{Ach} without change of the Hill coefficient, suggesting a noncompetitive inhibition. In addition, THB also inhibited caffeine- or strychnine-induced outward K^+ currents. It is concluded that THB inhibits Ach-induced K^+ current underlay the blockade of the membrane K^+ channels. Supported by National Natural Sciences Foundation of China (No. 39270775).

142.7

ANANDAMIDE, THE ENDOGENOUS CANNABINOID RECEPTOR AGONIST, IS RELEASED IN RAT HIPPOCAMPUS AND DECREASES THE POTASSIUM M-CURRENT. P. Schweitzer*, S.G. Madamba, G.R. Siggins and D. Piomelli[1]. Neuropharmacology, The Scripps Research Instit, La Jolla, CA 92037, [1]The Neurosciences Institute, San Diego, CA 92121.

Cannabinoids have powerful psychoactive properties and alter many physiological processes. Such effects are believed to be mediated through specific binding sites in the brain, with one of the highest densities found in the hippocampus. Anandamide (arachidonylethanolamide) is described as an endogenous ligand for cannabinoid receptors, but its existence and role in adult brain tissue is still poorly documented. Therefore, we assessed anandamide formation in adult rat hippocampus by incubating transverse slices with the calcium ionophore ionomycin (1 μM; 10 min) in the presence of PMSF (100 μM), an inhibitor of the enzyme that degrades anandamide. We subjected the incubation media and slices to lipid extraction and fractionated the extracts by HPLC, and analyzed the HPLC fractions by GC/MS. Anandamide, identified by its GC retention time and full mass spectrum, was present in the slices and incubation media, and its mass was increased by ionomycin treatment. We used the hippocampal slice preparation to perform intracellular voltage-clamp recordings in the presence of 1 μM tetrodotoxin. Superfusion of R-1 methanandamide (M-AEA), a non-degradable form of anandamide, onto CA1 pyramidal neurons decreased the non-inactivating voltage-dependent potassium M-current (I_M) by about 30% (1 μM; n = 3) to 50-60% (5 μM; n = 3). We also observed an I_M decrease with the cannabimimetic drug WIN 55,212-2 (0.5-2 μM; n = 6). Prior treatment of the slices with the cannabinoid receptor antagonist SR141716A (1 μM) prevented the effect of M-AEA (n = 3) and WIN (n = 4) on I_M.

Our results provide evidence for the formation and release of anandamide in the adult rat hippocampus, and show that such cannabinoid substance can post-synaptically modulate the excitability of CA1 pyramidal neurons.

Supported by NIH (MH 44346 and AA 06420) and Neurosciences Res. Foundation.

142.8

LITHIUM BLOCK AND PERMEATION OF DOPAMINE-MODULATED K^+ CHANNELS ON RAT STRIATAL NEURONS. Yong-Jian Lin*[1], Xueguang Chen, and Jonathan E. Freedman. Dept. Pharmaceutical Sciences, Northeastern Univ., Boston, MA 02115 (and [1]Dept. Anesthesiology, Cornell Medical Center, New York, NY 10021).

Using cell-attached patch-clamp recording from freshly dissociated rat caudate-putamen neurons, we have studied the blockade properties of an inwardly rectifying 85 pS K^+ channel, which is activated by D_2-like dopamine receptors. Previous studies showed that this channel was sensitive to block by cesium ions. In addition, Cs^+ weakly permeated the channel at voltages near the K^+ reversal potential, introducing an anomalous negative slope conductance into the current-voltage relationship. Here, we have studied the effects of 0.5-1.0 mM LiCl within the patch pipette upon this channel, and report results similar to those for CsCl. These concentrations of Li^+ partially blocked 85 pS channel currents near the resting membrane potential, and also carried small currents near the K^+ reversal potential. Li^+ block was flickery and voltage-sensitive, and nearly complete at 1 mM. Notably, Li^+ displayed these effects at concentrations corresponding to tissue levels attained at therapeutic dosages, rather than at the higher, toxic concentrations needed to block many other ion channels. These results raise the possibility that effects on this dopamine receptor-modulated K^+ channel may contribute to lithium's mechanism of action in the treatment of bipolar disorder. (Supported by NIH grant MH-48545.)

142.9

κ-CONOTOXIN PVIIA, A *CONUS* PEPTIDE TARGETED TO POTASSIUM CHANNELS. R. Jacobsen[1], M. Stocker[2], H. Terlau[2], K. Shon[3], M. Grilley[1], W. R. Gray[1], W. Stühmer[2] and B. M. Olivera[1]* Dept. of Biology, Univ. of Utah, Salt Lake City, UT, 84112; Molekulare Biologie Neuronaler Signale, Max-Planck-Inst. für Exp. Medizin, D-37075, Göttingen, Germany; Dept. of Physiology and Biophysics, Case Western Reserve Univ., Cleveland, OH, 44106.

A novel 27 amino acid *Conus* peptide with 3 disulfide bonds, κ-conotoxin PVIIA, was recently purified and characterized from *Conus purpurascens* venom (Terlau, et al., *Nature*, 381:148-151, 1996). The peptide inhibits the *Shaker* K^+ channel, but has no effects on any voltage-gated Na^+ or Ca^{2+} channel tested. κ-Conotoxin PVIIA has been chemically synthesized; the synthetic material is identical to the natural material by both chemical criteria and biological activity. Although the peptide rapidly inhibits the *Shaker* K^+ channel, the rat brain Kv1.1 K^+ channel is resistant to the toxin. Chimeras between the *Shaker* and Kv1.1 channel reveal that the peptide is generally targeted to the region of the channel between the fifth and sixth transmembrane domains. For the fish-hunting *Conus purpurascens*, κ-conotoxin appears to be part of an excitotoxic shock strategy generally used by venomous predators to very rapidly immobilize their prey. For rapid prey immobilization to occur, both the κ-conotoxin and a δ-conotoxin, which delays voltage-gated Na channel inactivation, are required. (Work supported by Grant PO1 GM 48677 NIGMS and SFB 406.)

142.10

THE REGULATION OF HUMAN (*hslo*) CA^{2+}-ACTIVATED K^+ CHANNELS BY REDOX REAGENTS. T. J. DiChiara* & P.H. Reinhart, Dept. of Neurobiology, Duke Univ. Medical Center, Durham, NC 27710.

Cloned human Ca^{2+}-activated K^+ channels (*hslo*) channels contain at least 25 cysteine residues, raising the possibility that reduction or oxidation of disulfide bonds in the channel protein could have functional consequences. We have investigated the influence of sulfhydryl redox reagents on *hslo* (hbr5) channels in a stably transfected HEK293 cell line using inside-out macropatch analysis. Intracellular application of the reducing agent dithiothreitol (DTT, 1 mM) has three effects: first, the voltage of half-maximal activation ($V_{0.5}$) is shifted an average of 18.3 ± 1.8 mV (n=8) to more negative potentials without affecting the slope of the voltage dependence. The time course of this potentiation can be fit with a single exponential having a mean time constant of 7.5 ± 0.6 min (n=8). Second, reduction by DTT consistently slows the rate of channel "run-down," measured as a right-shift in macroscopic conductance-voltage curves, from an average of 2.2 mV/min to 0.17 mV/min (n=13). Third, DTT increases current activation kinetics by 32.7 ± 4.2% (n=8). In contrast to DTT treatment, application of the reduced form of glutathione has no significant effect on *hslo* currents (n=2), while the oxidized form shifts $V_{0.5}$ values to more positive potentials (n=3). A similar shift is observed upon oxidation with 5-5'-dithio-bis(2-nitrobenzoic acid) (DTNB, 1 mM). These data show that *hslo* Ca^{2+}-activated K^+ channels can link cellular metabolism to excitability by means of one or more redox modulatory site(s) that significantly influence channel function. Supported by NIH grants to PHR (NS31253) and TJD (MH10930).

142.11

THE A-CURRENT IS NOT BLOCKED BY INTRACELLULAR CESIUM IN GUINEA PIG LATERODORSAL TEGMENTAL NEURONS. R.M. Sanchez*, A. Surkis, and C.S. Leonard, Center for Neural Science, New York University, 4 Wash. Pl., NY, NY 10003.

LDT neurons exhibit a prominent transient potassium current (I_A). I_A has been reported to be blocked by intracellular Cs^+ in various neurons, including rat LDT neurons. Unexpectedly, whole-cell voltage-clamp recordings of guinea pig LDT neurons in a brain slice preparation revealed an apparent I_A which persisted despite internal Cs^+ dialysis. LDT neurons recorded with Cs^+-based pipette solutions exhibited a transient inward current which resembled a T-type Ca^{++}-current as previously reported (Kamondi et al. 1992, J Neurophys, 68:1359). However, this current reversed near -35 mV and it was inhibited by 4-aminopyridine (4-AP), suggesting that it was I_A after cesium dialysis had shifted E_K positively, rather than a T-current. Increasing $[K^+]_o$ caused a further positive shift in the reversal potential for the transient current, and Ringer containing 0 Ca^{++}/4mM EGTA/TEA did not reduce the current, although the voltage-dependence of activation shifted negatively, resulting in larger inward 4-AP-sensitive currents. The mean time constant of inactivation removal was 15.5 ms, indicating that the Cs^+-resistant current was not I_D. Boltzmann fits were used to estimate the voltage-dependence of I_A and indicated an activation $V_{1/2}$ of -43mV and an inactivation $V_{1/2}$ of -59mV. The influence of dendritic filtering and series resistance on these estimates was also examined by computer simulation of morphologically realistic LDT neurons. These data indicate that guinea pig LDT neurons express a novel I_A which has a Cs-resistant ion permeation mechanism. It will be of interest to learn if this feature is correlated with other functional properties, such as sensitivity to neuromodulators. Further study may also reveal this property to be diagnostic of a particular K_V gene subfamily. Supported by NS27881

142.12

ETHANOL HAS OPPOSITE EFFECTS ON THE ACTIVITY OF TWO CLONED LARGE CONDUCTANCE, Ca^{++}-ACTIVATED K^+ (*bslo* AND *mslo*) CHANNELS. A.M.Dopico and S.N. Treistman*. Dept. Pharmacol. & Mol. Toxicol., and Program in Neurosci., Univ. Mass. Med. Ctr. Worcester, MA 01655.

The activity of large conductance, Ca^{++}-activated K^+ (BK) channels regulates arterial tone. Ethanol (EtOH) either increases or decreases arterial tone, depending upon the vascular net considered. We previously found that 10-200mM EtOH increased the activity (NP_o) of mouse brain (*mslo*) BK channels expressed in *Xenopus* oocytes. However, 50mM EtOH does not activate BK channels in aortic smooth muscle. To examine whether this differential effect of EtOH on BK channels is attributable to differences between the two BK channel proteins or to other components of the membrane, we report here the effects of EtOH on BK channels (*bslo*) cloned from aortic smooth muscle, and expressed in the oocyte system, using single channel recordings from inside-out patches. *Bslo* has been reported to be >99% identical to *mslo* (Biophys. J. 68: A29). *Bslo* channels showed a reversal potential of 0.8 and 30 mV in symmetric 145 mM $[K^+]$ and 145/45 mM $[K^+]_o/[K^+]_i$ respectively. The main unitary conductance was 270 pS in symmetic 145 mM $[K^+]_i$ with at least one subconductance state. NP_o increased when $[Ca^{++}]_i$ was increased and/or at more positive potentials (59 mV per e-fold change in NP_o, at low P_o). EtOH (50-100mM) reversibly decreased *bslo* channel NP_o (-66%), without modifying unitary conductance, reversal potentials, or voltage-sensitivity. Since *mslo* and *bslo* channels were embedded in the same oocyte membrane, these results suggest that the differential action of EtOH is attributable to differences between the channel proteins.

Supported by grants from ABMRF (AMD) and NIH AA-08003 (SNT).

142.13

EFFECT OF CHARYBDOTOXIN, A POTASSIUM CHANNEL BLOCKER, ON OVERT BEHAVIOUR AND LOCAL CEREBRAL GLUCOSE UTILISATION. S.M.Cochran* and J.A.Pratt. Dept. of Physiology and Pharmacology, University of Strathclyde, Glasgow, G1 1XW, U.K.

Charybdotoxin (CTX) is a polypeptide neurotoxin isolated from scorpion venom *Leiurus quinquestriatus*. It is a high affinity blocker of voltage- and Ca^{2+} -dependent K^+ channels, although it is thought to predominantly block transient "A" channels within the rat brain (Schweitz *et al., Biochem 28:9708-9714, 1989*). A dose response trial was designed in order to establish sub-convulsive doses of CTX after bilateral *icv* administration. A pilot study demonstrated that a dose of 50pmoles/5µl caused convulsions. In the present study, no seizures were observed at a dose of 25 pmoles/5µl, or lower (12.5 and 6.25pmoles/5µl).

The 2-Deoxyglucose Method (Sokoloff *et al, J. Neurochem. 28:897-916, 1977*) was then used to investigate the effects of CTX on functional activity, as reflected by changes in local cerebral glucose utilisation (LCGU). The structures investigated were the hippocampus, related limbic structures and the basal ganglia. At a dose of 12.5pmoles/5µl (bilateral *icv* injection) CTX evoked heterogenous changes in LCGU, with significant increases seen in 18 out of 26 structures (One-Way Anova, $p < 0.05$). Significant increases were seen, for example, in the perforant pathway, with LCGU increasing from 71±2 to 96±2 µmol/100g/min (n=4, mean±SEM ; saline vs CTX treated groups) in the entorhinal cortex, and from 88±3 to 103±1 µmol/100g/min in the molecular layer of dentate gyrus. In the basal ganglia, LCGU increased from 87±3 to 107±3 µmol/100g/min in the caudate nucleus but no change was seen in the globus pallidus (57±2 vs 55±1 µmol/100g/min). Preliminary data indicates that CTX may improve functional activity in selected structures rather than cause a global increase in activity throughout the brain.

This work was supported by The Smith's Trust and SHERT.

142.14

APAMIN, A BLOCKER OF CALCIUM ACTIVATED POTASSIUM CHANNELS INDUCE NEURODEGENERATION OF PURKINJE CELLS IN CEREBELLUM. C. Mourre*, B. Soumireu-Mourat. CNRS-URA 372, Lab Neurobiologie des Comportements, F-13388-Marseille, France.

Apamin induces characteristic epileptiform seizures. We used convulsive doses of apamin to determine a possible neuronal damage. Patterns of cell loss and neurodegeneration were studied using a Fink-Heimer silver staining procedures.

Following acute and unilateral intracerebroventricular injection (icv) of 1 ng apamin, the rat brains presented, after 48h survival times, a bilateral damage in cerebellum exclusively. The argyrophilic cells were Purkinje cells in floculus, parafloculus and paramedian lobules. No neurodegeneration was found in the others lobules and cerebellar nuclei. After 0.7 ng apamin icv injection, no seizures and no neuronal damage were observed.

Three doses of apamin were used for chronic apamin injections with osmotic pumps (Alzet, 0.5µl/h, 14 days) : 0.2, 0.4, 0.6 ng/µl. At 0.2 ng/µl apamin dose, no damage were found but animals were hypersensitive to noise especially. During 0.4 and 0.6 ng/µl apamin infusions, the rats showed typical seizures during five first days. They appeared restless during the time course of the infusions. The two highest doses of apamin induced a neurodegeneration only in Purkinje cells of the cerebellum. The pattern was identical with that observed after acute icv 1 ng apamin injection.

In conclusion, these data demonstrate that the inactivation of small conductance Ca^{2+} -activated K^+ potassium channels by apamin induce a particular neuronal damage in rat brain that was very different from neurodegenerations, frequently limbic, induced by convulsive drugs like kainate acid and dendrotoxin, a blocker of type A potassium channels.

Supported by DRET Grant 91 1465

142.15

RESTING ('LEAK') K^+ CHANNELS IN RAT HIPPOCAMPAL PYRAMIDAL NEURONS INHIBITED BY EXTERNAL Ba^{2+} AND INTERNAL Ca^{2+}. A.A.Selyanko, J.A.Sim* and D.A.Brown. Dept.Pharmacol., University College, London, WC1E6BT, UK.

Channels responsible for setting the resting potential of most neurons remain elusive. In hippocampal pyramidal neurons the resting ('leak') conductance is inhibited by Ba^{2+} (Storm & Heliessen,1989: Soc.Neurosci.Abstr.,15,77). We have identified corresponding Ba^{2+}-sensitive single channels in CA1/CA3 pyramidal neurons dissociated from 7 day-old rats and cultured in vitro for 8-25 days (Alger et al., 1994: Neurosci.Lett., 168,23). Whole-cell recording revealed a voltage-insensitive component of membrane current between -120 and -50 mV inhibited by 1 mM Ba^{2+} and reversing at -105 mV in 2.5 mM $[K^+]_{out}$. Single K^+ channels active at rest potential, with a slope conductance of 12.2 ± 2.0 pS (n=5; $[K^+]_{out}$ = 2.5 mM), were detected in 11 cell-attached patch recordings. When excised into outside-out mode channels were inhibited 91.1 ± 1.1 % (n = 4) by 1 mM Ba^{2+} but were unaffected by 1 mM TEA or 0.5 mM 4-AP. Channel activity persisted and increased on excision of patches into inside-out mode and was then reversibly inhibited by internal Ca^{2+} (IC_{50} 118 nM). This effect persisted in MgATP-free solution so probably did not involve phosphorylation/dephosphorylation. Channel activity in cell-attached patches was enhanced in Ca^{2+}-free bathing solution and was inhibited during spontaneous spiking in Ca^{2+}-containing solution, leading to a Ca^{2+}-dependent post-spike inward current. Inhibition of these channels may therefore be responsible for spike after-depolarization.

Supported by the U.K.Medical Research Council.

142.16

HERG CURRENTS ARE REDUCED BY KETOCONAZOLE, ERYTHROMYCIN, AND TERFENADINE, SUGGESTING MULTIPLE MECHANISMS FOR CARDIOTOXICITY. M.-L. Roy*, J. Kiehn, R. Dumaine, and A.M. Brown. Rammelkamp Center for Research, MetroHealth Med. Center, Case Western Reserve University Sch. of Med., Cleveland, OH 44109.

The HERG potassium channel, first identified in hippocampus, has also been reported in various cardiac tissues. Terfenadine (Seldane), a widely-used antihistamine, has been associated with arrhythmias when simultaneously administered with fungicides (i.e. ketoconazole) or macrolide antibiotics (i.e. erythromycin). We have recently shown that nanomolar concentrations of terfenadine block HERG currents as expressed in *Xenopus* oocytes (*Circulation*, in press).

We heterologously expressed Kv1.5 and HERG channels in *Xenopus* oocytes to compare their sensitivities to ketoconazole and erythromycin. Currents were recorded using the two-electrode voltage clamp technique. Ketoconazole reduced HERG and Kv1.5 currents with comparable K_d values of 30 µM and 60 µM, respectively, without use- or voltage-dependent block for either channel. These values are physiologically significant, as plasma concentrations of ketoconazole have been reported in the low micromolar range. In the whole oocyte, erythromycin (1- 1000 µM) did not alter either HERG or Kv1.5 currents, whereas application of erythromycin to inside-out patches blocked HERG current with an estimated K_d value of 150 µM. Our results indicate that, in addition to slowing the metabolism of terfenadine, ketoconazole and erythromycin may provoke cardiovascular complications. Thus, as HERG and Kv1.5 are expressed in neuronal and glial cells, respectively, similar compounds may alter these channels in the CNS.

Supported by A.H.A. (Northeastern Ohio Affiliate) to M.L.R.; Deutsche Forschungsgemeinschaft to J.K.; Fonds de la Recherche en Sante du Quebec to R.D.; and NS 23877 to A.M.B..

EXCITATORY AMINO ACIDS: ANATOMY AND PHYSIOLOGY I

143.1

MORPHOLOGICAL AND MORPHOMETRIC ANALYSIS ON IMPAIRED PARALLEL FIBER-PURKINJE CELL SYNAPSE FORMATION IN GluRδ2-KNOCKOUT MICE.

H. Kurihara, M. Watanabe, M. Mishina[1], and Y. Inoue*. Dept. of Anat., Hokkaido Univ. Sch. of Med., Sapporo 060, and [1]Dept. of Pharmacol., Tokyo Univ. Faculty of Med., Tokyo 113, Japan.

The glutamate receptor channel subunit δ2 is localized specifically in the Purkinje cell (PC) spines, and plays important roles in development of PC synapses, long-term depression, and motor coordination. In the present study, we quantitatively analyzed the parallel fiber-Purkinje cell (PF-PC) synapses in the vermis of the GluRδ2-mutant and wild-type cerebellum at 5 weeks of age. The number of the PCs in the mutant were almost comparable to that in the wild-type (98% of the wild-type). However, the numerical density of PC spines in the mutant was reduced to 75% of that in the wild-type. Serial electron micrographs demonstrated that 63% of the PC spines in the mutant were in contact with the PF terminals, whereas the rest failed to form synaptic contacts. These free or "naked" spines were completely surrounded by sheets of the Bergmann astrocytes. In the wild-type, on the other hand, all PC spines were contacted with the PF terminals. Based on these data, it was estimated that the total number of the PF-PC synapses per PC in the mutant mouse was remarkably reduced to 47% of the wild-type mouse. Moreover, the mean number of contacted spines per PF terminal was significantly reduced: 1.10 ± 0.03 in the mutant and 1.45 ± 0.03 in the wild type ($p<0.0005$). These findings suggest that the δ2 subunit may be involved in stabilization process of the PF-PC synapses, leading to a successful integration of all PC spines formed during development into synaptic structure.

143.2

CELLULAR DISTRIBUTION OF NMDA GLUTAMATE RECEPTOR SUBUNITS IN THE HUMAN CEREBELLUM. C.R. Scherzer, J.A. Kerner, D.G. Standaert, Z.R. Hollingsworth*, J.B. Penney, Jr., A.B. Young, G.B Landwehrmeyer Dept. of Neurology, Mass. Gen. Hosp., Boston, MA 02114.

NMDA receptors are composed of proteins from two families: NMDAR1, which are required for channel activity, and NMDAR2, which modulate properties of the channels. We have used a quantitative *in situ* hybridization method with human ribonucleotide probes to examine the regional and cellular distribution of the NMDA receptor subunit mRNA in the human cerebellum.

Purkinje cells showed intense labeling for NMDAR1 mRNA and NMDAR2A mRNA probes, whereas labeling for the NMDAR2D mRNA probe was moderate. Granule cells showed high hybridization signals for the NMDAR1 and NMDAR2C mRNA and moderate signals for the NMDAR2D mRNA. In addition, intense labeling for the NMDAR2B probe was observed in medium sized neurons with chromophilic cell bodies in the upper part of the granule cell layer, most likely representing Golgi cells. Neurons in the molecular layer, i.e. basket cells and stellate cells, showed high hybridization signals for NMDAR1 and NMDAR2D, moderate signals for NMDAR2B and low signals for NMDAR2C. While the NMDAR2D mRNA probe was found to label all neurons in the molecular layer equally, the expression of the other subunit mRNAs in this cerebellar layer seemed to be heterogenous.

NMDAR composition in the cerebellum appears to be distinctly different from composition in the cortex, with the NMDAR2C expression prominent in the cerebellum in contrast to the NMDAR2B expression prominent in the cortex. We conclude that each type of neuron analyzed displays a distinct NMDAR2 subunit profile. Supported by USPHS grant AG11337.

143.3

GLUTAMATE RECEPTORS POSTSYNAPTIC TO CORTICAL TERMINALS IN CORTEX AND THALAMUS. V.N.Kharazia*, K.Phend, R.J.Weinberg and A.Rustioni Dept. of Cell Biology & Anatomy, UNC, Chapel Hill, NC 27599.

Excitatory corticocortical and corticothalamic projections are both from axons of pyramidal cells. Though both appear to use glutamate as transmitter, postsynaptic effects may differ in these two projections. We here report an electron microscopic study of receptors postsynaptic to these projections. To supplement tentative morphological identification of cortical terminals, anterograde tracers were used, including WGA-HRP, PHA-L, and biotinylated dextran, injected into deep layers of rat S-I cortex. Labeled synapses were identified in ipsi- and contralateral S-I cortex, and in the ipsilateral ventrobasal thalamus. Anesthetized rats were perfused with mixed aldehydes. Following histochemistry for tracer, tissue was embedded and processed for postembedding immunogold (Phend et al.,'95) for AMPA, NMDA and metabotropic glutamate receptors. Analysis of immunostained material revealed that in both cortex and thalamus all three types of receptors could be found at synapses made by cortical terminals. AMPA receptors appeared to be more often postsynaptic to cortical terminals in cortex than in thalamus, whereas NMDA was more prominent in thalamus than in cortex. This work was supported by NIH awards 16264 (to AR) and 29879 (to RJW).

143.4

GLUTAMATE RECEPTORS POSTSYNAPTIC TO THALAMOCORTICAL TERMINALS IN SI. R.J.Weinberg* and V.N.Kharazia Dept. of Cell Biology & Anatomy, UNC, Chapel Hill, NC 27599

Pharmacological and immunocytochemical studies suggest that thalamocortical afferents use glutamate as neurotransmitter. We have used immunocytochemistry for ionotropic glutamate receptors to study thalamocortical transmission in primary somatic sensory cortex (SI) of rats. LM immunocytochemistry of barrel cortex suggests that thalamocortical transmission is mainly via NMDA receptors, whereas electrophysiology indicates that AMPA receptors play the primary role. Since LM cannot unambiguously identify synaptic receptors, we used immunogold EM techniques to study glutamate receptors postsynaptic to thalamocortical terminals in SI. Terminals were identified both on morphological grounds and by EM anterograde transport methods. Histochemistry for tracer was followed by an embedding procedure designed to optimize antigen retention (Phend et al., '95). Both AMPA and NMDA receptor subunits could be found at synapses made by thalamocortical fibers; however AMPA subunits were more often postsynaptic to thalamocortical terminals than were NMDA subunits. This evidence suggests that thalamocortical transmission is predominantly via AMPA receptors. This work was supported by NIH award 29879 (to RJW).

143.5

AMPA AND NMDA RECEPTORS IN MOTONEURONS OF THE SPINAL CORD AFTER SCIATIC NERVE TRANSECTION A. Rustioni*, A. Popratiloff, V.N. Kharazia, and R.J. Weinberg, Dept. of Cell Biology & Anatomy, UNC, Chapel Hill, NC 27599.

Transverse sections through the fourth and fifth lumbar segments of rat spinal cord were immunocytochemically processed using antibodies specific for AMPA receptor subunits GluR1-4, and for the NMDA receptor subunit NMDAR1. Changes in immunostaining for glutamate receptors in the ventral horn were observed after 3-14 days on the side ipsilateral to a lesion of the sciatic nerve. GluR2/3 staining was markedly decreased in injured motoneurons, which were concentrated in dorsolateral lamina IX. No obvious changes in staining of motoneurons were detected for the other AMPA subunits tested. Changes in GluR4 expression were conspicuous: numerous GluR4-positive microglial cells appeared in on the lesioned side within 3 days after the lesion and became more prominent after longer survival. Many of these intensely GluR4-positive microglial cells enveloped motoneurons. NMDAR1 staining became apparent in glia close to motoneuronal somata after 14 days survival. These NMDA-positive cells were identified as astrocytes, on the basis of their intense staining for GFAP. Down regulation of glutamate receptors in motoneurons may be associated with the loss of synaptic input known to occur after axotomy. Supported by NIH grant NS 12440.

143.6

LOCALIZATION OF GLUTAMATE RECEPTORS IN THE LOCUS CERULEUS OF RHESUS MACAQUES. H.F. Urbanski* AND S.G. Kohama. Division of Neuroscience, Oregon Regional Primate Research Center, 505 N.W. 185th Avenue, Beaverton, Oregon, 97006.

Noradrenergic innervation of major brain areas, such as the hypothalamus and cerebral cortex, originates in part from the locus ceruleus (LC) located in the hindbrain. The LC is theoretically under the control of excitatory glutamatergic afferents, since this area can be stimulated by exogenous glutamate. Therefore, this study examined the localization of ionotropic glutamate receptors in the LC of perfused fixed rhesus monkey brains (*Macaca mulatta*). Both the NMDA and AMPA classes of receptors were examined by immunocytochemistry (ICC) and *in situ* hybridization (ISH). The LC was localized initially in coronal sections by ICC for tyrosine hydroxylase (Boehringer-Mannheim), the rate limiting enzyme in the catecholamine synthesis pathway. Neighboring sections were then immunostained for NR1, the NMDA receptor subunit found in all NMDA receptor complexes, and for GluR1 and GluR2/3, major subunits of AMPA receptors (Chemicon Int.). In all cases, immunostaining for NR1, GluR1 and GluR2/3 subunits were found in neurons of the LC. To corroborate these results, ISH for NR1, GluR1, GluR2 and GluR3 subunits were performed on sections containing the LC. Coronal sections were initially postfixed, digested with proteinase K, acetylated, then hybridized overnight at 60 ° C with 35-S-labelled cRNA probes generated from rat cDNA templates. Sections were subsequently washed, treated with RNase A, then further rinsed, with a final stringency wash at 70° C in 0.1 X SSC. The LC was visualized on exposed β-max films (Amersham) and emulsion-dipped slides (Kodak). Again, all subunits examined were found in the LC, with NR1 and GluR1 giving the most robust signals. Both GluR2 and GluR3 mRNA were also found in LC neurons, corroborating the immunostaining seen with the GluR2/3 antibody.
Grant Support: NIH HD-29186 and RR00163

143.7

REGIONAL CONCENTRATIONS OF AMPA RECEPTOR SUBUNIT mRNAS SUGGEST RECEPTOR CLASSES. S.J. Gold[1]*, J. Ambros-Ingerson[1,3], J.R. Horowitz[2], G. Lynch[1,3], C.M. Gall[1,2]. Depts. of [1]Psychobiology, [2]Anatomy & Neurobiology and [3]CNLM, Univ. of Calif., Irvine, CA 92717.

In situ hybridization was used to assess regional concentrations of AMPA receptor (AMPAR) subunit mRNAs in adult rat brain to determine if subunit mRNA ratios, suggestive of different AMPAR subunit compositions, recur across brain areas. Four patterns of subunit mRNA content were observed and named AMPAR-1, AMPAR-2, AMPAR-2,3 and AMPAR-1,2 (numbers indicate subunit mRNAs most enriched for each class). Categories were resolved by agglomerative hierarchical clustering and statistically validated. Subunit mRNA ratios were of the AMPAR-1 type in habenula and lateral mammillary bodies; the AMPAR-2,3 type in thalamic sensory relay and pontine nuclei; and the AMPAR-2 type in piriform cortex, neocortex, and medial mammillary bodies. GluR2 mRNA represented a substantial fraction of total GluR mRNA in all regions, but was highest in the AMPAR-2 class where it attained 57% of total GluR mRNA. The AMPAR-1,2 class, expressed in neuronal layers of hippocampus, ventromedial hypothalamus and numerous amygdaloid nuclei, was the most prevalent pattern across regions surveyed. These results suggest four regionally recurring AMPAR subunit stoichiometries. The relative GluR concentrations presented here should 1) aid in identifying pathway-specific AMPAR features, and 2) provide a template for recombinant modeling of native AMPARs (supported by AG00538 and BNS9024143).

143.8

DISTRIBUTION OF mRNA CODING FOR AMPA RECEPTOR SUBUNITS DURING OVINE CNS DEVELOPMENT. D.T. Theophilopoulos, T.S. McGraw, M.S. Sapper, J. J. Mitchell, D.J. Burchfield, and K.J. Anderson*. Departments of Pediatrics, Physiological Sciences and Neuroscience, University of Florida, Gainesville, FL 32610.

Introduction: The AMPA receptor is one of three major ionotropic glutamate receptors that has been cloned and consists of four subunits (GluR-A-D) that are expressed in alternatively spliced isoforms termed flip and flop. AMPA receptors containing predominantly flip isoforms are associated with enhanced responses to L-glutamate and thought to play a role in excitotoxicity. Glutamate receptors are present in both fetal humans and fetal sheep, but not in fetal rodents, the most commonly used model for excitotoxicity. **Hypothesis:** Early in ovine CNS development, the flip isoforms of AMPA receptors predominate which may render the immature brain more vulnerable to excitotoxicity. **Methods:** *In situ* hybridization using ^{35}S labeled probes to all four subunits and both flip and flop isoforms was performed on sheep brains at 0.8 term, term, 2 days, 10 days and adult ages to determine the distribution and density of the different AMPA receptor subunit mRNAs. **Results:** In the neocortex and cerebellum, mRNA for the flip isoforms of all four subunits is prominent prenatally and then declines into adulthood. In the hippocampus, mRNA for the flip isoforms of GluR-A and GluR-D is also prominent prenatally and similarly declines into adulthood. The flip isoform of GluR-D, previously reported only in the cerebellum of adult rats, is also found in the neocortex prenatally. Flop isoforms are expressed in a constant fashion, prenatally through adulthood. **Conclusion:** The flip isoforms of the AMPA receptor, which are associated with an enhanced response to L-glutamate, are prevalent prenatally and may render the immature brain more vulnerable to excitotoxicity. Supported by AG08843 and The Children's Miracle Network.

143.9

CALCIUM SIGNALING PATHWAYS LINKED TO AMPA RECEPTOR ACTIVATION IN CULTURED PURKINJE NEURONS. D.L. Gruol*, J.G. Netzeband, and K.L. Parsons. Dept. Neuropharmacol., The Scripps Research Institute, La Jolla, CA 92037.

We have used FURA-2 microscopic Ca^{2+} imaging and electrophysiological techniques to investigate the contributions of extracellular and intracellular Ca^{2+} to the α-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA)-induced Ca^{2+} signal or voltage response of cultured rat Purkinje neurons (> 16 days in vitro). Rapid microperfusion of AMPA (10 μM; 1 s) elicited an immediate depolarization (35±1 mV, n=15), whereas the Ca^{2+} signal peaked (soma: 74±6 nM, n=66; dendrites: 40±4 nM, n=44) 6-11 s after stimulation. The peak Ca^{2+} signal corresponded in time to a plateau in the depolarization, but both the Ca^{2+} levels and the depolarization recovered within about 1 min following AMPA application. The depolarization was followed by a smaller but more prolonged hyperpolarization (peak = 13±1 mV; duration = 490±55 s). Removal of extracellular Ca^{2+} prolonged the depolarization by 92% and shortened the hyperpolarization by 50% (n=5). In contrast, similar treatment abolished the Ca^{2+} signal to AMPA (n=12). The Ca^{2+} signal was also greatly attenuated by the P-type Ca^{2+} channel antagonist ω-agatoxin-IVA (200 nM; n=19). Intracellular ryanodine-sensitive Ca^{2+} stores also contributed to the AMPA-mediated Ca^{2+} signal as the ryanodine-receptor antagonist dantrolene (10 μM) decreased the Ca^{2+} signal by 70% (n=9). Similar data were obtained with caffeine (20 mM, n=31) which depletes ryanodine-sensitive Ca^{2+} stores. In contrast, DTBHQ (10 μM), an inhibitor of the Ca^{2+}-ATPase responsible for Ca^{2+} re-uptake, had no effect on the peak Ca^{2+} signal. Interestingly, DTBHQ did alter the time course of the electrophysiologic response (n=6), whereas dantrolene did not (n=6). These data show that both extracellular and intracellular Ca^{2+} contribute to the AMPA-induced Ca^{2+} and voltage signals in Purkinje neurons. *Supported by AA 06665 and AA 05421.*

143.10

CALCIUM SIGNALING PATHWAYS INVOLVED IN THE RESPONSE TO METABOTROPIC GLUTAMATE RECEPTOR-1 (mGLUR1) AGONISTS IN CULTURED PURKINJE NEURONS. J.G. Netzeband*, K.L. Parsons, D.D. Sweeney and D.L. Gruol. Dept. Neuropharmacol., The Scripps Research Institute, La Jolla, CA 92037.

The purpose of the present studies was to investigate the role of extracellular and intracellular Ca^{2+} on both the Ca^{2+} signal and electrophysiologic response to mGluR1 agonists. Both inositol trisphosphate (IP3)-gated and ryanodine-gated intracellular Ca^{2+} stores were examined. FURA-2 microscopic Ca^{2+} imaging or current clamp recordings were used to record the Ca^{2+} signal or voltage response of cultured rat Purkinje neurons (>16 days in culture) to the mGluR1 agonists (1*S*,3*R*)-1-aminocyclopentane-1,3-dicarboxylic acid (ACPD; 300 μM), 3,5-dihydroxyphenylglycine (DHPG; 200 μM), glutamate (50 μM), and quisqualate (Quis; 1-5 μM). The mGluR1 agonists were applied by rapid microperfusion (1 s). In Ca^{2+}-free solution, Quis (n=27) and glutamate (n=6) elicited small, but reproducible increases in Ca^{2+} levels in both the soma and dendrites. Similar results were obtained with a concentration of La^{3+} (100 μM) which blocks all Ca^{2+} channel activity. These data show that mGluR1 agonists mobilize Ca^{2+} from intracellular stores. Ca^{2+} signals to Quis were attenuated (40-50%) by 2,5-di(*t*-butyl)-1,4-hydroquinone (DTBHQ; 10 μM; n=33) or thapsigargin (1 μM; n=11), inhibitors of the Ca^{2+}-ATPase responsible for re-uptake of Ca^{2+} into IP3-gated stores. Similarly, the ryanodine receptor antagonist dantrolene (10 μM; n=9) reduced the peak Ca^{2+} signal to ACPD by 40-65%. In contrast, thapsigargin (but not dantrolene) reduced the electrophysiological signal to ACPD. Thus, both IP3- and ryanodine-gated stores contribute to the Ca^{2+} signal to mGluR1 agonists in cultured Purkinje neurons. However, the data also suggest that the electrophysiological response is more heavily dependent on Ca^{2+} release from IP3-gated stores. *Supported by AA 06665 and AA 05421.*

143.11

AMPA RECEPTOR SUBUNITS IN RAT SUBSTANTIA GELATINOSA AFTER PERIPHERAL NERVE INJURY A. Popratiloff*, R.J. Weinberg and A. Rustioni, Dept of Cell Biology & Anatomy, UNC, Chapel Hill, NC 27599.

Increased excitability and decreased inhibition of superficial laminae of the spinal cord may contribute to the abnormal pain consequent to peripheral injury. We here report initial results on changes in AMPA receptors after peripheral nerve injury. Three rats with 14 d survival were used for light microscopy. Tissue was processed for immunofluorescence; digitally captured images from superficial laminae were analysed. Quantitative analysis suggested that staining with antibody to sequence shared by GluR2 and GluR3 subunit (GluR2/3) was stronger on the side ipsilateral to the lesion. Another group of rats were perfused with mixed aldehydes 7-60 days after transection of the sciatic nerve; tissue was prepared for electron microscopy and processed for postembedding immunogold. Gold particles coding for GluR2/3 subunits were counted from active zones at type II primary afferent terminals (underlying small myelinated fibers). GluR2/3 labeling was significantly increased on the side ipsilateral to the lesion. This increase could be detected at 7 d survival and became more prominent with 14 d. These results suggest that increased GluR2/3 expression may produce increased excitability of superficial laminae neurons after peripheral nerve injuries. Supported by NIH grant NS 12440.

143.12

THE RELATION OF THE HETEROMERIC CONSTRUCTION OF GluR SUBUNITS AND THE NEURON SPECIFIC CALCIUM BINDING PROTEINS IN THE MURINE CEREBRAL CORTEX M. Kondo[1,2], H. Kamogawa[2]*, R. Sumino[2] and H. Okado[1].

[1]Dept. of Neurobiology, Tokyo Metropolitan Inst. for Neurosci., Fuchu 183; [2]Dept. of Physiology,. Sch. of Dent., Nihon Univ., Chiyoda 101, Japan.

The AMPA receptors in the central nervous system play an excitatory synaptic transmission mechanism. The AMPA receptors in the majority of neurons are weakly permeable to Ca^{2+}, while the absence of GluR2 subunit increases the Ca^{2+} permeability. To determine the distribution of the type 1 (with GluR2 subunit) and type 2 (without GluR2 subunit) neurons in the cerebral cortex of the rat, we performed double stainings, combining nonradioactive *in situ* hybridization and immunocytochemistry. In addition, we analyzed the immunoreactivity of the neuronal calcium binding proteins, parvalbumin and calbindin D-28k in these two types of neurons. The type 2 neurons, which were immunoreactive with anti-GluR1 antibody and lacked GluR2 mRNA, were almost nonpyramidal cells, whereas the type 1 neurons, which contained GluR2 mRNA, were mainly pyramidal ones and a part of neurons were nonpyramidal ones. Some type 2 neurons, which showed nonpyramidal shape, were stained for parvalbumin immunoreactivity. The pyramidal type 1 neurons with weak level expression of GluR1 were lightly stained for calbindin D-28k immunoreactivity and the nonpyramidal type 1 ones with intensive expression of the GluR1 revealed the strong calbindin D-28k immunoreactivity. It was suggested that the varied combinations of GluR subunits were related to the expression of the differential calcium binding proteins.

143.13

HIPPOCAMPAL-, SUBSTANCE P-, ENKEPHALIN- AND CALRETININ-INNERVATION OF AMPA RECEPTOR-CONTAINING NEURONS IN THE RAT LATERAL SEPTUM. F. [1]Varoqueaux and C. [1,2]Leranth*, [1]Dept. of Obstetrics & Gynecology and [2]Section of Neurobiology Yale University, New Haven, CT 06520

Neurons of the lateral septum (LS) receive glutamatergic inputs from the hippocampus and from supramammillary calretinin (CR)-containing neurons. They are also innervated by other supposedly excitatory inputs from Leu-enkephalin (L-Enk)-containing axons originating in the perifornical area and Substance-P (SP)-immunoreactive fibers arriving from the laterodorsal tegmental area.

This study investigated the presence of ionotropic AMPA receptor subunits GluR1 and GluR2/3 in LS neurons targeted by these pathways. Single immunostaining for GluR1 and GluR2/3 was performed on septal sections of fimbria-fornix transected animals; double immunostaining for AMPA subunits and SP, L-Enk, and CR was carried out on septal sections of intact rats.

Neurons containing GluR1 and GluR2/3 were abundant and homogeneously distributed in the LS. GluR1 was most intensively expressed in dendrites and somatic and dendritic spines, while GluR2/3 was exclusively associated with the soma and proximal dendrites. Hippocampal, SP, and CR fibers terminated on both GluR1- and GluR2/3-containing neurons. All enkephalinoceptive neurons contained both GluR1 and GluR2/3. Degenerated hippocampo-septal boutons formed asymmetric synapses on GluR1-containing spines. In contrast, only a very few hippocampal fibers were seen in contact with GluR2/3 stained profiles. SP-, L-Enk-, and CR-immunoreactive boutons established asymmetric synaptic contacts with the soma and dendritic shafts of both GluR1- and GluR2/3-immunoreactive neurons.

Regarding the differential intracellular distribution of AMPA receptor subunits, these observations suggest that hippocampal input on GluR1-containing spines can elicit a much higher calcium influx than the ascending hypothalamic and tegmental afferents, that terminate on the predominantly GluR2/3-containing perisomatic area. *Supported by NS 26068*

143.14

AMPA RECEPTOR SUBUNIT EXPRESSION IN PARVALBUMIN AND CALRETININ POSITIVE NEURONS OF THE HIPPOCAMPUS. M.V. Catania[1], M. Bellomo[2], R. Giuffrida *[2], and V. Albanese [1].[1]Istituto di Bioimmagini e Fisiopatologia del Sistema Nervoso Centrale (IBFSNC), CNR, Piazza Roma 2, 95123 Catania and [2]Istituto di Fisiologia Umana, Univ. Catania, 95125 Catania, Italy.

Recent studies suggest a functional diversity of native AMPA receptors (AMPARs) with respect to their Ca^{2+} permeability and kinetic properties. In interneurons, AMPARs are characterized by higher Ca^{2+} permeability and faster kinetics than AMPARs in principal cells, possibly due to a low GluR-B and a high GluR-D subunit expression, respectively. We studied the mRNA expression profile of AMPAR subunits in the hippocampal parvalbumin (PV) and calretinin (CR) positive cells, which are different populations of non-principal cells. A double-labeling approach was used: non-radioactive *in situ* hybridization and immunohistochemistry.

The majority of PV-positive neurons were characterized by a predominant expression of GluR-A, -C and -D mRNAs and low or undetectable expression of GluR-B mRNA throughout the hippocampus. CR-positive cells were characterized by a different regional expression of AMPAR subunits. In the hilus and CA3 region, large CR-positive neurons exhibited high levels of GluR-A and -D, whereas in the other regions CR-positive neurons were moderately or weakly labeled for GluR-A and -D. CR-positive neurons were generally weakly labeled for both GluR-B and -C, although some big hilar neurons occasionally exhibited moderate levels of GluR-B and -C subunit.

This study indicates that the coexistence of GluR-A, -C and -D is a common feature of hippocampal PV positive cells and suggests the presence of AMPARs with fast kinetics and high Ca^{2+} permeability. On the contrary, AMPAR subunit expression was dissimilar in CR positive neurons suggesting the existence of at least two cell populations with different location and electrophysiological properties.

This work was funded by IBFSNC, CNR.

143.15

TETANIC STIMULATION OF EXCITATORY SYNAPTIC INPUTS TO CA3 STRATUM RADIATUM INTERNEURONS

F. Laezza* and R. Dingledine. Dept. of Pharmacol., Emory Univ., Atlanta, GA.

In the CA3 str. radiatum of the rat hippocampus two types of GABAergic interneurons can be identified based on the properties of the AMPA receptor mediated EPSCs. In type I neurons, EPSCs show linear I-V relationships and are not affected by the polyamine toxins Joro spider toxin (JSTX) or N-(4-Hydroxy-phenyl-propanoyl)-spermine (NHPP), while in type II cells EPSCs are characterized by inwardly rectifying I-V relationships and are suppressed by JSTX or NHPP, suggesting a prominent expression of Ca^{2+}-permeable AMPA receptors in type II neurons. To determine whether Ca^{2+} entry through synaptic AMPA receptors triggers forms of synaptic plasticity, we studied the effect of tetanic stimulation of excitatory synaptic inputs to type I and II neurons. EPSCs were evoked by electrical stimulation of CA3 pyramidal cells in the presence of 10 μM bicuculline and 50 μM D-APV, in the hippocampal slice preparation. Biocytin (~0.4%) was added in the intracellular solution for morphological verification following whole cell recording. After 5-15 minutes of low frequency (0.16 Hz) control stimulation, tetanic stimulation (usually 3x100 Hz, 0.3s) was applied while the neuron was voltage clamped at -70/-80 mV. High frequency stimulation was followed in type II neurons by synaptic depression (n=3) lasting up to 50 minutes and short or long term potentiation (n=2). In two type II neurons no clear effect was detectable, although a trend to synaptic depression was present. In contrast, no change in EPSC amplitude was detected after high frequency stimulation in type I neurons (n=3). JSTX or NHPP (10 μM) applied in the bath solution 40-60 minutes after tetanic stimulation reduced EPSC amplitude >35% in type II (n=3) and < 12% in type I (n=2) neurons. These results suggest that type II neurons might possess some forms of synaptic plasticity induced by high frequency stimulation, probably mediated by Ca^{2+} flux through AMPA receptors. This work was supported by NS27452, NS17771.

143.16

METABOTROPIC GLUTAMATE RECEPTORS SUPPRESS EXCITATORY SYNAPTIC INPUTS TO DENTATE INTERNEURONS.

J. Doherty*, and R. Dingledine. Dept. of Pharmacol., Emory Univ., Atlanta, GA.

The effects of subtype selective metabotropic glutamate receptor (mGluR) agonists on interneurons at the granule cell/hilar border in the dentate gyrus (DG) were examined under whole-cell voltage clamp using slices of neonatal rat hippocampus. We have previously shown that the mGluR antagonist (+) MCPG (500 μM) attenuates hypoxic suppression of excitatory input to DG interneurons. Therefore, we investigated which mGluRs were capable of mediating suppression of excitatory inputs to DG interneurons. Individual interneurons were morphologically identified with Hoffman modulation contrast optics and confirmed by a biocytin staining protocol following electrophysiological study. Minimal electrical stimulation of either DG granule cells or CA3 pyramidal cells generated AMPA and NMDA receptor mediated excitatory postsynaptic currents (EPSCs) in DG interneurons. Excitatory input from DG granule cells was suppressed by the nonselective mGluR agonist, ACPD (10-100 μM) and selective group I (DHPG, 20-50 μM), group II (DCG-IV, 1 μM), and group III (L-AP4, 100 μM) agonists. In contrast, excitatory input from CA3 was suppressed by selective group I and III agonists, but not the group II agonist. 1S,3R-ACPD and DHPG evoked inward currents in dentate interneurons and increased the frequency of spontaneous EPSCs. Suppression of excitatory input to DG interneurons by mGluRs, as occurs during transient hypoxic episodes, may modulate synaptic inhibition in the DG by regulating the output of feedback inhibitory interneurons. The differential sensitivity of input from the CA3 and DG granule cells to group II mGluRs may provide a mechanism to selectively enhance the effectiveness of feedback inhibition from the CA3 to DG during conditions when mGluRs are active. This work was supported by NS17771.

143.17

IMMUNOHISTOCHEMICAL LOCALIZATION OF METABOTROPIC GLUTAMATE RECEPTORS (mGluRs) IN THE RAT BASAL GANGLIA

C.M. Testa*, S.W. Weiss and D.G. Standaert. Dept. of Neurology, Mass. Gen. Hosp., and Harvard Medical School, Boston, MA 02114.

MGluRs, which couple glutamate to second messengers, have important roles in basal ganglia function. We used two polyclonal antisera to investigate mGluR1 and mGluR2/3 localization in rat basal ganglia. Using confocal laser microscopy, mGluRs were visualized in combination with tyrosine hydroxylase (TH), MAP2 (dendritic marker), or SV2 (presynaptic marker) immunoreactivity (ir). In neostriatum, punctate mGluR1-ir was present in the neuropil. This staining did not colocalize with MAP2 or SV2, and was not altered by decortication or unilateral 6-OHDA lesions. In the globus pallidus (GP) and substantia nigra pars reticulata (SNpr), however, mGluR1-ir was tightly clustered along large, MAP2-ir dendrites. In contrast, punctate mGluR2/3-ir staining was observed within the neuropil of all basal ganglia structures. In striatum these puncta were abundant; some colocalized with SV2-ir. Striatal mGluR2/3-ir puncta were markedly reduced in number after decortication, but not altered by 6-OHDA lesions. Unlike mGluR1, mGluR2/3-ir in the GP and SNpr was not associated with MAP2-ir dendrites. Neither mGluR1-ir nor mGluR2/3-ir could be detected in TH-ir soma within substantia nigra pars compacta, or in TH-ir striatal terminals.

Overall mGluR2/3-ir is similar in pattern across the basal ganglia whereas mGluR1 has distinct subcellular localizations, and therefore possibly distinct functions. These data support a presynaptic location for mGluR2/3 in striatum, and a postsynaptic location for mGluR1 on large GP and SNpr dendrites. *Supported by the National Parkinson Foundation & NS31579.*

143.18

SUBTYPE-SPECIFIC IMMUNOHISTOCHEMICAL LOCALIZATION OF METABOTROPIC GLUTAMATE RECEPTORS 2/3, 5 AND 7 IN THE RAT OLFACTORY BULB.

Y. Sahara*[1], T. Kubota[2], N. Noro[3], M. Ichikawa[4] and Y. Nakamura[1]. [1]Dpt. Physiol., Fct. Dent., [2]Schl. Allied. Hlth. Sci., Tokyo Med. & Dent. Univ., [3]Dpt. Pharmacol., Schl. Med., Univ. Tokyo, Tokyo 113, [4]Dpt. Anat. & Embryol., Tokyo Metropol. Inst. Neurosci., Tokyo 181 Japan.

Anti-peptide antibodies directed to metabotropic glutamate receptors (mGluRs) 2/3, 5 and 7 were used to study the precise distribution of mGluRs-immunoreactivities in the rat olfactory bulb. Each antibody was subtype-specific and selectively stained different elements in the olfactory bulb. In the main olfactory bulb, light microscopic analysis revealed intense mGluR2/3 and mGluR5 staining in the periglomerular region and the external prexiform layer, while cell bodies of the mitral cell and the granule cell lacked of staining. In electron microscopic analysis, immunoreactivities of mGluR2/3 and mGluR5 were recognized in dendrites of the granule cell. The mitral cell bodies were strongly immunoreactive for mGluR7, which was associated with endoplasmic reticulum and Golgi apparatus. In the accessory olfactory bulb, the mitral/tufted cell layer as well as the granule cell layer were intensely stained with mGluR2/3 and mGluR5 antibodies, and were also immunoreactive for mGluR7. Cell bodies of the mitral/tufted cell were not immunoreactive. In electron microscopy, immunoreactivities of mGluR2/3, 5 and 7 were recognized in dendrites of the granule cell. The glomerular layer was immunoreactive for mGluR 5. These results suggest that each subtype of mGluR plays a specific functional role in olfaction. Supported by Uehara Memorial Foundation.

143.19

DETERMINATION OF DISTRIBUTION OF mGLUR4a AND mGLUR7 DURING DEVELOPMENT WITH SUBTYPE-SPECIFIC ANTIBODIES.

S. R. Bradley*, A. I. Levey, A.I. Uyoe, and P. J. Conn. Departments of Neurology and Pharmacology, Emory University School of Medicine, Atlanta, GA 30322.

Polyclonal antibodies that specifically react with two subtypes of metabotropic glutamate receptors (mGluRs), mGluR4a or mGluR7, were produced and characterized by immunoblot analysis and immunocytochemistry. Antibodies were generated against the C-terminal domains of mGluR4a and mGluR7 using synthetic peptide immunogens. Both antibodies recognized native proteins in rat brain with molecular weights similar to the molecular weights of the bands in mGluR-transfected cell lines. Immunoblot analysis revealed that the levels of mGluR7 are differentially regulated in different brain regions during development. For instance, in the hippocampus, cortex, and midbrain, mGluR7 immunoreactivity is higher at postnatal days 7, 14, and 21 than in adults. In contrast, mGluR7 immunoreactivity is high at postnatal day 7 in cerebellum and pons/medulla, but is relatively low at postnatal days 14, 21, and in adults. Immunocytochemistry and western blot analysis revealed that mGluR4a and mGluR7 are widely but differentially distributed throughout the rat brain, and also that they are localized in specific cellular sites. Strong mGluR4a immunoreactivity was observed throughout the brain whereas the distribution of mGluR7 immunoreactivity was more varied, with heavy staining in cortical and limbic regions and light staining in pons/medulla and cerelbellum. Analysis at the electron microscopy level revealed that mGluR7 immunoreactivity is largely presynaptic, though postsynaptic mGluR7 staining was also observed. In contrast, mGluR4a immunoreactivity was present on both presynaptic and postsynaptic elements. These data are consistent with the postulated role of mGluR4 and mGluR7 as presynaptic autoreceptors in a variety of brain regions and suggest that mGluR4 may also have a postsynaptic function. Supported by an NIH NRSA postdoctoral fellowship (SRB) and NIH NINDS grant NS31373 (PJC).

143.20

EXPRESSION OF GROUP I METABOTROPIC RECEPTORS BY POPULATIONS OF NEURONS IN THE RAT NEOSTRIATUM.

J.A. Kerner, D.G. Standaert, J.B. Penney, Jr., A.B. Young*, and G.B. Landwehrmeyer. Dept. of Neurology, Mass. Gen. Hosp., Boston, MA 02114.

In the striatum, metabotropic glutamate receptors (mGluRs) couple to second messenger systems via G-proteins. The mGluRs can be divided into three groups based on homology and pharmacology. We studied expression of group I mGluRs (mGluR1 and mGluR5) in rat striatum using a quantitative RNA probe *in situ* hybridization method. Radiolabeled probes for mGluR 1 or 5 were hybridized with digoxygenin probes for somatostatin (SOM), preproenkephalin (ENK), preprotachykinin (SP), glutamic acid decarboxylase 67 (GAD67), parvalbumin (PARV), or choline acetyltransferase (ChAT).

MGluR5 mRNA was very abundant in the striatum. Projection neurons were intensely labeled, SP neurons more than ENK neurons. Of the interneuron populations studied, only GAD67 positive cells exhibited a significant mGluR5 signal; the intensity was about half that seen in the projection neuron populations. The intensity of the mGluR1 signal in the striatum was much less than that of mGluR5. The strongest signal was seen in SP-positive neurons. ENK-positive projection neurons as well as ChAT, PARV, GAD67 and SOM positive interneurons were also labeled by the probe for mGluR1 mRNA.

Group I mGluR's have been implicated in excitotoxicity because of their ability to regulate intracellular calcium. The differential expression of mGluR1 and mGluR5 by distinct types of striatal neurons may contribute to their selective vulnerability in neurodegenerative disease and neural injury. *Supported by USPHS grants NS31579 and AG11337.*

144.1

Differential cellular and regional distributions of the glutamate transporters GLT1 and rEAAC1: an in situ hybridization analysis in the rat brain. Torp, R.*, Danbolt, N.C., Storm-Mathisen, J., Ottersen, O.P. Dep. of Anatomy, University of Oslo, P.O. Box 1105, N-0317 Oslo 3, Norway.

The cellular and regional distribution of mRNA encoding the newly cloned glutamate transporter rEAAC1 (the rat homologue of rabbit EAAC1) was investigated by nonautoradiographic in situ hybridization using digoxigenin labelled riboprobes. The distribution of this mRNA species was compared with that of mRNA coding for GLT1. The two transporters showed very different expression patterns. The probe recognizing rEAAC1 mRNA labelled exclusively neuronal cells. GLT1 mRNA was predominantly glial, but some neuronal populations were labelled, including many thalamic and neocortical neurons. The neurons containing the GLT1 transcript exhibited a distribution that was different from, and at some sites complementary to, the distribution of neurons containing rEAAC1. In the subiculum, for example, neurons positive for GLT1 and rEAAC1 were found in the deep and superficial cell layers, respectively, while in the parietal neocortex GLT1 predominated in layer VI and rEAAC1 in layer V. Very few neuronal populations, most notably cells in hippocampal subfields CA3 and CA4, and in layer II in the entorhinal cortex, appeared to be equipped with both transcripts. In the cerebellum, GLT1 mRNA was restricted to astrocytes, while rEAAC1 was found in granule cells and occurred in particularly high concentrations in some Golgi neurons and neurons in the cerebellar nuclei. It can be concluded that the two glutamate transporters show highly differentiated distributions in rat brain and that they may prove useful targets in future attempts to modify glutamatergic transmission in select brain regions and nerve pathways.

144.2

THE EFFECTS OF GLUTAMATE ON THE DENDRITE OUTGROWTH OF CULTURED HIPPOCAMPAL NEURONS: ELECTRON MICROSCOPIC EXAMINATION OF THE CYTOSKELETON. Charles H. Keith* and Mark T. Wilson. Cellular Biology, University of Georgia, Athens, GA 30602.

Glutamate-induced increases in Ca^{2+} have been shown to cause dendrite retraction in cultured hippocampal neurons. This effect of glutamate is dendrite specific and may be important for modulating dendrite outgrowth during neuronal development in the hippocampus. Various cytoskeletal changes must occur after glutamate exposure, however, previous studies have not examined these changes at the ultrastructural level.

In the present study, hippocampal neurons were dissected from E17-18 rat (*sprague dawley*) embryos and co-cultured for 2 days with astrocytes before exposure to glutamate. The axons and dendrites of control and experimental neurons were measured and then fixed for electron microscopic examination.

After 4 hours of glutamate exposure dendrites stopped growing. Twelve hours after glutamate exposure dendrites were reduced in length, as compared to pre-glutamate dendrite lengths. Axons continued to grow after both 4 and 12 hours of glutamate exposure. Ultrastructural examination of the dendrites from control and experimental neurons displayed no obivous difference in microtubule morphology or distribution. The lack of changes in dendritic microtubules after glutamate exposure may implicate the actin filaments as the primary targets of glutamate-induced dendrite retraction.

144.3

ATP-INDUCED RELEASE OF EXCITATORY AMINO ACIDS FROM CULTURED ASTROCYTES IS CALCIUM-DEPENDENT. S. Jeftinija* and K. Jeftinija. Department of Veterinary Anatomy and Neuroscience Program, Iowa State University, Ames, IA 50011, USA.

The specific objective of this study was to study the mechanism by which ATP releases excitatory amino acids (EAA) from cultured astrocytes. Disassociated cortical glia cultures were grown on polylysine coated coverslips. Cultures were mounted in a perfusion chamber and perfused at a rate of 200µl/min, with gassed Ringer solution at 36±1°C. Quantification of amino acids was performed by high performance liquid chromatography utilizing fluorescence detection and pre-column OPA-derivatization. Following a period of culture equilibration, 1min, 200µl samples were collected. Baseline concentrations of Asp and Glu in astrocyte cultures were 28±12 nM (mean±SD; n=12) and 58±18 nM, respectively. Perfusion application of 100µM ATP for 2min to astrocytes resulted in an 179±4% increase of Asp and 212±10% increase of Glu release. A second applications of ATP 10 min after first application resulted in a increase in the release of both EAA s that was at the level of 64% of that from the first application. Ten min application of ATP to glial resulted in a peak increase in the release followed by a decline to a plateau significantly higher than baseline release. Bath application of adenosine (100µM) was without effect on release of EAA suggesting involvement of P2 receptors. Suramine, a competitive antagonist at P2 receptors, produced a block of the response to ATP. The release of EAA evoked by ATP was not abolished in low Ca -EGTA solution. Pretreatment of the astrocyte cultures with 50µM BAPTA-AM abolished the effect pf ATP. U73122, a PLC inhibitor, abolished stimulatory effect of ATP suggesting involvement of an IP3-sensitive calcium store. An anion transpor blocker, furosemide, blocked ATP-induced glutamate release. Our results show that ATP selectively evokes the release of EAAs from cultured glia by activating intracellular calcium stores.

144.4

THE EAAT4 IS A PURKINJE CELL-SPECIFIC, POST-SYNAPTIC GLUTAMATE TRANSPORTER IN THE BRAIN. K. Yamada, M. Watanabe, T. Shibata*, K. Tanaka[1], K. Wada[1], Y. Inoue. Dept. of Anat., Hokkaido Univ. Sch. of Med., Sapporo 060. [1]Dept.of Degenerative Neurological Diseases, National Institute of Neurosci., NCNP, Kodaira 187, Japan.

The glutamate transporter plays important roles in termination of the glutamate receptor activation and in protection of neurons from the glutamate excitotoxicity. The EAAT4 is the fourth subtype of the glutamate transporter, with properties of ligand-gated chloride channel activities. To clarify the expression and localization, in situ hybridization and immunohistochemistry were applied to the mouse brain. Antisense oligonucleotide probes labeled with ^{33}P-dATP demonstrated its specific expression in cerebellar Purkinje cells. The transcripts were detected in Purkinje cell layer of the posterior cerebellum as early as embryonic day 15. Immunohistochemistry with anti-peptide antibody revealed that the distribution of the EAAT4 was restricted to the molecular layer of the cerebellum. In the layer, the strong immunoreactivity was observed as numerous punctate stainings, while perikarya and thick dendrites of the Purkinje cells were labeled only weakly. No immunoreactivity was detected in the axons nor terminals . By immunoelectron microscopy, the antibody labeled dendritic spines of the Purkinje cells. These findings indicate that the EAAT4 is a Purkinje cell-specific glutamate transporter localized at the post-synapse. Taken together with previous results on dense localization of the GluT-1 and GLT1 on the Bergmann astrocytes, the Purkinje cell synapses are thus provided with distinct transporter subtypes at discrete synaptic elements.

144.5

DETERMINATION OF CALCIUM (Ca^{+2})-INDEPENDENT COMPONENT OF GLUTAMATE (GLU) AND GABA RELEASE BY MINIMIZATION OF PRE-SYNAPTIC Ca^{+2} INFLUX. **S.M. Lasley* and M.C. Green. Dept. Biomed. and Therapeutic Sci., U. Illinois Coll. of Medicine, Peoria, IL 61656.**

The amounts of GLU and GABA measurable in brain extracellular fluid (ECF) emanate from multiple sources. Ca^{+2}-dependent release was defined as the difference between GLU/GABA measured in the presence of Ca^{+2} (total release) and its absence (Ca^{+2}-independent release). This experiment was designed to accurately identify the Ca^{+2}-*independent* component by microdialysis perfusion of brain tissue with Ca^{+2}-free solutions in awake rats. Bilateral guide cannulae were stereotaxically implanted under anesthesia in dorsal hippocampus, and dialysis initiated 2-4 days later by insertion of probes that extended 2 mm beyond the cannula tips. Animals were perfused with a modified Ringer's solution on one side (for total release) and the same solution with Mg^{+2} replacing Ca^{+2} with or without Ca^{+2} channel blockers on the opposite side. After establishing basal levels of ECF GLU and GABA, release was stimulated by switching perfusion to Ringer's containing 150 mM K^+ (no Na^+ to maintain isotonicity). 40 min later perfusion was returned to the initial solution. Removal of Ca^{+2} from the perfusate significantly elevated basal levels of GLU and GABA and further enhanced K^+-stimulated release by 20-30% compared to rats dialyzed with modified Ringer's. Addition of 1 mM Cd^{+2} to the Ca^{+2}-free perfusate effectively diminished K^+-evoked release but markedly increased basal levels of GLU (28-fold) and GABA (11-fold). Inclusion of 1 mM methoxyverapamil (MVP) in the Ca^{+2}-free medium significantly decreased basal levels of GABA and blocked K^+-stimulated GLU and GABA release. With use of MVP the proportion of total release that was Ca^{+2}-dependent was found to be 82% for GLU and 95% for GABA. These results suggest that dialysis with simple Ca^{+2}-free solutions is insufficient to identify Ca^{+2}-independent release, and indicate that the preferred Ca^{+2} channel blocker is one that does not also support exocytosis. (Supported by NIH ES06253)

144.6

EXTRACELLULAR GLUTAMATE INDUCES LEUKOCYTE ADHERENCE TO PIGLET PIAL VENULES. A.R. Shah, J.M. Gidday, J.W. Beetsch, E.R. Gonzales, Y.-B. Lee, R.G. Maceren, M.J. Noetzel*, T.S. Park. Departments of Neurology and Neurological Surgery, and St. Louis Children's Hospital, Washington University School of Medicine, St. Louis, MO 63110

Glutamate release is important in the induction of several neurochemical cascades that promote tissue injury following cerebral ischemia. An inflammatory response to cerebral ischemia with leukocyte recruitment is gaining recognition as another important factor contributing to ischemic injury. We tested the hypothesis that glutamate can initiate an inflammatory response in the cerebral circulation. Anesthetized newborn pigs were equipped with closed cranial windows for epifluorescent videomicroscopy of rhodamine 6G-labelled leukocyte dynamics. Glutamate (1µM, 10µM, or 100µM in CSF) was superfused (50 µl/min) continuously through the window in separate animal groups (n=4 each), and leukocyte adherence was measured after 30, 60, and 120 minutes of superfusion. Relative to a control group (n=8), we found no increases in adherence with 1µM or 10µM glutamate, but 100µM glutamate caused a progressive, significant increase in adherence over time. The extent of adherence induced by 100µM glutamate did not differ from that observed in response to complete global ischemia (n=5). These findings indicate that extracellular glutamate, at a concentration similar to that measured in brain following ischemic insults, can induce an inflammatory response in cerebral venules that, in turn, is associated with increases in vascular permeability (see ER Gonzales et al., this volume). Leukocyte adherence may be secondary to glutamate-induced oxygen free radical formation and/or stimulated production of other inflammatory mediators. This mechanism, and its role in secondary brain injury following ischemia, have yet to be determined. (NINDS 21045 and 32568).

144.7

A1, mGluR2 Auto AND GABAB HETERORECEPTORS COMODULATE PURINE AND EAAs RELEASE FROM RAT HIPPOCAMPAL SYNAPTOSOMES A. Poli,* P. Di Iorio , F. Caciagli , R. Ciccarelli , P. Giuliani , P. Ballerini , R. Lucchi and F. Nicoletti^, Dept Biology, Univ. Bologna, Italy. *Inst. Pharmacology, Univ. Chieti, Italy. ^Inst. Pharmacology, Univ. Catania, Italy.

Previous our findings indicate that presynaptic A1 adenosine (Ado) receptor and class II metabotropic glutamate receptors (mGluR2 and 3) co-operate in inhibiting the evoked release of purines and excitatory amino acids (EAAs) from slices and synaptosomes of rat hippocampus. In the present study, we evaluated wheter presynaptic A1, mGluR2 as well as the GABAB receptors are involved in this modulatory mechanism for the purine and EAA release as well as that of GABA from rat hippocampal synaptosomes likely through a common signal transduction mechanism. K-induced release of endogenous Ado, glutamate (Glu) and GABA was increased in a concentration-dependent fashion. The fluorescent probe bisoxonol (0.5 μM) confirmed that K^+ caused a dose-depend membrane depolarization, which was significantly reduced by the pretreatment with CCPA, DCG-IV and (±)baclofen respectively. All these agonists reduced the evoked release of Ado and Glu in a dose-dependent fashion (IC_{50} 25 mM for CCPA, 4μM for DCG-IV and 70 μM for baclofen), and this effect was less than additive when the drugs were used in combination. On the contrary, K^+-evoked GABA release was unaffected either by CCPA or by DCG-IV, whereas (±)baclofen was able to reduce GABA release only at doses 200 μM. The A1 and mGluR2 antagonists DPCPX (100 nM) and MCCG-I (300 μM) counteracted the inhibitory effects of the respective agonists on the K^+-evoked release of both Ado and Glu. Phaclofen (1 mM), the GABAB autoreceptor antagonist, did not modify the baclofen-induced inhibition of K^+-evoked Glu and Ado release, whereas completely prevented that of GABA release. These findings suggest that presynaptic A1 Ado receptor and mGluR2 as well as GABAB heretoreceptor exert an ihnibitory control on the release of both purines and EAAs.

144.8

IMMUNOCYTOCHEMICAL CHARACTERIZATION OF HOFMANN NUCLEI NEURONS.

A.L. Eide*, R. Torp Inst. Basic Medical Siences, University of Oslo, Norway.

Hofmann Nuclei (HN) neurons are commissurally projecting neurons in the spinal cord of birds. Recent tracing studies have established the detailed projection pattern of these neurons and their anatomical input (Eide, 1996). The cells have been suggested as a useful model for studies of intersegmental connectivity in the spinal cord; physiologically as well as developmentally. Yet little is known about the function of the neurons.

We have made an attempt to characterize the transmitter substances involved in these spinal circuits by use of antibodies directed to some of the well established amino acid transmitters. The separate location of the HN neurons in segmentally iterated clusters attached on the lateral surface of the spinal cord make the cells a favourable population in which to use immunocytochemical methods.

Chicken embryos at d18 were fixed and prepared for immunocytochemistry. For both LM and EM neighbouring sections were incubated in primary antisera to glutamate, GABA, and glycin.

The HN soma stained positive for glutamate and glycin indicating that these amino acids are colocalized within the neuron.

Punctuate staining around the cells at LM level indicated that the cells received glutamatergic input. This was confirmed with an immunogold technique at EM level where identified terminals making contact with the soma were enriched with glutamate like immunoreactivity.

144.9

GLUTAMATE, ASPARTATE AND CALBINDIN IN THE MEDIAL THALAMUS: AN IMMUNOHISTOCHEMICAL STUDY IN THE RAT, M. Bentivoglio[1]*, C. Frassoni[2], R. Spreafico[2], [1]Inst. Anat. Histol., Univ. Verona, 37134 Verona, [2]Neurological Institute "C. Besta", 20133 Milan, Italy.

Topographical and quantitative features of medial thalamic neurons in which aspartate (ASP) or glutamate (GLU) may serve a neurotransmitter role were investigated in the rat. The calcium binding protein calbindin D-28k (CB) was exploited as a marker of neuronal subsets, thus allowing also to study the relationships between the CB-containing thalamic neurons and those immunoreactive (ir) to excitatory amino acids. Double immunocytochemistry of ASP and CB, or GLU and CB, was performed in 40 μm thick sections. The three markers were distributed, with regional variations, in the thalamic midline and medial nuclei, where ASP-ir neurons appeared more numerous than the GLU-ir ones; ASP-CB or GLU-CB double immunostained neurons were evident. ASP-, GLU-, CB-ir cells were then quantitatively evaluated in 5 μm thick consecutive sections. Interindividual variations and different anti-ASP and anti-GLU antibodies did not result in significant differences. ASP and GLU were not co-localized. Single ASP- or GLU-ir neurons accounted for 60% of the total number of immunostained cells; ASP-ir cells represented more than half of these neurons. Half of the CB-ir cells were double immunostained. The proportion of double CB-ASP-ir neurons was sevenfold higher than that of the CB-GLU-ir ones. These results suggest that ASP may act as excitatory neurotransmitter in a relatively high proportion of medial thalamic neurons, in which ASP frequently coexists with CB. About 50% of the CB-ir cells did not contain either ASP or GLU, suggesting that some medial thalamic neurons may utilize a different excitatory neurotransmitter.

144.10

QUANTITATION OF BLOOD CONTRIBUTIONS TO QUINOLINIC ACID CONCENTRATIONS IN BRAIN AND SYSTEMIC TISSUES. M.P. Heyes*, K.E. Beagles, M.A. Zito, M.G. Proescholdt, S.P. Markey and P.F. Morrison. Section on Analytical Biochemistry, Laboratory of Clinical Science NIMH, and Biomedical Engineering, NCRR, Bethesda MD 20892-1262.

The source of the neurotoxin quinolinic acid (QUIN) in brain and systemic tissues under normal and pathologic circumstances reflects either *de novo* synthesis from L-tryptophan and other precursors, or entry of QUIN itself from the blood. To quantify the relative contributions of blood versus tissue-derived QUIN, [$^{13}C_7$]-QUIN was infused in gerbils via osmotic pumps placed subcutaneously (30 mM at 0.55 μL/h), and the fraction of QUIN in tissue (Ti; measured in tissue homogenates) derived from blood (Bl; measured in serum) was calculated by the formula ([$^{13}C_7$]-$QUIN_{Ti}$ / $QUIN_{Ti}$) / ([$^{13}C_7$]-$QUIN_{Bl}$ / $QUIN_{Bl}$). In normal gerbils, blood QUIN contributed 40-50% of total QUIN in brain, 70% in CSF, >95% in spleen, lung, liver and intestine, and between 40-70% in kidney, heart and skeletal muscle. Systemic endotoxin (450 μg/Kg) administration increased blood, brain, CSF and systemic tissue QUIN levels at 24 h. The relative proportion of QUIN derived from blood in brain, spleen, lung and intestine was unchanged by endotoxin, but increased in kidney, heart and skeletal muscle. In contrast, cerebral ischemic injury (10 min carotid artery occlusion) resulted in an increase in regional brain QUIN concentrations at four days, with a proportional increase in the amount of QUIN derived endogenously by brain. In the hippocampus for example, brain QUIN levels increased from 48±4 to 1571±453 pmol/g, and 94.6 % of QUIN was synthesized by tissue. There were no changes in the post-ischemic gerbils in systemic tissue or blood QUIN levels, or changes in the relative proportions of blood- versus tissue-derived QUIN. These results establish that the brain normally synthesizes QUIN, and that the rate of this formation increases in conditions of brain and systemic immune activation. The design of drugs to attenuate QUIN accumulations as potential therapeutic strategies needs to target appropriate tissue QUIN sources.

CATECHOLAMINES: DOPAMINE I

145.1

IONTOPHORESIS OF AMPHETAMINE IN THE NEOSTRIATUM AND NUCLEUS ACCUMBENS OF AWAKE, UNRESTRAINED RATS. E.A. Kiyatkin* and G.V. Rebec, Program in Neural Science, Dept. Psychology, Indiana University, Bloomington, IN 47405.

The ability of amphetamine (AMPH) to enhance dopamine (DA) transmission in the neostriatum and nucleus accumbens is believed to play a critical role in the behavioral and psychogenic actions of this drug. To assess the direct effects of AMPH on neostriatal and accumbal neurons under naturally occurring behavioral conditions, single-unit recording was combined with iontophoresis in awake, unrestrained rats. Brief (15 - 20 s) applications of AMPH inhibited both spontaneously active and glutamate (GLU)-activated neurons, but the inhibition was stronger on the latter group. The inhibitory effect of AMPH occurred at low ejection currents (5 - 10 nA) and was dose-dependent. Onset (4 - 12 s) and offset (6 - 24 s) latencies were relatively brief. When applied for prolonged periods (2 - 4 min), AMPH exerted a powerful Inhibition of the GLU response, resulting in a complete blockade of the response at higher currents (≥ 20 nA). Although systemic AMPH is known to activate many neostriatal and accumbal neurons in ambulant rats, this effect appears to require GLU afferents from cerebral cortex (Tschanz et al., Eur. J. Pharmacol., 257:161, 1994). AMPH iontophoresis, in contrast, may selectively increase the local accumulation of DA. Comparisons with iontophoretic DA obtained in previous work (Kiyatkin and Rebec, J. Neurophysiol., 75:142, 1996), however, indicate that AMPH is more potent than DA in terms of number of responsive cells, threshold currents, and magnitude of inhibition. This difference may reflect an action of AMPH on other monoaminergic systems, including serotonin. It appears, therefore, that local applications of AMPH do not mimic the systemic actions of this drug on neostriatal and accumbal neurons. Supported by NIDA (DA 02451).

145.2

$GABA_B$ RECEPTORS MODULATE THE FIRING PATTERN OF DOPAMINE NEURONS IN THE VENTRAL TEGMENTAL AREA

J.M. Mathé*, K. Chergui, G. Engberg and T.H. Svensson Dept. of Physiology and Pharmacology, Div. of Pharmacology, Karolinska Institutet, 171 77 Stockholm, SWEDEN

Our previous studies demonstrate the fundamental role of the firing pattern, rather than the average firing rate, of dopamine (DA) neurons for the regulation of DA release as well as in the the DA effect on postsynaptic neurons. Here we have analyzed the role of $GABA_B$ receptors in the control of the firing pattern of DA neurons in the ventral tegmental area (VTA). Extracellular single cell recordings were obtained from VTA DA neurons in male chloral hydrate anesthetized SD rats. Discriminated action potentials were recorded by computer for analysis of firing rate, burst firing and regularity of firing, as assessed by the variation coefficient. Systemic administration of relatively low doses of baclofen (<8 mg/kg, i.v.), a selective $GABA_B$ agonist, dose-dependently reduced burst firing and increased the regularity of firing in VTA DA neurons. However, the firing rate was significantly reduced only after administration of high doses (≥8 mg/kg) of baclofen. The above effects were effectively antagonized by pretreatment with the selective $GABA_B$ antagonist CGP 35348 (100 mg/kg, i.v.), which did not affect the firing pattern of VTA DA cells when given alone. Local, microiontophoretic application on VTA DA cells of the above compounds induced essentially identical effects on the firing pattern as seen after systemic administration. Thus, $GABA_B$ receptors, similarly to NMDA receptors, can specifically control the firing pattern of mesolimbic and/or mesocortical DA neurons, without affecting their average firing rate. Since we have shown that specific alterations in firing patterns of midbrain DA neurons change gene expression in their target areas, $GABA_B$ receptors probably exert a prominent influence upon information processing in DA circuitries in brain. Supported by the Medical Research Council of Sweden (4747) and Karolinska Institutet.

145.3

WHOLE CELL RECORDINGS FROM VISUALLY IDENTIFIED MESOCORTICAL DOPAMINE NEURONS IN BRAIN SLICES. W.-X. Shi*, P. Zheng and B. S. Bunney. Departments of Psychiatry and Pharmacology, Yale University School of Medicine, New Haven, CT 06510

Mesocortical dopamine (MC DA) neurons may play a key role in the development and treatment of schizophrenia. However, our knowledge of MC DA cells is still vary limited, partially due to the technical difficulties of studying these cells. Thus far, MC DA cells have only been recorded *in vivo* with extracellular electrodes. To study MC DA cells in more detail and to overcome some of the limitations associated with an *in vivo* preparation, we developed a brain slice preparation in which MC cells in the VTA were first retrogradely labeled *in vivo* with fluorescent microspheres and then visually identified and recorded in slices under an upright fixed-stage fluorescence microscope. We made whole cell recordings from 16 identified MC cells. Half of them showed a hyperpolarization response to DA. Preliminary data suggest, however, that these cells are less sensitive to DA than nigral DA cells. Like SN DA cells, MC DA-responding cells had broad action potentials, large AHPs, and a strong time-dependent inward rectification during hyperpolarization. Unlike nigral DA cells, however, they had a much higher input resistance and longer membrane time constant. Most of them also lacked the transient outward rectifier and showed a fast rising LTS-like potential at the end of a hyperpolarization step. Most strikingly, MC DA-responding neurons were capable of firing at a much higher rate than SN DA cells without developing depolarization inactivation. Compared with MC DA-responding cells, MC DA non-responding neurons had a shorter membrane time constant and a lower input resistance. These cells also showed an inward rectifier during hyperpolarization. However, compared with that observed in MC DA-responding cells, the inward rectifier in MC DA non-responding cells was much faster in developing, thus giving rise to only a moderate degree of sag in response to a hyperpolarization step. The DA status of these two populations of MC cells is currently under investigation using double-staining for tyrosine hydroxylase.

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145.4

L-ARGININE AND NITRIC OXIDE MODULATION OF NMDA-INDUCED BURST FIRING OF DOPAMINE NEURONS IN RAT MIDBRAIN SLICE. B.A. Cox* and S.W. Johnson, Department of Physiology and Pharmacology, OHSU, Portland, OR, 97201.

Intracellular recordings of dopamine (DA) neurons in rat midbrain slices were performed to examine the effects of L-arginine (3-10 mM) and nitric oxide donor diethylamine/nitric oxide complex (DEA/NO, 0.05-1 mM) on NMDA (30 μM)/apamin (100 nM)-induced burst firing.

L-arginine caused a hyperpolarization (5-10 mV) which inhibited oscillations so that burst firing switched to slower single spike firing and eventually quit firing (n=4). L-arginine, applied together with $GABA_B$ antagonist CGP35,348 (300 μM) and $GABA_A$ antagonist bicuculline (30 μM, n=3), depolarized the cell 2-3 mV and inhibited firing, but had no effect on burst-related oscillations.

DEA-NO caused DA neurons to switch from burst firing to rapid single spike firing followed by a large depolarization (10-25 mV) which inhibited firing (n=3). However, DEA-NO, applied to the bath with CGP35,348 and bicuculline, resulted in a hyperpolarization (4-24 mV) which subsequently inhibited both firing and oscillations (n=4).

The results indicate that L-arginine inhibition of burst firing is partially due to L-arginine-induced potentiation of GABA synaptic currents (Cox, *et al.* Soc. Neurosc. Abstr., 21:1088, 1995), and that another mechanism is also involved. Additionally, the data suggest that there is a GABA component to DEA-NO modulation of burst firing.

Supported by RO1MH40416, * by Tourette Syndrome Association and Scottish Rite Benevolent Foundation's Schizophrenia Research Program.

145.5

D-AMPHETAMINE INHIBITS THE SPONTANEOUS ACTIVITY OF TWO- TO THREE-WEEK-OLD NIGRAL DOPAMINE NEURONS IN THE MIDBRAIN SLICE. **Amy J. Barron*, Mark W. Lewis and David K. Pitts. Department of Pharmaceutical Sciences, College of Pharmacy & Allied Health Professions, Wayne State University, Detroit, MI 48202.**

Previous *in vivo* studies of nigral dopamine neuron responses to D-amphetamine (AMP) during the early postnatal period have suggested that AMP either exerts anomalous effects (Trent, Nakamura and Tepper, Eur. J. Pharmacol. 204:265, 1991) or has more typical adult-like inhibitory effects on spontaneous activity (Pitts and Zhang, Soc. Neurosci. Abstr. 19: 928, 1993). However, Pitts and Zhang (1993) reported that the long-loop inhibitory forebrain feedback pathway that is activated by AMP in adult animals, and in part responsible for the inhibitory response, may not be fully functional in 2-week-old animals. The *in vitro* response to AMP has not been studied in the midbrain slice preparation of young animals, where the long-loop feedback pathway is not present. Under these conditions, the major effects of AMP are likely to be exerted through the release of DA from nigral dendrites and the subsequent stimulation of somatodendritic DA autoreceptors, i.e. short-loop feedback. In the present study the response of nigral DA neurons from 2- or 3-week-old midbrain slices to 5 μM AMP was examined. In all cases 5 μM AMP elicited inhibitory responses which ranged in magnitude from 39% to 100% (n=7). These results strongly suggest that the short-loop feedback loop is operational during this early postnatal period. Additional studies will further characterize the postnatal development of this response and determine the mechanism. (Supported by MH47857 to DKP).

145.6

SPONTANEOUSLY ACTIVE NIGRAL DOPAMINE NEURONS ARE BOTH EXCITED AND INHIBITED BY 8-BROMO-CYCLIC-AMP IN THE MIDBRAIN SLICE. **Mark W. Lewis*, Amy J. Barron and David K. Pitts. Department of Pharmaceutical Sciences, College of Pharmacy & Allied Health Professions, Wayne State University, Detroit, MI 48202.**

Little is known about the influence of cAMP on the pacemaker activity of nigral dopamine neurons. The membrane permeable analog of cAMP, 8-Bromo-cAMP (1-2 mM), was administered via the artificial CSF perfusate into a brain slice chamber, and the electrical activity of single nigral DA neurons was monitored using standard extracellular electrophysiological recording techniques. Brain slices from rats aged 2- (n=16), 3- (n=18), 4- (n=10) and 5-weeks-old (n=2) were examined and nigral DA neuron responses categorized as: excitation (50.0%), inhibition (28.3%), biphasic (excitation/inhibition: 19.6%) or none (2.1%). No age-dependent effects were detected, however, the 4- and 5-week-old sample sizes are small. The predominant response was excitatory, and the relative proportion of excitatory responses to inhibitory or biphasic responses was found to be concentration dependent (log-linear analysis: response category x concentration interaction, $P<0.05$). The excitatory response predominated at the lower concentration (80% at 1 mM; 35.5% at 2 mM). There was an increase in inhibitory responses at the higher concentration (13.3% at 1 mM; 35.5% at 2 mM). Biphasic responses (29.0%) were only seen with the 2 mM concentration. The mechanism for these effects is currently under investigation. Preliminary evidence suggests that stimulation of adenylate cyclase by 5 μM forskolin elicits excitatory and inhibitory responses similar to that of 8-Bromo-cAMP. (Supported by MH47857 to DKP).

145.7

REVERSIBLE BLOCKADE OF AN APAMIN-SENSITIVE AFTERHYPERPOLARIZATION BY (+)-TUBOCURARINE INDUCES BURSTING ACTIVITY IN NIGRAL DOPAMINE NEURONS IN VITRO. S.T. Connelly, H-X. Ping and P.D. Shepard*, MD Psychiatric Res. Cntr. & Univ. of MD. School of Med., Baltimore, MD 21228.

SK-type, Ca^{2+}-activated K^+ channels are responsible for the medium and slow components of the post-spike afterhyperpolarization (AHP) exhibited by mesencephalic dopamine (DA)-containing neurons *in vitro*. Apamin (APA), a potent antagonist of this conductance, increases neuronal excitability and leads to the appearance of bursting activity resembling that observed spontaneously *in vivo*. Although APA-induced bursting has been attributed to its ability to suppress the AHP, the irreversible nature of the blockade has made it difficult to establish the specificity of the toxin's actions. In the present series of experiments, (+)tubocurarine (d-TC), a neuromuscular blocking agent with affinity for the APA binding site, was tested for its effects on the (1) AHP complex and (2) firing pattern exhibited by DA neurons in nigral brain slices. d-TC (500 μM) blocked or reduced the APA-sensitive component of the AHP (61.0 ± 6.3% decrease) without affecting spike width. Extracellular recordings revealed a marked alteration in neuronal activity during d-TC application. The coefficient of variation (CV = standard deviation/mean), computed from interspike interval distributions, was used to assess drug-induced alterations in firing pattern. Of the 55 control cells analyzed, 53 exhibited CVs < 18% (mean ± SEM: 11.3 ± 0.5%) indicative of a pronounced pacemaker-like discharge. By contrast, the majority of cells treated with d-TC (38/64) exhibited CVs ≥ 18% (mean ± SEM: 40.0 ± 4.6%). Bursting activity was detected in 17% of the d-TC treated cells. d-TC induced alterations in both the AHP complex and firing pattern were fully reversed within 45 minutes of washout from the recording chamber. These data strongly suggest that alterations in firing pattern observed following APA and d-TC result from *selective* blockade of the post-spike AHP. Supported by USPHS grant MH-48543.

145.8

IONIC MECHANISMS UNDERLYING BURSTING PLATEAU POTENTIALS IN NIGRAL DOPAMINE NEURONS IN VITRO. H-X. Ping* and P.D. Shepard, MD Psychiatric Res. Cntr. & Univ. of MD. School of Med., Baltimore, MD 21228.

Mesencephalic dopamine (DA)-containing neurons exhibit a Ca^{2+}-dependent, sinusoidal oscillation in membrane potential that underlies the ability of these cells to maintain spontaneous activity in the absence of afferent synaptic input. Recent studies have shown that an SK-type Ca^{2+}-activated K^+ channel is principally responsible for the falling phase of the autogenous pacemaker oscillation (Ping and Shepard, NeuroReport, 1996). Apamin-induced blockade of this conductance results in the emergence of a voltage-sensitive plateau potential capable of driving a rhythmic bursting discharge similar to that observed spontaneously *in vivo*. In the present series of experiments, sharp electrode intracellular recording techniques were used to investigate the ionic mechanisms contributing to generation of the bursting plateau potentials exhibited by DA neurons in nigral brain slices. Depolarizing current pulses applied in the presence of TTX (2 μM) and apamin (100-200 nM) evoked a ramp shaped, slow depolarization with a trajectory identical to that preceding spike generation in control media. When applied from negative holding potentials (-64 mV), low intensity current pulses were followed by a plateau potential exhibiting all-or-none characteristics. Increases in stimulus strength (duration or amplitude) *reduced* the duration of the voltage response without significantly affecting its amplitude. Addition of TEA (20-30 mM) to bathing solutions containing TTX and apamin increased the input resistance of the cell and the duration of the plateau potential; however, TTX + TEA were incapable of evoking a similar response in the absence of apamin. Bath application of Cd^{2+} (200-400 μM) or removal of Ca^{2+} from the bathing media fully blocked the plateau potential without affecting the initial ramp-shaped component of the voltage response. These data suggest plateau potentials underlying apamin-induced bursting in vitro are mediated by a voltage-sensitive Ca^{2+} conductance which may possess self-limiting properties. This research was supported by USPHS grant MH-48543.

145.9

MICRODIALYSIS PROBE IMPLANTATION INTO STRIATUM DISRUPTS HALOPERIDOL-INDUCED DOPAMINE CELL DEPOLARIZATION BLOCK

C.L. Todd, H.Moore, D.G. Harden and A.A. Grace, Depts. of Neuroscience and Psychiatry, Univ.of Pittsburgh, Pittsburgh PA 15260.*

Depolarization block (DB), the cessation of spontaneous activity due to sustained hyperexcitatation, develops in midbrain dopamine (DA) neurons during repeated administration of neuroleptics. Although DB has been well-characterized electrophysiologically, microdialysis studies have provided inconsistent data regarding changes in DA levels in the striatum during DB. Such variability could reflect regulation of extracellular DA by compensatory mechanisms as well as by lowered DA cell activity. An additional possibility is that implantation of the dialysis probe disrupts striatal cell activity, an effect which as been shown to reverse DA cell DB.This study examined the effects of striatal implantation of a microdialysis probe on DA cell activity in the SN in rats that had been treated repeatedly with haloperidol (HAL). Intact rats treated with HAL for 21-22 days showed a marked reduction in the number of spontaneously-active cells in the substantia nigra (SN). Five to 90 min after lowering the dialysis probe into the striatum, the number of active DA cells in the ipsilateral SN was increased. An increase in the number of active DA cells was also apparent at 24-48 hours following probe implantation. The number of DA cells that had apparently recovered from DB depended on the interval between implantation and testing, and whether or not HAL was withdrawn during this interval. These results show that implantation of a microdialysis probe can reverse DA cell DB. Thus, although microdialysis may be useful in determining the effects of DB on the extracellular levels of DA in the striatum, the experimental design, particularly the timing of probe implantation relative to withdrawal of treatment and dialysis measurement may be critical. *Supported by USPHS MH 45156, Howard Hughes Undergrad. Award 71192-514202 to C.L.T. and an NRSA Fellowship to H.M.*

145.10

INHIBITION OF DOPAMINE RE-UPTAKE: SIGNIFICANCE FOR THE FIRING RATE AND FIRING PATTERN OF NIGRAL DOPAMINE NEURONS. A. Elverfors*, G. Engberg, J. Jonason and H. Nissbrandt, Department of Pharmacology, Göteborg University, Medicinaregatan 7, S-413 90 Göteborg, Sweden.

In the present study the effects of inhibition of the re-uptake of dopamine (DA) were analysed with respect to DA release and to the firing pattern of DA neurons in the substantia nigra (SN). Intravenous administration of GBR 12909 (0.5 - 8 mg/kg), a specific and potent inhibitor of DA re-uptake, was found to dose-dependently increase the DA concentration both in the SN and in the striatum, as measured by microdialysis. However the drug failed to significantly affect the firing rate of the nigral DA neurons. In contrast, GBR 12909 dose-dependently induced a regularisation of the firing pattern, concomitant with a reduction in burst activity. An acute hemisection of the brain, which by itself produced a slight regularisation of the firing pattern of the nigral DA neurons without changing the firing rate or the ability of the DA neurons to fire in bursts, was found to prevent the the regulatory action of GBR 12909. Pretreatment with the selective $GABA_B$-receptor antagonist CGP 35348 (200 mg/kg, i.v., 5 min) did not significantly affect the firing rate, the regularity of the DA neurons, or their ability to fire in bursts. However CGP 35348 markedly antagonised the ability of GBR 12909 to induce pacemaker-like firing or a decrease in burst activity of the nigral DA neurons.

The results of the present study suggest that a striatonigral projection may serve to control the activity of nigral DA neurons not primarily by regulating the firing rate, but, preferably, by modulating the firing pattern of the neurons. In this regard, activation of somatodendritic $GABA_B$-receptors may form the final link in this feedback inhibitory control system.

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145.11

OPTICAL IMAGING OF EXTRACELLULAR DIFFUSION IN SUBSTANTIA NIGRA AND VENTRAL TEGMENTAL AREA. C. Nicholson*, S. J. Cragg, L. Tao and M. E. Rice. Dept. Physiology & Neuroscience, NYU Medical Center, 550 First Avenue, New York, NY 10016.

Dendritic release of dopamine (DA) and other neurochemicals in the substantia nigra (SN) and ventral tegmental area (VTA) represents a novel form of cell-cell communication. DA in particular is thought to diffuse from release sites to mediate volume transmission in these areas. We determined regional differences in diffusion properties of the SN pars compacta (SNc), pars reticulata (SNr), and VTA in slices of guinea pig midbrain. Fluorescent-labeled 3 kDa dextran was co-diffused with DA after pressure microejection. The dextran was monitored with integrative optical imaging while DA was simultaneously detected using fast scan cyclic voltammetry at a carbon fiber microelectrode 100 µm from the source. This technique permitted visualization of isotropic and anisotropic diffusion and evaluation of how regional variations in uptake influence the diffusion of DA (see Abstract by Rice et. al.). We found significant regional differences in the geometric parameter, tortuosity ($\lambda = (D/D^*)^{0.5}$ where D is the free, and D^* the apparent, diffusion coefficent in brain). Diffusion in SNc ($\lambda = 1.76$) was less restricted than in VTA ($\lambda = 2.03$) or SNr ($\lambda = 1.95$) ($P < 0.001$). Regional differences in λ had similar effects on the diffusion of both DA and dextran. Optical imaging of dextran migration showed that extracellular diffusion in VTA was isotropic. By contrast, dextran diffusion in SNc was anisotropic, with a preferred direction along the intrinsic lateral dendrites and channeling along dendrites extending into the SNr. These data suggest that the structure of the extracellular microenvironment in the SNc, SNr and VTA will influence how DA and other neurochemicals mediate volume transmission in each region. Supported by NS-28642 (CN) and NS-28480 (MER).

145.12

DIFFUSION AND UPTAKE OF DOPAMINE IN SUBSTANTIA NIGRA AND VENTRAL TEGMENTAL AREA. M. E. Rice*, S. J. Cragg, S. A. Greenfield and C. Nicholson. Dept. Physiology & Neuroscience, NYU Medical Center, 550 First Avenue, New York, NY 10016.

Dopamine (DA) released from somatodendritic sites in the substantia nigra pars compacta (SNc) and ventral tegmental area (VTA) may reach receptors through extrasynaptic communication (volume transmission). These regions contain few DA synapses and so the time course and sphere of influence of DA will be determined by the diffusion and uptake properties of the extracellular microenvironment. We examined DA uptake properties in SN and VTA in guinea-pig *in vitro* following local pressure microejection of DA. DA was detected using fast-scan cyclic voltammetry at a carbon-fiber microelectrode 100 µm from the source. Most DA diffusion curves were adequately fitted using with linear uptake (i.e. uptake = k' x concentration), though an improvement is expected with Michaelis-Menten kinetics. Overall k' was similar in SNc ($k' = 0.10\ s^{-1}$) and VTA ($k' = 0.10\ s^{-1}$) but significantly less in SN pars reticulata ($k' = 0.02\ s^{-1}$). Inhibition of DA uptake with GBR 12909 significantly decreased k' in SNc and VTA, but to a rate in SNc ($k' = 0.03\ s^{-1}$) that was half that in VTA ($k' = 0.06\ s^{-1}$) ($p < 0.05$). Inhibition of uptake at the norepinephrine transporter with desipramine slightly decreased k' in SNc ($k' = 0.07\ s^{-1}$) and VTA ($k' = 0.09\ s^{-1}$). While overall kinetics were similar, the data reflected regional heterogeneity in uptake characteristics which may have important implications for the role of DA in the physiology and pathophysiology of DA cells. Uptake via the DA transporter is more avid in SNc than VTA; in parallel, populations of SNc are more vulnerable to degeneration than VTA in idiopathic and MPTP-parkinsonism, the latter of which depends on DA uptake for toxin accumulation.

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145.13

DOPAMINERGIC TRANSMISSION IN THE RAT RETINA: EVIDENCE FOR VOLUME TRANSMISSION

Börje Bjelke[1]*, Menek Goldstein[2], Barbro Tinner[1], Cecilia Andersson[1], Susan R. Sesack[3], Harry W.M. Steinbusch[4], Jow Y. Lew[2], Xi He[2], Stan Watson[5], Björn Teneroth[6] and Kjell Fuxe[1]

[1]Department of Neuroscience, Karolinska Institutet, 171 77 Stockholm, Sweden, [3]Dept Behavioural Neuroscience, University of Pittsburgh, Crawford Hall, Pittsburgh, PA 15260, USA, [6]St. Eriks Eye Hospital, 112 82 Stockholm, Sweden, [2]Neurochemistry Research Unit, New York University, Medical Centre, 560 First Avenue, New York, N.Y. 100 16, USA, [4]European Graduate School for Neuroscience in Brain Behaviour, University of Limburg, Dept. Psychiatry and Neuropsychology, Maastricht, The Netherlands, [5]Mental Health Research Institute, The University of Michigan, Ann Arbor, Michigan 48109, USA

The study was designed to determine whether dopaminergic neurotransmission in the retina can operate via volume transmission. Using double immunolabelling, a mismatch as well as a match was demonstrated between tyrosine hydroxylase (TH) and dopamine (DA) immunoreactive (ir) terminals and cell bodies and DA D2 receptor-like ir cell bodies and processes. The match regions were located in the inner nuclear and plexiform layers. The mismatch regions were located in the ganglion cell layer, the outer plexiform layer, and the outer segment of the photoreceptor layer. Analyzing D1 receptor like ir processes versus TH ir nerve terminals, mainly a mismatch could be demonstrated, with the D1 like ir processes present in the outer plexiform layer and the outer segment. The demonstration of a mismatch between the localization of the TH terminal plexus and the DA D2 and D1 receptor subtypes suggests that DA may reach the D2 and D1 mismatch receptors via diffusion in the extracellular space. After injecting DA into the corpus vitreum, DA diffuses through the retina, and strong catecholamine (CA) fluorescence appears in the entire inner plexiform layer and outer plexiform layer where DA probably is bound to D1 and D2 receptors. The DA receptor antagonist chlorpromazine fully blocks the appearance of the CA fluorescence. Thus, the amacrine and/or interplexiform DA cells can operate via volume transmission to influence the outer plexiform layer and the outer segment, as well as other layers such as the ganglion cell layer. Supported by grants from St Eriks Eye Hospital, (04X-715) the Swedish Medical Research Council, USPHS MH50314, Marianne and Marcus Wallenberg foundation, the American Parkinson Disease Association.

145.14

CHARACTERISTICS OF EXTRACELLULAR DOPAMINE FORMED FROM EXOGENOUS L-DOPA IN THE INTACT AND DOPAMINE-DENERVATED STRIATUM OF THE RAT. D.W. Miller* and E.D. Abercrombie. Center for Molecular and Behavioral Neuroscience, Rutgers University, Newark, NJ 07102.

In vivo microdialysis was used to examine the effects of exogenous L-DOPA on extracellular dopamine (DA) in the striatum of intact and DA-depleted rats. Extensive unilateral lesions (>95%) of the nigrostriatal DA neurons were accomplished by injecting 6-OHDA (6µg) into the medial forebrain bundle. A microdialysis probe was implanted into the ipsilateral striatum 2 weeks following the lesion. Previous work from this group suggested that in intact rats, L-DOPA (50mg/kg) induces a small increase in striatal DA which is impulse-independent. However, DA uptake in the intact striatum has been shown to mask the effects of L-DOPA on striatal DA efflux (Wachtel & Abercrombie, Neurosci. Abstract 126.18, 1994). The present studies examined the effect of impulse activity blockade on DOPA-induced striatal DA efflux in two experimental paradigms. The first condition incorporated DA uptake blockade via systemic administration of GBR-12909 (20mg/kg) in intact rats. The second condition examined the effect of impulse blockade in the DA-denervated striatum where DA uptake does not exert a strong influence on DOPA-induced extracellular DA efflux. Impulse activity was blocked via infusion of 1µM tetrodotoxin (TTX) through the microdialysis probe. A small TTX-insensitive component was revealed in both cases. We hypothesize that this component is similar to the small amount of DA released in the intact striatum following L-DOPA administration in absence of DA uptake blockade. In both conditions, there also exists a larger component that is TTX-sensitive, as revealed in the intact rat when DA uptake is blocked. Thus, there are two components of DOPA-induced striatal DA efflux (a small impulse-independent component and a large impulse-dependent component) in the intact striatum when DA uptake is blocked and in the DA-denervated striatum. The source of the regulated (TTX-sensitive) release is yet to be determined. [Supported by USPHS Grant NS 19608]

145.15

EFFECT OF WITHDRAWAL DURATION AFTER CHRONIC AMPHETAMINE ON STRIATAL ACETYLCHOLINE RESPONSE TO ACUTE AMPHETAMINE CHALLENGE Michael J. Bickerdike* & Elizabeth D. Abercrombie. Center for Molecular & Behavioral Neuroscience, Rutgers University, Newark, NJ 07102.

Chronic intermittent amphetamine (AMPH) administration to rats results in behavioral sensitization to a subsequent AMPH challenge. Behavioral sensitization develops during chronic treatment and is apparent to a varying degree after any period of withdrawal. However, potentiated dopamine (DA) release in response to AMPH is consistently reported only after withdrawal of a week or more. The present microdialysis study assessed the affect of short (2 day) and long (2-3 week) withdrawal from chronic AMPH (4mg/kg i.p.; b.i.d; 12 days) on striatal acetylcholine (ACh) release in male rats administered an AMPH challenge (4mg/kg i.p.). Behavioral studies of locomotion showed that sensitization developed throughout chronic AMPH treatment - this effect was greatest after long withdrawal, but clearly apparent at short withdrawal. Systemic AMPH did not significantly affect striatal ACh efflux in naive or chronic saline treated rats, either after short or long "withdrawal". However, AMPH challenge resulted in a significant and prolonged increase in striatal extracellular ACh after both short and long withdrawal from chronic AMPH. After short withdrawal, striatal ACh increased from 69.5 fmol/20µl (basal) to a maximal level of 97.5 fmol/20µl after 135 min. After long withdrawal, striatal ACh increased from 65.6 fmol/20µl to a maximal level of 118.8 fmol/20µl after 120 min. Preliminary results indicate that DA release in response to AMPH is not significantly affected by long withdrawal from chronic AMPH (87.3 pg/20 µl increase post AMPH, versus an 87.8 pg/20 µl increase in chronic saline controls), while after short withdrawal, striatal DA efflux in response to AMPH was attenuated (61.6 pg/20 µl increase post AMPH, versus 103.8 pg/20 µl for controls). Taken together these data suggest that the behavioral sensitization observed in these studies, particularly at the short withdrawal time point, may relate to increased ACh release in striatum. Further studies are underway to investigate the hypothesis that an increase in glutamate drive to striatum is important in mediating these alterations in striatal ACh function. (Supported by NIDA 08086)

145.16

ONTOGENETIC EFFECTS OF NOMIFENSINE ON DOPAMINERGIC MODULATION OF STRIATAL ACETYLCHOLINE RELEASE IN MALE RATS. C.A. Bolanos*, S.L. Abel and D. Jackson. Psychology Department, Northeastern University, Boston, MA 02115.

Previous research has shown that periadolescent rats approximately 30- to 40-days old are generally less sensitive to pharmacological challenges when compared with younger or older animals. For example, studies have illustrated that periadolescent rats exhibit attenuated responses to the behavioral effects of dopaminergic drugs such as cocaine, amphetamine, or apomorphine. The neurobiological basis for these periadolescent effects are still unresolved; but several possibilities have been proposed, including the development of dopamine (DA) autoreceptors. In the present study, the lack of sensitivity shown in the periadolescent rats was further assessed by measuring DA inhibition of acetylcholine (ACh) release in striatal slices. It was hypothesized that superfusion with 10μM nomifensine, a DA reuptake blocker, would not alter the evoked ACh efflux from periadolescent rats (PD35), while it would inhibit the electrically-evoked ACh release in striatal slices prepared from young (PD20) and adult (PD80) animals. ACh release studies were conducted with slices (350μm) that had previously been labeled with ^{3}[H]choline. Basal and electrically-evoked tritium efflux were used as measures of ACh release. Surprisingly, superfusion with nomifensine resulted in an inhibition (-61 ± 18%) of electrically-evoked ACh release in striatal slices prepared from the PD35 rats. The magnitude of this effect was not significantly different from that observed in the PD20 (63 ± 15%) or the PD80 (-56 ± 19%) rats. In summary, periadolescent rats show the same magnitude of DA reuptake when compared with PD20 and PD80 animals. Future studies will examine the effects of lower doses of nomifensine (0.1 and1μM) as well as the effects of other DA reuptake blockers (cocaine or amphetamine) and DA receptor agonists in order to assess saturation of the DA transporter and to examine the potential differences in autoreceptor sensitivity in the three age groups, respectively.

145.17

DOPAMINE MODULATES BOTH EXCITATORY AND INHIBITORY SYNAPTIC TRANSMISSION IN THE SUPERFICIAL LAYERS OF RAT PREFRONTAL CORTEX. G.M. DiBlasi*, G. Gonzalez-Burgos and G. Barrionuevo *Department of Neuroscience and Center for the Neural Basis of Cognition, University of Pittsburgh, Pittsburgh, PA.*

In the rat, the prelimbic/infralimbic cortices of the frontal lobes receive dorsomedial thalamic nucleus projections as well as a dense subcortical dopamine (DA) innervation; therefore, this region of rat cortex is a good model of the primate medial prefrontal cortex (mPFC). The objective of these studies was to characterize the effects of exogenous dopamine on synaptic transmission within the local mPFC circuitry at the cellular and synaptic levels. To further simplify the cortical circuitry, we used the rat mPFC cortical slice preparation for the *in vitro* study of dopaminergic neuromodulation. Synaptic responses were evoked by bulk stimulation of the underlying white matter alternating with stimulation of the parallel local gray matter. Complex postsynaptic potentials (PSPs) were recorded using sharp-electrode intracellular techniques. The waveforms were evoked as the postsynaptic membrane potential (Vm) was altered by constant injections of current. PSP-Vm plots reveal multiple components to the synaptic responses which can be separated based on their reversal potentials. Responses were collected before and during the application of 50 µM dopamine in the bath.

The hyperpolarizing components of the responses evoked at depolarized membrane potentials increased with dopamine treatment (n=4) as was predicted based on the *in vivo* literature. However, the apparent depolarizing components of the responses showed a either increases or decreases (n=2 each). Pharmacological manipulations are currently being used to investigate the affects of dopamine on the individual components of the synaptic waveform. CNQX (1-10 µM) will be used to eliminate the bulk of glutamatergic transmission, and bicucculine (1-5 µM) will be used to eliminate the bulk of fast inhibitory transmission.

This work was supported by MH45156 and an Andrew Mellon Foundation predoctoral fellowship (GMD).

145.18

DOPAMINE MODULATION OF HIPPOCAMPAL INPUT TO THE RAT PREFRONTAL CORTEX. D.B. Carr*, S.R. Sesack, and A.A. Grace. Departments of Neuroscience and Psychiatry, University of Pittsburgh, Pittsburgh, PA 15260.

Afferents to the prefrontal cortex (PFC) from the hippocampal formation and from dopamine neurons in the ventral tegmental area (VTA) have been implicated in working memory processes and in the pathogenesis of schizophrenia. Previous anatomical investigations in our laboratory have demonstrated that both hippocampal and dopamine terminals synapse on dendritic spines of presumed pyramidal cells in the PFC. In addition, these afferents are often in direct apposition to one another. In order to determine whether dopamine afferents modulate hippocampal input to individual pyramidal neurons, we have examined the responses of PFC neurons, recorded extracellularly in anaesthetized rats, to fornix stimulation before and after stimulation of ascending dopamine afferents from the VTA. Our preliminary results indicate that single-pulse stimulation of the fornix produces both short and long latency excitatory responses in PFC neurons. Prior stimulation of the VTA (10 Hz train, 300 ms duration) attenuates the excitatory response of some PFC neurons to hippocampal stimulation. Pharmacological studies are in progress to examine the dopamine receptor subtype involved in this modulatory response This work is supported by USPHS grants MH45156 and MH50314.

145.19

DOPAMINE DEPRESSES EXCITATORY SYNAPTIC TRANSMISSION ONTO LAYER V PYRAMIDAL CELLS IN RAT PREFRONTAL CORTEX, A.T. Gulledge* and D.B. Jaffe, Div. of Life Sci., Univ. of Texas at San Antonio, San Antonio, TX 78249.

The prefrontal cortex is an area of associational cortex important for working memory function. The PFC of both primates and rodents is highly innervated by dopaminergic fibers and has elevated dopamine receptor expression when compared to other cortical areas.

We have been examining the effects of dopamine on excitatory synaptic transmission onto prefrontal layer V pyramidal cells. Excitatory postsynaptic potentials (EPSPs) were evoked in disinhibited slices containing medial prefrontal cortex obtained from the brains of 14 to 28 day old rats. The stimulating electrode was placed in layer V, within 75 μM of the soma of the pyramidal cell to be recorded.

Bath application of dopamine (30 or 60 μM) decreased the initial slope of EPSPs 21% ± 3.5 (mean ± SEM, n=10). Reduction in EPSP slope was reversible in 4 of 5 cells in which washout was attempted.

Excitatory postsynaptic currents recorded under voltage-clamp displayed paired-pulse depression (PPD). Application of 100 μM dopamine decreased the magnitude of PPD 20.43% ± 9.98 (n=8). These data suggest that dopamine may be acting presynaptically to reduce excitatory synaptic transmission.

This work was supported by a Faculty Research Award from UTSA.

146.1

IDENTIFIED 5-HT-LABELLED FIBERS AND THE LATERAL GIANT INTERNEURON OF CRAYFISH. B.E. Musolf and D.H. Edwards* Dept. of Biology, Georgia State University, Atlanta, GA 30302-4010

The Lateral Giant (LG) interneurons form a tightly coupled ladder-like network that provides a command system for the tailflip escape response in crayfish. In each abdominal hemiganglion, the dendrites of an LG neuron receive inputs from mechanosensory afferents and interneurons. The LG axon projects to the next rostral ganglion where it excites both the LG in that hemiganglion and the fast flexor (FF) motorneurons. The LG is excited through a strong axo-axonic electrical connection, and the FFs are excited both directly and indirectly, through an intervening interneuron, the segmental giant (SG).

We found earlier that LG's response is modulated by superfused serotonin according to the social dominance status of the animal (Yeh, *et al.*, 1996, Science **271**: 366). Recently we identified a pair of 5-HT-immunoreactive fibers that project caudally from the thorax to the terminal abdominal ganglion. Each 5-HT fiber has a set of short lateral branches in each ganglion that end in terminal varicosities along the ventral surface of the adjacent LG axon, where the axon excites SG and the FF motorneurons. The ganglionic LG neuron receives few contacts at its intial axon segment and none in the dendrites. We also found that superfused serotonin enhances the FF response to an LG spike in dominant crayfish. The 5-HT fibers are likely candidates for modulating FF responses to LG spikes, and also for modulating LG's responses to mechanosenosory inputs. Supported by grants from NIH and NSF to DHE.

146.2

CHARACTERISATION OF THE SEROTONERGIC NEURONES IN THE FIRST ABDOMINAL GANGLION OF THE CRAYFISH. **Linda J. Anderson*, Michael Hörner & Donald H. Edwards. Dept. of Biology, Georgia State University, Atlanta, GA 30302.**

Previous work in our laboratory has shown that the crayfish escape tailflip is modulated by serotonin, and the modulation is affected by the social status of the animal (Yeh *et al*, Science **271**, 366-369, 1996). Immunohistochemical experiments have identified a pair of serotonin-immunoreactive (5HT-IR) neurones in the first abdominal ganglion (a1). In lobster, homologous neurones receive input from the escape circuit (Hörner et al, this meeting). The crayfish neurones may contribute to the serotonin responsible for the modulation of the tailflip. Therefore, this study was undertaken to examine the synaptic connections between these neurones and elements of the tailflip escape circuitry. Electrical stimulation of the terminal abdominal ganglion afferents evoked either an EPSP or an IPSP in the 5HT-IR neurones; the latencies of these responses differed, suggesting two discrete pathways. Similarly, stimulation of the first root of a1, which contains the axon of the segmental giant, an interneurone which receives input from the command neurone responsible for the tailflip (lateral giant, or LG), also produced either an EPSP or an IPSP. However, in the majority of preparations (70 %), stimulation of LG did not evoke a response in the 5HT-IR neurones. In the remaining preparations, a small EPSP was evoked. Experiments are continuing to discover further connections in this system.

This work was supported by the National Science Foundation.

146.3

A LIBRARY SCANNING CLONING PROCEDURE FOR THE ISOLATION OF BIOGENIC AMINE RECEPTOR GENES FROM THE HONEY BEE. P. R. Ebert*, J. Rowland, B. Meadows. Depts. of Biochemistry and Entomology, University of Queensland, St. Lucia, QLD 4072 Australia.

Degenerate, biogenic amine specific primers were used to isolate receptor genes from the honey bee. The utility of PCR-based gene cloning was greatly enhanced by performing PCR reactions on 200 individual subpools of both genomic and cDNA libraries containing approximately 100 clones each. By this method, a total of seven biogenic receptor genes have so far been isolated. Standard PCR reactions using total head cDNA as template only produced two receptor clones while genomic DNA template failed to produce any amplification product. DNA homology suggests that the cloned genes represent serotonin, dopamine, tyramine/octopamine and noradrenergic receptors. Gene regulation with respect to social and caste-specific behaviour is currently being investigated.

146.4

POTENTIATION OF B38-EVOKED CONTRACTIONS BY 5HT AND THE SCPS HAS A SIGNIFICANT POSTSYNAPTIC COMPONENT. L. E. Fox* and P. E. Lloyd Committee on Neurobiology, University of Chicago, Chicago, IL. 60637

Aplysia anterior buccal muscle 3 (I3a) is innervated by 2 excitatory glutamatergic motor neurons (B3 and B38). B38 also expresses the SCPs while B3 expresses FMRFamide. I3a is also innervated by a modulatory serotonergic neuron C1 (or the MCC). Intracellular stimulation of B38 evokes contractions of I3a that are potentiated by both 5HT and the SCPs. While the short-term effects of 5HT and the SCPs are similar, there is a dramatic difference in the duration of their modulatory effects. The effects of the SCPs reverse on wash out while the effects of 5HT persist for several hours after wash out. The short-term potentiation of B38-evoked contractions by 5HT and the SCPs as well as the persistent effects of 5HT appear to be due, in part, to postsynaptic actions of the modulators. 5HT and the SCPs potentiated contractions of isolated I3a muscle evoked by the bolus application of glutamate. SCPs (1 μM) increased the excitability of the muscle and increased the bolus-evoked contractions ~4 fold. 5HT (1 μM) increased the excitability of I3a dramatically, usually causing the muscle to contract rhythmically. Lower concentrations of 5HT (0.1 μM) also increased the excitability of the muscle and increased the bolus-evoked contractions ~5 fold. Again, the effects of the SCPs reversed on wash out while the effects of 5HT persisted several hours after wash out.

B38-evoked excitatory junction potentials (EJPs) were also persistently potentiated by 5HT. Methiothepin (30 μM) a 5HT antagonist known to act on *Aplysia* 5HT receptors coupled to phospholipase C, inhibits the persistent potentiation of EJPs, but not short-term potentiation produced by 5HT. Supported by F31 MH10656 and NSF IBN 9418815.

146.5

LIGAND-SPECIFIC COUPLING OF A NOVEL CLONED *DROSOPHILA* DOPAMINE RECEPTOR WHEN EXPRESSED IN *XENOPUS* OOCYTES. P.D.Evans[1]*, V.Reale[1], F.Hannan[1], G.Feng[2] and L.M.Hall[2]. [1]The Babraham Institute Lab. Molecular Signalling, Dept. Zoology, Univ. Cambridge, Cambridge, CB2 3EJ, U.K. and [2]Dept. Biochem. Pharmacol., State Univ. of New York, Buffalo, USA.

In insects dopamine receptor subtypes may be involved in memory and learning. Dopamine receptors belong to the G-protein coupled receptor superfamily of seven transmembrane domain proteins. Five distinct classes of dopamine receptor have been identified by cloning in mammals. These fall into two broad pharmacological categories D1-like and D2-like. A D1-like receptor affecting adenylyl cyclase activity has already been identified in *Drosophila* (Gotzes et al., 1994, Receptors and Channels 2:131-141; Sugamori et al., 1995, FEBS Lett. 362:131-138). We have recently cloned a novel G-protein coupled dopamine receptor (DopR99B), expressed in *Drosophila* heads, which may define a novel class of dopamine receptors (Feng *et al.*, 1996, J.Neurosci., in press). We report here that when expressed in *Xenopus* oocytes the activated DopR99B increases intracellular Ca^{2+} levels and also increases cyclic AMP levels. The rank order of potency for biogenic amines in stimulating both second messenger effects is dopamine > norepinephrine > epinephrine > tyramine. Octopamine and 5-hydroxytryptamine are not active at concentrations up to 100μM. The pharmacological profile for synthetic antagonists on both second messenger responses was identical and suggested that DopR99B is a D1-like dopamine receptor. The pharmacological profile for synthetic agonists again suggests a D1-like dopamine receptor, but was different for the two second messenger systems assayed. Thus, DopR99B exhibits the "ligand-specific" form of agonist-specific coupling to different second messenger pathways, a phenomenon exhibited by a range of other aminergic and peptidergic G-protein coupled receptors from both invertebrates and vertebrates (see Evans *et al.*, 1995, Prog. Brain Res. 106:259-268).

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146.6

DEPLETION OF ENDOGENOUS DOPAMINE ALTERS THE DENSITY OF D1-LIKE DOPAMINE RECEPTORS IN THE BRAIN OF THE HONEY BEE, *APIS MELLIFERA*. M.T. Purnell, I.C. Kokay, D.J. Taylor and A.R. Mercer* Department of Zoology and Centre for Neuroscience, University of Otago, Dunedin, NZ.

Two distinct dopamine (DA) receptor subtypes have been identified in the brain of the honey bee (I. Kokay and A. Mercer (1996) *Brain Research* **706:** 47-56). Both are present in the brain early in metamorphic adult development and exhibit significant changes in density during the lifetime of the bee (I. Kokay and A. Mercer (1995) *Soc. Neurosci. Abstr.* **21:** 632). The effects of depleting brain DA levels on the density of one of these two DA receptor subtypes, namely the D1-like DA receptor in the brain of the bee, have now been examined. We have found that brain DA levels can be reduced significantly by a single 10μl (250μg) injection of α-methyl-p-tyrosine (AMT) into the haemolymph of the bee. Maximum depletion of DA was observed 3 h after treatment. DA levels in the brain of the bee returned to normal 24 h after AMT injection. Injection of saline alone into the haemolymph of the bee did not alter the levels of DA in the brain. *In vitro* assays using the D1-specific DA receptor radioligand ^{3}H-SCH 23390 were used to monitor the effects of amine depletion on D1-DA receptor densities. Non-specific binding was defined as binding in the presence of 5 x 10^{-6} M cis-(Z)-flupentixol. No significant change in DA receptor density was apparent 30 min after treatment with AMT. However, 3 h after injecting this drug, depletion of DA caused a significant reduction in the density of D1-like DA receptors in the brain of the bee. [Supported by ORG MFZB77]

146.7

AGE DEPENDANT DOPAMINE DISTRIBUTION IN THE CNS GANGLIA OF APLYSIA CALIFORNICA. D. Daniels, J. M. Flinn, & V. Chandhoke*. Department of Psychology & The Shared Research Instrumentation Facility, George Mason University, Fairfax, Virginia.

Previously, we have demonstrated age dependant changes in the dopamine levels of the ring and abdominal ganglia of *Aplysia californica*. The relationship between age and dopamine levels is complex with a maximum at five months and a secondary peak at ten months. This study seeks to investigate the distribution of the dopamine in the individual ganglia of *A. californica* as a function of age.

Twenty animals in each of five age groups (five, six, seven, eight, and ten months post-hatch) were obtained from The *Aplysia* Facility at the University of Miami. The animals were anaesthetized, dissected and the CNS removed. The following ganglia were isolated for examination: cerebral, buccal, abdominal, left pleural-pedal, and right pleural-pedal. Individual ganglia were then weighed and suspended in TBS. The protein content of each sample was measured by a BioRad protein assay. Dopamine levels were measured by reverse phase HPLC and normalized by the protein content. Preliminary data show that the pattern of dopamine distribution in the six month animals was different from the distribution in the ten month animals. At six months, the left pleura-pedal ganglia had the highest levels, followed by the abdominal, cerebral, right pleural-pedal, and buccal ganglia respectively. The dopamine levels of the left pleural-pedal ganglia were much greater than that of the right pleural-pedal. At ten months, the left pleural-pedal still had the highest values, followed by the right pleural-pedal, buccal, cerebral, and abdominal ganglia respectively. The left pleural-pedal and the abdominal ganglia, which had the highest dopamine levels at six months, showed the greatest decrease between six and ten months.

146.8

OCTOPAMINE IMMUNOREACTIVITY IN THE STOMATOGASTRIC GANGLION OF *HOMARUS AMERICANUS, PANULIRUS INTERRUPTUS,* AND *CANCER BOREALIS,* V.L. Kilman* and E. Marder. Volen Center for Complex Systems, Brandeis University, Waltham, MA 02254.

Octopamine powerfully modulates a wide range of behaviors in decapod crustacea. One example of this is the modulation of the motor pattern generating networks responsible for feeding in the stomatogastric ganglion (STG) of *P. interruptus*, *H. americanus*, and *C. borealis*. While central octopamine-containing neurons have been described in the ventral nerve cord and brain of *H. americanus*, and biochemical studies have shown octopamine in the STG of *P. interruptus*, the source of octopamine input to the STG has not been determined. In this study we immunocytochemically localized octopamine in sections of paraffin- and plastic resin-embedded STG of crabs (*C. borealis*) and lobsters (*H. americanus, P. interruptus*). While the neuropil of *C. borealis* and *P. interruptus* STG contain octopamine immunoreactivity, the STG of *H. americanus* does not. As octopamine modulates rhythmic activity in the STG of *H. americanus*, the lack of octopamine immunoreactivity in *H. americanus* neuropil suggests that octopamine reaches STG targets in this animal *via* neurohemal release. In contrast, octopamine immunoreactivity in *P. interruptus* and *C. borealis* suggests that the source of some octopamine input is local release from the fibers stained in the ganglion. Experiments are in progress to determine what other modulators may be colocalized with octopamine.

Supported by NS17813

146.9

CLONING AND SEQUENCING OF TYRAMINE BETA-HYDROXYLASE IN *MANDUCA SEXTA*. K. Mace and H. K. Lehman*. Dept. of Biology, Univ. of Nevada, Las Vegas, NV 89154.

Tyramine beta-hydroxylase (TβH) is the putative rate-limiting enzyme controlling the biosynthesis of octopamine in the insect nervous system. TβH appears to be developmentally regulated in the terminal abdominal ganglia of *Manduca*, and the increase in TβH activity during metamorphosis is coincident with the appearance of a subset of octopaminergic neurons. Furthermore, the steroid hormone 20-hydroxyecdysone is necessary and sufficient for the developmental increase in TβH activity. To better understand the role of steroid hormones in the regulation of TβH, we have attempted to clone and characterize TβH from the nervous system of *Manduca sexta.*

We have performed RT-PCR using degenerate primers based on conserved regions of mammalian dopamine beta-hydroxylase to amplify and clone TβH from terminal abdominal ganglia poly (A)+ RNA taken from late pupa. Following PCR amplification and agarose gel analysis, several products of the approximate expected size were cloned using a TA cloning kit (Invitrogen). Sequence analysis (Sequenase, Amersham) of several products revealed a single clone 578 bp in length with high homology (50% identity) to mammalian dopamine beta-hydroxylase at the amino acid level. Northern blot analysis is currently underway to verify that this sequence is expressed in the nervous system and in-situ hybridization will be used to determine the tissue distribution of this sequence within the nervous system. Supported by the NSF (IBN-9496168).

146.10

OCTOPAMINERGIC MODULATION OF SYNAPTIC TRANSMISSION AT THE *DROSOPHILA* LARVAL NEUROMUSCULAR JUNCTION. M.J. Coleman* and L.C. Griffith. Brandeis University, Dept. of Biology & Volen Center, Waltham, MA 02254.

Glutamate is the major excitatory transmitter at the larval neuromuscular junction of the fruit fly, *Drosophila melanogaster*. Several neuromodulators, including octopamine, have been immunocytochemically localized to subsets of the nerve terminals innervating these muscle fibers. However, the physiological function of these modulators is not well-understood.

Octopaminergic modulation of evoked excitatory junctional potentials (EJPs) in larval body wall muscle fibers was examined by bath-application of octopamine. In muscles that are innervated by octopaminergic motor neurons (muscle 12), octopamine increased the EJP amplitude by 14±0.1% relative to control ($p<0.01$), but did not significantly alter the decay rate of the EJP (N=3). In addition, octopamine appeared to reversably decrease the input resistance of individual muscle fibers by 32±0.1% ($p<0.05$). Interestingly, we found that octopamine also modulated EJPs in muscles that are not innervated by octopaminergic motor neurons (muscle 6). In these muscles, although octopamine did not produce an increase in EJP amplitude, it increased the EJP decay rate by 20±0.1% ($p<0.01$, N=4). These data suggest that at the *Drosophila* larval neuromuscular junction, octopamine may act both as a modulatory transmitter and as a neurohormone.

To begin examining the second messenger pathways involved in octopaminergic modulation, we are studying the effects of octopamine in a mutant with decreased CaM kinase activity. We found that the amplitude of EJPs in muscle 6 of this mutant was 19±0.1% larger than in control ($p<0.01$), but the decay rate was not significantly different (N=4). These data suggest that octopaminergic modulation of decay rate may involve CaM kinase activity.

LCG is an Alfred P. Sloan Fellow. Work supported by NSF grant IBN9421360 (LCG) and NIH grant NS07293 to Brandeis (MJC).

146.11

DEVELOPMENT OF THE DOPAMINERGIC SYSTEM IN THE ANTENNAL LOBES OF THE BRAIN OF THE HONEY BEE

B.S. Kirchhof[1]* U. Homberg[2] and A.R. Mercer[1]. [1]Department of Zoology and Centre for Neuroscience, University of Otago, Dunedin, New Zealand [2]Institut für Zoologie, Universität Regensburg, 93040 Regensburg, Germany

Dramatic growth and reorganization of the primary antenno-sensory centres (antennal lobes, AL) of the brain is associated with the metamorphic adult development of the honey bee, *Apis mellifera*. In the adult worker bee, the AL neuropil is innervated by a small population of dopamine-immunoreactive (DA-IR) neurons (S Schäfer and V Rehder (1989) *J Comp Neurol* **280**:43-58). We have used immunohistochemistry and HPLC, respectively, to examine the development of DA-IR neurons in the AL and changes in endogenous DA levels associated with the development of the AL neuropil. The DA antibody used in this study was generously supplied by Dr. R.M. Buijs, Netherlands Institute for Brain Research, Amsterdam. DA-IR somata in the brain could first be detected at pupal stage 1 (P1) of the 9 stages of metamorphic adult development, but no immunoreactive processes were observed in the AL neuropil at this stage. DA-IR processes first became prominent in the developing neuropil of the AL at pupal stage 3, coinciding with the onset of protoglomerular formation in the AL. During subsequent stages of metamorphosis, the density of DA-IR processes increases. Although most of these processes remain restricted to the inner margins of the glomeruli, some extend also into the glial borders that surround each glomerulus. Changes in endogenous DA levels correlate well with the appearance of DA-IR in the AL. DA was first detected in the developing AL at pupal stage 3 (P3), and peaked at pupal stage 4 (P4). Previous studies have shown that the percentage of pupal AL neurons *in vitro* that express D2-like DA receptors peaks also at P4 (B Kirchhof and A Mercer (1995) *Soc Neurosci Abstr* **21**:406). The early appearance of DA and DA receptors in the AL of the bee brain is consistent with the possibility that DA plays a role in regulating the development of the highly structured AL neuropil. [Supported by ORG MFZB77]

147.1

cAMP-GATED Na^+ CURRENT IN MOLLUSC CNS: MODULATION OF MOTOR NETWORKS, COINCIDENCE DETECTION, AND IN VIVO ENZYMOLOGY. R. Gillette, L. Sudlow, L.L. Moroz, J.Jing, and M. Gillette*. Dept. of Molecular and Integrative Physiology, Univ. of Illinois, Urbana IL 61801

Sodium currents gated directly by cAMP ($I_{Na,cAMP}$) are the most prominent ion currents stimulated by cAMP in many molluscan neurons. $I_{Na,cAMP}$ is found in effector neurons and interneurons of the motor networks for feeding, gill movement, locomotion and escape swimming, where it may mediate excitatory neuromodulatory effects lasting seconds or more. $I_{Na,cAMP}$ shows different characteristics in different neurons. In neurons of the feeding network of *Pleurobranchaea*, $I_{Na,cAMP}$ is increased with depolarization from the resting membrane potential in the pacemaker region via lifting of a depolarization-sensitive Ca^{2+} block, is markedly enhanced by applications of nitric oxide (NO), and is sensitive to changes in pH_i, pentylenetetrazol and oxidized glutathione. In contrast, $I_{Na,cAMP}$ in locomotor neurons is reduced by depolarization via a mechanism dependent on intracellular Ca^{2+}, and is not sensitive to NO, pH_i, pentylenetetrazol, nor thiol oxidant. The sensitivity of the feeding neurons' $I_{Na,cAMP}$ to NO confers coincidence detection ability and specifically suggests a role in arousal of feeding behavior. Kinetic characters of $I_{Na,cAMP}$ lend it to quantitative analysis of cAMP metabolism in living neurons, such that the effects of 5-HT and NO on cAMP synthesis, degradation and concentrations can be precisely measured with conventional voltage clamp methods. Supported by NIH (RO1 NS26838) to RG.

147.2

THE NO/cGMP PATHWAY MEDIATES MUSCARINIC RESPONSES IN THE CNS OF *Manduca sexta*. B. A. Trimmer* and S. Qazi Dept. of Biology, Tufts University, Medford, MA 02155.

Biochemical and physiological studies were performed to describe the role of nitric oxide (NO) in the muscarinic stimulation of cGMP levels in whole abdominal nerve cords of *Manduca sexta* larvae. Incubations were all performed in the presence of 3-isobutyl-1-methylxanthine (IBMX), to inhibit phosphodiesterase breakdown, this resulted in a 3 fold increase in basal accumulation of cGMP (136 ± 23, fmoles/nerve cord (fm/nc), n=14) measured by radioimmunoassay. Addition of a muscarinic agonist, oxotremorine-M (oxo-M) further increased cGMP levels (214 ± 42 fm/nc, n=11; one way anova, contrast, $F_{(1,113)} = 5.84$, $p = 0.017$). Treatment of nerve cords with the nitric oxide synthase inhibitor, nitroarginine (NA, 1mM) reduced the basal levels of cGMP to 37 ± 1.6 fm/nc (n=6, contrast, $p<0.0001$). Furthermore, NA blocked the oxo-M evoked increase in cGMP (NA+oxo-M = 43 ± 3.0 fm/nc, $p<0.0001$). The suppression of cGMP levels was not caused by inhibition of GC, because direct activation of GC with a NO donor compound, sodium nitroprusside in the presence of NA resulted in high levels of cGMP (945 ± 87 (n=6) fm/nc). Therefore the muscarinic activation of guanylate cyclase (GC) appears to require NO. To determine the physiological role of the NO/cGMP pathway during muscarinic activation of abdominal motoneurons, extracellular recordings were made of activity in the lateral branch of the ventral nerve (VNL). Instantaneous spike frequencies were averaged over a 40 sec recording period. Perfusion with 1μM oxo-M resulted in a significant increase in VNL activity (34 ± 5.7 to 134 ± 15.2 spikes/sec; n=10; one way anova, $F_{(1,36)}=21$, $p<0.0001$). When ganglia were pretreated with NA, VNL activity was suppressed (NA + oxo-M = 39 ± 17 spikes/sec, n=6). This suggests that muscarinic activation of NO/cGMP pathway modulates motoneuron activity.
Supported by a Whitehall Foundation grant and NIH Grant NS 30566 to BAT.

147.3

NITRIC OXIDE AND ORTHOGRADE NEUROTRANSMISSION IN THE CNS OF THE SNAIL *LYMNAEA STAGNALIS*. Michael O'Shea* and Ji-Ho Park, Sussex Centre for Neuroscience, School of Biological Sciences, University of Sussex, Brighton, East Sussex, BN1 9QG, UK

Nitric oxide (NO) is synthesized in the snail by a calcium/calmodulin activated nitric oxide synthase (NOS) that has been localised to identifiable neurons in the buccal ganglion. One of these is the giant B2 motor neuron. This cell is peptidergic and cholinergic (Paul Benjamin, pers. comm) but also contains NOS. Another identified motor neuron, B7, is excited by the B2 neuron. Stimulation of a train of action potential in B2 produces a constant latency (~ 200ms) slow depolarisation in B7 but not in other neurons. The B2 induced EPSP in B7 cannot be blocked by hexamethonium but it is reversibly blocked by the NO scavenger haemoglobin at 500μM. Met-haemoglobin, which cannot scavenge NO, has no effect. Also the EPSP is eliminated by the NOS inhibitor L-NAME (1 mM) but is unaffected by D-NAME. Solutions of NO and NO donors were pressure ejected from a micropipette onto neurons in the buccal ganglion. This causes depolarisation of B7 leading to action potentials but the B2 neuron does not respond to local pressure ejection of NO donors or NO solutions. The B7 cell is the only neuron in the vicinity of B2 to respond to the NO ejection. When the solutions of NO donors are de-gassed using a 24hr delay between preparation and the use of the solution and nitrogen airation, there is no depolarisation. Together this evidence indicates that there is a central synaptic interaction between the B2 and the B7 interneuron and the transmitter is NO. In previous experiments we demonstrated that NO can activate the central pattern generator for feeding (Elphick et al, J Neurosci, 15(11): 7653-7664). It may also be involved in synaptic interactions between motoneurons that are driven by the feeding CPG. Supported by BBSRC Grant GR/J33234 to MO'S.

147.4

NITRIC OXIDE ENHANCES cAMP-GATED NA^+ CURRENT IN NEURONS OF THE FEEDING NETWORK OF THE PREDATORY SEA-SLUG *PLEUROBRANCHAEA CALIFORNICA*. L. Sudlow*, L.L. Moroz and R. Gillette Dept. of Molecular and Integrative Physiology, Univ. of Ill, Urbana IL 61801

cAMP-gated Na^+ current ($I_{Na,cAMP}$) is a prominent mediator of neuromodulatory arousal in motor networks of feeding, locomotion and escape swimming. These same motor networks exhibit strong histochemical evidence of nitric oxide synthase (NOS) activity (Moroz and Gillette, 1996, J.Comp.Neurol. 367:607). Earlier, it was shown that nitric oxide (NO) donors stimulate buccal motor output both in intact animals and isolated CNS. We sought to examine possible interactions between $I_{Na,cAMP}$, cAMP and NO. Here we find that NO gas and NO donors (spermine/NO, DEA/NO, SNAP, S-Nitrosocysteine, final concentrations of 0.1 - 1 mM) markedly potentiate the $I_{Na,cAMP}$ response to injected cAMP in neurons of the buccal ganglion. DEA/NO caused a 4-fold increase in the maximum $I_{Na,cAMP}$ and a 30% decrease in the K_m of the current for cAMP. Application of 8-Br-cGMP did not mimic the effects of NO donors on $I_{Na,cAMP}$. V_{max} and K_m of phosphodiesterase (PDE) in the buccal neurons were also altered as a result of application of NO donors. PDE V_{max} decreased by 50% after application of DEA/NO while the K_m decreased by 40%. In contrast, NO donors did not enhance $I_{Na,cAMP}$ in the pedal ganglion G-cluster neurons. It was previously shown that $I_{Na,cAMP}$ also differs between the feeding and locomotor neurons in voltage dependence and sensitivity to Ca^{2+}, H^+, and thiol reagents. $I_{Na,cAMP}$ appears to serve as a coincidence detector for cAMP and NO via a cGMP-independent mechanism. Supported by NIH RO1 NS26838 to RG.

147.5

PRODUCTION OF NITRIC OXIDE (NO) IN INVERTEBRATE CENTRAL NERVOUS AND PERIPHERAL TISSUES: AN ELECTRON PARAMAGNETIC RESONANCE STUDY.
L. L. Moroz*, S. W. Norby, R. B. Clarkson and R. Gillette. Dept. Molecular and Integrative Physiology, EPR Center, Univ. Illinois, Urbana, IL, 61801.

We measured NO formation in CNS and peripheral tissues by combining electron paramagnetic resonance and spin trapping techniques for eight molluscan genera chosen for diverse ecological adaptations and systematic relationships (Gastropoda: *Pleurobranchaea*, *Aplysia*, *Tritonia*, *Phestilla*, *Lymnaea*, *Planorbarius*; Cephalopoda: *Octopus*, *Loligo*), for an annelid (*Lumbricus*), two arthropods (*Homarus*, *Limulus*) and two poriferans (*Terpios*, *Lencetla*). A spin trap complex of sodium N-methyl-D-glucamine dithiocarbamate with $FeSO_4$ was employed for intact tissues and homogenates. We have found: 1) NO is present and its production is L-arginine-dependent in CNS and some peripheral tissues of all genera investigated; 2) There is a correlation between NO synthesis rate and NADPH-diaphorase activity for all tissues investigated, with the exception of the *Tritonia* CNS; 3) Estimated endogenous NO concentrations in CNS and some peripheral tissues were 0.4-10 μM; 4) The NO synthase inhibitors L-NAME, L-NA, and L-NMA did not suppress NO signals, and in some cases enhanced them; 5) The seemingly paradoxical effects of the NO synthase inhibitors are explainable by observations that the inhibitors themselves act as substrates for non-enzymatic NO production in the presence of the reducing compounds NADPH, DTT, L-cysteine and L-ascorbate. Supported by NIH RO1GM42208 grant to R.B.C. and NIH RO1NS26838 grant to R.G.

147.6

NITRIC OXIDE SYNTHESIS IN CENTRAL AND PERIPHERAL TISSUES OF THE TROPICAL SEA SLUG, *Phestilla sibogae* (Nudibranchia, Aeolidiida). D.Yu. Budko[1], L. L. Moroz[2], M. Hadfield[1] and R. Gillette[2]*. [1]Kewalo Marine Lab., Univ. Hawaii, Honolulu, HI, 96813; [2]Dept. Molecular and Integrative Physiology, Univ. Illinois, Urbana, IL, 61801.

Nitric oxide (NO) production and NO synthase (NOS) localization was studied in the CNS and peripheral tissues of the nudibranch *P. sibogae*. Electron paramagnetic resonance (EPR) of specific NO spin traps was used to measure NO, while NOS was localized using NADPH-diaphorase (NADPH-d) histochemistry in the buccal mass, esophagus, rhinophores, tentacles and gills. NADPH-d activity was strongly resistant both to glutaraldehyde and paraphormaldehyde fixation, but distribution patterns of NADPH-d activity were dissimilar in the different fixatives, suggesting the presence of at least two distinct isoforms of NOS. Only a few cells in the CNS were labelled; the majority were localized in the peripheral tissues. Hundreds of intensely stained multipolar neurons were observed in the buccal mass and esophagus. *P. sibogae* appears to be alone among more than 30 molluscan species examined (Moroz and Gillette, 1995) in possessing a plexus of NADPH-d reactive neurons in the buccal mass. We suggest that these cells are modulator and/or motor neurons. EPR detected a high level of NO production in both buccal mass and esophagus. NO is a potent neuromodulator of the feeding program in molluscs; thus, NO production might underly the virtually chronic feeding activity characteristic of *P. sibogae*. Moderately labelled NADPH-d reactive neurons were observed in peripheral sensory structures (rhinophores, tentacles, mouth area) as well as in the rhinophoral ganglia, where NO signals were also detected by EPR, suggesting an involvement of NO in chemosensory processing. Supported by NIH RO1NS26838 grant to R.G. and by Office of Naval Research grant NOOO14-94-10524 to M.H.

147.7

LOCALIZATION OF NITRIC OXIDE SYNTHASE BY NADPH-DIAPHORASE HISTOCHEMISTRY AND IMMUNOCYTOCHEMISTRY IN THE BRAINS OF THE FLY, *SARCOPHAGA BULLATA*. M.K. Walsh and H. Itagaki*. Dept. of Biology, Kenyon College, Gambier, OH 43022.

Nitric oxide (NO), a free radical gas, has been shown to play a major signalling role in the vertebrate immune, vascular, and nervous systems. It is synthesized from L-arginine by a family of enzymes, the nitric oxide synthases (NOS), that also has NADPH diaphorase activity (Hope *et al.*, 1991). By using either antisera to NOS, or a histochemical method to detect the NADPH diaphorase activity, it is possible to localize NOS in various tissues.

Among invertebrates, other investigators have found evidence of NOS in the central nervous systems of a variety of Mollusca, as well as insects (*e.g. Drosophila*, locusts, honeybees). In addition, the *Drosophila* NOS gene has recently been cloned and sequenced by Regulski and Tully (1995).

In this study, we used both histochemical and immunocytochemical methods to localize NOS in the brain and subesophageal ganglia of adult *Sarcophaga bullata*, the blowfly. We found heavy NOS activity in the antennal lobes and in the central bodies, while we found little staining in the mushroom bodies. Our results are congruent with the results of the earlier work on *Drosophila* (Müller and Buchner, 1993). However, the NOS activity patterns are different from the work on locusts (Elphick *et al.*, 1995) in that we found little activity in the mushroom bodies. Our results also support the hypothesis that NO is involved in olfactory processing in insects.

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147.8

INVOLVEMENT OF NITRIC OXIDE IN THE SYMBIOTIC RELATIONSHIP BETWEEN DINOFLAGELLATE ALGAE AND ANTHOZOANS. H. Trapido-Rosenthal*[1], L. Fraser-Smith[1], H. Holder, C. Morrall[1,2], and Z. Billinghurst[1,3]. ([1]Bermuda Biological Station for Research, Bermuda, University of Plymouth[2], UK, and [3]University of York, UK).

Cnidarians of the class anthozoa, such as anemones and corals, can live in symbiotic relationships with dinoflagellate algae (zooxanthellae) of the genus *Symbiodinium*. Such symbioses are at least in part chemically mediated (Gates *et al.*, PNAS, 1995). When living in a symbiotic relationship, the free amino acid pools of zooxanthellae are dominated by arginine, the cells have a high arginine transport capacity; and they possess an inducible nitric oxide synthase (NOS) activity (Bester *et al.*, Chem. Senses, in press).We report here the results of investigations into the NOS activity present in the tissue of *Aiptasia pallida*, an organism that maintains populations of *Symbiodinium* in its tentacles.

We assayed for NOS activity by monitoring the ability of *Aiptasia* homogenates (from which algae had been removed) to convert arginine to citrulline. We found that *Aiptasia* possess NOS activity, and that this activity is localized in the soluble fraction of homogenates. Calcium, calmodulin, and NADPH are required for maximal activity, which is inhibited by N-arginine methyl ester and by nitroarginine, but not by valine.

In response to stresses such as heat shock or mechanical agitation, anemones retract their tentacles by contracting their muscles. Such stressed animals have lower NOS activity than do unstressed controls. Treatment of *Aiptasia* with NOS inhibitors can also cause tentacular retraction, while treatment with the NOS substrate arginine can inhibit this response to stress. Taken together, our results suggest that (1) NO may be involved in the anemone's control of tentacular deployment, and that (2) NO derived from symbionts has the potential to contribute to the behavior of the host organism.

Supported in part by a contract from Boehringer-Mannheim Corporation and a grant from the NIH (1-R03-DE11916-01).

147.9

EFFECTS OF NITRIC OXIDE ON AFFERENT RESTING ACTIVITY IN THE CEPHALOPOD (*SEPIA OFFICINALIS*) STATOCYST. Y. Tu, T.D. Snell and B.U. Budelmann*. Marine Biomedical Inst., Univ. Texas Medical Branch, Galveston, TX 77555-1163.

The effects of nitric oxide on the resting activity (RA) of afferent crista fibers were studied in isolated statocysts (n = 98) of the cephalopod cuttlefish *Sepia officinalis* by bath application. L-Arginine (precursor of nitric oxide; threshold 10^{-6}M) caused either a decrease of RA, or first a short increase and then a long lasting decrease, or in a few cases an increase only. Application of D-Arginine had no effect. Similar effects to L-Arginine were seen after application of 10^{-6}M to 10^{-4}M 8-Bromo-cGMP sodium (permeable analog of cyclic GMP), and the nitric oxide sources Sodium nitroprusside (SNP, 10^{-6}M to 10^{-4}M), Diethylamine sodium (10^{-5}M to 10^{-4}M), and 3-Morpholinosydnonimine (SIN-1, 10^{-5}M to 10^{-4}M). When nitric oxide synthase was blocked by 10^{-5}M to 10^{-3}M N^G-Nitro-L-arginine methyl ester (L-NAME) or 10^{-5}M to 10^{-4}M N^G-Nitro-L-arginine (L-NOARG), 10^{-4}M L-Arginine was no longer effective, but the effects of 8-Bromo-cGMP sodium and the above nitric oxide sources still occurred. After application of 10^{-4}M Methylene blue or 10^{-4}M Cystamine, L-Arginine and the above nitric oxide sources had only excitatory effects.

These data suggest that in the cuttlefish statocyst a Nitric oxide-cyclic GMP pathway exists with a mainly inhibitory effect on RA. Additional data will be presented on a possible interaction between the cyclic GMPand cyclic AMP systems.

Support by the John Sealy Memorial Endowment Fund.

147.10

DOES NITRIC OXIDE INHIBIT METAMORPHOSIS IN A LARVAL MOLLUSC? S.Froggett and E.M. Leise* Dept. of Biol., U. of N. Carolina Greensboro, Greensboro, NC 27412.

In metamorphically competent larvae of the gastropod *Ilyanassa obsoleta*, the apical ganglion maintains a higher level of staining for NADPH-diaphorase than all other ganglia throughout pre-metamorphic development (Lin, M. and Leise, E. M., J. Comp. Neurol. in press). Staining intensities increase in all neuropils during this stage, however neuropil staining decreases in all ganglia at metamorphosis.

To elucidate the function of nitric oxide (NO) during metamorphosis, we bath-applied NO donors and a nitric oxide synthase (NOS) inhibitor, in combination with a known metamorphic inducer, serotonin, to competent larvae. NO donors or the NOS inhibitor were applied once or replaced every 6 hrs to maintain high activity during the experiment. Metamorphosis was scored at 24 and 48 hrs.

The NO donor SNAP inhibits metamorphosis at 10^{-3} M to 10^{-5} M at 24 hrs and 10^{-3} M to 10^{-4} M at 48 hrs. The NOS inhibitor L-NAME does not affect metamorphic induction. Our results suggest that NO may be an endogenous inhibitor of metamorphosis. The lack of significant results with L-NAME suggests that this compound is not actively taken up by larvae. Injection experiments and further use of other NO donors are needed to confirm the SNAP results.

This research was supported by grants from UNCG.

TRANSPORTERS I

148.1

EFFECTS OF VARIOUS ANTIDEPRESSANTS ON GABA TRANSPORTER SUBTYPES EXPRESSED IN COS7 CELLS. M. Nakashita*, K. Sasaki, C. Matsuyama, N. Sakai and N. Saito, Laboratory of Molecular Pharmacology, Biosignal Research Center, Kobe University, Kobe 657, Japan

The discovery of the tricyclic and tetracyclic antidepressants, which are known to inhibit the reuptake of two biogenic amines, noradrenaline and serotonin, has revolutionized the therapy of the depression, but the molecular machanism of these drugs has not yet been elucidated. Molecular cloning of neurotransmitter transporters revealed that the tricyclic and tetracyclic antidepressants act on the presynaptic serotonin and noradrenaline transporters, and that the antidepressants has different affinity for the two biogenic amine transporters. It was also shown that GABA uptake is also altered by antidepressants and some antidepressant have anticonvulsive effects. We here examined the effects of various antidepressants on the GABA uptake through three subtypes of GABA transporters (GAT1, GAT2 and GAT3) expressed in COS7 cells.

IC50 value of 11 antidepressants for serotonin transporter (SET) and three GABA transporters was examined. Desipramine, maprotilline, amitriptyline and nortriptyline proved to have similar inhibitory potency for GAT1 and GAT3 to that for SET. According to the previous uptake study using synaptosomal fraction, amitriptyline has similar value of IC50 for serotonin uptake to noradrelnaline uptake among these four antidepressants. These results suggested that amitriptyline acts on three neurotransmitter transporters (GABA, noradrenaline and serotonin transporters) at the similar concentration and that the inhibitory effect of amitriptyline on GABA uptake should be mentioned clinically.

148.2

EXPRESSION OF GABA TRANSPORTERS IN THE RAT THALAMUS. S. De Biasi*, L. Vitellaro-Zuccarello and N. Brecha°. Dip. Fisiol. Biochim. Gen, Sez. Istologia e Anatomia Umana, Univ. Milano, Milano, Italy; °Dept. Neurobiology, UCLA, Los Angeles, CA 90095-1763, USA.

GABAergic transmission is terminated by the uptake of GABA into neuronal and glial processes through GABA plasma membrane transporters. The three GABA transporters (GAT-1, GAT-2 and GAT-3) isolated from the rat nervous system share structural similarities but have distinct pharmacological properties and tissue distributions.

The localization of GAT-1 and GAT-3 in rat thalamus has been studied by immunocytochemistry with anti-peptide antibodies raised in rabbits against the C terminal portion of the transporters (Minelli et al., 1995, 1996). Vibratome sections of the thalamus of rats perfused under anesthesia with mixed aldehydes were processed for the visualization of GAT-1 and GAT-3 immunoreactivity (GAT ir) by a standard ABC-DAB procedure. Representative sections containing the reticular, ventrobasal and dorsal lateral geniculate nuclei were also processed for electron microscopy.

At light microscopy the two antisera labeled the thalamic neuropil with a distinct pattern of intensity, as GAT-1 ir was generally faint whereas GAT-3 ir was very intense throughout the thalamus. At the electron microscope GAT-1 ir and GAT-3 ir were always confined to astrocytic cell bodies and their proximal and distal processes. Astrocytic processes were scattered througout the neuropil, where they surrounded blood vessels and unlabeled neuronal profiles, including axonal terminals making either symmetric or asymmetric synapses. The present results indicate that in rat thalamus both GABA transporters are involved in the uptake of GABA into glial cells.

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148.3

INFLUENCE OF STRUCTURAL ANALOGUES ON MOUSE BRAIN SYNAPTOSOMAL TRANSPORT OF γ-HYDROXYBUTYRATE. G. Tunnicliff, S.J. McCormick and R.D. Stith* Laboratory of Neurochemistry, Department of Biochemistry & Molecular Biology, Indiana University School of Medicine, Evansville, IN 47712.

γ-Hydroxybutyrate is a metabolite of γ-aminobutyric acid (GABA), the prime inhibitory neurotransmitter in the brain, and has pronounced neuropharmacological actions, such as the induction of somnolence and general anesthesia. Further, it inhibits neuronal activity in cultured cells, and induces a generalized absence seizure-type EEG activity in experimental animals. In addition to possessing a specific receptor site in the brain, γ-hydroxybutyrate possesses a high-affinity transport system in synaptosomes which might represent an uptake mechanism responsible for removing γ-hydroxybutyrate from the synaptic gap. Tliis transport system displayed saturation kinetics and was substantially activated in the presence of pyridoxal 5'-phosphate.

One way to characterize a transport system is to study the effects of inhibitors. The structure of these inhibitors and their mechanism of action can shed light on the nature of the recognition site on the transporter. This study measured the action of several γ-hydroxybutyrate and GABA structural analogues, as well as the Na^+ antagonist, harmaline. The GABA transport inhibitors nipecotic acid, guavacine and β-alanine had no effect on γ-hydroxybutyrate synaptosomal uptake. However, the γ-hydroxybutyrate analogues 2-hydroxycinnamic acid, furylacrylic acid and citrazinic acid were all competitive inhibitors of transport, with K_i values of 74 (± 14) μM, 130 (±16) μM and 222 (± 35) μM, respectively. Harmaline was a competitive inhibitor with respect to Na^+. These results show that the γ-hydroxybutyrate transporter is pharmacologically distinct from the GABA transporter, and that structural analogues can compete for binding at the γ-hydroxybutyrate recognition site on the transport protein. Funded by IUSM

148.4

CHARACTERIZATION OF GLYCINE TRANSPORT IN CULTURED MÜLLER CELLS FROM THE RETINA. A. Gadea, M. Romo-De-Vivar* and A. M. López-Colomé. Instituto de Fisiología Celular, Universidad Nacional Autónoma de México, Apartado Postal 70-253, México, D.F., 04510, México.

Rapid termination of the synaptic action of glutamate (glu) and glycine (gly) is achieved by uptake into the presynaptic terminal and glial cells. In the vertebrate CNS, gly acts both, as an inhibitory neurotransmitter and as a glu modulator or coagonist at postsynaptic N-methyl-D-aspartate receptors. We have previously described NMDA receptors in Müller cells of chick retina coupled to the phosphoinositide cascade, the entry of calcium and the activation of PKC (López-Colomé et al., 1993). A colocalization of gly transporters and NMDA receptors has been reported in brain tissue (Smith et al., 1992); since the concentration of gly could participate in the modulation of glu excitatory transmission in the vertical pathways of the retina, transport of gly in confluent monolayer cultures of Müller cells from 7-day-old chick embryos was studied. Uptake was measured at 37 °C in Krebs-Ringer Bicarbonate buffer containing [^{3}H]-gly/gly 1:25000. Gly transport into an osmotically-sensitive compartment reaches equilibrium at 30 min. Process is energy-requiring, since iodoacetate (1mM), ouabaine (200 μM), and potassium cyanide (1mM), inhibited transport 23%, 58% and 15% respectively; 2,4-dinitrophenol (50 μM) had no effect. Sarcosine (1mM) competitively inhibited transport, whereas 1mM taurine, D-serine, GABA and β-alanine were ineffective. Gly transport was pH-sensitive with an optimum at pH 7.4. Kinetic analysis of the saturation curve for gly in a concentration range from 0.01 mM to 2 mM ([^{3}H]-gly/gly 1:5000), revealed two transport systems: low affinity with a Km 579 μM, V_{max} 2.3 nmol/min/mg protein, and high affinity with a Km of 43.8 μM, V_{max} 0.55 nmol/min/mg protein. Ion dependence was investigated for both systems, replacing NaCl with choline chloride or LiCl in the case of Na^+, or with Na^+ gluconate in the case of Cl^-. Both systems were highly dependent on the presence of Na^+; the high-affinity system proved also dependent on Cl^- ions in the external medium. This work was partially supported by Grant 3375-N from CONACYT.

148.5

RXT1, AN «ORPHAN» Na^+/Cl^--DEPENDENT TRANSPORTER, IS LOCATED ON AXON TERMINALS OF CORTICO-STRIATAL GLUTAMATERGIC NEURONS IN THE RAT BRAIN. S. El Mestikawy[(1)]*, J. Masson[(1)], L. Kerkerian[(3)], P. Kachidian[(3)], Z. Aïdouni[(1)], P. Gaspard[(2)] and M. Hamon[(1)]. [(1)] INSERM U288 and [(2)] INSERM U109 Institut Fédératif des Neurosciences de la Pitié-Salpêtrière. PARIS 75013, France; [(3)] CNRS-UPR 9013, MARSEILLE 13402, France.

Rxt1 was cloned thanks to its sequence homology with the well characterized Na^+/Cl^--dependent transporters for neurotransmitters such as the dopamine-, norepinephrine-, serotonine- and , GABA- transporters. However, its transported substrate has not yet been identified. The development of specific probes (cRNA, polyclonal antibodies) allowed the demonstration that Rxt1 is expressed exclusively by central neurons whose distribution in the rat brain suggests that they may correspond mainly to those using glutamate as neurotransmitter. In particular, a high concentration of the Rxt1 protein but only low levels of the Rxt1 mRNA were found in the striatum as expected of the addressing of the «orphan» transporter to the terminals of cortico-striatal glutamatergic projections. Several complementary approaches were used in order to directly assess this possibility. Firstly, electrocoagulation was applied to the cerebral cortex for inducing a massive degeneration of cortico-striatal afferents. This electrolytic lesion resulted in the parallel decrease in both the density of striatal glutamatergic terminals (quantified by the measurement of synaptosomal [^{3}H]glutamate uptake) and the striatal levels of the Rxt1 protein (estimated by western blot). Secondly, cortical neurons projecting to the striatum were retrogradely labeled by fluorogold injections into the striatum. Combined in situ hybridization with a specific cRNA probes showed that the vast majority of retrogradely labeled cortico-striatal neurons contained Rxt1 mRNA. Thirdly, immunolabeling at the electron microscope level indicated that the Rxt1 protein was present in axon terminals of cortico-striatal projections identified by the anterograde transport of HRP-tracer. Altogether, these data demonstrated that Rxt1 is located on the striatal terminals of glutamatergic neurons originating in the cerebral cortex. Therefore, Rxt1 may play a key role in glutamate-dependent modulations of striatal (motor and cognitive) functions.

148.6

CONTROL OF NMDA RECEPTOR ACTIVATION BY A GLYCINE TRANSPORTER. S. Supplisson* and C. Bergman. Laboratoire de Neurobiologie, Ecole Normale Supérieure, 75005 Paris, France.

Cotransporters accumulate specific substrates inside the cells using ionic electrochemical gradients. They can also lower the substrate concentration outside the cell membrane if the passive diffusion from the bulk solution towards the membrane does not compensate the uptake. Such a depletion could be of physiological importance for the regulation of the synaptic glycine concentration at glutamatergic synapses and the control of N-Methyl-D-Aspartate receptors (NMDAR). To study the capacity of a glycine transporter (GLYT1b) to control NMDAR, we coexpressed both proteins in *Xenopus* oocytes. We previously reported that in such a system stopping the superfusion flow in the presence of glycine and glutamate resulted in a dramatic decrease of the NMDA-induced current. This inhibition was interpreted as resulting from a local (juxtamembrane) decrease of [Gly] due to glycine uptake. We show now that even in a fast perfusion mode, the activity of GLYT1b is sufficient to lower significantly the extracellular glycine. This conclusion is drawn from the comparison of concentration-response curves for glycine activation of the NMDAR in oocytes expressing NMDAR with or without GLYT1b. In the presence of GLYT1b, the glycine EC_{50} of NMDAR is shifted from 2.2μM to 8-10μM. That this shift is only apparent is indicated by the fact that the EC_{50} of D-serine (a non transported glycine agonist of NMDAR) remained unchanged. We calculate that even in the fast perfusion mode, the transporter can lower the glycine concentration at the membrane by a factor of 4 to 5. *Supported in part by EC grant BMH4CT950571.*

148.7

ALTERATIONS IN HIGH AFFINITY CHOLINE UPTAKE IN RAT BRAIN SECTIONS DUE TO 17β-ESTRADIOL. M.L. Caspers*, B.E. Deverman and M.J. Fu. Dept. of Chemistry, Univ. of Detroit Mercy, Detroit, MI 48219.

We have shown that a decrease in high affinity choline uptake occurs in ovariectomized (OV) rats exposed to either 0.5 or 5 mg 17β-estradiol pellets for 2 or 3 weeks (Soc. Neurosci. Abstr. 21 (1995) 2064). In this study, young (2 mo) OV rats were sacrificed 5 or 8 wks after implantation of 17β-estradiol (0.5 or 5 mg) or placebo pellets, 24μm frozen brain sections were prepared and treated with [^{3}H]hemicholinium-3 (HC-3), a competitive inhibitor of the high-affinity choline transporter. Autoradiographic studies with computer-assisted densitometry indicated an 18.6% ($P<0.001$) and a 13.7% ($P<0.0005$) decrease in HC-3 binding in the caudate putamen (CP) of rats treated with 0.5 mg 17β-estradiol pellets for 5 and 8 wks, respectively. In rats receiving 5 mg 17β-estradiol pellets, an 11.1% ($P<0.04$) and 12.7% ($P<0.001$) increase in HC-3 binding in the CP was noted after 5 and 8 wks, respectively. After 3 wks, non-OV and OV, old (15 mo) rats treated with 0.5 mg estradiol pellets had a 7.1% and an 8.4% ($P<0.003$) decrease in HC-3 binding, respectively. After 5 wks of exposure to 5 mg 17β-estradiol pellets, a 29.1% ($P<0.002$) decrease and a 9.9% ($P<0.001$) increase in HC-3 binding was noted in non- and OV old rats, respectively. (Supported by an Amer. Fed. Aging Res. grant).

148.8

DIFFERENCES IN THE DEVELOPMENTAL EXPRESSION OF THE VESICULAR ACETYLCHOLINE TRANSPORTER AND CHOLINE ACETYLTRANSFERASE IN THE RAT BRAIN. Thomas Holler[1]*, Brygida Berse[1], Jennifer Marie Cermak[1], Marie-Françoise Diebler[2] and Jan Krzysztof Blusztajn[1]. [1]Department of Pathology, Boston University School of Medicine, Boston, MA 02118; [2]Département de Neurobiologie Cellulaire, CNRS, 91190 GIF-sur-Yvette Cedex, France

The neurotransmitter acetylcholine (ACh) is synthesized by the enzyme choline acetyltransferase (ChAT) and then transported into synaptic vesicles by the vesicular acetylcholine transporter (VAChT). Since the VAChT gene is located within the first intron of the ChAT gene, it is likely that expression of the two genes is coregulated. We compared the developmental expression of VAChT and ChAT mRNA and protein in rat brain. ChAT mRNA and enzyme activity increased by almost 10-fold from embryonic day 19 to adulthood, with the most pronounced increase occurring after birth. In contrast, VAChT mRNA increased by only about 2-fold from late embryonic stages to adult levels. However, VAChT protein followed the developmental pattern of ChAT activity, revealing a large excess of VAChT mRNA over VAChT protein during early stages of development. The results are suggestive of differential mechanisms of ChAT and VAChT regulation during brain development, and of possible translational control of VAChT expression.

(Supported by AG09525 from NIA, National Institutes of Health)

148.9

β-AMYLOID INHIBITS GLUCOSE UPTAKE IN CULTURED RAT ASTROCYTES. A.Parpura-Gill*, C. Martens, D. Beitz and E. Uemura. Dept. of Veterinary Anatomy, Neuroscience Program and Dept of Animal Science, Iowa State University, Ames, IA 50011.

Astrocytes possess cytoplasmic membrane glutamate uptake carrier which normally keeps extracellular levels of glutamate below neurotoxic concentrations. The process of glutamate uptake is energy dependent, and the capacity of astrocytes to continue with this function during pathologic conditions may have significant consequences on neuronal survival. Glycolysis is the major source of that energy. Any inhibition of this ATP-producing process could result in diminished glutamate uptake in astrocytes. Investigation of the cerebral glucose metabolic rate in AD patients determined that glucose oxidation in these patients was significantly lower than in normal, age- matched patients. This suggests lower ATP production in AD patients which might have direct effect on astrocytic glutamate uptake. In this study, we investigated β-amyloid effect on astrocytic glucose uptake. Purified astrocyte cultures were prepared from hippocampi of P2-5 Sprague-Dawley rats. Cells were plated onto tissue culture plates coated with β25-35 peptide (1mg/ml). At postplating day 7 ^{14}C-glucose was added. The reaction was stopped after 1 hour, and radioactivity was measured in cell lysates . We found that β25-35 peptide significantly inhibits glucose uptake in astrocytes. Our data suggest that β-amyloid might play a role in neuronal cytotoxicity by lowering efficacy of glutamate uptake carrier via decreasing glucose uptake, thus disrupting energy production essential for the glutamate carrier functioning. Supported by Alzheimer's Association #93048.

148.10

DIFFERENTIAL DISTRIBUTION OF LACTATE TRANSPORTERS AND LACTATE DEHYDROGENASE (LDH) ISOZYMES: EVIDENCE FOR AN ASTROCYTE-NEURON LACTATE CYCLE. G.Pellegri, P.G.Bittar, J.-L.Martin and P.J. Magistretti* Institut de Physiologie, Université de Lausanne, Switzerland.

There is experimental evidence indicating that lactate and pyruvate are adequate substrates for brain tissue and that lactate is produced by astrocytes and utilized by neurons. This metabolic exchange requires the presence of a lactate transport system and a selective distribution of LDH isoenzymes. Two monocarboxylate transporters have been cloned in peripheral tissues (MCT1 and MCT2). We have used cDNA probes to study the regional and the cellular distribution of MCT mRNAs in the mouse brain. The expression of MCT1 and MCT2 mRNAs is developmentally regulated, reaching a peak during the second postnatal week (P15). In *situ* hybridization experiments revealed a differential distribution of MCT1 and MCT2 mRNAs in P15 brain. Thus, MCT1 is particularly expressed in the vascular endothelial cells, while MCT2 labelling is observed in hippocampal and cortical neurons. In contrast, in the adult mouse brain, MCT1 and MCT2 mRNAs are expressed throughout the whole brain, with no endothelial labelling and no colocalization with GFAP; this suggests that in the adult brain MCT1 and MCT2 are predominantly expressed in neurons.The cellular distribution of the MCT1 and MCT2 mRNAs was also examined in primary cultures of cortical astrocytes and neurons. Northern blot analysis indicates that MCT1 mRNA is predominantly localized in astrocytes, while MCT2 is more abundant in neurons. Further suggesting the existence of an astrocyte-neuron lactate cycle is the selective distribution of LDH isoenzymes. Indeed, the A subunit, which is predominantly found in lactate-producing tissues, is exclusively detected in certain populations of astrocytes in the adult human brain while neurons are labelled exclusively by antibodies raised against the B subunit which is enriched in lactate-consuming tissues. Our findings suggest that lactate produced in astrocytes may passively diffuse into the extracellular space and be avidly taken up by neurons possessing MCT1 and MCT2, thus providing an energy substrate for those cells.
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148.11

EXCITATORY AMINO ACIDS STIMULATE GLYCOLYSIS IN ASTROCYTES VIA ACTIVATION OF THE Na^+/K^+ ATPase
L. Pellerin* and P.J. Magistretti. Laboratoire de Recherche Neurologique, CHUV et Institut de Physiologie, Université de Lausanne, Switzerland.

Several lines of evidence indicate that astrocytes may play a pivotal role in coupling neuronal activity with energy metabolism (Magistretti & Pellerin *Cerebral Cortex* **6**:50-61 1996). We have observed that excitatory amino acids (EAAs) like glutamate and aspartate increase 2-deoxyglucose (2-DG) uptake and phosphorylation by mouse cortical astrocytes in culture. The stimulatory effect of EAAs is mediated by a Na^+-dependent glutamate transporter. Since the effect on 2DG uptake can be prevented by ouabain, an inhibitor of the Na^+/K^+ ATPase, we have engaged in a more direct demonstration of the involvement of the pump. Using ^{86}Rb uptake as an index of the activity of the Na^+/K^+ ATPase, we have found that L-glutamate increases Rb uptake into astrocytes in a concentration-dependent manner with a K_m of 72 μM. Both D- and L-aspartate, but not D-glutamate exert a similar effect, further indicating a transporter-mediated effect. In addition, the possibility of a massive Na^+ influx due to opening of voltage-dependent Na^+ channels can be excluded since TTX is without effect. As for 2-DG uptake, the effect of L-glutamate is prevented by 100 μM ouabain, further supporting a funçtional link between these two processes. Since EAAs also increase lactate release by astrocytes, these observations also strongly support the view that EAAs stimulate glycolysis in astrocytes via activation of the Na^+/K^+ ATPase. When viewed within their physiological context, these data suggest that upon cortical activation, EAAs released by activated neurons provide a direct signal for astrocytes to take up glucose and metabolize it to lactate, which can be used by neurons as an energy source.
(Supported by FNRS grant 31-40565.94 to PJM)

148.12

DEVELOPMENTAL EXPRESSION OF ORPHAN TRANSPORTERS rB21a AND v7-3 mRNAS IN EMBRYONIC RATS. K. Inoue[1], S. Shimada[1], K. Sato*[1], G. R. Uhl[2] and M.Tohyama[1]. [1] Dept. of Anat. & Neurosci., Osaka Univ. Med Sch., Osaka, 565. JAPAN. [2]Mol. Neurobiol. Br., IRP, NIDA, NIH, Dept. Neurol. & Neurosci., JHUSM, Balto., MD21224

Orphan transporters rB21a and v7-3 are members of a new subfamily of Na^+, Cl^- dependent neurotransmitter transporters with two large extracellular loops. We have investigated developmental expression of orphan transporters rB21a and v7-3 mRNAs in rats by *in situ* hybridization histochemistry. Both mRNAs exhibited unique regional and temporal developmental expression profiles. As a characteristic feature, rB21a mRNA was expressed strongly in liver during development, but diminished markedly to undetectable levels around birth. Intense expression of v7-3 mRNA in choroid plexus also faded around birth. In the adult rat brain, rB21a mRNA was localized in pineal gland, pia matter and choroid plexus, whereas v7-3 was widely distributed in central nervous system neurons. These developmental expression patterns may suggest possible candidate substrstrates for the orphan transporters that could even differ from embryonic development to adulthood.

148.13

The Mammalian Brain-Specific, High-Affinity, L-Proline Transporter: Identification of Cysteine Residues Important for Transport. JW Miller*, SE Renick, RT Fremeau, Jr. Dept. of Pharmacol., Duke Univ. Med. Ctr., Durham, NC 27710.

The cloned, mammalian brain-specific, high-affinity L-proline transporter (PROT) is a member of the gene family of Na^+- (and Cl^-)-dependent plasma membrane transport proteins that includes transporters for several neurotransmitters, osmolytes, and metabolites. We report that the sulfhydryl reagent *p*-hydroxymercuriphenylsulfonic acid (pHMBS) is a potent inhibitor of high-affinity L-proline uptake in PROT-transfected HeLa cells (IC_{50} ~5 μM). pHMBS is an organomercurial compound which is membrane impermeable and covalently modifies cysteine residues. Therefore, one or more externally accessible cysteine residues within the PROT protein may be important for substrate binding and/or translocation. To investigate this hypothesis, we used site-directed mutagenesis to individually replace each of eleven cysteine residues conserved between the human and rat PROT homologs with isosteric serine residues. We then examined whether any individual cysteine residue is required for the transport function of mammalian brain PROT. The eleven $C_{10}S_1$ mutants were transiently transfected into HeLa cells and ^{3}H-L-proline uptake was measured. Three mutants (C50S, C54S, C431S) exhibited ≤50% L-proline uptake as compared to the wild type PROT; four mutants (C86S, C220S, C231S, C547S) exhibited 60-80% uptake; and four mutants (C218S, C470S, C483S, C501S) exhibited 100% uptake. We are currently investigating the molecular basis of the transport defects in those mutants expressing reduced transport. We are also determining the effect of each mutation on transport inhibition by pHMBS. Preliminary data indicate that one mutant is less sensitive to pHMBS inhibition than wild type PROT. This residue may be located in the PROT permeation pathway. Grant support: Neurobehavioral Sciences Training Grant PHS MH15177, and NIH NS32501.

148.14

EFFECTS OF ENDOGENOUS MONOCARBOXYLATES (PYRUVATE & LACTATE) ON THE PRESERVATION OF RAT HIPPOCAMPAL SLICES UNDER HYPOGLYCEMIC CONDITIONS A. M. Benz, Y. Izumi, D.B.Clifford*, & C.F. Zorumski, Depts. of Psychiatry and Neurology, Washington Univ., School of Med., St.Louis, MO 63110.

In rat hippocampal slices, dark cell appearance is a characteristic histological change during glucose deprivation. We have previously shown that the dark cell appearance by glucose deprivation was prevented by the application of alternative energy substrates other than glucose (NeuroReport 1994;5:617. Society for Neuroscience 1994;20:1320.). Among these substrates, lactate and pyruvate from glia may function as endogenous energy substrates in the central nervous system. If this is the case, monocarboxylates are particularly important for the preservation of neuronal integrity when glucose is unavailable. To address the question of whether endogenous monocarboxylates participate in the preservation of neurons during hypoglycemia, we examined the effects of cytochalasin B (CCB) and α-cyano-4-hydroxycinnamate (4CIN) in hippocampal slices. CCB and 4CIN are inhibitors of glucose- and monocarboxylates-transport, respectively.

Administration of 200 μM 4CIN for 90 min in the presence of 10 mM glucose did not produce histological change (N=6), suggesting that monocarboxylates are not essential energy substrates for neurons when glucose is available. However, the combination of 50 μM CCB and 200 μM 4CIN produced remarkable shrinkage in pyramidal cells (N=6). Although 50 μM CCB alone for 90 min revealed some dark cell appearance, the degree of changes was less compared to the effects of the combination (N=6). This result indicates that when glucose utilization is impeded endogenous monocarboxylates play an important role in neuronal preservation.

Supported by the Diabetes Research and Training Center at Washington University and Alzheimer's Disease and Related Disorders Program at University of Missouri.

148.15

CHANGES IN EXTRACELLULAR [KCl] ALTERS GLUCOSE UTILIZATION IN CEREBELLAR GRANULE NEURONS. E.M.Koehler-Stec*, T.M.Davies-Hill, I.A.Simpson, EDMNS, DB, NIDDK, NIH, Bethesda, MD 20892

Cerebellar granule neurons (CGN) are normally cultured in high KCl [25mM] media to allow for optimal growth and differentiation. Under these conditions, the expression of glucose transporters (GLUTs) 1 and 3 and the rate of glucose transport/utilization are maximal. If CGNs are grown in serum-free low KCl [5.6mM] media, the rate of differentiation and GLUT expression are slower. Transferring differentiated CGNs from a high to low KCl environment leads to apoptotic cell death. In this study, the effect of acute exposure of differentiated CGNs, grown in media containing high KCl and serum, to a low KCl solution on glucose transport/utilization was measured by the 2-deoxyglucose (2DG) method. Transfer of CGNs (day 8 *in vitro*) to low KCl Lockes buffer increased the apparent rate of 2DG uptake which was maximal after 9 min and remained elevated for up to 30 min. Addition of energy substrates (i.e. lactate (1mM), pyruvate (1mM) or glucose (100μM)) blunted the apparent increase in 2DG uptake. In contrast, transfer of CGNs to high KCl Lockes buffer did not induce an increase in the rate of 2DG uptake but rather decreased uptake over time; addition of energy substrates appeared to block this effect. To determine if the increase in the apparent rate of 2DG uptake in cells incubated in the low KCl buffer resulted from an increase in glucose transport activity or GLUT translocation, 3-O-methylglucose transport as well as photoaffinity labelling techniques were used, respectively. There was no difference in 3-O-methylglucose uptake in cells incubated for 0 or 9 min in low KCl buffer +/- 100μM glucose nor was there any change in the number of GLUTs present on the cell surface after 30 min incubation in the low KCL buffer. Thus, the apparent increase in 2DG uptake is not a result of GLUT activation and/or translocation but rather reflects the ambient energy status of the cells following ionic manipulations which results in an increased phosphorylation of 2DG. (Supported by NIGMS and NIDDK)

148.16

GLUCOSE TRANSPORTER, GLUT 5 EXPRESSION IN CULTURED MICROGLIAL CELLS OF RAT. J. Payne*, I. Simpson and P. Davies. Dept. of Pathology, Albert Einstein College of Medicine, Bronx, N.Y. 10461; NIDDK, NIH, Bethesda, MD 20892.

Glut 5 is a member of a group of related facilitative glucose transporter proteins that are involved in enhancing transportation of glucose and/or fructose across plasma membranes. Although some isoforms are present in non-CNS tissue, GLUT 5 is one of three isoforms present in human and rat brain. We have shown, using immunocytochemical techniques, that Glut 5 is expressed exclusively in microglial cells of human and rat brain. In the present study we examined the expression of Glut 5 in cultured microglial cells from rat brain. Antibody to Glut 5 was raised to a 20 amino acid synthetic peptide specific for the C terminal region of the rat Glut 5 sequence. The anti- rat Glut 5 antibody was affinity purified. Mixed cell cultures were prepared from 4 day postnatal rats, and microglia were isolated from day 7-9 cultures. For immunocytochemistry, purified microglial cells were grown on glass coverslips and treated with the following antibodies: 1) OX 42, a microglial marker, 2) ISO B4, a lectin that labels all forms of microglia, 3) Anti-rat Glut 5. Protein from the membrane and cytosol fractions of cultured microglial cells was used for Western blotting. Control experiments included no primary antibody as well as peptide absorption experiments. The results show that Glut 5 is expressed in cultured microglial cells of rat. Western and immunocytochemical data show that the protein is found not only in the plasma membrane but also seems to be expressed in the cytosol. This is a novel finding
because other glucose transporters (Glut 1-4) are present in the plasma membrane (Glut 1-3) or in cellular vesicles (Glut4). The regulation of this transporter is currently being investigated. Supported by NIMH 38623

148.17

CALCIUM AND CALMODULIN DEPENDENT PROTEIN KINASE II REGULATION OF BRAIN MICROSOMAL Ca^{2+} UPTAKE MEDIATED BY Mg^{2+}/Ca^{2+} ATPase. J. T. Parsons*[1], S. B. Churn[2], and R. J. DeLorenzo[1, 2, 3], Departments of Biochemistry[1], Neurology[2], and Pharmacology[3], Medical College of Virginia, Richmond, VA 23298.

Multifunctional Calcium and Calmodulin Dependent Protein Kinase II (CaMKII) regulates a multitude of brain functions. CaMKII is also a ubiquitous enzyme that has been shown to stimulate cardiac microsomal Ca^{2+} uptake by phosphorylation of Mg^{2+}/Ca^{2+} ATPases. In order to determine whether CaMKII regulates brain Mg^{2+}/Ca^{2+} ATPases, we examined the effect of various kinase inhibitors on endogenous microsomal CaMKII activity and Ca^{2+} uptake mediated by Mg^{2+}/Ca^{2+} ATPase. Microsomes were isolated from rat brain homogenates by differential centrifugation. Endogenous CaMKII activity was measured by ^{32}P incorporation into the 50 kDa alpha subunit (autophosphorylation) of the enzyme. Mg^{2+}/Ca^{2+} ATPase mediated Ca^{2+} uptake was measured by scintillation counting of $^{45}Ca^{2+}$ accumulation in the microsomes. Ca^{2+} uptake was performed under conditions that resulted in significant CaMKII autophosphorylation activity. The calmodulin antagonists chlorpromazine and W-7 resulted in 62 % and 71 % inhibition respectively of CaMKII activity. The nonspecific kinase inhibitor H-7 caused 81 % inhibition of CaMKII autophosphorylation. In addition, the CaMKII specific calmodulin antagonist peptide [CPI(290-309)] inhibited 64 % of CaMKII autophosphorylation activity. These compounds also had similar inhibition of microsomal Ca^{2+} uptake. Chlorpromazine and W-7 caused 41 % and 78 % decrease in Ca^{2+} uptake respectively. H-7 resulted in microsomal Ca^{2+} uptake inhibition of 64 %. Finally, CPI(290-309) demonstrated an inhibition of Ca^{2+} uptake of 32 %. The data suggest that CaM Kinase II either directly or indirectly regulates neuronal microsomal Ca^{2+} uptake mediated by Mg^{2+}/Ca^{2+} ATPases. This work was supported by NIH grant R01-NS23350.

148.18

AGMATINE IS TRANSPORTED BY A SPECIFIC UPTAKE SYSTEM INTO RAT BRAIN SYNAPTOSOMES. M. Sastre*, S. Regunathan and D.J. Reis. Div. of Neurobiol., Dept. of Neurol. & Neurosci., Cornell University Medical College, New York, NY 10021.

Agmatine, a polyamine is synthesized by decarboxylation of arginine in rat brain (Li et al., *Science*, 1994). The facts that agmatine is stored in specific neuronal systems and is released from synaptosomes (Regunathan et al., *Soc. Neurosci. Abstr.*, 1996) suggest it has properties of a novel neurotransmitter/neuromodulator. We investigated whether neurons contain an uptake system for agmatine. The uptake of guanido ^{14}C-agmatine (4.2 μM) was measured (P_2 fractions) for 10 min at 37°C in Krebs buffer in synaptosomes prepared from rat brain. The reaction was stopped by centrifugation, pellets washed extensively and radioactivity counted. The uptake of agmatine was temperature-dependent and saturable only at high concentrations (K_m of 18.8±3.3 mM and V_{max} of 4.78±0.67 nmol/mg prot/min). Treatment with ouabain (a Na^+/K^+ ATPase inhibitor) or replacement of extracellular Na^+ did not attenuate the uptake. Agmatine uptake was not inhibited by various amino acids, polyamines or monoamines. Of various ion-channel modulators, only Ca^{2+} channel blockers inhibited whereas reduction of extracellular Ca^{2+} increased uptake. Some drugs acting at imidazoline receptors, e.g. idazoxan (Ki= 0.24 mM) and phentolamine (Ki= 0.22 mM) were strong non-competitive inhibitors of uptake. We conclude that synaptosomes can take up and concentrate agmatine by a mechanism differing from common amino acid, polyamine or monoamine transporters and might be mediated/regulated by calcium channel activity.

148.19

THE SITES OF ^{3}H-HISTIDINE UPTAKE WITHIN AN ARTHROPOD EYE RAISE QUESTIONS ABOUT THE SYNTHESIS OF HISTAMINE, THE PHOTORECEPTORS' NEUROTRANSMITTER. J.R.Morgan and A.E.Stuart*, Dept. of Physiology, Univ. of North Carolina, Chapel Hill, NC 27599-7545.

Histamine (HA), the proposed neurotransmitter at arthropod photoreceptor (PR) synapses, is synthesized from histidine (Hd) by decarboxylation. We examined the uptake of ^{3}H-Hd into barnacle PRs and surrounding glia using autoradiography and biochemistry. No cellular domain of the PRs (somata, axons, or synaptic terminals) labeled after incubation in ^{3}H-Hd (0.2, 2. or 20 µM, 15 min, 15^0, flashing light). Glia labeled intensely, however, as did non-PR axons in the ocellar nerve. To determine whether ^{3}H-Hd was converted to ^{3}H-HA in the glia and supplied to the PRs, preparations were incubated in ^{3}H-Hd (15 min), washed, and either homogenized or fixed for autoradiography 1, 5, or 24 hrs later. Even after 24 hrs, radioactivity ran with Hd and not HA in thin layer chromatography, indicating that ^{3}H-HA was not synthesized by glia. Autoradiographic label remained confined to glia and non-PR axons. Attempts to deplete the PRs of HA by stimulating release with high $[K^+]_o$ while blocking HA uptake with chlorpromazine did not change the pattern of ^{3}H-Hd label. Since the PRs do not take up ^{3}H-Hd (under our incubation conditions), the pathway by which their HA is supplied remains a puzzle. Supported by NIH grant EY03347 to AES.

148.20

Effects of Inorganic Phosphate Concentration on ATP Metabolism in Cultured Fetal Rat Cortical Neurons. M. Glinn*, B. Ni and S. M. Paul. Lilly Research Laboratories, Lilly Corp. Center, Indpls, IN 46285

Our laboratory has recently characterized saturable Na^+-dependent P_i import into cultured fetal rat cortical neurons, and shown that a substantial fraction of the P_i so accumulated is incorporated into ATP. We now report that the ATP content of cortical neurons after incubation with extracellular P_i ($[P_i]_e$) strongly correlates with the intracellular P_i level ($[P_i]_i$). Both $[P_i]_i$ and [ATP] were dependent upon $[P_i]_e$ and rose significantly after incubation with $[P_i]_e \geq 10$ µM and 80 µM respectively. Both increases were maximal above 160 µM $[P_i]_e$. In the absence of $[P_i]_e$, both [ATP] and $[P_i]_i$ declined with time. [ATP] strongly correlated with $[P_i]_i$ over time in the presence of $[P_i]_e$; however, in the absence of $[P_i]_e$, the correlation decreased in parallel with absolute metabolite levels. The ratio of [ATP]:$[P_i]_i$ also rose after incubation with $[P_i]_e$, indicating a stimulation of ATP biosynthesis which was abolished when glucose was omitted from the uptake medium. Omission of Ca^{2+}, which inhibits P_i uptake (Glinn et al., 1995), resulted in a rapid decline in [ATP] which was not ameliorated by addition of $[P_i]_e$; nevertheless, the correlation between [ATP] and $[P_i]_i$ remained strong in the presence of $[P_i]_e$ independently of the presence of external Ca^{2+}. These results demonstrate that $[P_i]_e$ effects a concentration-dependent stimulation of ATP biosynthesis which is not solely a result of increased substrate availability, and that $[P_i]_i$ correlates with [ATP] after incubation with $[P_i]_e$ independently of the presence of extracellular Ca^{2+}.

149.1

MUTATIONAL ANALYSIS OF THE VESICULAR MONOAMINE TRANSPORTERS IDENTIFIES RESIDUES THAT INFLUENCE SUBSTRATE AFFINITY AND DRUG SENSITIVITY. J.P. Finn III, D. Peter, T. Vu, and R.H. Edwards*. Departments of Neurology and Physiology, UCSF School of Medicine, San Francisco, CA 94143.

Vesicular monoamine transporters (VMATs) are required for the packaging of monoamine neurotransmitters into vesicles. Two VMATs (VMAT1 & VMAT2) have recently been cloned and show a high degree of sequence similarity (62% identity) but substantial differences in apparent substrate affinity and drug sensitivity. VMAT2 has a higher affinity for all amine transmitters, especially histamine, and a greater sensitivity to the inhibitor tetrabenazine (TBZ). Functional chimeras have identified two domains of VMAT2 responsible for these differences, one from transmembrane domain 5 (TMD5) to the beginning of TMD8 and another from the end of TMD9 through TMD12. Mutational analysis of these two domains now identifies specific residues responsible for histamine recognition and TBZ sensitivity.
(Supported by NIH)

149.2

TARGETING OF THE HUMAN VESICULAR MONOAMINE AND ACETYLCHOLINE TRANSPORTERS TO SECRETORY ORGANELLES IN RAT PC-12 CELLS
H. Varoqui and J.D. Erickson*. Sec. Mol. Neurosci., Lab. Cell Biology, NIMH, Bethesda, MD 20892.

The neuroendocrine PC-12 cell line is useful to study the molecular basis for preferential targeting of the vesicular monoamine transporter isoforms (VMAT1 and VMAT2) and the vesicular acetylcholine transporter (VAChT) to large dense core vesicles (LDCV's) and small synaptic vesicles/synaptic-like microvesicles (SLMV's), respectively (*PNAS 93, 3547*).

We have generated stable PC-12 cell lines which express the human neuronal VMAT2 isoform (hVMAT2) or human VAChT (hVAChT) and compared their subcellular distribution with that of the endogenous endocrine-specific VMAT1 isoform (rVMAT1) and VAChT (rVAChT). Expression of the human transporters in PC-12 cells could be distinguished from the rat vesicular transporter proteins using species-specific antisera. In addition, the VMAT2 isoform selectively binds ^{3}H-dihydrotetrabenazine. Homogenates from control and transfected PC-12 cells were fractionated on equilibrium sucrose density gradients and assayed by biochemical methods. In control PC-12 cells, transport of ^{3}H-5HT was predominantly in a heavy fraction which contained secretogranin, a marker of LDCV's. The binding of ^{3}H-vesamicol was found in a lighter fraction which contained synaptophysin, a marker of small synaptic vesicles, as well as in intermediate fractions (presumably endosomal). The expression of hVMAT2 and hVAChT paralleled that of the endogenous rVMAT1 and rVAChT with the preferential targeting of the VMAT isoforms to LDCV's and VAChT to SLMV's.

Chimeras between hVMAT2 and hVAChT have been constructed to identify structual domains which direct the trafficking of these proteins in PC-12 cells. The intralumenal glycosylated loop and cytoplasmic tails, poorly conserved between VMATs and VAChT, may contain targeting information. Unique restriction sites have been added to conserved regions of these proteins by site-directed mutagenesis and the chimeras tested for functional activity in a transient expression assay in CV-1 fibroblasts. Stable PC-12 cell lines expressing these chimeras have been made to determine what role the glycosylated loop or the cytoplasmic tails of these proteins play in the preferential targeting of these proteins to LDCV's or SLMV's. *(Support: NIMH Intramural Research Program)*

149.3

CHEMICAL MODIFICATION OF DOPAMINE TRANSPORTER (DAT): ROLE OF HISTIDINE RESIDUES IN COCAINE ANALOG BINDING, AP Patel*, TA Kopajtic, and MJ Kuhar. Food and Drug Administration, Office of Generic drugs, Rockville, MD 20855, NIDA/IRP, Balto., MD 21224, Yerkes Primate Center, Emory University, Atlanta GA 30322.

The rat DAT gene predicts the presence of 12 histidine residues, 5 of which are in the large second extracellular loop, one each in extracellular loops 4, 5, and 6, two in intracellular loop 4 and two in the C-terminnus tail. We tested if these residues were involved in (125-I)RTI-55 binding by treating LLC-PK1 cells that stably espress DAT with diethylpyrocarbonate (DEPC), a reagent that specifically modifies histidine residues, prior to binding studies.

DEPC inhibited DAT ligand binding in a time- and concentration-dependent fashion. Half maximal inhibition occurred with 3 mM DEPC at room temperature after a 5 min treatment, and >90% inhibition occurred after 60 min. The inhibition was dependent on pH of the media, and slightly greater inhibition occurred at pH 7.4 than at 6.0. These results suggest that histidine residues are part of the ligand binding domain of DAT. Supported by NIDA/IRP.

149.4

DOPAMINE TRANSPORTERS WITH TRANSMEMBRANE DOMAIN PHENYLALANINE MUTATIONS. Z. Lin , R. Revay, T. Kopajtic, & G.R. Uhl* Molec. Neurobiol., NIDA IRP & Dept. Neurol. & Neurosci., JHUSM, Baltimore, MD 21224

Dopamine transporter (DAT) recognition of cocaine, cocaine analogs such as carboxyflurotropane (CFT) and dopamine may involve hydrophobic interactions. Aromatic DAT transmembrane domain (TM) residues could play roles in DAT recognition of catecholamine catechol groups, cocaine phenyl groups, and the halogenated phenyl group of CFT. They could also orient DAT TMs, helping to direct the correct amino acids toward ligand- and substrate-recognizing pockets. To address possible roles played by TM aromatic residues in such interactions, we have constructed DATs with alanine (A) substitutions for more than 10 of the 26 phenylalanines (F) lying in postulated TMs. TM 1 and 2 F-A substitutions reduced dopamine uptake and [^{3}H]-CFT binding. In transiently-expressing COS cells, these receptor mutants as well as mutants in TM domains yielded perinuclear DAT immunoreactivity indicating abnormal cellular trafficking of even these single amino acid mutants. Mutants in TMs 4, 7, 8, and 11, on the other hand, produced nearly-normal patterns of cellular expression. These studies elucidate contributions of aromatic amino acids to the correct DAT folding necessary for plasma membrane targeting, and may also identify residues essential for cocaine recognition and/or dopamine uptake.

149.5

DETERMINATION OF NOREPINEPHRINE TRANSPORTER AMINO-ACIDS THAT ARE INVOLVED IN TRICYCLIC ANTIDEPRESSANT BINDING. C. Roubert, M. Hamon* and B. Giros. INSERM U-288, 91, Bd. de l'Hôpital, 75013, Paris, France.

Transporters for biogenic amines regulate the concentration of neurotransmitters in the synaptic space by a process of rapid and efficient reuptake. Therefore, blockade of the uptake mechanisms will potentiate the nerve transmission, with profound *in vivo* consequences. Widely used in the clinic, tricyclic antidepressants (TA) are specific blockers of the norepinephrine- and serotonin-transporters (NET and 5-HTT), but they have no action at the dopamine transporter (DAT). Taking advantage of this observation, and on the recent molecular cloning of these transporters, we constructed a series of chimeric proteins between NET and DAT, which allowed to localize the binding domain of TA to transmembrane domains (TM) 6 to 8 of the NET (Giros et al., *J. Biol. Chem.*, 1994, 23, 15985-15988). In order to have more precise and relevant informations of the structure-activity relationships of the TA binding sites on NET, we have constructed a set of mutated NET, using a PCR-aided mutagenesis strategy. All the amino-acids contained in TM domains 6 to 8 of NET were systematically mutated toward their counterpart of the DAT. The 10 mutants are : Mut1 (Ser288>Ile, Asn289>Asp, Asn 292>Arg), Mut2 (His296>Ser), Mut3 (Lys303>Cys), Mut4 (Phe316>Cys), Mut5 (Asp336>Thr), Mut6 (His370>Gln, Glu371>Lys, Lys372>Ser, Asn374>Pro), Mut7 (Glu376>Gly, Glu381>Asp, Ala383>Pro), Mut8 (Ser395>Ala, Ser398>Pro, Gly399>Leu), Mut9 (Phe402>Ala) and Mut10 (Ser419>Ala). All these mutants will be transiently expressed in LLCPK cells, and their pharmacological profile will be compared with those of the native DAT and NET. As a consequence of this study, we should be able to determine which amino-acid in the NET are important for high affinity binding of the TA.

149.6

THE FUNCTIONAL EFFECT OF THE SECOND TRANSMEMBRANE LEUCINE ZIPPER MOTIF OF THE DOPAMINE TRANSPORTER.
F. R. Sallee, N. S. Vrindavanam, M. George*, S. A. Hamamdzic, J. X. Ma. Dept of Psychiatry, MUSC, Charleston, SC 29425

The dopamine transporter [DAT] is a dopamine reuptake protein found exclusively on dopaminergic neurons. The DAT cDNA has been cloned, expressed and studied. Potential regulatory regions identified in sequence data of the DAT include two leucine zipper motifs, one in the second transmembrane region and the other in the ninth transmembrane region. The third leucine of the second transmembrane region was mutated to an alanine. This mutant form was then cloned into an eukaryotic expression vector pcDNA3 and expressed in COS-7 cells. Expression of the mutant form was confirmed by Northern and Western blots. Radiolabelled substrate uptake and ligand binding assays were performed on these cells and compared to non mutated forms. The mutation had a differential effect on dopamine [DA] uptake and WIN 35,428 binding. It is noted that the mutation changes the functional characteristics of the transporter by making it at least thirty percent more efficient in substrate uptake and decreasing ligand binding by approximately the same margin. The results suggest that the leucine zipper mutation makes the transporter more efficient for substrate transport and decreases WIN 35,428 binding. This suggests that the second transmembrane leucine zipper motif may play a significant role in the functional regulation of the transporter involving substrate translocation and also in ligand binding. Source of funding: NIDA Grant# 5-RO1-DA06881

149.7

TYROSINE-533 IS CRITICAL FOR THE FUNCTION OF THE RAT DOPAMINE TRANSPORTER. S. Kitayama*, C. Mitsuhata, G.R. Uhl# and T. Dohi. Dept. of Pharmacology, Hiroshima Univ. Sch. of Dent., Hiroshima 734, Japan, #Mol. Neurobiol. Branch, ARC/NIDA/NIH, Baltimore, MD21224.

Sodium- and chloride-coupled dopamine transporters (DAT) located in the plasma membrane of dopaminergic nerve terminals act to terminate synaptic transmission by reaccumulating released dopamine (DA). We have investigated the role of polar amino acids located in putative transmembrane regions (TM). Using site-directed mutagenesis we have demonstrated that serines and tyrosine located in 11th TM are involved in substrate discrimination [S. Kitayama et al., Synapse 15, 58-62(1993)]. In this region, the rat DAT contains tyrosine-533 while corresponding amino acid in human is phenylalanine. Since transport activities of the human and rat DATs and their sensitivities to various substances including drugs of abuse differ subtly, we have investigated the role of tyrosine-533 of rat DAT in substrate transport and in sensitivity to drugs including cocaine. Tyrosine-533 replacement by alanine caused a marked increase in ability to transport DA and 1-methyl-4-phenylpyridinium (MPP^+), a Parkinsonism-inducing neurotoxin. Cocaine's potency in inhibiting DA uptake was unchanged. Replacement by phenylalanine yielded small increases in DA and MPP^+ uptake, but increased cocaine's potency in inhibiting DA uptake. Expression levels and targeting to the plasma membrane of the mutant DATs appeared normal, as indicated by unchanged Bmax values for binding [^{3}H]CFT, a cocaine analogue, to expressing cells. Tyrosine-533 is critical for the DAT function , and may be implicated in species differencies of transporter functions including differential sensitivities to cocaine in human and rat.

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149.8

CHARACTERIZATION OF DRUGS WHICH DISCRIMINATE BETWEEN UPTAKE AND LIGAND BINDING SITES AT THE DOPAMINE TRANSPORTER PROTEIN

Carol W. Tiffany, Jennifer L. Olkowski, Kevin L. Tays, Keith M. Maclin, Paul F. Jackson, and Barbara S. Slusher. Guilford Pharmaceuticals, Inc. 6611 Tributary Street, Baltimore, MD 21224*

Dopamine uptake and cocaine binding occur at distinct sites on the dopamine transporter protein (DAT), making it possible to design drugs which specifically inhibit cocaine recognition by the DAT while permitting the transporter to maintain its function of dopamine accumulation. To explore this possibility, we analyzed the potencies of several drug classes in inhibiting [^{3}H]dopamine uptake (Ki_{uptake}) and [^{3}H]CFT binding (Ki_{bind}) in CHO cells stably expressing the human DAT. Because others have shown that Ki_{uptake}/Ki_{bind} ratios different than unity can result when the assays are conducted under different conditions, we conducted the two assays under identical conditions of time, buffer and temperature. Using these assay parameters, we identified several series of compounds having Ki_{uptake}/Ki_{bind} significantly greater than unity including local anesthetics (procaine, dibucaine, tetracaine, dyclonine, oxethazine, diperodone, lorcainide), antipsychotic agents (desipramine, imipramine), methylphenidate, and diuretics. Under these assay parameters, GBR 12909 was found to have a Ki_{uptake}/Ki_{bind} ratio of two. By chemically dissecting GBR 12909 into distinct regions, we have determined which moieties are important for binding at the cocaine site, and which were important for dopamine uptake inhibition. Several novel derivatives of this compound were found to be more selective than GBR 12909 at binding to the cocaine site when compared to their ability to inhibit dopamine uptake. The utility of compounds with Ki_{uptake}/Ki_{bind} ratios greater than unity are currently being explored in animal models of addiction.

149.9

MUTUALLY EXCLUSIVE BINDING DOMAIN FOR WIN 35,428 AND MAZINDOL ON THE CLONED HUMAN DOPAMINE TRANSPORTER. C. Xu and M.E.A. Reith, Dept. of Biol., Illinois State Univ., Normal, IL 61790; Dept. of Biomedical and Therapeutic Sci. Univ. of Illinois Coll. Med., Peoria, IL 61656.

It has been suggested that cocaine and mazindol bind to separate sites on the dopamine transporter. The present study addresses this issue by examining the inhibition by mazindol of the binding of the cocaine analog [^{3}H]WIN 35,428 ([^{3}H]CFT), and the inhibition by CFT of [^{3}H]mazindol binding to membrane preparations of the cloned human dopamine transporter expressed in C6 glioma cells. Inhibition curves were constructed at 6 widely spaced radioligand concentrations, enabling the distinction between the nonlinear hyperbolic competition (i.e., negative allosteric) model and the apparent competitive (i.e., mutually exclusive binding) model, as opposed to the common approach using a single inhibitor/radioligand concentration where negative allosteric interactions can masquerade as simple competitive inhibition. Nonlinear computer curve fitting analysis indicated that the data were described equally well by the hyperbolic model with an allosteric constant β of ~ 100 or greater and the mutually exclusive model ($F_{1,33-39} < 1.1$). Among data sets, there was a strong correlation between equilibrium dissociation constants fitted by the allosteric model and those fitted by the mutually exclusive model ($r=0.999$, $P < 0.00001$), and the average equilibrium dissociation constant was 13 nM for [^{3}H]CFT and 7 nM for [^{3}H]mazindol. The present results show that when negative allosterism becomes extreme (β of 100 or more), it is indistinguishable from mutually exclusive binding ($\beta = \infty$). Complexities in cocaine/mazindol interaction are more likely to be found at the level of substrate translocation and transporter reorientation than at the level of recognition.

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149.10

DOPAMINE TRANSPORTER (DAT) LIGANDS: N-SUBSTITUTED PHENYLTROPANES (NSPTs) AS LIGANDs FOR RAPID PET/SPECT STUDIES, T Kopajtic*, U Scheffel, J Lever, P Abraham, K Parham, WB Mathews, FI Carroll, and MJ Kuhar. NIDA/IRP, The Johns Hopkins Univ, Balto MD, Research Triangle Institute, Raleigh-Durham NC, and Yerkes Primate Cent, Emory Univ, Atlanta GA.

Phenyltropanes are superb binding ligands for DAT. In this study, we examine NSPT deriviatives of RTI-55 as in vivo binding ligands. The methyl group on the nitrogen was subtituted with propyl (RTI-310), allyl (RTI-311), butyl (RTI-312) or fluoropropyl (RTI-313) groups. In vitro binding revealed nanomolar potency at DAT with reasonable high potency at 5HT and NE transporters. Maximal in vivo Striat./cerebel. ratios in mice were about 5 for RTI-311, RTI-312 and RTI-313, and about 6.4 for RTI-310. The ratios peaked most rapidly for RTI-311 and RTI-313. Pharmacological studies indicated that these compounds were binding to DATs. These findings suggest that NSPTs may be excellent in vivo binding ligands for rapid PET/SPECT studies. Supported by NIDA/IRP, Guilford Pharm, DA06309, and DA08870.

149.11

INDUCED EFFLUX AT THE NOREPINEPHRINE TRANSPORTER BY TYRAMINES AND PHENYLETHYLAMINES K.S.Danek*, J.W.Kable, J.B.Justice,Jr. Department of Chemistry, Emory University, Atlanta , GA 30322.

Rotating Disk Electrode Voltammetry (RDEV) was utilized to measure the transport of catecholamines and other nonelectroactive substrates in suspensions of LLC-NET cells. Dopamine (DA) (1μM) or dihydroxybenzylamine (DHBA) (1μM) was initially added to a suspension of cells and the clearance of the substrate from solution was monitored by a glassy carbon electrode at an applied potential of +450mV vs. Ag/AgCl. Subsequent addition of a second nonelectroactive substrate induced immediate efflux of DA or DHBA. The data were expressed as the ratio of initial rate of efflux to initial rate of uptake. β-Phenylethylamine (10μM) induced efflux of DA at 45% of the initial rate of DA uptake (n=8). However, the D-and L-α-Phenylethylamines produced almost no DA efflux. β-Phenylethylamine induced efflux of DHBA at only 13% of its initial uptake rate (n=7). These results indicate that shortening the side chain length reduces transport. D-and L-Amphetamine had DA efflux/uptake ratios of 22% and 45%, respectively (n=6). Meta-Tyramine (10μM) induced efflux of DA at 19% of the uptake rate (n=4) whereas para-Tyramine was slower (13%) (n=3) at this and 1μM and 100μM added concentrations. This suggests that the presence of the hydroxy group on the phenyl ring also causes a decrease in transport. The distance between the amine and phenyl functionalities appears to be more influential than the hydroxy ring substituent in the transport process.

149.12

SYNTHESIS AND EVALUATION OF METHYLPHENIDATE ANALOGS AS POTENTIAL TREATMENT AGENTS FOR COCAINE ABUSE. M.M.Schweri*, S.G.Holtzman#, Q.Shi, Z.Liu, and H.M.Deutsch. *Mercer Univ. Sch. Medicine, Macon, GA 31207; #Emory Univ. Sch. Medicine, Atlanta, GA 30322; Sch. Chem. Biochem., GA Tech, Atlanta, GA 30332-0400.

Cocaine (CC) is thought to exert its reinforcing effects by inhibiting carrier-mediated neuronal re-uptake of dopamine (DA). Derivatives of the stimulant drug *threo*-(±)-methylphenidate (MP) containing modifications of the piperidine, ester, and/or aromatic ring functions were synthesized as part of a program aimed at developing a site-directed antagonist of CC which will block the binding of CC to the DA transporter, yet spare DA uptake. The potency of the test compounds to inhibit synaptosomal [^{3}H]DA uptake and [^{3}H]WIN 35,428 binding to the CC receptor were determined using rat striatal tissue. Conversion of the aromatic ring to a napthyl ring increased potency in both assays. Replacement of the ester function by -CH_2OH, -CH_2OCOCH_3, or -$CONH_2$ caused parallel 6- to 20-fold losses in potency against both uptake and binding. While all of the N-methyl substituted compounds also underwent absolute losses in potency, the relative potency of N-Me, *p*-Me MP to inhibit [^{3}H]WIN 35,428 binding increased to more than x 7 its potency to inhibit [^{3}H]DA uptake.

Most of the MP analogs tested were found to substitute for CC in rat drug discrimination studies, although N-Me,p-Me MP exhibited a unique biphasic dose-response curve. Due largely to the differential effect of N-Me,*p*-Me MP on binding and uptake, the ED_{50}'s of the compounds for CC discrimination correlated better with their potency to block [^{3}H]DA uptake than with their potency to block [^{3}H]WIN 35,428 binding.

This data suggest that synaptic DA contributes to the discriminatory properties of CC in the rat, and that it is feasible to synthesize a CC antagonist which can block CC binding and spare DA uptake.

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149.13

TRIHEXYPHENIDYL: A DOPAMINE-SPARING COCAINE ANTAGONIST WITH SPECIFIC STRUCTURE-FUNCTION REQUIREMENTS D.E. Dar@*, S. Kitayama$, P. Abraham#, M. Thiruvazhi#, F.I. Carroll#, T.A. Kopajtic@, T. Dohi$, & G.R. Uhl@. @Molec. Neurobiol., NIDA-IRP, NIH; #Dept. Neurol. & Neurosci., JHUSM, Balto., MD 21224, #RTI, Research Triangle Pk, NC 27709-2194, & $Dept of Pharm., Hiroshima Univ. Sch. Dent., Hiroshima, Japan

Mutagenesis of the dopamine transporter (DAT), the principle site for cocaine reward and euphoria, provides evidence that sites for cocaine and dopamine may overlap but may not be identical. Candidate compounds that might block cocaine, minimally interfere with dopamine transport, and thus provide useful cocaine antagonism were selected based on DAT modeling and tested for potencies in blocking dopamine uptake and inhibiting binding of the cocaine analog carboxyflurotropane (CFT). Trihexyphenidyl (THP) displayed dopamine transport sparing cocaine antagonism with up to 7-10-fold greater potency in CFT binding inhibition than in uptake blockade. Structure-activity studies reveal THP features important for its differential potencies. Both substitutions for the THP piperidine ring, including replacement with diethylamine, and halogenation of the THP phenyl enhance potency in dopamine uptake inhibition and thus reduce the compounds' selectivities for blocking CFT binding in rat striatal and/or expressed recombinant human DATs. Phenyl substitution for THP's cyclohexyl ring, conversely, exerts modest effects on potency ratios. The prototype dopamine sparing cocaine antagonist, THP highlights the strict structural requirements at several positions likely to be required of a pharmacologically-useful cocaine antagonist.

149.14

KINETIC ANALYSES OF STRIATAL DOPAMINERGIC TRANSPORT *IN VITRO*: LINKAGE TO METABOLISM AND VESICULAR SEQUESTRATION. Jennifer J. Parish and James O. Schenk*. Depts. of Biochemistry/Biophysics and Chemistry, Programs in Pharmacology/Toxicology and Neuroscience, Washington State Univ., Pullman, WA 99164.

The membrane dopamine transporter functions to take up dopamine (DA) into the cytosolic compartment of a dopaminergic neuron following neurotransmission. Once inside the cell, dopamine can be packaged into vesicles, remain in the cytoplasm or become metabolized. We suspected that the rate at which dopamine is transported into the cell may be related to these intracellular processes. Specifically, this research focused on the kinetic relationship of dopamine uptake to: 1) transport into synaptic vesicles or 2) metabolism to 3,4-dihydroxyphenylacetic acid (DOPAC) by monoamine oxidase. These data indicate that these two intracellular processes did not affect initial rate measurements (<30 s) in apparent *zero trans* entry experiments, but did affect the kinetics of dopamine reuptake examined during longer time intervals (30s<t<200s) when intracellular dopamine was allowed to accumulate in *low to infinite trans* experiments. In the latter experiments removal of the synaptic vesicles, as well as inhibiting vesicular sequestration, caused an increase in the K_m and V_{max} of the transporter. Inhibition of monoamine oxidase caused a decrease in the K_m and V_{max} of the transporter. Thus, under certain conditions these two cytosolar biochemical processes were found to be linked to the kinetic activity of the transporter and may alter the magnitude and timing of chemical neurotransmission. (Supported by National Institutes on Drug Abuse (NIDA) grant, DA07384. J.O.S. is also the recipient of a NIDA Research Scientist Development Award, KO2 DA00184 and J.J.P. was supported by the Howard Hughes Foundation and a Barry Goldwater Fellowship.)

149.15

INVOLVEMENT OF CATECHOL SUBSTRATE RELATED PROTON TRANSFER REACTIONS IN THE STRIATAL TRANSPORT OF DOPAMINE. Tamera Stobb*, Martin T. Morocco, and James O. Schenk. Depts. of Chemistry and Biochemistry/Biophysics, Programs in Pharmacology/Toxicology and Neuroscience, Washington State University, Pullman, WA 99164.

Deuterium oxide solvent isotope effects on the transport velocities of a series of catechol derivatives (dopamine, 4-ethylcatechol, and 3-methoxytyramine) were measured by rotating disk electrode voltammetry to determine that a rate limiting step in the dopamine transporter-mediated translocation involved a catechol proton transfer. Acid-base titrimetry with linear potential sweep voltammetry and glass electrode potentiometry was also conducted to assess the relative pKa values of catechol- and amine-derived protons on dopamine. The results suggest that the catechol protons of dopamine are at least as strong as the ammonium protons on the side chain and when looked at in the absence of side chain basic functionality may be stronger by about 0.8 pKa units than the ammonium form of the ethylamine. These results lend credence to the possibility that catechol proton transfer is an important mechanistic feature of recognition and translocation of dopamine by the striatal transporter for dopamine. (Supported by National Institutes on Drug Abuse (NIDA) grant, DA07384. J.O.S. is also the recipient of a NIDA Research Scientist Development Award, KO2 DA00184.)

149.16

MEASUREMENTS OF DOPAMINERGIC TRANSPORTER-MEDIATED OUTWARDLY-DIRECTED TRANSPORT OF DOPAMINE (DA) BY PROGRAMMED ROTATION RATE AT THE ROTATING DISK ELECTRODE. Cynthia Earles* and James O. Schenk. Depts. of Chemistry and Biochemistry/Biophysics, Programs in Pharmacology./Toxicology and Neuroscience, Washington St. Univ., Pullman, WA 99164.

The outward function of the DA transporter in reserpine- and pargyline-treated striatal tissue from the rat *in vitro* was investigated by measuring the current responses for the oxidation of DA as a function of rotation rate at a glassy carbon rotating disk electrode. The release of DA via the transporter and its subsequent electrochemical detection is analogous to a chemical reaction - electron transfer (CE) kinetic model in electroanalytical chemistry. Data was found to fit the CE mechanism and a psuedo-first order rate constant of dopamine release was measured. Amphetamine, a drug known to inhibit DA uptake as a substrate analog, increased the outwardly-directed transporter rate constant in a concentration dependent manner. In contrast, cocaine, a compound not thought to be a substrate analog, had no effect on the outwardly directed transporter rate constant. The results of these experiments suggest that: (a) the apparent outward rate constant of the dopamine transporter can be kinetically resolved and measured and (b) the differing mechanisms of action of two different drugs of abuse can be observed. (Supported by National Institute on Drug Abuse grants, DA07384 and KO 2 DA07384, to J.O.S.).

149.17

RELATIONSHIPS BETWEEN CATECHOL SUBSTRATE BINDING SITE AND AMPHETAMINE (AMP) AND COCAINE (COC) BINDING SITES IN A KINETIC MODEL OF THE STRIATAL TRANSPORT OF DOPAMINE (DA). Hollie. K. Wayment* and James O. Schenk. Depts of Chem. and Biochem./Biophys., Washington State Univ., Pullman, WA 99164.

Previously this laboratory (*J. Neurochem.* **63**: 1683-1692, 1994) described results of kinetic studies designed to test whether a number of inhibitors of DA transport bind at the same site as COC on the striatal DA transporter. Experiments were not conducted to determine how COC or S(+)-AMP binding sites relate to each other and the catechol substrate site mediating translocation of DA by the transporter. Here we address this question by showing that *m*-tyramine (TYR) and S(+)-AMP act as substrate analogs for DA by competitively inhibiting inwardly-directed, striatal DA transport as measured by rotating disk voltammetry. Both compounds caused release of DA from intracellular stores at concentrations ≥ ten-fold larger than those observed to inhibit inwardly-directed transport of DA from the extracellular compartment. In separate studies it was found that S(+)-AMP and *m*-TYR inhibited the transport of DA by competing for a common binding site whereas COC was found to inhibit the transport of DA by binding to a site separate from that of the substrate analogs. However, binding sites of COC and substrate analogs were found to be mutually interactive with an equilibrium constant in the μM range suggesting that the COC binding site on the DA transporter is distinct from that of hydroxyphenethylamines. These results suggest that an antagonist for COC binding may be useful in blocking COC binding while minimizing inhibition of DA transport. This may be accomplished by developing agents which maximize the negative interaction between inhibitor binding sites and minimize the action at the catechol substrate site. However, it leaves unresolved the question of how the action of the AMP's and other drugs of abuse that act as substrate analogs may be antagonized. (Supported by NIDA grants, DA07384 and DA00184, to J.O.S.)

149.18

A THERMODYNAMIC STUDY OF THE DOPAMINE TRANSPORTER ION-COUPLING MECHANISM. H. H. Gu* and G. Rudnick. Department of Pharmacology, Yale University School of Medicine, New Haven, CT 06510

We previously described the stable expression of dopamine (DA), norepinephrine, serotonin, and gamma-aminobutyric acid (GABA) transporters (DAT, NET, SERT and GAT, respectively) in LLC-PK_1 cells and determined the ion coupling stoichiometry for NET in plasma membrane vesicles from those cells using SERT and GAT as internal controls. A preparation of plasma membrane vesicles from MDCK cells expressing DAT now allows us to employ the same approach to study the transport mechanism for DAT. DA accumulation into DAT vesicles was stimulated by inward Na^+ and Cl^- gradients and required external Na^+ and Cl^-, suggesting that these ions are co-transported with DA. An outward K^+ gradient did not stimulate transport, and the complete removal of K^+ from both sides of the vesicle membrane had little effect on DA accumulation, suggesting that K^+ is not involved in DAT-mediated transport. Addition of valinomycin to vesicles with an outward K^+ gradient resulted in a 5 to 6 fold increase of substrate accumulation into DAT vesicles, compared to a 1 to 2 fold increase in GAT and NET vesicles. This result suggests that DAT transport is electrogenic and that at least two charges are moved across the membrane in each transport cycle (GAT and NET each transports a single charge per cycle). The Na^+ gradient dependency of DAT was more similar to that of GAT than to that of NET, suggesting a Na^+:DA ratio of two. In addition, the K_M for DAT transport was not significantly affected by changing external pH from 6.5 to 7.5 (increasing the neutral form of DA 10-fold), suggesting that cationic DA was the predominant substrate. Therefore, we conclude that DAT co-transports one DA^+ molecule with one Cl^- and at least two Na^+ ions, that K^+ is not involved, and that two (or more) net positive charges are transported with each molecule of DA.

This work is supported by a grant from NIDA.

150.1

TRANSLOCATION OF γ-SUBTYPE OF PROTEIN KINASE C -DIRECT VISUALIZATION IN LIVING CELLS USING FUSION PROTEIN WITH GREEN FLUORESCENT PROTEIN. N.Sakai*, S. Sumioka, C. Hasegawa, K. Sasaki , H. Obata and N. Saito. Lab. of Mol. Pharmacol., Biosignal Res. Ctr., Kobe Univ., Kobe 657, Japan

The protein kinase C-γ(PKC-γ), a neuron specific subtype of PKC, is known to be translocated from cytosol to membrane by various stimulus including phorbol ester and Ca^{2+}. The green fluorescent protein (GFP), isolated from jellyfish *Aequorea victoria*, have fluorescent activity without additional substrates and co-factors. To directly observe the translocation of PKC-γ in living cells, a cDNA, encoding a GFP sequence following the 3' end of the PKC-γ, was constructed using PCR, subcloned into expression plasmids and PKC-γ-GFP fusion protein was expressed in various cell lines. The significant fluorescence of PKC-γ-GFP fusion protein was observed in the cytosol of cells using confocal lazar scanning fluorescent microscope. The treatment of 5 μM TPA produced the translocation of PKC-γ-GFP fusion protein to plasma membrane within 30 min. A23187, a Ca^{2+} ionophore, first translocated PKC-γ-GFP to membrane more rapidly than TPA and finally patchy signals were observed in both membrane and perikarya.These findings revealed the diverse mechanism of the translocaion of PKC in the difference of activators.

150.2

THE TARGET OF PKC INDUCED SUPPRESSION OF ADENOSINE INHIBITION OF GLUTAMATE EXOCYTOSIS FROM SYNAPTOSOMES IS DOWNSTREAM OF THE A1 RECEPTOR AND G-PROTEIN COUPLING. M.McLaughlin, D.C. Budd and D.G. Nicholls*. Neurosciences Institute, Dept. of Pharmacology, Ninewells Medical School, Dundee, DD1 9SY, UK

Glutamate exocytosis from isolated cortical terminals from rat brain (synaptosomes) can be inhibited by the activation of the adenosine A1 receptor. The inhibitory action is mediated by decreasing the influx of Ca^{2+} after clamped depolarization with high K^+, suggesting a Ca^{2+} channel locus. Adenosine inhibition can be suppressed by activation of PKC with phorbol esters and also with the physiological activator diacylglycerol produced by metabotropic receptor activation. This study explored the mechanism(s) by which suppression of the inhibitory pathway by PKC is/are achieved.

Synaptosomes were prepared and aliquots used to confirm phorbol dibutyrate (PDBu) induced suppression of adenosine A1 inhibition of glutamate exocytosis. The remaining synaptosomes were incubated with vehicle (control) or PDBu and the membranes and cytosol fractionated. Western blot analysis of various PKC isoforms demonstrated a positive translocation of the PKC(s) from the cytosol to the membrane in PDBu treated samples. PDBu treatment however failed to alter basal or A1 agonist induced stimulation of GTPgammaS binding and GTPase activity associated with heterotrimeric G-proteins.

These findings suggest that the A1 receptor coupling to the G-protein and the G-protein activation/deactivation cycle are not compromised by kinase action. Thus, the locus of PKC action appears to be downstream of heterotrimeric G-proteins.

(This work was supported by the MRC and an EU network grant)

150.3

EFFECTS OF ESTRADIOL ON PROTEIN KINASE C ACTIVITY IN FEMALE RAT BRAIN. M. A. Ansonoff* and A. M. Etgen. Depts. Neurosci. & Psychiat., Albert Einstein Coll. Med., Bronx, NY 10461

Previously we demonstrated that α_{1b}-adrenergic receptor mRNA is elevated in hypothalamic, but not cortical tissue of ovariectomized, estrogen-treated female rats (J. Neuroendocrinol, in press). Protein kinase C (PKC) has been shown to affect α_{1b}-adrenergic receptor mRNA levels (JBC 268:3610-3615, 1993) and to be estrogen-regulated in some estrogen-sensitive tissues (Lab. Inv. 68:472-480, 1993). Because PKC may be important in regulation of the α_{1b}-adrenergic receptor mRNA, PKC activity was measured in hypothalamic and cortical extracts. This was accomplished by quantifying ^{32}P incorporation into a synthetic peptide substrate containing the optimal consensus sequence for PKC-mediated phosphorylation. PKC activity increased approximately two-fold in the hypothalamus of ovariectomized female rats treated with 2 μg of estradiol benzoate (EB) at 24 and 48 hr prior to sacrifice in comparison to ovariectomized female rats treated with peanut oil ($n = 5$, $p < 0.01$). In cortical tissue, PKC activity was much greater than in hypothalamus, and EB treatment did not affect PKC activity ($n = 5$, $p > 0.10$). Estrogen enhancement of PKC activity may thus influence α_{1b}-adrenergic receptor mRNA levels in female rat hypothalamus. Future studies will determine if this enhancement of PKC activity is due to an increase in the amount of PKC enzyme or an enhancement of enzyme activity. Furthermore, it must be determined if estrogen's enhancement of PKC activity is isoform-specific.

Supported by grants MH 41414, RSDA MH 00636 and T32 AG00194

150.4

EFFECT OF CHRONIC TREATMENT WITH DEXAMETHASONE ON [^{3}H]PHORBOL DIBUTYRATE BINDING AND EXPRESSION OF PROTEIN KINASE C ISOZYMES IN THE RAT CORTEX. Y. Dwivedi* and G.N. Pandey. Psychiatric Institute, Department of Psychiatry, University of Illinois at Chicago, Chicago, IL 60612

The mechanisms of the upregulation of serotonin ($5HT)_{2A}$ receptors in the postmortem brain of suicide victims and in platelets of depressed patients are unclear. Since dysregulation of functions of the hypothalamic-pituitary-adrenal (HPA) axis has been observed in depressed and/or suicidal patients, it is possible that increased levels of cortisol may cause the upregulation of $5HT_{2A}$ receptors. Another possible mechanism may be increased phosphorylation of $5HT_{2A}$ receptors by protein kinase C (PKC). In an earlier study, we observed that corticosterone or dexamethasone (DEX) treatment caused a significant increase in $5HT_{2A}$ receptors in the rat brain. To further investigate the mechanism of upregulation of $5HT_{2A}$ receptors, we studied the effects of DEX on [^{3}H]PDBU binding and the expression of PKC isozymes (α, β, γ, δ, and ε) in membranal and cytosolic fractions of the rat cortex. Male Sprague-Dawley rats were treated with DEX (1.5 mg/kg) or vehicle subcutaneously for 10 days. The animals were sacrificed on the 10th day of injection. [^{3}H]PDBU binding was determined by the radioligand binding technique, whereas expression of PKC isozymes was determined by the Western blot technique. We observed that DEX treatment caused a significant increase in B_{max} of [^{3}H]PDBU binding to cytosolic PKC but had no effect on membranal PKC. Immunolabeling results showed that the increase in cytosolic PKC is isozyme specific. DEX treatment caused a significant increase in levels of only the γ and the ε isozyme in the cytosolic fraction but had no effect on the expression of PKC isozymes in the membranal fraction. These results thus suggest that PKC is involved in the mechanism of upregulation of $5HT_{2A}$ receptors and plays an important role in depression and suicidal behavior.

150.5

BIOCHEMICAL AND BEHAVIORAL CORRELATES OF CHRONIC CONSUMPTION OF LOW DOSES ETHANOL: FOCUS ON PROTEIN KINASE C ACTIVITIES. A. Pascale, M. Persichella§, F. Battaini°*, V. Cuomo§ and S. Govoni#. Inst. of Pharmacol. Sci., Univ. of Milano; §Inst. of Pharmacology, Univ. of Bari; °Dept. Exptl. Med. and Biochem. Sci., Univ. of Roma Tor Vergata; #Inst. of Pharmacology, Univ. of Pavia, Italy.

Several studies have been focused on detecting biochemical and physiological alterations produced by ethanol. Multiple receptor functions and signalling pathways are modulated by this substance, among these, the PLC-mediated hydrolysis of phosphoinositides seems to be particularly sensitive to ethanol. Along this line the related protein kinase C (PKC) system has been reported to be affected at brain level by chronic ethanol intake. We have previously demonstrated that chronic low doses ethanol (3% v/v in drinking water for two months) does not induce tolerance or dependence. The lack of toxicity is indicated by no changes in body weight and other vital parameters (bilirubin, γ-glutamil transferase, serum transaminases). In addition treated rats perform better than controls in a two-way avoidance task [*Govoni et al., Alcohol, 11: 241-246, 1994*]. Few data are available on the neurobehavioral and neurochemical consequences of chronic consumption of low doses of ethanol. The aim of the present study was to explore the effect of this treatment on both calcium-dependent and -independent PKC activities in rat cortex and hippocampus. We also investigated whether ultrasonic calls (UCs), a significant parameter for evaluating emotional and motivational states of the animal, correlate with PKC activities. The results indicate that ethanol exposure produces a reduced vocalization, an effect reminescent of that of benzodiazepines, and a decrease in PKC activities both in the cortex and in the hippocampus. In addition, only at cortical level, in ethanol-treated rats we observed a correlation between calcium-dependent activity and UCs. Our data suggest that low doses ethanol affect PKC activity and that this enzyme may be an important target of the ethanol action in modulating emotional and motivational behaviours.

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150.6

PB^{2+} BOTH ACTIVATES AND INHIBITS PROTEIN KINASE C PURIFIED FROM RAT BRAIN AND IN NEURONAL CELL CULTURE. W.R. Mundy*, T.J. Shafer and T.R. Ward. Neurotoxicology Division, NHEERL, U.S. EPA, RTP, NC 27711.

Environmental exposure to the toxic metal Pb^{2+} has been identified as a major hazard to the health of children in industrialized nations. Although Pb^{2+} is a well-recognized neurotoxin which can produce neurobehavioral deficits at blood levels as low as 10 μg/dl (0.5×10^{-6} M), the molecular target(s) is unclear. Identifying these targets is essential for the development of biologically based dose-response (BBDR) models. Several lines of evidence suggest that Pb^{2+} neurotoxicity may result from interference with intracellular signaling systems which depend on Ca^{2+}. Pb^{2+} has been reported to either activate or inhibit the calcium- and phospholipid-dependent enzyme protein kinase C (PKC) in a number of different systems. We examined the effects of a wide range of Pb^{2+} concentrations on highly purified (>90%) PKC from rat brain. Free Pb^{2+} concentrations of 10^{-13} to 10^{-6} M activated PKC ($K_m = 1.7 \times 10^{-12}$ M, maximal activity = 295 nmol/mg protein/min) as compared to activation by free Ca^{2+} concentrations of 10^{-8} to 10^{-4} M ($K_m = 6.3 \times 10^{-7}$ M, maximal activity = 475 nmol/mg protein/min). Concentrations of free Pb^{2+} greater than 10^{-6} M inhibited PKC activity. Ca^{2+}-stimulated (5×10^{-4} M) PKC activity was also inhibited by Pb^{2+} at concentrations of 10^{-7} to 10^{-4} M ($IC_{50} = 1.5 \times 10^{-6}$ M). In rat primary cortical cell cultures the binding of [^{3}H]phorbol ester was used to estimate the translocation of PKC from the cytosol to the membrane *in situ*. Pb^{2+} (1-10 μM total concentration) stimulated phorbol ester binding in both the absence and presence of Ca^{2+} in the media. When phorbol ester binding was stimulated by ionomycin, Pb^{2+} inhibited this binding with an IC_{50} of 1.3 μM. These results suggest that low concentrations of Pb^{2+} can interfere with PKC activity both *in vitro* and in cell culture.

150.7

THE PROTEIN KINASE C PATHWAY IS ENDOGENOUSLY ACTIVE IN EXPIRATORY NEURONS OF CATS A. Haji, O. Pierrefiche, P.M. Lalley* and D.W. Richter II. Inst. Physiol., Univ. Göttingen, Humboldtallee 23, 37073 Göttingen, FRG

We studied the functional role of intracellular signal pathways involving protein kinase C (PKC) in the process of respiratory pattern generation in expiratory neurons of anesthetized, paralyzed and vagotomized cats. Measurements in current- and voltage-clamp were performed with fine-tipped electrodes while the specific PKC blocker (NPC 15-437) was intracellularly injected before and after extracellular application of TEA. Intracellular blockade of PKC reversibly hyperpolarized expiratory neurons throughout the respiratory cycle, reduced synaptic drive potentials and spontaneous action potential discharge. Neuronal input resistance was decreased during inspiration and increased during expiration. Blockade of PKC decreased spontaneous excitatory synaptic drive currents. This effect became more prominent after K^+ conductances were blocked by TEA. Under such conditions, injection of NPC 15-437 evoked a decrease of both excitatory and inhibitory synaptic drive currents. Stimulation induced post-synaptic currents and potentials were decreased after blockade of PKC, with or without TEA application.

These results demonstrate that PKC is endogenously active and acts throughout the respiratory rhythm by modulating three different currents: 1) it upregulates Cl^--mediated inhibitory synaptic currents and 2) it upregulates excitatory synaptic currents, 3) it downregulates persistent K^+ currents.

This work was supported by SFB 406 and the Graduiertenkolleg: "Organisation und Dynamik Neuronaler Netzwerke".

150.8

DYNAMIC REGULATION OF CALMODULIN AVAILABILITY BY CAM-KINASE II: COMPUTER SIMULATION ANALYSIS. M. D. Mauk* and M. N. Waxham. Department of Neurobiology and Anatomy, University of Texas Medical School, Houston, TX 77030

Given the numerous and often opposing cellular processes regulated by calcium/calmodulin (CaM), how are these processes activated selectively for different calcium influxes? Until recently it was generally held that activation of different CaM-dependent enzymes is controlled simply by the level of calcium influx. However, evidence suggests that *i*) the diffusion of CaM is severely restricted, *ii*) autophosphorylation of calcium/CaM-dependent protein kinase (CaMK-II) dramatically decreases the off-rate of CaM, effectively "trapping" CaM, and *iii*) CaMK-II is abundant in neurons. These observations suggest that CaM-trapping by CaMK-II can regulate the local availability of CaM for binding to other enzymes in a complex and time-dependent manner. This may favor the selective activation of particular CaM-dependent enzymes depending on the pattern, not just the level, of calcium influx. We have used simulations of CaMK-II to test these and related hypotheses. Results suggest that the frequency and magnitude of calcium influx can influence the activation of CaM-dependent enzymes competing with CaMK-II for a limited supply of CaM. Results also suggest that the ability of different enzymes to compete with CaMK-II for CaM is extremely sensitive to the on- and off- rates that comprise the enzymes' affinities for CaM, even when the affinities are identical. Thus, CaMK-II regulation of CaM availability may allow selective activation of other CaM-dependent enzymes depending on the specific calcium dynamics.

Support: R29 MH46904

150.9

SUBSTRATE SPECIFICITY AND GENOMIC STRUCTURE OF CALCIUM/CALMODULIN-DEPENDENT PROTEIN KINASE I (CaM KINASE I) M. R. Picciotto*, J. Elliott, A.J. Czernik and A. C. Nairn Dept. of Psychiatry & Peptide Synthesis Facility, Yale Univ. Sch. of Med., New Haven, CT 06510. & Lab. of Mol. and Cell. Neurosci., Rockefeller Univ., N.Y., NY 10021.

CaM kinase I was originally identified in rat brain based on its ability to phosphorylate site 1 of synapsin I. In order to define a consensus phosphorylation site for the enzyme we have used purified bovine brain CaM kinase I, bacterially produced recombinant CaM kinase I and cAMP-dependent protein kinase (PKA) to phosphorylate a number of peptide substrate analogs based on the phosphorylation site of synapsin I. We have also used site directed mutagenesis to identify a residue in CaM kinase I that is important for substrate interaction and overall enzyme activity. The CaM kinase I cDNA was used to clone the genomic DNA encoding the enzyme in order to identify alternatively spliced exons that might encode CaM kinase I variants. The intron-exon boundaries and overall genomic structure were determined. Preliminary experiments are underway to define the CaM kinase I promoter, with the aim of elucidating the regulatory elements that control CaM kinase I expression in neurons as well as in other expressing tissues. These experiments will enhance our understanding of the mechanisms underlying the interaction of CaM kinase I and its substrates, and could contribute to the identification of new substrates and regulatory mechanisms for the enzyme.

Supported by Yale University and Rockefeller University funds.

150.10

INHIBITION OF CaM KINASE CASCADE BY cAMP DEPENDENT PROTEIN KINASE **Gary A. Wayman, Hiroshi Tokumitsu and Thomas R. Soderling* Vollum Institute, Oregon Health Sciences University, Portland, Oregon 97201**

Ca^{2+}/Calmodulin dependent protein kinase IV (CaM KIV), and CaM kinase kinase (CaM KK) are neuronally expressed members of the CaM kinase family that are involved in transcriptional regulation. Phosphorylation of CaM KIV by CaM KK increases the total as well as the Ca^{2+}/CaM independent activity of the enzyme. In this study we examined the regulation of the CaM kinase cascade by cAMP dependent protein kinase (PKA). Here we report that phosphorylation of CaM KK by PKA *in vitro* as well as *in vivo* inhibits CaM KK and thereby inhibits activation of CaM KIV. PKA phosphorylates purified rat brain CaM KK on multiple residues, but does not phosphorylate CaM KIV *in vitro*. Phosphorylation of CaM KK inhibits the ability of CaM KK to activate CaM KIV *in vitro*. Activators of PKA also inhibit CaM KK activity *in vivo* . CaM KK expressed in COS-7 cells is inhibited by both forskolin as well as Sp-5,6-DCI-cBIMPS, an analog of cAMP. Likewise, the endogenous CaM KK in both PC12 cells and primary hippocampal neurons is also inhibited by activation of PKA. The inhibition of CaM KK *in vivo* is blocked by H89, further supporting a role for PKA in the inhibition. Omission of protein phosphatase inhibitors from the homogenization buffer reverses forskolin induced inhibition of CaM KK by PKA in PC12 cells. These data suggest that CaM KK/CaM KIV signaling cascade may be inhibited by the activation of the cAMP/PKA signaling pathway *in vivo*. This crosstalk may allow neurons to fine tune transcriptional activity depending on the pairing or sequence of signals received. (supported: NIH GM41292)

150.11

ASSOCIATION OF SRC-FAMILY TYROSINE KINASE LYN WITH GANGLIOSIDE GD3 IN RAT BRAIN.
K. Kasahara*, Y. Watanabe, T. Yamamoto#, Y. Sanai.
Department of Biochemical Cell Research, Tokyo Metropolitan Institute of Medical Science, Bunkyo-ku, Tokyo, Japan, #Institute of Medical Science University of Tokyo, Japan

Gangliosides are membrane-bound glycosphingolipids containing sialic acids that are found in high concentrations on the central nervous system. The carbohydrate moieties of gangliosides undergo profound changes during development and differentiation, suggesting that they may play fundamental roles in these processes. To clarify the function of gangliosides, we have undertaken identification of ganglioside-specific binding proteins.

In the present study, we demonstrated that monoclonal antibody (mAb) to ganglioside GD3 coimmunoprecipitated src-related protein tyrosine kinase Lyn in rat brains. In vitro kinase assay of the mAb immunoprecipitates there were phosphorylated proteins of 53/56 kDa. The phosphoamino-acid residue of p53/56 was tyrosine. p53/56 were identified as Lyn by reimmunoprecipitation with anti-Lyn antibody. The mAb also coprecipitated Lyn in rat cultured cerebellar neurons. This identification was confirmed using the cDNA expression system in CHO cells which express sole ganglioside GM3, the enzymatic substrate of ganglioside GD3 synthase. Cotransfection with GD3 synthase and lyn expression plasmids resulted in the association demonstrated by coimmunoprecipitation. These ganglioside GD3-Lyn tyrosine kinase interactions could be the mechanism by which ganglioside modulate cellular functions.

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150.12

REGULATION OF CYCLIC AMP-DEPENDENT PROTEIN KINASE IN CATH.A AND SH-SY5Y CELL LINES. V. A. Boundy*, J. S. Chen, and E. J. Nestler. Laboratory of Molecular Psychiatry, Depts. of Psychiatry and Pharmacology, Yale University School of Medicine, New Haven, CT 06508.

Increasing evidence indicates a role for chronic morphine-induced upregulation of the cAMP pathway in mediating aspects of opiate tolerance, dependence, and withdrawal in locus coeruleus neurons. This upregulation is manifested by increases in adenylyl cyclase levels, cAMP-dependent protein kinase (PKA) activity, and phosphorylation of specific protein substrates. *In vivo*, the alterations of PKA activity are associated with increases in immunoreactivity of the catalytic subunit (Cα/β) and regulatory subunit RII (α/β) as well as in the message levels of catalytic subunit Cβ. In an effort to understand the mechanisms underlying the regulation of PKA in the locus coeruleus, the effects of perturbations of the cAMP pathway on PKA were examined in the LC-like Cath.a cell line and the human neuroblastoma SH-SY5Y cell line. Exposure of Cath.a cells to forskolin (5μM) resulted in a decrease in the immunoreactivity of Cα/β. This downregulation occurred in a time-dependent fashion with a 25% decrease seen after 2 hours and a recovery to near basal levels by 48 hours. Exposure of SH-SY5Y cells to forskolin also resulted in a time-dependent decrease in the immunoreactivity of Cα/β with a maximal decrease of 50-60% seen by 12-18 hours and maintained up to at least 48 hours. Following differentiation of SH-SY5Y cells to a more neuronal state with retinoic acid (10μM), exposure to forskolin resulted in a biphasic curve of Cα/β immunoreactivity similar to that seen for Cath.a cells. The 40% decrease in Cα/β immunoreactivity detected at 4-6 hours was not attenuated by pretreatment of the SH-SY5Y cells with the protein synthesis inhibitor, cycloheximide (100μg/ml, 1 hour), suggesting that new protein synthesis is not required for the observed downregulation. We are currently investigating the effects of perturbations of the cAMP pathway on the regulatory subunit proteins, RI and RII, as well as on the message levels of the PKA subunits. (USPHS DA 07290 and 08227.)

150.13

CHRONIC HYPOXIA DECREASES cAMP-DEPENDENT PROTEIN KINASE ACTIVITY IN PC12 CELLS. D. Beitner-Johnson* and D. Millhorn. Department of Cellular and Molecular Physiology, University of Cincinnati, Cincinnati, OH 45267-0576.

The intracellular signaling pathways involved in hypoxia are poorly understood, and very little is known about the effects of long-term hypoxia. Acute hypoxia is known to increase synthesis and release of dopamine. PC12 cells are oxygen-sensitive catecholaminergic cells that express dopamine D_1 and D_2 receptors and thereby provide a useful system to study the effects of oxygen deprivation. To evaluate the effect of long-term hypoxia on intracellular signaling mechanisms, PC12 cells were exposed to 5% oxygen for various times between 0 and 48 hours. Cells were then harvested and assayed for cAMP-dependent protein kinase activity. Chronic (24 and 48 hours), but not acute hypoxia decreased cAMP-dependent protein kinase activity by ~30% from control levels. Both basal and 8-bromo-cAMP stimulated cAMP-dependent protein kinase activity were decreased by chronic hypoxia, suggesting that prolonged oxygen deprivation down-regulates the total amount of the enzyme. This effect was not due to hypoxia-induced alterations in cell number or cell viability. These data suggest that down-regulation of the cAMP signaling pathway is one mechanism by which PC12 cells adapt to hypoxia. Further studies are currently underway to evaluate the role of dopamine receptor function in hypoxia. This work was supported by HL33831 and HD28948.

SECOND MESSENGERS: cAMP

151.1

ALTERATION IN CALCIUM/CALMODULIN-SENSITIVE ADENYLYL CYCLASE ACTIVITY IN THE CEREBRAL CORTEX OF STROKE-PRONE SPONTANEOUSLY HYPERTENSIVE RATS. S. Hatta*[1], H. Togashi[2], H. Ozawa[3], H. Saito[2], and H. Ohshika[1]. Depts. of [1]Pharmacology and [3]Neuropsychiatry, School of Medicine, Sapporo Medical University, [2]First Dept. of Pharmacology, Hokkaido University School of Medicine, Sapporo 060, Japan

Stroke-prone spontaneously hypertensive rats (SHRSP) are a genetic animal model of severe hypertension with cardiovascular and cerebrovascular complications. Recently, we have reported that SHRSP exhibited behavioral impairment in the passive avoidance task and dysfunction in the hippocampal cholinergic system, suggesting that SHRSP is a useful animal model for the study of vascular dementia. Several recent studies have suggested that Ca^{2+}/calmodulin-sensitive type I adenylyl cyclase (AC) may be important for neuroplasticity and spatial memory. In the present study, the function of adenylyl cyclase was examined in the cerebral cortex of SHRSP. Male SHRSP (25-week-old) was used for the experiment, and age-matched Wistar-Kyoto rats (WKY) were used as genetic control. GppNHp-stimulated and forskolin-stimulated AC activities were not different between WKY and SHRSP. There were no significant differences in the amount and function of Gs, estimated by immunoblotting and guanine nucleotide photoaffinity labeling, respectively, between WKY and SHRSP. However, Ca^{2+}/calmodulin-sensitive AC activity was slightly but significantly decreased in SHRSP compared with that in WKY. These results suggest the possibility that the function of Ca^{2+}/calmodulin-sensitive, presumably type I, AC was impaired in the brain of SHRSP. (Supported by a grant from the Hokkaido Government, Japan.)

151.2

OPIATE-INDUCED ADENYLYL CYCLASE SUPERACTIVATION: STUDY IN TRANSFECTED CELLS, I. Nevo, Z. Vogel*, R. Levy, D. Saya, M. Bayewitch and T. Avidor-Reiss. Dept. of Neurobiology, Weizmann Institute of Science, Rehovot 76100, Israel.

Acute stimulation of opiate receptors inhibits adenylyl cyclase (AC) and reduces cAMP levels in the cell. Chronic activation of opiate receptors leads to a progressive increase in cAMP. This phenomenon, particularly manifest upon withdrawal of the opiate agonists, is referred to as AC superactivation and was previously observed in several neuronal cultures. We reconstituted the ability of opiates to induce AC superactivation using CHO and COS-7 cells transfected with opiate receptors. Such cells can serve as a useful model for studying the signal transduction of opiates, especially the modulation of AC activity. We found that AC superactivation is due to prolonged activation of the receptor and is gradually reversed by exposure to antagonist. AC superactivation seems to be the primary mechanism for the apparent morphine tolerance in chronically treated cells. AC superactivation is mediated via pertussis toxin-sensitive G proteins ($G_{i/o}$). $G\beta\gamma$ dimers, which dissociate from $G\alpha$ following GTP-binding protein activation, have an important role in this process. AC superactivation can be obtained following chronic stimulation by several other inhibitory agonists, demonstrating the generality of the phenomenon. We are currently investigating the AC isozymes involved in the process. *Supported by NIDA (DA6265), Israeli Ministry of Health, and Forschheimer Center for Molecular Genetics.*

151.3

THE EFFECTS OF CTOP AND NALTRINDOLE ON DYNORPHIN A_{1-17}-INDUCED INHIBITION OF BASAL AND FORSKOLIN STIMULATED ADENYLYL CYCLASE ACTIVITY IN RAT CAUDATE PUTAMEN. L.H. Clave, A. Ho, and M.J. Kreek*. The Rockefeller University, New York, NY 10021.

Adenylyl cyclase activity is used as one functional measure of opioid receptor-mediated signal transduction. The naturally occurring opioid peptide, Dynorphin A_{1-17}, is the primary endogenous ligand of the κ opioid receptor. Previous studies using the selective κ opioid receptor antagonist, nor-BNI, have suggested that dynorphin's effects are not solely κ receptor-mediated. The aim of the present study was to determine the extent to which Dyn A_{1-17} exerts its κ-mediated effects on cyclase activity in rat caudate putamen, an area known to contain a heterogeneous population of opioid receptors. Adenylyl cyclase activity was determined by measuring cAMP production in membranes prepared from caudate putamen of naive male Fischer 344 rats using a cAMP radioligand binding assay. Dyn A_{1-17} (10^{-9} - 10^{-4} M) produced a significant dose-dependent inhibition of both basal and forskolin-stimulated (10^{-5} M) adenylyl cyclase activity. Although the forskolin-stimulated values were approximately 9-10 fold higher than basal values as was expected, the percentage changes which were negligible in this assay were similar. The ability of CTOP (10^{-8} - 10^{-5} M) and naltrindole (10^{-8} - 10^{-5} M), selective μ and δ antagonists, respectively, to attenuate Dyn A_{1-17} -induced inhibition of cyclase was determined. Preliminary findings show that both CTOP (10^{-5} M) and naltrindole (10^{-5} M) are able to block, in part, the inhibition of cyclase caused by Dyn A_{1-17}. [Supported by The Aaron Diamond Foundation and NIDA Research Center Grant, P-50-05130 (MJK)]

151.4

$\alpha_{2A/D}$-ADRENOCEPTOR COUPLING TO ADENYLATE CYCLASE II, III AND IV: ENZYME TYPE-SPECIFIC MODIFICATION BY PROTEIN KINASE C ACTIVATION. A. Marjamaki* and S.M. Lanier, Depts of Pharmacol., Univ. of Turku, Finland and MUSC, Charleston, SC USA.

$\alpha_{2A/D}$-adrenoceptor (α_2-AR) coupling to adenylate cyclase (AC) was examined in DDT1-MF2 smooth muscle cells (stably transfected with α_2-AR) before and after ectopic expression of AC types II, III or IV. AC expression was determined by mRNA analysis and functionality. Relative to control cells, basal activity was increased in only ACII transfectants (5-fold), whereas maximal enzyme activity (FSK/GTPγS/Mn^{2+}) was increased by 7 (ACII), 4 (ACIII) and 2 (ACIV) fold. In intact cells, FSK stimulation was augmented 4 (ACII), 3 (ACIII) and 3 (ACIV) fold versus control, whereas isoproterenol-induced (100μM) increase in cellular cAMP levels were minimally altered. However, in ACII and ACIV transfectants the effect of α_2-AR activation was biphasic, inhibiting isoproterenol stimulation at lower α_2-AR agonist concentrations. The stimulatory and inhibitory effects of α_2-AR activation were blocked by pertussis toxin and the overall effect likely represents a balance between inhibitory input ($G\alpha_i$) and enzyme stimulation by $G\beta\gamma$ synergizing with $G\alpha_s$. Protein kinase C (PKC) activation did not modify α_2-AR coupling to AC III. However, PKC activation identified distinct properties of ACII and ACIV relative to their regulation by Gi/Go-coupled receptors. PKC activation blocked α_2-AR inhibition of isoproterenol effects in ACII transfectants, but not ACIV transfectants. Although types II and IV share several regulatory properties, these data suggest that PKC phosphorylation differentially effects stimulatory and inhibitory input to these two enzymes. (Supported by NS 242821/CTR 2235).

151.5

PERFUSION OF NEOCORTICAL SLICES WITH PROGESTERONE AFFECTS CYCLIC AMP AND $GABA_B$ RECEPTORS. R. H. Thalmann*, M. I. Al-Dahan and M. H. Jalilian Tehrani, Baylor Coll. of Med. , Houston,TX 77030.

Baclofen inhibition of cyclic AMP may be enhanced by proestrus levels of plasma progesterone in vivo (Al-Dahan et al, this meeting). We have attempted to study the effects of progesterone in vitro upon $GABA_B$ receptors and this messenger system. neocortical slices of rats that had been ovariectomized at least one week were incubated in a Krebs buffer (bubbled with $95\%0_2,5\%CO_2$). Exposure of slices to progesterone (1/2 max 30nM for 4 hours) increased baclofen binding to GABAb receptors up to two fold. Although 100nM progesterone did not affect basal levels of cyclic AMP, it did facilitate (3uM)forskolin stimulated cyclic AMP levels when introduced along with forskolin. However, when forskolin was introduced 45minutes following progesterone, there was no such facilitation. Thus progesterone seems to produce a short-latency, transient facilitation of forskolin-stimulated cyclic AMP that would seem to require an unconventional or membrane progesterone receptor. This effect may reflect a progesterone interaction with this messenger system that could couple progesterone to cellular effects such as the increase in GABAb receptors that progesterone produces.Supported by National Institutes of Health grant NS21713

151.6

CYCLIC AMP AND PROTEIN KINASE A IN THE PONTINE RETICULAR FORMATION CONTRIBUTE TO CHOLINERGIC RAPID EYE MOVEMENT (REM) SLEEP GENERATION. M.L. Capece*, M.A. Fleegal, and R. Lydic. Department of Anesthesia, Pennsylvania State University, College of Medicine, Hershey, PA 17033.

Microinjection of the cholinergic agonist carbachol into the medial pontine reticular formation (mPRF) of cat produces a REM sleep-like state by way of a signal transduction pathway involving muscarinic receptors (*Neuropsychopharm 2:67, 1989*), pertussis toxin sensitive G-proteins, and adenylate cyclase *(Am. J. Physiol. 269:R308, 1995)*. The present study is testing the hypothesis that cAMP and protein kinase A (PKA) regulate cholinergic REM sleep generation. Four cats received unilateral microinjections (n) of saline (n=17); carbachol (n=18); 8-bromo-cAMP (n=9), a cAMP analog; 8-bromo-cAMP as a pretreatment to carbachol (n=9); Sp-cAMPS (n=3), an activator of PKA; Sp-cAMPS as a pretreatment to carbachol (n=6); Rp-cAMPS (n=4), an inhibitor of PKA; and Rp-cAMPS as a pretreatment to carbachol (n=6). After each microinjection, 2 hr recordings were made of percent time spent in waking, non-REM sleep, and REM sleep. Carbachol enhanced REM sleep (67.8%) and this enhancement was significantly decreased by pretreatment with 8-bromo-cAMP (48.8% REM sleep) or Sp-cAMPS (40.3% REM sleep). Rp-cAMPS did not decrease the carbachol-induced REM sleep-like state. These data suggest that stimulation of the cAMP/PKA pathway can significantly decrease cholinergically-induced REM sleep. The results are consistent with previous studies which microinjected dibutyryl cAMP, pertussis toxin, and forskolin into the mPRF. Taken together, these data suggest that REM sleep generation involves a muscarinically-activated cAMP/PKA pathway.

Support: HL-40881 (RL), Departments of Anesthesia and Neuroscience & Anatomy.

151.7

CIRCADIAN RHYTHM IN THE CALCIUM-INHIBITABLE ADENYLYL CYCLASE ACTIVITY OF THE RAT STRIATUM. Y. Chern*, E. H. Y. Lee, H.-L. Lai, H.- L. Wang, Y.-C. Lee and Y.-H. Ching. Division of Neuroscience, Institute of Biomedical Sciences, Academia Sinica, Taipei 11529, Taiwan, R. O. C.

To elucidate the neuronal significance of the Ca^{2+}-inhibitable adenylyl cyclase (AC), we investigated the circadian regulation of the Ca^{2+}-inhibitable AC in the rat striatum. Under our experimental conditions, almost 90 % of the forskolin-evoked AC activity in the striatum was Ca^{2+}-inhibitable. Intriguingly, the Ca^{2+}-inhibitable AC activity in the striatum exhibited a daily oscillation with a peak occurring around 10:00. This circadian fluctuation of the Ca^{2+}-inhibitable AC activity remained largely unchanged in those cyclase assays in which GDPβS and $MnCl_2$ were substituted for GTP and $MgCl_2$. Therefore, regulation of the effector enzyme itself, but not Gsα protein, may lead to the circadian variation of the Ca^{2+}-inhibitable AC activity. A circadian fluctuation of the AC activity evoked by an A2a adenosine-selective agonist (CGS21680) was also observed in the striatum. Intrastriatal injection of an A2a-selective adenosine agonist or antagonist at the interval (9:30- 10:30) in which the Ca^{2+}-inhibitable AC activity was at its peak, resulted in a more significant alteration of locomotor activity than those observed at a later interval (16:30-17:30) when the Ca^{2+}-inhibitable AC activity was relatively low. Taken together, we have characterized a marked circadian variation in the Ca^{2+}-inhibitable AC activity localized in the striatum which appears to cause a circadian fluctuation of at least one neuromodulator's action. This work was supported by grants from National Science Council (NSC 84-2331-B-001-029), Taiwan.

151.8

SYNERGISTIC ACTIVATION OF ADENYLYL CYCLASE IS DEPENDENT UPON PHOSPHOLIPASE C-MEDIATED PROCESSES IN HUMAN NEUROBLASTOMA SK-N-BE(2)C CELLS. Kyong-Tai Kim* and Byung-Chang Suh Department of Life Science, Pohang University of Science and Technology, Pohang, 790-784, Republic of KOREA

U-73122, an inhibitor of processes involved in the activation of phospholipase C (PLC), was used to assess the role of PLC activation in the synergistic elevation of cAMP induced by carbachol and prostaglandin E_2 (PGE_2) in human neuroblastoma SK-N-BE(2)C cells. Pretreatment of the cells with U-73122 resulted in inhibition of carbachol-induced intracellular Ca^{2+} ($[Ca^{2+}]_i$) rise and inositol 1,4,5-trisphosphate ($InsP_3$) generation, with maximal and half maximal inhibition (IC_{50}) occurring at approximately 15 μM and 3.2 μM, respectively. U-73122 also inhibited the synergistic enhancement of cAMP accumulation induced by carbachol and PGE_2 in a concentration-dependent manner with maximum and IC_{50} at 12 ± 4 μM and 3.4 ± 0.3 μM, respectively. However, U-73122 does not significantly inhibit PGE_2-induced production. While BAPTA/AM treatment decreased the synergistic cAMP accumulation by 28%, addition of U-73122 further decreased it down to complete inhibition. Furthermore, GTPγS- and AlF_4^-- induced $InsP_3$ generation in digitonin-mediated permeabilized cells was also inhibited by U-73122 treatment. Pretreatment of the cells with neomycin, another blocker of the PLC pathway, also resulted in inhibition of the synergistic cAMP accumulation. These results suggest that the increase in $[Ca^{2+}]_i$ and the coupling process between muscarinic receptor-linked G protein and PLC are important for the synergistic activation of adenylyl cyclase in SK-N-BE(2)C cells.(Supported from the POSTECH and the KOSEF)

151.9

CHARACTERISATION OF CB1 RECEPTORS ON RAT NEURONAL CELL CULTURES: BINDING AND FUNCTIONAL STUDIES USING THE SELECTIVE ANTAGONIST, SR 141716A. M. Jung, R. Calassi, M. Rinaldi-Carmona, P. Chardenot, G. Le Fur*, P. Soubrié and F. Oury-Donat. Sanofi Recherche, 34184 Montpellier, France.

This study was undertaken to provide a further characterization of central cannabinoid receptors on rat primary neuronal cell cultures from selected brain structures. Using [^{3}H]-SR 141716A, the specific CB1 receptor antagonist, we demonstrated the presence of a relatively high density of specific binding sites (B_{max} = 139 ± 9 fmol/mg of protein) displaying a high affinity (K_d = 0.76 ± 0.09 nM) on cortical neurones. Furthermore, the two cannabinoid receptor agonists, CP 55940 and WIN 55212-2, inhibited in a concentration-dependent manner cyclic AMP production induced by either 1 μM forskolin or isoproterenol with EC_{50} values in the nanomolar range (4.6 and 65 nM on forskolin and 1.0 and 5.1 nM on isoproterenol for CP 55940 and WIN 55212-2, respectively). Moreover, on striatal neurones and cerebellar granule cells, CP 55940 also reduceD the cyclic AMP accumulation induced by 1 μM forskolin with a potency similar to that observed on cortical neurones (EC_{50} values of 3.5 and 1.9 nM on striatum and cerebellum, respectively). SR 141716A antagonized the CP 55940- and WIN 55212-2-induced inhibition of cyclic AMP accumulation suggesting the CB1 receptor specific mediation of these effects on all primary cultures tested. Furthermore, CP 55940 was unable to induce MAP kinase activation either on cortical or on striatal neurones. In conclusion, our results showed nanomolar efficiencies for CP 55940 and WIN 55212-2 on cyclase activity with no effect on any other signal transduction pathway investigated on primary neurone cultures.

151.10

EFFECTS OF FORSKOLIN ADMINISTRATION ON BEHAVIORAL RESPONSE TO FORCED SWIMMING IN THE RAT H. Maeda,H. Ozawa,N. Amemiya[1],K. Kaneta*,T. Saito,N. Takahata. Dept. of Neuropsychiatry and Pharmacology[1], School of Medicine, Sapporo Medical University, Sapporo, Japan.

Our previous studies showed that GppNHp-stimulated adenylyl cyclase (AC) activity is increased subsequent to the chronic administration of tricyclic antidepressants (TCA), electroconvulsive shock and selective serotonin reuptake inhibitors (SSRIs) suggesting enhanced cAMP signal transduction system by theses treatments (Ozawa and Rasenick, Mol. Pharm. 1989, J. Neurochem. 1991, Ozawa et al, Neurosci Abst 95).

Forskolin increases intracellular cAMP formation in a neurotransmitter receptor-independent manner by direct activation of catalytic subunit of AC or stabilizing between Gs and AC. The Porsolt behavioral despair test or the forced swimming test (FST) is an animal behavior test sensitive to discover new types of antidepressants agents. The present study investigated the effect of forskolin on behavioral responses to FST compared to TCA. Wistar male rats were injected (i.p)with saline and amitriptyline (15mg/Kg), forskolin (15mg/Kg). Water depth 37 cm to prevent from supporting themselves with tail or hind limbs. The behaviors in the swim were recorded on videotape. Following a 15 min pretest, drugs were administrated 1hr prior to the 5 min swim test. Forskolin decreased ratings of immobility and increased swimming which is similar effects to amitriptyline treatment. In addition, water soluble forskolin also reduced immobility.

These data are consisted with hypothesis that elevation of the cAMP cascade system may have an important role in antidepressive effects .

151.11

FORSKOLIN EVOKES EXTRACELLULAR ADENOSINE ACCUMULATION IN RAT CORTICAL CULTURES. Y. Li, X. Gan*, and P.A. Rosenberg, Department of Neurology, Children's Hospital & Harvard Medical School, Boston MA 02115

Previous studies have shown that β-adrenergic receptor agonists increase extracellular adenosine by stimulating the transport of cyclic AMP from astrocytes into the extracellular space, where it is sequentially hydrolyzed to AMP and adenosine. In this study, we investigated the effect on extracellular adenosine concentration of direct and maximal activation of adenylyl cyclase by forskolin. Forskolin evoked intracellular and extracellular cAMP accumulation as well as extracellular adenosine accumulation. In order to test whether cAMP transport was required for forskolin stimulated extracellular adenosine accumulation, we attempted to block adenosine accumulation using probenecid, an inhibitor of cAMP transport. Probenecid at 1 mM blocked forskolin stimulated extracellular cAMP accumulation (90.4 ± 2.1 inhibition in 3 experiments), and also blocked forskolin stimulated adenosine accumulation (62 ± 10% inhibition of excess adenosine accumulation due to forskolin; 3 experiments). Next, we tested whether the accumulation of adenosine in response to forskolin could be blocked by the cyclic nucleotide phosphodiesterase inhibitor RO 20-1724. Adenosine accumulation due to forskolin in the presence of 180 μM RO 20-1724 was only 19 ± 11% of the value due to forskolin in the absence of this inhibitor (n = 3). Finally, the accumulation of adenosine in response to forskolin could also be blocked by the 5'-ectonucleotidase inhibitor GMP, at 500 μM. These results demonstrate that forskolin can increase extracellular adenosine levels, and suggest that this adenosine is ultimately derived from cAMP.

This work was funded by a grant from the NINDS (NS26830).

151.12

EXPRESSION OF THE bZIP DOMAIN TRANSCRIPTION FACTORS C/EBPs AND ATF-4 IN HIPPOCAMPAL NEURONS AND THEIR REGULATION BY THE CYCLIC AMP SIGNAL TRANSDUCTION PATHWAY. J.C. Lopez-Garcia*, D. Baranes, Y.-Y. Huang, H. Golan, T. Tanaka, K. Takeda, S. Akira and K. Yukawa. Center for Neurobiology & Behavior, Columbia Univ., New York, NY 10032 and Hyogo College of Medicine, Hyogo 663, Japan.

The *Aplysia* CCAAT enhancer-binding protein (ApC/EBP) has been found to be an essential transcription factor activated by the cAMP signal transduction pathway during the consolidation phase of long-term facilitation. By contrast, ApCREB2 acts as a repressor for other bZIP factors including CREB1 and C/EBP. To explore whether mammalian homologues of these bZIP transcription factors play a role in the molecular processes leading to long-term memory, we initially examined the expression of C/EBPs and ATF-4, a mammalian homologue of ApCREB2 and the inducibility of those bZIP factors by the cAMP pathway in hippocampal neurons. Among several C/EBP isoforms identified in mammals, only C/EBPβ and CHOP-10 are expressed in dissociated hippocampal neurons in culture. Expression of both C/EBPβ and CHOP-10 are augmented 2-4 hours after forskolin treatment. Immunocytochemical and *in situ* hybridization analysis similarly indicate that ATF-4 mRNA and protein are expressed in hippocampal neuron and are significantly increased by forskolin treatment. To directly examine the roles of these transcription factors in synaptic plasticity, we are beginning to analyze long-term potentiation and long-term depression in the hippocampus of C/EBPβ knock-out (k/o), CHOP-10 k/o and ATF-4 k/o mice. Supported by HHMI & grants from the Ministry of Education of Japan.

SECOND MESSENGERS AND PHOSPHORYLATION II

152.1

ROLE OF NEUROFIBROMIN 1 IN SIGNAL TRANSDUCTION MEDIATING NEUROPEPTIDE RESPONSE. F. Guo†, I. The, A. Bernards, I. Hariharan, Y. Zhong†* †Cold Spring Harbor Lab Cold Spring Harbor, NY 11724; MGH cancer center-Harvard Medical School, Charlstown, MA 02129

The NF1 gene, which is altered in patients with type 1 neurofibromatosis, encodes a protein homologous to the ras GTPase activating protein (GAP). To understand its physiological function, the mutants of *Drosophila* NF1 homolog were examined.

It has been shown that PACAP38-like neuropeptide induces a 100-fold enhancement of K^+ currents (Zhong and Pena; Neuron 14:527; 1995) by coactivation of the Ras/Raf and adenylyl cyclase/cAMP pathways at the larval neuromuscular junction (Zhong; Nature 375:588, 1995). This 100-fold enhancement of K^+ currents was eliminated in NF1 mutants and the defect was rescued by expression of the normal NF1 transgene. In contrast, the PACAP38 response was not affected by GAP mutations. This NF1 defect appears not result from an alteration of the Ras pathway rather from the failure of activation of adenylyl cyclase to synthesize cAMP. This is strongly supported by the observation that PACAP38 could induce a normal-like response in NF1 mutants if membrane permeable cAMP analogs or forskolin (stimulating adenylyl cyclase) was supplied. It is suggested that the NF1/Ras complex may be important in controlling activation of adenylyl cyclase by G protein .

152.2

MODULATION OF ION CHANNEL ACTIVITY BY TYROSINE PHOSPHORYLATION. S. Catarsi, L. Aniksztejn and P. Drapeau* Centre for Research in Neuroscience, McGill University and Montreal General Hospital, Montreal, PQ, Canada H3G 1A4.

Pressure (P) sensory neurons of the leech possess a cation channel whose activity is increased by serotonin through protein kinase C. Soon after a serotonergic Retzius neuron comes into contact with the P cell, the cation channel loses the serotonin modulation at the sites of contact. This event, which requires tyrosine phosphorylation, leads to the specific recognition of the two cells, to the selection of the correct serotonin response and eventually to the formation of an inhibitory synapse. We have examined the possible direct modulation of the channel by tyrosine phosphorylation. In cell-attached configuration, bath application of the tyrosine kinase inhibitor genistein, but not of its inactive analog daidzein, induced a 6 fold increase of the open probability of the channel (Po) without altering its conductance or its mean open time; this effect was blocked by sodium pervanadate, a membrane permeable inhibitor of tyrosine phosphatases. In inside-out configuration, a protein tyrosine phosphatase (TCΔC11.PTPase) induced a 10 fold increase of Po; this effect was totally blocked by the specific phosphatase inhibitor sodium orthovanadate. Incubation with either orthovanadate or pervanadate did not provoke any significant variation in the activity of the channel.

These data suggest that the cation channel is directly phosphorylated by a tyrosine kinase under resting conditions in order to maintain a low basal level of activity. Future experiments will try to clarify the possible interactions between tyrosine phosphorylation and protein kinase C as well as the role of tyrosine phosphorylation in modulation and synaptogenesis.

Supported by the MRC and FRSQ of Canada and by INSERM and Ministère de la Recherche Française.

152.3

NEFIRACETAM, A NOOTROPIC AGENT, INHIBITS Ro 5-4864 - INDUCED MITOCHONDRIAL DEPOLARIZATION AS EXAMINED BY RHODAMINE 123 FLUORESCENCE IN NG108-15 CELLS. S. Watabe[1,2], M. Yoshii[2], T. Nukada*[3], T. Shiotani[1] and M. Tanaka[1]. [1]Tokyo R & D Center, Daiichi Pharmaceutical Co., Ltd., Tokyo 134 and [2]Dept. of Neurophysiology, [3]Dept. of Neurochemistry, Tokyo Institute of Psychiatry, Tokyo 156, Japan.

We have recently reported that the peripheral-type benzodiazepine receptor (PBR) in brain has an affinity to nefiracetam, a nootropic agent (Watabe et al., *Soc. Neurosci. Abstr.* **21**, 2034, 1995). It remains to be seen, however, whether nefiracetam influences the membrane potential of mitochondria which might be regulated by the mitochondrial benzodiazepine receptor (MBR). In the present of study, mitochondrial membrane potentials (Ψ_m) were measured fluorometrically in NG108-15 cells using rhodamine 123 (5 μg/ml) and the effects of nefiracetam on Ψ_m were investigated. Ro 5-4864, a specific agonist for PBR, induced a depolarization of Ψ_m at concentrations higher than 25 μM. In contrast, PK 11195, a specific antagonist for PBR, exerted no effect on Ψ_m but prevented the agonist-induced depolarization. Similarly, nefiracetam (50 μM) did not affect Ψ_m but prevented Ro 5-4864-induced depolarizations. The results suggest that nefiracetam may act as a stabilizer of the mitochondrial membrane potential possibly by interacting with MBR.

152.4

AGONIST-INDUCED MU OPIATE RECEPTOR PHOSPHORYLATION IS DEPENDENT ON THE POTENCY OF OPIATE LIGANDS Y. Yu, L. Zhang@*, H. Sun, G. R. Uhl[1] and J. B. Wang Dept of Pharmacuetical. Sciences, Sch. of Pharmacy. Univ. of Maryland at Baltimore, Baltimore, MD 21201, @Lab. of Molecular and Cellular Neurobiology, NIAA/ NIH, Bethesda, MD 20892, [1]Molecular Neurobiology Branch, NIDA/NIH and Depts. of Neurol. and Neurosci., Johns Hopkins Univ., Sch. of Med., Baltimore, MD 21224.

Mu opiate receptor phosphorylation can be induced upon opiate agonist activation. In order to understand the mechanisms of this phosphorylation event, we examined the effects of a variety of opiate ligands on mu receptor phosphorylation, mu receptor mediated adenylyl cyclase inhibition, and mu receptor mediated activation of a G protein linked K^+ channel. Mu receptor phosphorylation was assessed by ^{32}P-labeling of Chinese hemster overay cells stably expressing the cloned human mu receptor (hμCHO) which was immunoprecipitated with a specific anti-mu receptor peptide polyclonal antibody. Among 22 tested opiate ligands, sufentanil, dihydroetorphin, etorphine, and DAMGO caused the strong mu receptor phosphorylation; methadone, morphine, β-endorphin$_{(1\text{-}13)}$, enkephalins and dynorphin A $_{(1\text{-}17)}$ produced a ranged intermediate effect; while the partial agonist buprenorphine and several other "mixed agonist-antagonists" had little effect on mu receptor phosphorylation. Correlation between the potencies and intrinsic activities of opiate ligands and receptor phosphorylation that they induced suggests that the extent of agonist-induced mu receptor phosphorylation relates to the nature or agonist activities at these receptors.

Supported by Sch. of Pharmacy, UMAB and NIH intramural program.

152.5

ENDOTHELIN (ET) STIMULATES PHOSPHORYLATION OF ETS-1 IN GLIAL CELLS. L.F. Fleischman and D.W. Fink, Jr.* Lab. of Neurotrophic Factors, Div. of Cytokine Biol., CBER/FDA, Bethesda, MD 20892.

The 51 kDa isoform of Ets-1, a putative transcription factor that binds the DNA target sequence GGAATA, has been detected in cells of glial lineage. Phosphorylation of Ets-1 is elicited by a variety of peptidergic and neurotransmitter substances that evoke increases in intracellular calcium. It is reported here that the potent vasoconstrictor, ET, stimulates Ets-1 phosphorylation in glial cells. In C6 glioma cells, incubation with ET-1 stimulated Ets-1 phosphorylation in a dose-dependent manner. Similarly, dose-dependent phosphorylation of Ets-1 was elicited by ET-2, whereas ET-3 was without effect. Induction of Ets-1 phosphorylation by ET-1 and ET-2, but not ET-3, suggests these effects are mediated through ETA-type receptors. ET-1 stimulation of Ets-1 phosphorylation could be detected at 5 min and was sustained for at least 15 min. Pretreatment with the cell permeant, myosin light chain kinase-selective inhibitor KT5926 prevents ET-1/ET-2-mediated Ets-1 phosphorylation. Addition of ET-1 to cultures of primary rat astrocytes derived from neonatal cortex resulted in a weak but discernible dose-dependent stimulation of Ets-1 phosphorylation. Increases in Ets-1 phosphorylation were also observed following addition of ET-1 and ET-2, but not ET-3, to RN-22 cells derived from a rat schwannoma. This effect of ET in RN-22 cells was inhibited by KT5926, analogous to observations made in C6 glioma cells. For all cell types tested, Ets-1 in untreated control cells was largely in the unphosphorylated 51 kDa form; thus, ET-stimulated increases in phosphorylation were assessed against backgrounds devoid of detectable endogenous Ets-1 phosphorylation. ET has been reported to promote DNA synthesis and stimulate neurotrophin expression in astrocytes. These data suggest Ets-1 regulated processes may be involved.

152.6

THE PHOSPHORYLATION STATE OF TYROSINE HYDROXYLASE AT SERINE 40 PARALLELS DOPAMINE AUTORECEPTOR-MEDIATED CHANGES IN DOPA BIOSYNTHESIS. C.W. Aretha†, M.P. Galloway†, M. Goldstein‡, and J.W. Haycock§. †Dept. Psychiat Behav Neurosci, Wayne State Univ Sch Med, Detroit, MI., ‡Neurochem Res Lab, NYU Med Ctr, New York, NY, §Dept Biochem Molec Biol, LSU Med Ctr, New Orleans, LA.

Dopamine (DA) agonists decrease the rate of basal and stimulated DA biosynthesis in corpus striatum and, in a previous study, these effects were associated with a decrease in overall ^{32}P incorporation into tyrosine hydroxylase (TH). Activation of the nigrostriatal pathway, however, increases the phosphorylation of striatal TH at multiple sites. Thus, in the present studies, we examined the effects of DA agonists upon DOPA biosynthesis and the phosphorylation state of TH-Ser19 and TH-Ser40 (using quantitative blot immunolabeling with site- and phospho-specific antibodies) in rat striatal slices. In control slices, forskolin (1μM, 5-40 min) increased the stoichiometry of Ser40, but not Ser19, phosphorylation while elevated K (+30 mM, 40min) increased both Ser19 and Ser40 phosphorylation. Both treatments increased DOPA synthesis. Pretreatment with DA agonists (7-OH-DPAT and Quinpirole, 1 μM) decreased DOPA synthesis in both treated and untreated slices. These reductions were associated with decreases in the phosphorylation state of Ser40, whereas Ser19 phosphorylation was relatively unaffected. Preincubation with the DA antagonist eticlopride (1 μM) essentially blocked the effects of quinpirole.[Supported by DA04210 and the Joe Young, Sr. Research fund (MPG), MH2717 and NS6801 (MG), NS25134 and MH00976 (JWH)]

152.7

ACUTE HALOPERIDOL INCREASES THE ACTIVITY AND PHOSPHORYLATION OF TYROSINE HYDROXYLASE AT SPECIFIC SERINE SITES IN *VIVO*. K. Harada*[1], J.Y. Lew[1], A. Garcia-Espana[1] J. Platt[1], M. Goldstein[1], J.W. Haycock[2] and A.Y. Deutch[3], [1]Neurochem. Res. Labs., NYU Med. Ctr., New York, NY, [2]Dept. Biochem. Molec Biol., LSU Med. Ctr., New Orleans, LA and [3]Dept. Psychiatry and Pharmacol., Yale Univ., Sch. of Med., New Haven, CT.

Acute administration of haloperidole is known to increase the activity of striatal tyrosine hydroxylase (TH), but it is not yet known whether this is associated with changes in the phosphorylation of the enzyme. Using antibodies that specifically recognize TH phosphorylated at different sites, namely anti-THpSer19 and anti-THpSer40, we have determined the effects of a single injection of haloperidol upon the *in vivo* phosphorylation of TH at Ser19 and Ser40 and correlated this with changes in TH activity. Striata were dissected from rats sacrificed at 10 and 60 min after treatment. Blot immunolabeling analysis revealed that haloperidol increased the phosphorylation of TH at both Ser19 and Ser40, and these increases were associated with a concomitant increase in TH activity. Immunohistochemical studies revealed that the anti-THpSer40 did not result in staining in the striatum of vehicle-treated rats, but did label dopamine terminals in the dorsal striatum of haloperidol-treated rats. These data indicate that haloperidol activates TH by phosphorylating the enzyme at both Ser19 and Ser40 sites, and thus suggests that acute activation of TH by the D2 antagonist occurs through both activation of CAM kinase II and PKA. These antibodies should prove useful in determining the degree to which depolarization-induced activation of TH is modified after chronic antipsychotic drug treatment. Supported by USPHS grants MH2717 and NS6801 (M.G.), NS25134 (J.W.H.), and MH45124 (A.Y.D.), and the National Parkinson Foundation Centers of Excellence at NYU and Yale Univ.

152.8

17-β ESTRADIOL PREVENTS CREB DECLINE IN THE RAT HIPPOCAMPUS FOLLOWING SEIZURE AND HYPOXIA. K.S. Panickar*[1,2], M.E. Hosford[1], M.A. King[3], G. Rajakumar[1], P. Persaud[1] & J.W. Simpkins[1]. Center for Neurobiology of Aging and Department of Pharmacodynamics[1]; Department of Pharmacology and Therapeutics[2], Department of Neuroscience[3], University of Florida, Gainesville, FL 32610.

We have reported that CREB concentrations in the male rat hippocampus is reduced at 90 minutes after hypoglycemia-induced seizure (Soc. for Neurosci. Abstr.1995, p.1947). In Experiment 1, of the present study, two groups of rats were ovariectomized (OVX) but one group received 17-β estradiol (E2) treatment for 1 week. Hypoglycemic seizure was induced by injecting Insulin (12.5 U/kg, IP) and the rats sacrificed at 90 minutes following seizure. When compared to E2-treated animals OVX animals revealed a 53% decrease ($p<0.05$) in the number of CREB immunoreactive neurons in the CA1 pyramidal cell layer and a 56% decrease ($p<0.05$) in the dentate granule cell layer of the hippocampal region. In the CA3 region there was a 30% decrease in OVX versus E2-treated rats (NS). In Experiment 2, hippocampal slices from OVX and E2-replaced animals were subjected to hypoxia for 30 minutes and slices were collected at different time points following hypoxia. Western Blot analysis revealed an initial decline in CREB protein levels by 60% and 25% in OVX and E2-replaced animals, respectively, with a recovery phase beginning at 2.5 hrs following hypoxia. More importantly, the recovery of CREB protein level was increased to 25% above the control level in the E2-treated animals. These results suggest that 17-β estradiol may be beneficial in preventing CREB decline and in facilitating CREB recovery following an insult. (Supported by NIH AG 10 485 to JWS).

152.9

TYROSYL-PHOSPHORYLATION IS ASSOCIATED WITH MORPHOLOGICAL PLASTICITY OF RG-2 ASTROGLIAL CELLS. K.D. Ramsell*, S.W. Watts, D.F. Matesic, and P. Cobbett. Depts. of Pharmacology & Toxicology and Pediatrics & Human Development and The Neuroscience Program, Michigan State University, East Lansing, MI 48824.

Serum modulates cAMP-dependent morphological changes of neurohypophysial astrocytes (Ramsell *et al.*, 1996) and glioma-derived astrocytes (Koshel and Tas, 1993) *in vitro*. Serum has also been shown to cause tyrosyl-phosphorylation of cytoskeletal elements in cultured fibroblasts (Chrzanowska-Wodnicka and Burridge, 1994). This study was designed to test the hypothesis that the effect of serum on astrocytic morphology is mediated by modulation of tyrosyl-phosphorylation of cytoskeletal proteins. Western blot analysis revealed that RG-2 cells incubated in serum (2-10%) containing medium exhibited strong tyrosyl-phosphorylation of proteins of approximately 125 KDa and 66 KDa, which correspond in molecular weight to the cytoskeletal proteins Fak125 and paxillin respectively. Tyrosyl-phosphorylation of these proteins was decreased by approximately 60% when cells were incubated in serum-free medium and was decreased to a greater extent when cultures were incubated in serum-free medium containing either forskolin (5 μM), or genistein (50 μM), a tyrosine kinase inhibitor. Cells in cultures with high levels of tyrosyl-phosphorylation of 125 KDa and 66 KDa proteins were mostly non-stellate (<2%). Cultures incubated in genistein or forskolin containing medium not only contained cells with low levels of tyrosyl-phosphorylation of these proteins but also had a significantly increased proportion of stellate cells. These data show that astrocytic morphology is not only dependent on cAMP levels but also on the status of tyrosyl-phosphorylation of presumed cytoskeletal proteins. Supported by NINDS (NS 28206).

152.10

THE RAS ACTIVATOR CDC25^{Mm}/RAS-GRF: EXPRESSION IN THE CENTRAL NERVOUS SYSTEM AND MECHANISM OF SIGNAL TRANSDUCTION. R. Zippel, E. Mancinelli, S.Denis-Donini*, E. Martegani, C. Ferrari, N. Gnesutta, S. Orecchia and E. Sturani. Dept. General Physiology and Biochemistry, University of Milan, 20133 Milan, Italy

CDC25^{Mm}/Ras-GRF is a guanine nucleotide exchange factor of Ras reported to be expressed in the brain.We show that it is highly expressed in different area of the brain and, at a reduced level, in the spinal cord. It is localized in neuronal cells of the CNS and absent both in glial cells and in dorsal root ganglia. Subcellular fractionation of mouse brain shows that CDC25^{Mm} is present in synaptosomes and highly enriched in postsynaptic densities. In primary cultures of hippocampal cells the p140 Ras-GRF is phosphorylated and associated with calmodulin. This association can be modulated by different stimuli. We have previously shown that the constitutive expression of CDC25^{Mm} in fibroblasts potentiate a signalling cascade mediated by pertussis toxin sensitive-Gi proteins (Zippel et al, Oncogene, 1996,in press). Investigations have been undertaken to elucidate the pathways in which Ras-GRF is involved in neuronal cells.
This investigation was partially supported by CEE BIO2CT-93005.

152.11

DISTRIBUTION OF THE mRNA FOR C-JUN NH_2-TERMINAL KINASE KINASE (JNKK/SEK1) IN THE ADULT AND DEVELOPING RAT BRAIN. L. Carboni, R. Carletti, S. Tacconi, E. Bettini, F. Ferraguti*. Dept. of Pharmacology, Glaxo Wellcome S.p.A., Medicines Research Center, 37135 Verona, Italy.

At least three different Mitogen Activated Protein Kinase (MAPK) pathways mediate the transduction of extracellular signals to cellular responses in mammalian cells. The activation of Stress Activated Protein Kinases (SAPKs), also named c-Jun NH_2-Tetminal Kinases (JNKs), a recently discovered subfamily of MAPKs, induces the phosphorylation and trans-activation of c-Jun and other transcription factors. SAPK/JNK is activated via Thr and Tyr phosphorylation mediated by a dual specificity kinase, named SEK1/JNKK. We studied the mRNA distribution of SEK1/JNKK in the adult rat CNS using *in situ* hybridization with 45mer radiolabeled oligonucleotide probes. Film images were analyzed by means of a computer-assisted image analysis system. Strong signal intensity was observed in the hippocampal formation, in the medial habenular nucleus, in the granular layer of the cerebellar cortex, in some brain stem nuclei; intermediate expression levels were detected in the cerebral cortex and in thalamic and hypothalamic nuclei; hybridization signal was low in the basal ganglia. SEK1/JNKK mRNA distribution was compared to the hybridization pattern obtained using a SAPK specific probe: the localization of the two mRNA species was overlapping. SEK1/JNKK mRNA distribution was also studied at days 1, 3, 6, 9, 12, 15, 18 and 21 of post-natal development and compared to that found in the adult. The pattern of expression during development appeared to be similar to the adult animal. mRNA levels were low at P1, but increased the following days to reach, at P21, values similar to the adult animal. This study demonstrate a discrete distribution of SEK1/JNKK and a co-localization of this kinase with its substrate SAPK/JNK, suggesting a physiological role of the SAPK/JNK pathway in rat CNS.

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152.12

REGIONAL DIFFERENCES IN CANNABINOID RECEPTOR CATALYTIC ACTIVATION OF G-PROTEINS IN RAT BRAIN. C. S. Breivogel*, L. J. Sim, and S. R. Childers. Department of Physiology and Pharmacology, Bowman Gray School of Medicine, Wake Forest University, Winston-Salem, NC 27157.

Cannabinoid receptor activation of G-proteins can be measured by WIN 55212-2 (WIN)-stimulated [^{35}S]GTPγS binding in brain membranes or sections with excess GDP. In autoradiographic studies, although receptor binding and agonist-stimulated [^{35}S]GTPγS binding were generally parallel, a lack of correlation was found between these two parameters in some regions, with some areas providing relatively less [^{35}S]GTPγS stimulation than predicted from receptor binding. To quantify these differences, membranes from different brain regions were assayed by Scatchard analysis of net WIN-stimulated [^{35}S]GTPγS binding and [^{3}H]WIN binding, to calculate catalytic amplification factors (ratio of activated G-protein B_{max} to receptor B_{max}) for each region. Results showed significant differences between regions, in agreement with the results from autoradiography. Cannabinoid receptors in frontal cortex and brain stem exhibited the greatest catalytic amplification, with approx. 1.6 G-proteins activated per receptor. The lowest levels of amplification were in the cerebellum and sensomotor cortex, with values of approximately 0.8 G-proteins per receptor. These results suggest that cannabinoid receptors may exhibit varying levels of amplification in different regions with respect to G-protein activation.

Supported by DA-06784 and DA-07246 from NIDA.

152.13

ISOFORM-SPECIFIC LOCALIZATION OF CALCINEURIN IN HUMAN CONTROL AND ALZHEIMER HIPPOCAMPUS T. Kawamata[1]*, T. Hashimoto[1], K. Maeda[1], P. L. McGeer[2], E. G. McGeer[2], and C. Tanaka[1] [1]Hyogo Institute for Aging Brain and Cognitive Disorders, Himeji 670, Japan, and [2]Kinsmen Laboratory of Neurological Research, Univ. of B. C., Vancouver V6T 1Z3, Canada

Calcineurin (CN), an ionized calcium (Ca^{++})/calmodulin-dependent protein phosphatase, has been recognized as a key element in cellular signal transduction pathways. It is enriched in the central nervous and immune systems. To determine whether it might be involved in the pathogenesis of Alzheimer disease (AD), we investigated the localization of both the Aα and Aβ subunits of the catalytic domain of CN in human brain tissues. Eight postmortem brains of AD cases were compared with 5 control brains. Polyclonal antibodies against the two subunits were raised in rabbits, affinity-purified, and used for immunohistochemistry.

The isoforms were differently distributed in the hippocampus. Strong CA1-subiculum neuron and mossy fiber zone staining was obtained with the CN-Aα antibody, with weaker staining of almost all neurons. Strong staining of granule and pleomorphic neurons, as well as mossy fiber zone, was obtained with the CN-Aβ antibody. CN-Aβ was more associated than CN-Aα with such neuritic pathology as neuropil theads, degenerative neurites and intracellular neurofibrillary tangles in AD brains. Some glia with astrocytic profiles immunoreactive for CN-Aα or CN-Aβ were prominent in AD hippocampus.

These findings indicate that the two subunits may also differ in both their normal physiological roles and their involvement in AD pathology.

152.14

DISTRIBUTION AND REGULATION OF ACTIVATED MAP KINASES ERK1 AND ERK2 IN RAT CNS. D. S. Cameron[1]*, J. W. Haycock[2], E. Schaefer[3], and A. Y. Deutch[1] [1]Depts. of Psychiat. and Pharmacol., Yale Univ. Sch. Med. and VA Medical Center, New Haven, CT 06516, [2]Dept. of Biochem., LSU Medical Center, New Orleans, LA 70119, and [3]Promega Corp., Madison, WI, 53711.

Extracellular signal-Related protein Kinases (ERKs) are widely distributed in brain, and mediate a broad spectrum of effects, ranging from mediation of growth hormone actions to activation of catecholamine biosynthesis. ERKs possess both phosphorylated Tyr (pY) and Thr (pT) residues. Although anti-pY antibodies have been used to identify activated ERK, phosphorylation of both residues is required for ERK activity. We used an antibody (Promega) that specifically recognizes the diphosphorylated forms of ERK1 and ERK2 to map the distribution and regulation of ERK in the rat. Diphospho-ERK-immunoreactivity (ppERK-ir) was regionally heterogeneous. Cortical neurons (layers II/III) were intensely labeled, particularly in the entorhinal and perirhinal cortices. Dense cytoplasmic staining resulted in full visualization of processes in these cells, and in lightly-stained sections nuclear ppERK-ir was apparent. Densely-stained neurons were also seen in the central amygdaloid (CAN) and hypothalamic paraventricular (PV) nuclei; few somata were seen in the hippocampus. Acute ECS treatment increased the numbers of cortical ppERK-ir neurons, but in contrast enhanced neuropil staining in the hippocampus. ECS resulted in the virtual disappearance of ppERK-ir somata in the CAN and PV; only a few weakly-stained nuclei were observed. An increase in neuropil staining in the hippocampus was also seen after icv infusion of the PKC activator phorbol 12,13 dibutyrate but not the inactive 4α-phorbol ester. These data suggest that ERK activation may result in association of ppERK with an intracellular substrate that masks ppERK antigenicity. The pattern of ECS-elicited changes suggests that ERK activation may be important in stress-elicited changes in cellular function. Supported by MH45124, MH00967, NS25134, NPF.

152.15

CLONING AND EXPRESSION PATTERN OF RAT G-PROTEIN COUPLED RECEPTOR KINASE 6 (GRK6) Ch. Fehr, S. Reuss§, Ch. Hiemke and N. Dahmen* *Departments of Psychiatry and Anatomy§, University of Mainz, 55131 Mainz, Germany.*

Phosphorylation of G-protein coupled receptors at intracellular amino acid residues by G-protein coupled receptor kinases (GRKs) is believed to be involved in receptor regulation. To facilitate studies on G-protein coupled receptor kinase 6 (GRK6) in rodents, we cloned rat GRK6 cDNA by a modified 5'and 3'-RACE technique. We obtained a 2817 bp rat GRK6 cDNA, with an open reading frame of 1731 bp and a partial termination site at position 2543. Sequence comparison with human GRK6 revealed a 77.8% identity of the cDNA in the 5'-UTR, 89.5% in the CDS, 77.5% in the 3'-UTR and 96.2% on the protein level. Comparison with other GRK family members showed a closer relationship to GRK5 (78.6% in the N-terminal, 87.4% in the catalytical and 80.3% in the C-terminal domain) than to GRK2 (47.6% in the N-terminal, 69,8% in the catalytical and 67,7% in the C-terminal domain).

RT-PCR experiments demonstrated the ocurrence of GRK6 mRNA in all samples investigated: brain, muscle, heart, aorta, liver, lung, thymus, stomach, uterus and kidney. To quantify brain expression of GRK6 in different brain regions we performed quantitative RT-PCRs with increasing amounts (10^2-10^8 copies) of a truncated in vitro transcribed GRK6 RNA standard added to the brain RNA samples (400 ng each reaction). These experiments demonstrated similar expression patterns of GRK6 (10^4-10^5 copies/ng RNA) in cortex frontalis, cortex occipitalis, striatum, thalamus, hypothalamus, pons, cerebellum and cervical spinal cord. Taken together, our findings suggest a role for GRK6 in receptor phosphorylation and regulation in various tissues and brain regions.

Supported by the Deutsche Forschungsgemeinschaft (grant Da 370/1-1)

152.16

STIMULATION OF MUSCARINIC M_1 RECEPTORS LEADS TO MULTIPLE SIGNALING EVENTS: EVIDENCE USING THE CYTOSENSOR® MICROPHYSIOMETER K. De Moor & S. Pitchford* Molecular Devices Corp., Sunnyvale, CA 94089.

The stimulation of many G-protein coupled receptors results in the initiation of multiple signaling pathways, often through the recruitment of different G-protein subunits. We used the Cytosensor Microphysiometer System, which measures the excretion of acid products of cell metabolism and homeostasis in real time, as a continuous monitor of the cellular response to muscarinic M_1 receptor activation while adding agents that interfere with different signaling pathways. Activation of the M_1 receptor transfected into CHO cells (classically known to couple via a Gq protein) with the agonist carbachol resulted in a biphasic increase in the extracellular acidification rate (ECAR) in a concentration-dependent manner (EC_{50}: 2.44 ± 0.19 μM). The initial response was completely inhibited by blockers of the sodium-hydrogen exchanger (NHE) and by the kinase inhibitor, staurosporine. The response was slightly attenuated in cells that had been treated for 18 hours with 100 nM PMA or pre-treated for 30 minutes with 25 μg/ml genistein. The response was unaltered, however, by separate pertussis toxin or KN-62 treatments and, more importantly, by treatment with U73122, an inhibitor of PLC. Treatment with wortmannin, an inhibitor of PI-3-kinase, resulted in an ECAR response larger than that to carbachol alone. This suggests that the coupling of the M_1 receptor to the extracellular acidification response may involve pathways other than the classical Gq/PLC path.

152.17

AN ESTROGEN RECEPTOR THAT NONGENOMICALLY UNCOUPLES G-PROTEIN COUPLED RECEPTORS. A.H. Lagrange*, Y. Tong, L. Yu, O.K. Ronnekleiv, and M.J. Kelly. Dept. of Physiol. & Pharm. OHSU, Portland OR 97201 and Dept. Genetics, IU. Sch. Med. Indianapolis, IN

μ-Opioid peptides (e.g. β-endorphin) hyperpolarize hypothalamic neurons by activating a K^+ inward-rectifier (GIRK). In a subpopulation of cells, 17β-estradiol (E_2) rapidly (≈ 10 min) activates PKA which uncouples this system, causing a four-fold increase in the EC_{50} of the μ-selective agonist, DAMGO. To study this estrogen receptor (ER), we used intracellular recordings of arcuate neurons from *in vitro* hypothalamic slices from ovariectomized guinea pigs. Inhibition of protein synthesis with 200 μM cycloheximide did not block the effects of E_2 (DAMGO EC_{50} = 123 nM ± 27 nM; E_2 alone = 115 ± 10 nM; controls = 59 ± 3 nM), thus implying that E_2 acts nongenomically. The EC_{50} for E_2's effect was 8 nM and the biologically inactive isomer, 17α-E_2 had no effect. E_2 appears to act at an intracellular receptor, since the membrane-impermeant conjugate BSA-E_2 did not alter DAMGO potency. E_2's effects were blocked by the antiestrogen ICI 164,384 and Schild analysis estimated that the affinity for ICI 164,384 was identical to the classical ER. Furthermore, E_2-sensitive cells were immunocytochemically stained for the classical ER. In addition, we expressed the μ-receptor, GIRK and the rat ER in oocytes. Using the same paradigm that we used for the slice experiments, the DAMGO EC_{50} was not altered by E_2 treatment (8.3 nM ± 0.6 in controls; 6.3 nM ± 0.5 after E_2). Therefore, these nongenomic actions may represent either a novel ER or a novel action of the classical ER that requires multiple components and/or subcellular organization that are not reproduced in the oocyte expression system. (Supported by DA05158, DA00192, MH10327)

152.18

Effects of pertussis toxin and Gα protein specific antibodies on phosphoinositide hydrolysis in rat brain membranes after cholinergic denervation and hippocampal sympathetic ingrowth. K.KOLASA*, D.PARSONS, M.ROBERSON AND L.E.HARRELL. VA Med.Ctr. and University of Alabama at Birmingham Med.Ctr.

Following cholinergic denervation (CD) of the hippocampus by medial septal lesions (MSL), an unusual neuronal reorganization occurs in which peripheral adrenergic fibers arising from the superior cervical ganglia grow into the hippocampus (hippocampal sympathetic ingrowth-HSI). We have previously reported that CD and HSI differentially affected cholinergically stimulated phosphoinositide (PI) turnover and the affinity of muscarinic acetylcholine receptors (mAChR) as measured with non-specific and specific antagonists. We have also reported that GTPγS- as well as GTPγS+carbachol-stimulated PI hydrolysis were differentially changed by CD and HSI, which would indicate an alteration in G proteins and/or the entire receptor function. To examine the type of G protein which may be involved in these effects, we have preincubated rat hippocampal membranes with pertussis toxin (PTX) in the presence of GTPγS and GTPγS+carbachol. Our results indicate that PTX reduced both GTPγS- and agonist-stimulated PI hydrolysis but significant effect was obtained only in CON and CD group. This may suggest that PTX-sensitive G proteins are involved in the mediation of PI hydrolysis. To confirm this hypothesis membranes were preincubated with monoclonal antibodies to G_{α}o,i1,i2 and with polyclonal antibody to Gq/11. It was found that the Go antibody significantly decreased GTPγS stimulated PI hydrolysis in all experimental groups . Go antibody also induced a significant decrease in GTPγS+agonist-stimulated PI, but only in HSI group. Impairment of GTPγS- and agonist- stimulated PI hydrolysis was also significant in all experimental groups when preincubated with a specific antibody to PTX-insensitive G protein- Gq/11.The results suggest that the mechanisms by which HSI and CD induced alterations in mAChR affinity and PI hydrolysis are probably mediated by changes in the coupling between AChR and possibly 2 different types of G proteins, or by their differential activation.

152.19

PERTUSSIS TOXIN ADP-RIBOSYLATION OF BRAIN AND SPINAL CORD INHIBITORY G-PROTEINS USING [^{3}H] NAD AS SUBSTRATE. **N.W. DeLapp*, D. E. Womer and H. E. Shannon. Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN 46285.**

Pertussis toxin (PTX) blocks inhibitory G-protein (Gi/0) function by ADP-ribosylation. ADP-ribosylation *in vitro* is usually determined using [^{32}P] NAD as substrate coupled with SDS-PAGE and autoradiography to identify labeled Gi/0. An alternative method is described.

Rat brain and mouse spinal cord membranes were solubilized by sonication in Tris buffer containing 1% sodium cholate followed by centrifugation to remove insoluble material. PTX was activated by incubation in 50 mM DTT. The ADP-ribosylation reaction (50 μl) contained 25 mM Tris HCl pH 8.0, 1 mM ATP, 100 μM GDP(β)S, 10 mM thymidine, 1 mM EDTA, 12.5 mM DTT, 0.025% SDS, 1.25 μgs activated PTX, 15 μM [^{3}H] NAD (3 Ci/mmole), and 20-100 μgs of protein. Reactions were carried out for one hour at 30 degrees C and samples were prepared for electrophoresis. G-proteins were separated by SDS-PAGE, transferred to nitrocellulose and immunoblotted with an anti-Gi/0 antibody. Immunoreactive bands on the blot corresponding to 40 Kd MW were cut out, dissolved in scintillation fluid, and counted. Incorporation of ADP-ribose was 20-30 fold greater using cholate-solubilized compared to suspended membrane preparations. A dose dependent increase in labeling of Gi/0 was obtained between 20-80 μgs of soluble protein/assay. ADP-ribosylation of spinal cord Gi/0 was reduced by 62% in samples taken from mice 7 days after intrathecal injection of 0.3 μgs/Kg of PTX. ADP-ribosylation of cholate-solubilized membranes using [^{3}H] NAD coupled with scintillation counting of bands identified by immunoblotting provides a rapid, quantitative method not requiring [^{32}P].

152.20

EXISTENCE OF PROTEIN VARIANTS OF RAT BRAIN 14-3-3 ζ ISOFORM. K. Murakami*, S.Y.Situ and F. Eshete Dept. of Biochemical Pharmacology, State University of New York at Buffalo, NY 14260

The 14-3-3 protein is highly enriched in the central nervous system, consisting of approximately 1% of the total cytosolic proteins in the brain. Despite such high abundance, the function(s) of the protein is poorly understood. Recent studies indicate that the 14-3-3 ζ mediates diverse cellular functions, including arachidonic acid-specific PLA2 activity, PKC regulation, and Raf-1 activation and translocation to the membrane. Increase in the immunoreactivity of Raf-1 during LTP has also been reported. These observations suggest that the 14-3-3 mediates a pivotal step in signaling pathways that involves arachidonic acid, PKC as well as MAP-kinase. However, substantial controversies exist regarding its PLA2 activity and effects on PKC regulation. To closely examine the contradictory issues, we have cloned the 14-3-3 ζ from the rat hippocampal cDNA library for expression studies in mammalian cells. Our DNA sequencing analysis of the clone shows that there are protein variants present in the 14-3-3 ζ. These are missense mutations, which lead ACG (Thr) to ATG (Met) and CGT (Arg) to GCT (Ala) conversions at amino acid residues 88 and 109, respectively. Respective amino acid residues of rat 14-3-3 ζ isolated from hippocampal, pineal and brain cDNA libraries are Met/Ala, Thr/Ala and Thr/Arg at these positions. It is thus apparent that rat 14-3-3 ζ has three distinct protein variations. Such mutation is an important indication for the diverse functions of this protein and may also contribute to the recent contradictory observations regarding the role of the 14-3-3 ζ subtype. (NIMH 48973 to KM)

SECOND MESSENGERS AND PHOSPHORYLATION III

153.1

MEASUREMENT OF MITOCHONDRIAL [Ca^{2+}] IN CULTURED RAT NEURONS TRANSFECTED WITH MITOCHONDRIAL-TARGETED AEQUORIN. R.A. Padua*, C. Campbell and S.A. Thayer. Dept. of Pharmacology, University of Minnesota, Minneapolis, MN 55455.

The mitochondrial Ca^{2+} concentration ($[Ca^{2+}]_{mt}$) was measured directly in cultured rat hippocampal and dorsal root ganglion neurons. Neurons were transfected with the gene encoding the Ca^{2+}-binding photoprotein apoaequorin fused to the mitochondrial targeting sequence of cytochrome C oxidase. Gene transfer was mediated by a replication-deficient adenoviral vector that carried the apoaequorin gene construct. In DRG neurons, high [K^+] and caffeine elicited cytosolic Ca^{2+} ($[Ca^{2+}]_i$) increases that produced concomitant $[Ca^{2+}]_{mt}$ transients as detected by aequorin bioluminescence. Both depolarization- and caffeine-elicited $[Ca^{2+}]_{mt}$ transients were blocked by application of the mitochondrial uncoupler FCCP although $[Ca^{2+}]_i$ was still increased. Furthermore, depolarization-induced $[Ca^{2+}]_{mt}$ transients were inhibited by combined addition of antimycin A1 and oligomycin B. In hippocampal neurons, NMDA elicited $[Ca^{2+}]_i$ transients that were paralleled by increases in $[Ca^{2+}]_{mt}$. Similar to DRG neurons, $[Ca^{2+}]_{mt}$ transients in hippocampal neurons were inhibited by FCCP. Taken together, these results suggest that apoaequorin is selectively expressed in the mitochondria of rat neurons and that these organelles take up Ca^{2+} when $[Ca^{2+}]_i$ is elevated by either influx or release from intracellular stores. Supported by the NIH and the NSF.

153.2

INHIBITORY ACTION OF NOOTROPIC NEFIRACETAM ON Ca^{2+} RELEASE FROM MITOCHONDRIA INDUCED BY Ro 5-4864 IN NG108-15 CELLS. M. Yoshii*[1], S. Watabe[1,2], T. Nukada[3], T. Shiotani[2] and M. Tanaka[2]. [1]Dept. of Neurophysiology, [3]Dept. of Neurochemistry, Tokyo Institute of Psychiatry, Tokyo 156 and [2]Tokyo R & D Center, Daiichi Pharmaceutical Co., Ltd., Tokyo 134, Japan.

We have recently shown that nefiracetam, a nootropic agent, has an inhibitory action on bradykinin-induced Ca^{2+} mobilization in NG108-15 cells (Yoshii et al, *Soc. Neurosci. Abstr.* **20**, 188, 1994). In the present study, we have further examined whether nefiracetam also exerts an inhibitory action on a Ca^{2+} release from mitochondria which might be induced by stimulation to the peripheral-type (or mitochondrial) benzodiazepine receptor (PBR or MBR). Cytosolic Ca^{2+} activities of NG108-15 cells were measured fluorometrically using fura-2/AM (5 μM). Ro 5-4864 (25-50 μM), a specific agonist for PBR, induced a sharp rise of Ca^{2+}. The Ca^{2+} response to Ro 5-4864 remained intact in a Ca^{2+}-free medium, even after repetitive stimulation by bradykinin (0.2-1 μM) was applied to deplete stored Ca^{2+}. The Ca^{2+} rise in response to Ro 5-4864 was prevented in the presence of PK 11195 (20 μM), a specific antagonist for PBR. Similarly, nefiracetam (50 μM) inhibited Ca^{2+} response to Ro 5-4864. The results suggest that nefiracetam might interact with MBR thereby inhibiting Ca^{2+} release from mitochondria.

153.3

PHARMACOLOGICAL CHARACTERIZATION OF A CLONED RAT EXTRACELLULAR CALCIUM SENSING RECEPTOR IN TRANSFECTED CHO CELL LINES. M. Ruat*, S. Ferry, B. Chatel, R. H. Dodd#, A.M. Snowman* and S.H. Snyder* CNRS, UPR9040 and # ICSN, 91198 Gif sur Yvette, France and (*) Dept. of Neuroscience, J.H.U. Sch. of Med., Baltimore, MD 21205.

The calcium sensing receptor (CaSR) belongs to the superfamily of G-protein coupled receptors and responds to extracellular calcium ions. In brain, immunohistochemistry revealed discrete punctate localizations reflecting nerve fibers and terminals (Ruat et al, 1995).

We have characterized a Chinese hamster ovary cell line (CHO(CaSR)) stably expressing rat CaSR (Ruat et al, 1996) and studied the transduction mechanisms and the pharmacology of rat CaSR. Western blot analysis of membrane preparations from these transfected cells using antibodies raised against a synthetic peptide based on the rat CaSR carboxyl domain, identified two highly glycosylated proteins with apparent molecular masses of 140 and 160 kDa. Stimulation of phosphatidylinositol (PI) hydrolysis and arachidonic acid (AA) release via phospholipases C and A2 respectively displayed highly cooperative responses to calcium and magnesium with a Hill coefficient of 4-5. These observations show that stimulation of the rat CaSR can trigger several major intracellular signals in response to extracellular calcium and magnesium.

In the presence of 1.5mM calcium, NPS 568, an organic compound, potently stimulated PI hydrolysis with an EC50 of 3 μM. In the presence of lower concentrations of calcium, the dose response curve was shifted to the right suggesting that the potency of the drug was dependent upon the concentration of external calcium. Experiments are underway in order to further characterize CaSR transduction mechanisms and to investigate any link between CaSR and neurotransmitter release.

153.4

LOW EXTRACELLULAR CALCIUM ACTIVATES PRODUCTION OF IP3 AND INTERCELLULAR CALCIUM WAVES IN GLIAL CELLS S.A. Zanotti, R.C. Collins* and A. C. Charles Dept. of Neurology, UCLA, Los Angeles, CA.90095

Changes in $[Ca^{2+}]_i$ in primary mixed glial cultures from mouse cortex were measured using fluorescence videomicroscopy and fura2. Replacement of the normal extracellular medium with medium containing no added calcium induced an increase in $[Ca^{2+}]_i$ in single cells which propagated as intercellular waves to groups of 10 to 100 cells. Intercellular calcium waves propagated at rates of 5-20 uM/sec and were similar in their temporal and spatial characteristics to those induced by mechanical stimulation of a single cell. The low extracellular calcium ($[Ca^{2+}]_o$) - induced calcium waves were abolished by pre-treatment with 1 uM thapsigargin and by the phospholipase C inhibitor U73122. The low $[Ca^{2+}]_o$-induced intercellular calcium waves did not occur when extracellular calcium was replaced by extracellular barium. Low $[Ca^{2+}]_o$ - induced intercellular waves were unaffected by pretreatment of cells with pertussis toxin, or by replacement of $[Ca^{2+}]_o$ with zinc. These studies provide evidence for coupling between $[Ca^{2+}]_o$, IP3, and $[Ca^{2+}]_i$ in glial cells. This coupling may be involved in the regulation of glial intracellular calcium stores, as well as in regulation of the extracellular space by glia. Supported by NIH Grants R29-NS32283 and PO1-NS2554-05.

153.5

INTERCELLULAR CALCIUM WAVES IN HIPPOCAMPAL SLICE EXPLANTS. A.C. Charles[1*], S.A. Frautschy [1,2,3], S.A. Zanotti[1] and M.E. Harris[2,3]. UCLA Departments of Neurology[1] and Medicine[2], Los Angeles, CA 90095 and GRECC, VAMC Sepulveda[3], Sepulveda, CA 91343.

Organotypic slice cultures provide an excellent system to study basic cellular physiology and cell-cell interactions. Hippocampal slice cultures have a well-preserved cellular organization and proper cell-cell contacts are maintained. We have utilized these slices, at 1 to 3 weeks *in vitro*, to examine calcium signaling activity. Spontaneous calcium waves occur in areas CA 1, 2 and 3. Intercellular calcium waves occur in at least 2 distinct patterns. Small, rapid calcium waves involving 5-20 cells and intracellular calcium concentrations of 100-200 nM occur in multiple areas of each slice region. Stimulation of the slices with NMDA or DNQX produces large intercellular waves involving in excess of 200 cells which often travel in spiral patterns. These large wave fronts travel at velocities ranging from 5 to 20 um/sec and intracellular calcium concentrations in these waves range from 200-400 nM. Simultaneous immunofluorescence labeling of neurons in these slices suggests that the majority of the intercellular waves are occurring in glial cells, although neurons may be capable of stimulating glial wave activity. These results indicate that glial cells in hippocampal slices are capable of extensive spontaneous and stimulus-evoked intercellular signaling. This work is supported by grants PO1-NS02808 (A.C.C.) and NS30195 (S.A.F.).

153.6

LOCALIZED CALCIUM RELEASE "CA^{2+} SPARKS" AND CALCIUM ACTION POTENTIALS RECORDED IN FLUO-3-LOADED CULTURES OF PITUITARY CORTICOTROPES USING CONFOCAL SCANNING LASER MICROSCOPY. J.F. Fiekers[1*] and T.J. Heppner[2], [1]Dept. of Anat. & Neurobiol.,[2]Dept. Pharmacology, Univ. of Vermont Coll. of Med., Burlington, VT 05405.

Spontaneous calcium action potentials increase $\{Ca^{2+}\}_i$ in a clonal pituitary cell line of corticotropes, AtT-20/D16v cells. The present study was undertaken to determine the characteristics of these action potentials in single corticotropes. Single cells were loaded with fluo-3/AM (2 μM) and examined with a BIORAD MRC-1000 CLSM equipped with a krypton/argon laser. Single line scans were made through the cell body as well as cellular processes extending from the cell body. Action potentials were recorded as uniform waves of increasing $\{Ca^{2+}\}_i$ which were initiated at random locations within the cell body. The duration of the waves of $\{Ca^{2+}\}_i$ ranged from 1.5-2.5 sec. Examination of processes from single cells demonstrated that "sparks" of calcium, which represent locally released calcium, were present along cell processes and extended to the terminal ends. The time constant of decay of individual Ca^{2+} sparks ranged from 79-200 msec. These results demonstrate that cytosolic calcium can be increased within these cells by (1) global waves of calcium action potentials or (2) localized release of calcium within cytoplasmic domains in the form of Ca^{2+} sparks. Future studies will explore the role of these two calcium signals in both release and translocation mechanisms in these neuroendocrine cells.

153.7

SIGMA RECEPTOR LIGANDS MODULATE PROTEIN PHOSPHORYLATION AND INTRACELLULAR FREE CALCIUM LEVELS IN RAT FOREBRAIN SYNAPTOSOMES

P.J. Brent, L. Herd, H. Saunders, A.T.R. Sim, B. Walmsley and P.R. Dunkley. The Neuroscience Group, University of Newcastle, NSW, Australia

We recently reported that sigma (σ) ligands inhibit the depolarisation-dependent changes in phosphorylation of the key proteins synapsin I and dynamin which modulate neurotransmitter release and vesicle recycling respectively. These effects were not a result of a direct action on protein kinases or phosphatases I, IIA or IIB, but were dependent on extracellular calcium (Ca^{2+}) and were inhibited by the σ receptor antagonist rimcazole. In the present study, intact synaptosomes (P2 fraction) isolated from rat forebrain were prelabelled with $^{32}P_i$, and preincubated with 1,3-di-O-tolylguanidine (DTG; 3-100 μM). Aliquots were incubated for 10s in control (5 mM K^+) or depolarising buffer (41 mM K^+) and samples were run on polyacrylamide-SDS gel electrophoresis (PAGE). Phosphopeptides were digested with V8 protease and fractionated using PAGE. Protease digestion of protein bands for synapsin Ia and Ib demonstrated that DTG inhibited depolarisation-dependent phosphorylation of phosphopeptide fragments of synapsin I which are phosphorylated separately by PKA and CaMPK-II, and of MARCKS protein, which is phosphorylated by PKC. In subsequent experiments, the effect of the σ ligands DTG, (+) and (-)pentazocine and BD1008 (1-100 μM) on basal intracellular free Ca^{2+} levels ($[Ca^{2+}]_i$) and the increase in $[Ca^{2+}]_i$ produced by depolarisation with KCl (45 mM),veratridine (25 μM) and 4-aminopyridine (4-AP, 1 mM) was investigated using a fura-2 assay. The σ ligands elicited concentration-dependent decreases in basal $[Ca^{2+}]_i$, and inhibited the rise in $[Ca^{2+}]_i$ produced by all the depolarising agents. All σ ligands were more potent to inhibit the rise in $[Ca^{2+}]_i$ after depolarisation with veratridine than with KCl and 4-AP. The effects on basal $[Ca^{2+}]_i$ were blocked in the presence of 2 mM EGTA, indicating dependence on extracellular Ca^{2+}, and inhibited by preincubation with rimcazole (25 μM) and the Ca^{2+}-channel antagonist, ω-conotoxin MVIIC (200 nM; N-type), but not by nifedipine (5 μM; L-type). The data suggests that activation of σ receptors leads to decreased entry of Ca^{2+} into synaptosomes resulting in changes to protein phosphorylation.

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153.8

2,2'-DICHLOROBIPHENYL INCREASES $[Ca^{2+}]_i$ IN CEREBELLAR GRANULE CELL CULTURES: TIME-COURSE, CONCENTRATION-RESPONSE, AND DEPENDENCE ON $[Ca^{2+}]_e$. T.J. Shafer*, W.R. Mundy and P.R.S. Kodavanti. Neurotoxicology Division, NHEERL, U.S. EPA, RTP, NC 27711.

Neuroactive polychlorinated biphenyls (PCBs) alter signal transduction processes in neurons. We have demonstrated previously that 2,2'-dichlorobiphenyl (DCB), a neuroactive PCB congener, increases intracellular free Ca^{2+} concentration ($[Ca^{2+}]_i$) in primary cultures of rat cerebellar granule cells as measured using the Ca^{2+}-sensitive dye, Fluo-3. In the present experiments, we examined the time-course, concentration-response and dependence on extracellular Ca^{2+} concentration ($[Ca^{2+}]_e$) of DCB effects on $[Ca^{2+}]_i$ in cerebellar granule cells using Fura-2. In buffer solution containing 2.5 mM $CaCl_2$, basal $[Ca^{2+}]_i$ in cerebellar granule cells was 43±8 nM. Exposure of granule cells to 25, 50, and 100 μM DCB for 15 min increased $[Ca^{2+}]_i$ by approximately 1.5, 3.2 and 15 fold, respectively. During a 60 min exposure to 25 or 50 μM DCB, $[Ca^{2+}]_i$ increased by 4 and 11 fold, respectively. By contrast, exposure to 100 μM DCB resulted in a biphasic response; in a typical experiment, $[Ca^{2+}]_i$ increased to a peak of 1749 nM following 30 min of exposure and declined to 1014 nM after 60 min of DCB exposure. When $[Ca^{2+}]_e$=0.75 mM, a 30 min exposure to 50 and 100 μM DCB increased $[Ca^{2+}]_i$ from 36±7 nM to 100±27 and 453±60 nM, respectively. In buffers nominally free of $[Ca^{2+}]_e$, exposure to 50 or 100 μM DCB resulted in a small, transient increase $[Ca^{2+}]_i$ in cerebellar granule cells. However, when the buffer solution was returned to 2.5 mM $CaCl_2$, $[Ca^{2+}]_i$ increased to greater than 750 nM in 100 μM DCB solutions and began a gradual increase in 50 μM DCB solutions. These results confirm our previous observation that DCB alters intracellular Ca^{2+}homeostasis in cerebellar granule cells. Furthermore, they provide quantitative measurement of the concentration-response and time-course of DCB effects, and demonstrate that the DCB-induced increase in $[Ca^{2+}]_i$ is largely dependent on extracellular Ca^{2+}.

153.9

MOBILIZATION MECHANISM OF CALCIUM IN THE EARTHWORM MEDIAN GIANT FIBER. K. Oka* and H. Ogawa. Dep. of System Design Engin., Fac. of Sci. and Tech., Keio Univ., Yokohama 223, Japan and Kawachi millibioflight project, ERATO, JRDC, Tokyo 153, Japan

In the ventral nerve cord of the earthworm, *Eisenia foetida*, a median giant fiber (MGF) and a pair of lateral giant fibers (LGFs) are the most major and multifunctional interneurons mediating various behavior from contact reflex to escape response. In the MGF, we have reported Ca^{2+} wave propagation (Ogawa et al. 1994). We further investigated the spatio-temporal patterns of intracellular Ca^{2+} concentration ($[Ca^{2+}]i$) change for elucidating Ca^{2+} mobilization mechanisms in the MGF by a confocal laser-scanning microscope and a fluorescent Ca^{2+} indicator, Indo-1. Depolarization by a high-K^+ saline induced localized elevation of $[Ca^{2+}]i$ in the MGF. Bath-application of high-K^+ saline containing 1 mM Mn^{2+} reduced the Indo-1 fluorescence, and the Mn^{2+}-quenching pattern was heterogeneous in the MGF. From these results, we suggested that voltage dependent Ca^{2+} channels (VDCCs) were heterogeneously located on the MGF. Caffeine, which evoked the Ca^{2+}-induced Ca^{2+}-release (CICR) from intracellular Ca^{2+} stores, continuously increased $[Ca^{2+}]i$ in all over the MGF even in a Ca^{2+} free saline. This result indicated that the CICR mechanism was homogeneously distributed over the MGF. From the results stated above, we speculate a model of the cellular mechanisms for Ca^{2+} wave propagation in the MGF. Electrical excitation of the MGF activates VDCCs heterogeneously located on the MGF. Then, influx of Ca^{2+} through VDCCs induces localized increase of $[Ca^{2+}]i$ around the areas of high VDCCs' density, and triggers Ca^{2+} release from homogeneously distributed CICR mechanism that propagates Ca^{2+} waves.

153.10

MODULATION OF NMDA RECEPTOR FUNCTION IN RAT STRIATUM-INTERPLAY OF KINASES AND PHOSPHATASES. T. Blank*, I. Nijholt, H. Behrsing and J. Spiess. Dept. Molecular Neuroendocrinology, Max Planck Institute Exp. Medicine, Hermann-Rein-Str. 3, 37075 Goettingen, Germany.

Ionotropic excitatory amino-acid receptors can be broadly classified as N-methyl-D aspartate (NMDA) or non-NMDA receptors. Recent studies demonstrated the critical, countervailing role of protein kinases and protein phosphatases in regulating receptor function.

The role of protein phosphorylation in modulating NMDA channels was explored in *Xenopus* oocytes injected with poly $(A)^+$ mRNA, which was isolated from rat striatal and hippocampal nervous tissue. Two electrode voltage clamp recordings showed that one minute incubation of oocytes with the PKC activator phorbol 12-myristate 13-acetate (PMA; 10 nM) caused comparable potentiation of striatal (264 ± 33%; n=6) and hippocampal (244 ± 32%; n=9) NMDA induced currents.

In contrast, incubation with the PKA activator forskolin (50 μM) for one minute potentiated NMDA-mediated responses only in the striatum (145 ± 3%; n=4) whereas no effect could be observed with hippocampal NMDA-induced currents.

Interestingly, in rat striatum the PKA-mediated enhancement was inhibited when oocytes were preinjected with 500 nM calyculin A, a nonselective inhibitor of protein phosphatases 1 and 2A. On the other hand, the PKC-induced potentiation of NMDA-mediated responses was not affected by calyculin A injection. When 8-Bromo-cAMP (10 μM), the membrane-permeable analog of cAMP, was applied, responses were enhanced in a similar way as observed in the experiments using forskolin. Further incubation with PMA (10 nM) for one minute produced additional potentiation to 413 ± 60% of control (n = 4). These results demonstrate that both PKA and PKC potentiate NMDA-mediated responses in the striatum, however, different mechanisms appear to be involved. *(Supported by the Max Planck Society)*

153.11

DOPAMINE-MEDIATED PHOSPHORYLATION OF NMDA-R1 IN THE RAT NUCLEUS ACCUMBENS. G. Snyder*, A. Fienberg, I. Dulubova, A.C. Nairn, and P. Greengard. Lab of Molecular & Cell. Neuroscience, The Rockefeller University, New York, NY 10021

There is considerable evidence to indicate that the neurotransmitters dopamine (DA) and glutamate interact to control the activity of medium spiny-type neurons of neostriatum and nucleus accumbens. The mechanism for this interaction involves the regulation of phosphorylation of receptors, ion channels, and membrane pumps. We have now obtained evidence for the regulation of glutamate receptor phosphorylation by dopamine.

Nucleus accumbens slices were prelabelled with γ32P-phosphate. NR1 was immunoprecipitated and phosphate incorporation quantified by PhosphorImager analysis. Forskolin (1-50μM) or DA (100μM) increase phosphorylation of NR1, an effect which was mimicked by the D1-type receptor agonist, SKF-82526 (1μM), but not the D2-type receptor agonist, quinpirole (1μM). DA-induced phosphorylation of NR1 was blocked by an inhibitor of protein kinase A (H-89, 0.5 μM), but not by calphostin C, a protein kinase C inhibitor (1μM). Moreover, the ability of DA to phosphorylate NR1 was strongly attenuated in mice lacking πthe gene for DARPP-32, a DA-activated inhibitor of protein phosphatase-1 (PP-1).

Taken together, these data provide evidence for the regulation of the glutamate receptor subunit NMDA-R1 by the D1/DARPP-32/PP-1 pathways in neurons of the basal ganglia. (Supported by USPHS Grant DA-10044 to PG and an APDA Research Grant to AF).

153.12

ROLE OF DARPP-32 IN THE REGULATION OF Na^+,K^+-ATPase ACTIVITY IN STRIATAL NEURONS. A. Nishi[1], G.L. Snyder[1], A. Fienberg[1], P. Allen[1], G. Fisone[1], A.C. Nairn*[1], A. Aperia[2] and P. Greengard[1]. [1]Lab. of Mol. and Cell. Neurosci., The Rockefeller Univ., NY, NY 10021; [2]Dept. of Woman and Child Health, Karolinska Institute, Stockholm, Sweden.

Na^+,K^+-ATPase (NKA) is a ubiquitous enzyme essential for the maintenance of electrolyte balance. In neurons, NKA maintains the ionic gradients of Na^+ and K^+ that underlie the resting and action potential. We have previously shown that dopamine inhibits NKA activity in striatal neurons. In this study, the regulatory mechanisms utilized by dopamine were investigated in acutely dissociated striatal neurons prepared from C57BL/6 mice. Dopamine (10 μM) and the D_1 agonist SKF82526 (1 μM) each inhibited NKA activity by 20%. The inhibitory effect of SKF82526 was blocked by the D_1 antagonist SCH23390 (1 μM). The fraction of NKA activity attributable to the α2 and α3 isoforms of the enzyme was significantly inhibited by SKF82526 whereas the activity of the α1 isoform was unaltered. The inhibitory effect of SKF82526 was mimicked by the cyclic AMP analog, 8-Br-cAMP (1 mM), and was blocked by the cyclic AMP-dependent protein kinase (PKA) inhibitor, H89 (2 μM). In the same cell preparation, dopamine and SKF82526 were found to stimulate the phosphorylation of DARPP-32 (dopamine- and cAMP-regulated phosphoprotein-32kDa), an inhibitor of phosphatase-1 (PP-1). The effect of dopamine on NKA activity was examined in DARPP-32 knock-out mice. Dopamine inhibited NKA activity in striatal neurons from wild type but not DARPP-32 mutant mouse. In contrast, in mice deficient in inhibitor-1, a PP-1 inhibitor closely related to DARPP-32, NKA activity was inhibited by dopamine in both wild type and mutant. The phosphorylation of NKA was also examined using PKA phosphorylation-site specific Ab. The basal level of phosphorylation of NKA found in control condition was not modulated by PKA stimulants such as SKF82526 or forskolin. In conclusion, the inhibitory effect of dopamine is mediated by activation of the PKA pathway, and DARPP-32 plays an essential role in the regulation of NKA activity.
(Supported by U.S.P.H.S. Grant MH-40899)

153.13

THE NMDA RECEPTOR NR2A/B SUBUNITS BIND TO THE SH2-DOMAINS OF PLCγ. J.W. Gurd*, N. Bissoon and R. Wenthold. Division of Life Sciences, University of Toronto at Scarborough, West Hill, Ont. M1C 1A4 and Lab. Neuro-Otolaryngology, NIH, Bethesda, MD 20892.

Src-homolgy 2 (SH2) domains bind to phosphorylated tyrosine residues of proteins which are involved in intracellular signalling pathways. The NMDA receptor has recently been shown to be phosphorylated on tyrosine residues (Lau and Huganir, 1995, J Biol. Chem. 270 20036-41). In order to determine if tyrosine phosphorylation of the NMDA receptor might be involved in signal transduction in the postsynaptic cell we have investigated the interaction of the receptor with the SH2 domains of PLCγ. Sequences corresponding to the N-proximal and C-proximal SH2 domains of PLCγ were expressed as GST-SH2 fusion proteins and were bound to glutathione-agarose (PLC SH2-agarose). The binding of the postsynaptic glycoprotein, GP180, which corresponds to the NR2B subunit of the NMDA receptor (Moon et al., 1994. Proc. Natl. Acad. Sci. 91 3954-58), to SH2 domains was analysed by incubating the con A-binding glycoprotein fraction from rat synaptic junctions with PLC SH2-agarose. Bound glycoproteins were detected by immunoblotting with anti-tyr (P) antibodies. Tyr(P)-GP180 bound to both the N and C-proximal SH2 domains. Incubation of SJs with ATP increased the tyrosine phosphorylation of GP180 and enhanced its binding to SH2 domains. Analysis of immunoblots of SH2-bound proteins with antibodies specific for NR2A and B subunits confirmed that these receptor subunits bound to the SH2 domains and that binding was sensitive to phosphorylation levels; 0.45 +/- 0.05% and 6.1 +/- 2.0% of NR2A/B immunoreactivity being recovered in the SH2-binding fraction before and after incubation of SJs with ATP respectively. The results suggest that tyrosine phosphorylation may serve to link the NMDA receptor to signalling pathways which involve PLCγ.

Supported by NSERC.

153.14

INDENTIFICATION OF THE PRINCIPAL PHOSPHORYLATION SITE FOR Ca^{2+}/ CALMODULIN-DEPENDENT PROTEIN KINASE II ON NMDA RECEPTOR SUBUNIT NR2B. R.V. Omkumar, M.J. Kiely, and M.B. Kennedy*, Division of Biology 216-76, California Institute of Technology, Pasadena, CA 91125.

The NMDA subtype of excitatory glutamate receptors is important for synaptic plasticity during development and in the mature nervous system. The receptor is a heteromultimer of a core subunit, NR1, and one or more regulatory subunits NR2A-D. Phosphorylation of the receptor has been shown to regulate its function in living neurons[1-3]. However, the full range of regulation by various kinases, and the sites on the receptor that govern various functions are still unknown. We have found that Ca^{2+}/ calmodulin dependent protein kinase II (CaMKII) phosphorylates the NR2B subunit when the kinase is activated in the postsynaptic density fraction *in vitro*. To identify the site of phosphorylation, we used a recombinant fusion protein containing the C-terminal region of NR2B as a substrate for CaMKII *in vitro*. Compared to previously known substrates for CaMKII, the fusion protein showed very high affinity for the kinase as reflected in its Km. We purified and sequenced phosphopeptides from the fusion protein, identifying a single principal phosphorylation site in the tail of NR2B. By comparison of phosphopeptide maps, we found that this site is also the principal site phosphorylated in the full length NR2B by CaMK II in the postsynaptic density fraction. Furthermore, this site is phosphorylated *in vivo* in organotypic hippocampal cultures.

1. Lieberman and Mody (1994) *Nature* **369**, 235-239.
2. Wang and Salter (1994) *Nature* **369**, 233-235.
3. Wang et al. (1994) *Nature* **369**, 230-232.
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154.1

Regulation of Mel 1a melatonin receptor expression in ovine pars tuberalis cells by protein kinase C. Perry Barrett*, Gary Davidson, David Hazlerigg#, Alison MacLean, Shaun Conway and Peter Morgan. Rowett Research Institute, Bucksburn, Aberdeen AB21 9SB, Scotland and #Scottish Agricultural College, Aberdeen.

The long exposure of the melatonin receptor to the melatonin signal during extended periods of darkness (16h) in winter, presents a unique case in regulation of receptor function for a G-protein coupled receptor. Previous studies of ours have demonstrated that the mRNA for the ovine Mel 1a receptor is by cAMP through forskolin treatment of ovine PT cells. This elevation can be elevated reversed by melatonin. Furthermore there is a spontaneous increase in Mel 1a mRNA and protein levels in PT cells which can be inhibited by melatonin. This spontaneous increase is not due to a spontaneous increase in cAMP levels. We therefore sought to try and determine the mechanism of the spontaneous increase in Mel 1a mRNA and protein levels and its inhibition by melatonin. Here we present evidence that activation of isoforms of PKC by PMA in ovine PT cells can reverse the induction of the forskolin stimulated increase in Mel 1a mRNA levels. In addition this reversal occurs within the same time frame as the reversal by melatonin. Prolonged treatment of PT cells with PMA depletes several isoforms of PKC. In these depleted cells, melatonin can still reverse the forskolin elevated mRNA levels suggesting that melatonin does not act through the PMA responsive (depleted) isoforms of PKC. However, the involvement of another isoform of PKC is not ruled out, as the PKC inhibitor RO388220 has the same effect as PMA on forskolin elevated mRNA levels. Thus the expression and regulation of the melatonin receptor in ovine PT cells may be regulated by multiple pathways involving different isoforms of PKC.

This work was funded by SOAEFD.

154.2

MITOCHONDRIA MODULATE Ca^{2+} WAVES IN OLIGODENDROCYTES. P.B. Simpson and J. T. Russell* LCMN, NICHD, NIH.

Methacholine (MCh, 0.1mM) evoked Ca^{2+} waves were investigated in cultured rat oligodendrocyte processes. Wave propagation was supported by several regions of enhanced Ca^{2+} release kinetics (increased amplitude and rate of rise of response) along these processes. Using dyes that specifically stain mitochondria (JC-1 and $DiOc_6(3)$), we observed that groups of 3 or more mitochondria were invariably found in the specialized Ca^{2+} release sites along each process. Mitochondria were also found in dense clusters in the cell body. By staining the cell after stimulation with MCh, we found that in the processes, the presence of groups of mitochondria closely correlated with the sites of elevated Ca^{2+} release kinetics. Mitochondria were absent in regions of the processes where elevated kinetics were not found. FCCP (1 μM, 2-5 min) inhibited (47/249 cells) or potentiated (97/249) Mch-evoked Ca^{2+} signals in different cells within the same field and antimycin (2 μg/ml, 30 min), inhibited MCh responses in most (27/37) cells. A quantitative comparison of the presence of mitochondria with the sites of enhanced Ca^{2+} release was carried out using cross-correlation analysis which showed significant positive correlation. Similar positive cross correlation was also found when the patterns of immunocytochemical staining with antibodies for inositol trisphosphate receptors, SERCA pumps and calreticulin were compared with local Ca^{2+} release kinetics. It, therefore, appears that both the presence and the activity level of mitochondria may be important for MCh-evoked Ca^{2+} waves in oligodendrocytes, and that the regions that support wave propagation can be identified by the presence of mitochondria, which may modulate the level of Ca^{2+} near release sites, and by elevated levels of membrane proteins involved in Ca^{2+} signaling.

154.3

CALCIUM SIGNALING RESPONSIBLE FOR CHOLINERGIC ACTIVATION OF PHENYLETHANOLAMINE N-METHYLTRANSFERASE EXPRESSION. K. Morita* and D.L. Wong. Dept. of Psych. & Beh. Sci., Stanford Univ. Sch. of Med., Stanford, CA 94305

Phenylethanolamine N-methyltransferase (PNMT) has been shown to be transcriptionally regulated through both neural and hormonal mechanisms. However, despite considerable evidence for the activation of PNMT mRNA expression by neural and hormonal factors in the adrenal chromaffin cell, the molecular mechanisms by which these factors regulate PNMT gene expression have not yet been completely elucidated. We have previously shown that a neural factor carbachol (CCh) activates the PNMT promoter as a consequence of stimulating Egr-1 expression, raising the possibility that Egr-1 is one intrinsic factors mediating the neural regulation of PNMT gene expression. To further investigate the mechanism of neural regulation, we examined the effects of various drugs affecting calcium mobilization on CCh-induced PNMT promoter activation in the RS1 cell using a transient transfection assay. The stimulatory action of CCh on the PNMT promoter was not inhibited by organic and inorganic calcium channel blockers, and was completely inhibited by pretreatment of the cells with an intracellular calcium chelator BAPTA/AM. Further studies showed that CCh action was inhibited by pretreatment of the cells with thapsigargin, but not ryanodine. These results suggest that CCh elevates the cytoplasmic concentration of calcium through the stimulation of calcium mobilization from inositol-1,4,5-triphosphate ($InsP_3$)-sensitive calcium pools, resulting in the activation of PNMT promoter in the adrenal medullary cell.

154.4

MECHANISMS OF ELEVATED INTRACELLULAR Ca^{2+} AND STIMULATION OF TYROSINE HYDROXYLASE GENE EXPRESSION. A. Menezes*, H. Hiremagalur, R. Zeman[1], E.L. Sabban. Depts of Biochem. and Molecular Biology and [1]Cell Biol. and Anatom. New York Med. Coll., Valhalla, NY 10595.

Elevations of $[Ca^{2+}]i$ by different mechanisms can activate various calcium sensitive signalling pathways and regulate expression of several genes. We have been investigating the relationships between elevations of $[Ca^{2+}]i$ and increased tyrosine hydroxylase (TH) gene expression in PC12 cells. Blockers of various types of Ca^{2+} channels were added to PC12 cells and their ability to inhibit the rise in $[Ca^{2+}]i$ and elevation of TH gene expression were monitored by ratioed Fura-2 fluorescence and by Northern blots. The nicotine (100 μM) mediated induction of TH gene expression was inhibited by the L-type channel blocker, nifendipine and was coupled to activation of adenylyl cyclase. Membrane depolarization with 50 mM K^+ elevated $[Ca^{2+}]i$ from about 50 to 120± 8 nM. A N- type channel blocker w-conotoxin GVIA or L- type channel blocker calciseptine as well as dihydropyridine blockers reduced the rise in calcium by about 60 %. The Q-type calcium channel blocker w-conotoxin MVIIC blocked almost 90 % of the elevation of $[Ca^{2+}]i$. In contrast to nicotine, L-type channel blocker did not prevent the rise in TH mRNA in response to 50 mM K^+, while the Q type blocker inhibited the induction of TH mRNA. These results point to local actions of calcium in the vicinity of the respective channel.

Increased TH expression was also observed with released intracellular calcium stores by 300 nM thapsigargin, a Ca-ATPase inhibitor, which raised $[Ca^{2+}]i$ to 250-260 nM. Various concentrations of the chelator of intracellular Ca^{2+}, BAPTA were loaded to gradually decrease, but not prevent rise in $[Ca^{2+}]i$. The results indicate that in the range of 75-175 nM $[Ca^{2+}]i$, the elevation of TH gene expression by thapsigargin is directly proportional to the rise in calcium (Grant 251 from Smokeless Tobacco Research Council and fellowship from NY Heart Assoc).

154.5

ALDEHYDE DEHYDROGENASE (ALDH): LOW EXPRESSION IN RATS THAT OVEREAT FAT AND BECOME OBESE IN A NUTRIENT CHOICE CONDITION C. Tang*, P. Zheng, J. Ng, J. Wang and S.F. Leibowitz. The Rockefeller University, New York, N.Y. 10021

Studies in Sprague-Dawley rats, allowed to select from 3 macronutrient diets, have distinguished groups that spontaneously consume a high amount of fat (>40%; HFE) and gain weight, versus others that eat relatively little fat and stay lean (<20%; LFE). In order to identify the genes underlying this difference, mRNA differential display was used to screen high-fat (n=29) and low-fat (n=39) eaters. cDNA was made from the medial hypothalamus dissected from each group. One partial cDNA fragment, showing a 5-fold higher expression in the hypothalamus of a LFE, was isolated, cloned and confirmed with RNase protection assay. This fragment was used to screen a rat hypothalamus cDNA library, and a cDNA of 1.2 kb was identified and sequenced. The sequence had 80% homology with human aldehyde dehydrogenase (ALDH). To test the possible involvement of ALDH in fat metabolism, we analyzed the expression of ALDH in brain of the above two groups of rats, by using a rat ALDH cDNA fragment (from Dr. Pitot at the U. Wisconsin). By using RNA purified from both hypothalamus and midbrain, where ALDH-synthesizing neurons are concentrated, Northern blot showed lower mRNA level of ALDH in HFEs compared to LFEs. Using in situ hybridization, ALDH expression, while dense in the midbrain in the area of the substantia nigra (cell number/mm^2 of 257±21 for HFE vs 320±20 for LFE, $p<0.05$), was also dense in the ependymal layer surrounding the third ventricle of the hypothalamus and, once again, lower in HFEs. The function of this enzyme in the brain in relation to dietary fat is being explored.

154.6

OVINE PLACENTAL SEROTONIN TRANSPORTER (oSERT): CLONING, AND EXPRESSION, J.F. Padbury*, Y.T. Tseng, B. McGonnigal, K. Penado, M. Stephan and G. Rudnick. Dept. Pediatrics, Brown University, Providence, RI 02905 and Dept Pharmacology, Yale University, New Haven, CT 06510

The placenta is reported to express plasma membrane transporters for biogenic amines. We have demonstrated transporter-dependent placental catecholamine uptake *in vivo*. To confirm the molecular basis for this uptake, we screened an ovine placental cDNA library with human norepinephrine transporter (NET) cDNA. Several clones isolated and partially sequenced showed >87% homology to rat and human SERT. A 5.2 kb clone contained an 1893 bp open reading frame with >90% amino acid identity to human and rat SERT as well as 500 bp 5' untranslated sequence and ~ 3kb 3' sequence. Expression of this oSERT clone was carried out in transiently transfected HeLa cells. Transport assays using ^3H labeled substrates showed high affinity uptake of ^3H-5HT and little or no transport of ^3H-NE or ^3H-DA. The K_d for competition with ^{125}I βCIT binding in membrane versicles prepared from transiently transfected HeLa cells is shown in the Table.

Transporter	Competitor	K_D	Competitor	K_D
ovine SERT	imipramine	6.6 ± 1.6 nM	serotonin	1 ± 0.2 μM
rat SERT	imipramine	47.8 ± 7.3 nM	serotonin	0.9 ± 0.1 μM

We conclude: The ovine placenta has abundant expression of the neuronal SERT gene which may play a unique role in fetal life and be important in the pathogenesis of the effects of drugs like cocaine which block neurotransmitter re-uptake.

Supported by DA-07753

154.7

ROLE OF CREB IN CHRONIC MORPHINE-INDUCED ADAPTATIONS IN LOCUS COERULEUS NEURONS. S.B. Lane, J. Pineda, V. A. Boundy, K.L. Widnell, G.K. Aghajanian*and E.J. Nestler . Departments of Psychiatry and Pharmacology, Yale Univ School of Medicine, New Haven, CT.

Activation of locus coeruleus (LC) noradrenergic neurons mediates many of the signs and symptoms of physical opiate withdrawal. Previous work has shown that this activation is mediated in part by up-regulation of the cAMP pathway in the LC. Based on the finding that chronic morphine also increases expression of CREB (cAMP response element binding protein) in the LC (Widnell et al., *Proc Natl Acad Sci USA* 91, 10947, 1994), we have hypothesized a role for this transcription factor in the chronic morphine-induced up-regulation of the cAMP pathway in this brain region. In the present study, we investigated this possibility directly using a recently established CREB antisense oligonucleotide approach (Widnell et al, *J Pharmacol Exp Ther* 276, 306, 1996).

CREB antisense oligonucleotide was infused (20 µg/day) for 5 days via osmotic mini-pumps into the LC unilaterally. This treatment resulted in a partial reduction in CREB levels. This reduction was fully reversible upon cessation of oligonucleotide infusion, and was not seen upon infusion of CREB sense or scrambled oligonucleotide. CREB antisense oligonucleotide infusion decreased levels of certain cAMP pathway proteins in the LC. It also reduced the basal firing rates of LC neurons as well as their responses to 8-bromo-cAMP, consistent with a reduction in cAMP function. In addition, CREB antisense oligonucleotide infusion attenuated the ability of chronic morphine adminstration to upregulate certain cAMP pathway proteins in the LC as well as to increase basal firing rates of these neurons. However, the usual elevation in firing rates was restored upon application of 8-bromo-cAMP, suggesting an effect of CREB antisense oligonucleotide and morphine at a step proximal to cAMP formation. Together, these studies support a role for CREB in the maintenance of LC neuronal function and in the response of these neurons to chronic morphine administration. (Supported by DA08227.)

154.8

MOLECULAR MECHANISMS IN THE CELLULAR SPECIFICITY OF CREB EXPRESSION. E.R. Coven, K.L. Widnell, J.S. Chen, ^W.H. Walker, ^J.F. Habener, and E.J. Nestler*, Laboratory of Molecular Psychiatry, Yale Univ. Sch. Med., New Haven, CT; ^Harvard Med. Sch., MGH, Boston, MA.

CREB, cAMP Response Element Binding Protein, belongs to a family of leucine zipper transcription factors that share certain structural motifs and bind DNA as dimers. Perturbation of the cAMP pathway has been shown to regulate transcription of the CREB gene. Activation of the cAMP pathway in primary rat Sertoli cells results in up-regulation of CREB expression, an effect mediated via two CRE motifs found in the CREB promoter (Walker et al., 1995). Previously, we have demonstrated a transcriptionally mediated down-regulation of CREB expression by activation of the cAMP pathway in a locus coeruleus-like (CATH.a) cell line (Widnell et al., 1994, 1996).

In the current study, we examined the effect of stimulation of the cAMP pathway in rat C6 glioma cells. Brief treatment with isoproterenol and IBMX transiently down-regulates levels of CREB immunoreactivity and CRE-binding activity, whereas treatment for 6 to 24 hours significantly increases these levels. These effects are opposite to those observed in CATH.a cells. To determine whether these alterations could be accounted for by an activation of CREB promoter activity, CAT assays were performed in C6 cells transiently transfected with a CREB promoter-CAT fusion plasmid. Treatment for 24 h with isoproterenol and IBMX increases levels of CAT activity in C6 cells transiently transfected with plasmids containing progressive 5' deletions of a 1240 bp fragment of the CREB promoter. Mutations of the CRE or the Sp-1 DNA binding sites in the CREB promoter do not affect this regulation in C6 cells; likewise these mutations do not affect the forskolin-induced decrease in CAT activity observed in CATH.a cells. However, in both cell lines these sites play an important role in basal CREB promoter-driven CAT activity. These results show that regulation of CREB expression is transcriptionally mediated, exhibits an interesting cellular specificity, and may not be explained solely by recognized DNA-binding sites. (Supported by DA 08227).

154.9

CONVERGENCE OF CALCIUM AND GROWTH FACTOR SIGNALS ON CREB PROTEIN PHOSPHORYLATION IN GLIA. M. Pende* and V. Gallo. Lab. Cell. Mol. Neurophysiol., NICHD, NIH, Bethesda, MD 20892.

In rat cortical oligodendrocyte progenitor (O-2A) cells, activation of distinct receptors, such as glutamate-gated channels, G protein-coupled muscarinic receptors and growth factor receptors, lead to immediate early gene transcription initiation within 15 minutes (Pende and Gallo, Soc. Neurosci. Abstr. 1995, vol.21, Abstr. 521.5). We are now interested in defining the molecular mechanisms which account for such convergence of signals on gene expression. The selective agonists kainate and carbachol, and the combination of platelet-derived and basic fibroblast growth factors (PDGF and bFGF, respectively) induced phosphorylation of the transcription factor CREB (cAMP response element-binding protein) on Ser-133, as assessed by immunoblotting with a phospho-specific antiserum. The effects of kainate and carbachol on CREB were maximal 5 minutes after receptor stimulation, required calcium influx through the membrane and were inhibited by down regulation of protein kinase-C (PKC). Differently, the effects of PDGF+bFGF on CREB phosphorylation had a later onset, were long-lasting and were not affected by removal of extracellular calcium, or by down regulation of PKC. In transient transfection experiments, both calcium (kainate and carbachol) and tyrosine kinase (PDGF+bFGF) signals stimulated the activity of exogenous GAL4-CREB fusion protein, as measured by luciferase reporter gene expression driven by a promoter containing 5 GAL4 binding sites. Our findings suggest that CREB is a common nuclear target for distinct extracellular signals and is sufficient to induce receptor-mediated gene expression. Ongoing experiments are aimed at identifying the calcium- and growth factor-activated CREB kinases in O-2A cells. Supported by NIH.

154.10

EFFECTS OF AFTERDISCHARGE (AD) ON SYNTHESIS OF EGG-LAYING HORMONE (ELH) AND EXPRESSION OF PRO-ELH MRNA. W. Lee* and N. L. Wayne. Dept. of Physiology, UCLA Sch. Med., Los Angeles, CA 90095.

ELH of the marine mollusk, *Aplysia*, is a peptide cleaved from a larger prohormone, pro-ELH. It is synthesized by neuroendocrine bag cells and its secretion is triggered by an electrical AD. We hypothesize that AD stimulates ELH synthesis to replenish peptide loss through secretion and degradation. Previous work showed that depolarization of bag cells with high K^+ increased synthesis of ELH (Berry and Arch, 1981). To investigate whether AD has similar effects, pairs of bag cell clusters (n=3) were dissected from abdominal ganglia and separated from one another. One cluster was electrically stimulated; the other served as an unstimulated control. After initiation of AD, both clusters were incubated in medium containing 100 µCi/ml ^{3}H-leucine for 4 hr. Clusters were then homogenized and ELH peptide immunoprecipitated. Radioactivity of tritium was determined with a liquid scintillation counter. The result showed that AD stimulated a 2-fold increase in incorporation of ^{3}H-leucine into ELH. To determine whether this increase was due to increased expression of pro-ELH mRNA, bag cell clusters were dissected and electrically stimulated to AD; clusters from other animals were used as unstimulated controls. The clusters were homogenized 15, 30, 60, 120, and 240 min (n=3 each) after initiation of AD. Total RNA was isolated and subjected to Northern blotting analysis, using a ^{32}P-labeled ELH cDNA probe. The result showed that AD did not have a consistent effect on the level of pro-ELH mRNA at any of the time points compared to controls. These results indicate that AD stimulates ELH synthesis and this effect may occur mainly at the translational level. Supported by NIH-NS33548 (NLW).

154.11

Na^+/Ca^{2+} EXCHANGER ISOFORM EXPRESSION IN DIFFERENT REGIONS OF RAT BRAIN. L. Yu, L.C. Wince*, and R.A. Colvin. Program in Neurobiology, Ohio University College of Osteopathic Medicine, Athens, OH 45701.

The Na^+/Ca^{2+} exchanger (NCX) is a transmembrane protein that functions to maintain intracellular Ca^{2+} homeostasis in neurons and glia . There are three distinct isoforms cloned from rat brain, named NCX1, NCX2, and NCX3. The present study has determined the regional isoform distribution of NCX by RT-PCR and Northern analysis. RT-PCR was performed to amplify the three isoforms from brain stem, cerebellum, frontal cortex, striatum/septum, and hippocampus. Digoxin-11-dUTP labeled probes specific to NCX1, NCX2, and NCX3 were made and each was used to probe a Northern blot consisting of poly(A)$^+$ RNA isolated from the five regions. RT-PCR showed that all three isoforms were present in each region studied. Northern analysis showed that NCX1 had two transcripts with the size of 15 and 6kb, the 6kb transcript was predominant in brain stem and cerebellum. The 4.8kb NCX2 transcript was most abundant among the three isoforms in all regions except in brain stem where NCX2 expression was lowest. The 6kb NCX3 transcript was also present in each region and the amount was much less than NCX2. It is concluded that all of three isoforms of Na^+/Ca^{2+} exchanger are expressed in brain stem, cerebellum, frontal cortex, striatum/septum, and hippocampus. NCX2 expression is much more abundant than NCX1 and NCX2 indicating this isoform might be responsible for most of the protein transport activity in rat brain. Supported by grants from the Alzheimer's Association.

154.12

DISTRIBUTION OF mRNA FOR THE LIM-ONLY PROTEIN GENES *Lmo1, 2, 3* AND DIFFERENTIAL REGULATION IN THE MOUSE HIPPOCAMPUS FOLLOWING KAINATE-INDUCED SEIZURES. G. L. Hinks*, B. Shah, S. J. French, J. A. Poat and M. V. Sofroniew. Parke-Davis Neuroscience Research Centre and MRC Centre for Brain Repair, Cambridge University Forvie Site, Robinson Way, Cambridge, CB2 2QB, U.K.

LIM-only proteins contain cysteine-rich domains involved in protein-protein interactions. Studies suggest these interactions may be with transcription factors hence regulating transcriptional activity. In the present study we determined the expression pattern of the LIM-only protein genes *Lmo1*, *Lmo 2* and *Lmo 3* in the untreated adult mouse brain and following seizure activity, using radiolabelled oligonucleotide probes and a standard *in situ* hybridization protocol. *Lmo3* mRNA was found in many brain regions most notably throughout the hippocampus, striatum and cortex. *Lmo1* was expressed throughout the hippocampus and striatum, while *Lmo2* was predominately expressed in all regions of the hippocampus. Administration of the excitotoxin kainic acid (30 mg/kg i.p.) resulted in upregulation of *Lmo1* expression in the hippocampus. This was maximal 6 hours after injection returning to basal levels by 24 hours. In contrast, expression of *Lmo2* and *Lmo3* mRNA was reduced throughout the hippocampus with a similar timecourse. These studies suggest a role for lim-only proteins in the adult CNS, and that changes in *Lmo1, 2* and *3* expression are triggered by the cascade of events following excitotoxicity. Supported by the Wellcome Trust, MRC and Parke-Davis & Co. Ltd.

154.13

COMPUTER-ASSISTED IMAGE-AVERAGING STRATEGIES FOR THE TOPOGRAPHIC ANALYSIS OF *IN SITU* HYBRIDIZATION AUTORADIOGRAPHS . W. Zhao*, M.D. Ginsberg, J.T. Singer, O.F. Alonso, Y. Loor-Estades, W.D. Dietrich, M.Y.-T. Globus and R. Busto Cerebral Vascular Disease Research Center, Dept. of Neurology (D4-5), Univ. of Miami, Sch. of Med., Miami, FL, 33101.

In situ hybridization autoradiography is being increasingly applied to the topographic analysis of messenger RNA (mRNA) expression both in the normal brain and in brains of animals subjected to pathological insults. Conventional analysis of macroscopic (whole-section) autoradiographs typically involves densitometric region-of-interest measurements in individual brains; grand means may then be produced for multiple animals. We applied computer-assisted image-averaging strategies (Zhao et al, J Cereb Blood Flow Metab, 15:552-565) to the quantitative topographic analysis of whole-section *in situ* hybridization autoradiographs. The approach is based upon our recent development of a powerful method, termed "disparity analysis", for the alignment and mapping of sequential autoradiographic material. We mapped corresponding coronal sections from different animals into a preselected template to generate a "mean" section and a corresponding "S.D." (standard deviation) section. By setting a thresholding value, one can convert each section into a binary format. Mapping of these binary sections then yields a "frequency" map on which each pixel represents the number of animals affected. The application of these techniques to *in situ* hybridization autoradiographic analysis offers several advantages: a) quantitative mapping of *group trends* in a series of replicate animals by image-averaging; b) three-dimensional visualization of individual and averaged image data sets; c) quantitative topographic comparisons of mean trends in two or more series of replicate brains hybridized with the same probe but studied under differing physiological or pathological conditions; and d) feature-comparison of *in situ* autoradiographs derived from reaction of adjacent sections of the same brain with several different probes. This method has been applied to brain trauma study in our laboratory.

This work was supported by USPHS Grant NS 05820.

154.14

HIGH LEVEL EXPRESSION OF THE HUMAN HISTAMINE1 RECEPTOR IN SF9 CELLS: PHARMACOLOGICAL AND FUNCTIONAL COMPARISON TO THE MAMMALIAN CHO SYSTEM. K. Josson, M. Ercken, G. Van Hecke, M. Jurzak, P. Lijnen, K. L. De Loore, W. H. M. L. Luyten and J. E. Leysen*, Janssen Research Foundation, Department of Biochemical Pharmacology, 2340 Beerse, Belgium

The baculovirus expression system allows high levels of protein expression in insect cell lines. Functional coupling and/or posttranslational modifications for mammalian proteins, however, might be impaired. The coding region of the human histamine1 (H1) receptor was subcloned into the baculo expression vector pBacpak9. Expression levels in virus infected Sf9 cells were analyzed using [^{3}H]pyrilamine binding. Bmax values increased with post-infection time up to 137 pmol/mg protein after 48-72 h; the K_D-value of [^{3}H]pyrilamine was 1.8 nM. Binding competition experiments using various H1-antagonists did not show significant pharmacological differences compared to the human H1 receptor expressed in CHO cells (Bmax values of 7 pmol/mg protein). Functional responses to histamine, however, were more marked in transfected CHO cells. In inositol phosphate measurements a 10-fold and 3-fold rise over basal levels were obtained with 100 µM histamine in CHO and Sf9 cells, respectively. Measurements of intracellular Ca^{++} ($[Ca^{++}]_i$) in fura2 loaded cells revealed a 4-fold smaller peak of $[Ca^{++}]_i$ in the insect cells compared to the CHO cells. The most striking difference was found in measurements of [^{3}H]arachidonic acid (AA) release. Histamine stimulated the AA release in CHO cells up to 20-fold, but AA release could be detected in Sf9 cells. Therefore, the insect cell system proved to be useful for binding experiments but suboptimal for functional studies.

154.15

REGIONAL DISTRIBUTION, AGE AND SPECIES DIFFERENCES OF BRAIN PHOSPHOLIPASES A_2. L. A. Horrocks*, H.-C. Yang and A. A. Farooqui. Dept. Med. Biochem., The Ohio State Univ., Columbus, OH 43210.

Phospholipases A_2 (EC 3.1.1.4, PLA_2) are involved in signal transduction and neurodegeneration. Cytosol fractions from bovine, horse, dog, pig, human and rat brains contain two different Ca^{2+}-independent PLA_2 (PtdEtn-selective and PlsEtn-selective PLA_2) that are separable on a Sephadex G-75 column. These enzymes are present in frontal, parietal, occipital, and temporal cortices and hippocampus. The occipital cortex from dog brain shows the highest activity of PtdEtn-selective and PlsEtn-selective PLA_2. Activities of these enzymes increase rapidly in rat brain during development. Polyclonal antibodies against purified PlsEtn-selective PLA_2, prepared in rabbits, do not cross-react with types I, II, or III, the 85 kDa $cPLA_2$ or the Ca^{2+}-independent PLA_2 from P388D_1 macrophage-like cells. The PlsEtn-selective PLA_2 in rat is present only in brain and spinal cord. Western blotting experiments confirmed the presence of PlsEtn-selective PLA_2 immunoreactivity in cytosolic fractions, but not in membrane fractions, from horse, dog, pig, human and rat brains. The presence of these phospholipases A_2 in various mammalian brains suggests that they are involved in the regulation of arachidonate release and eicosanoid formation in the CNS.

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154.16

Expression of Elk-1 mRNA and protein in the central nervous system of the rat. J.Caboche, P. Vanhoutte, M. Rogard, C. Pagès, and M.J. Besson* Lab. Neurochimie-Anatomie, IDN, UPMC, URA 1488, 9 Quai St Bernard, 75005 Paris.

The transcription factor Elk-1 plays an important role in the induction of c-*fos* by proliferative and CNS-specific signals that activate MAP kinase cascades. The activated MAP kinases translocate to the nucleus, where one of their major targets is Elk-1, which is part of the protein complex assembled on the c-*fos* SRE. The subsequent phosphorylation converts Elk-1 into a potent transactivator of transcription, thus Elk-1 is a key mediator of signal-directed gene activation.

We have analyzed the expression of Elk-1 in the rat central nervous system at both the mRNA and protein levels. Rat brain sections were probed with a ^{33}P-cRNA specific for the 3' region of mouse Elk-1. Elk-1 mRNA was highly expressed in neurons of the piriform cortex, the olfactory tubercules, the hippocampus and the cerebellum. Lower levels of Elk-1 hybridization were found in the cerebral cortex, the basal ganglia, and the amygdala. Elk-1 protein levels, revealed by immunocytochemistry using an antibody directed against the C-terminal region of the protein, showed a good correlation with the mRNA signals. One exception was the substantia nigra pars reticulata (SNr), where we detected strong immunoreactivity with the Elk-1 antibody in spite of low mRNA levels. Interestingly we observed immunolabelling in both nuclear and cytoplasmic compartments in all the regions investigated. Furthermore some dendrites also showed strong labeling. These results suggest that, in the central nervous system, Elk-1 might change its subcellular compartmentalization upon activation and/or may have other functions in addition to gene regulation. Intriguingly Western blot analyses using specific N-terminal or C-terminal Elk-1 antibodies reveal two isoforms of the protein in nervous tissues. One corresponds to the full length protein (52 kD) found in PC12, Cos7, or NHI3T3 cells, and the other appears to be a novel, smaller protein (45 kD) that is presently under investigation.

154.17

THE ZINC FINGER PHOSPHOPROTEIN ETO AND THE RIBOSOMAL PHOSPHOPROTEIN P2 LOCALIZE TO RIBOSOMES PRIMARILY IN SPINE SYNAPSES IN THE ADULT RODENT BRAIN. R. S. Lasher* and P.F. Erickson†. Depts. of C & S Biology and Medicine†, Univ. Colorado Med. Sch., Denver, CO 80262.

The zinc finger phosphoprotein ETO is part of a fusion protein produced by the 8;21 translocation in acute myelogenous leukemia [Erickson et al., 1996. *BLOOD*, in press]. Relatively high levels of ETO are found in developing brain suggesting that it could be involved in the regulation of some aspect of neural development [Erickson et al., 1994. *Cancer Res.* 54:1782]. Using an affinity-purified polyclonal antibody to ETO we found by Western blotting that ETO is present in synaptic junctions. We then investigated the localization of this protein in adult rat brains by the ABC method. Immunohistochemically, ETO is seen in nuclei and as a fine punctate pattern in regions of neuropil. Preembedding immuno-electron microscopic analysis of ETO localization in the neuropil indicated that it is seen to be associated with 20 nm particles primarily in the postsynaptic elements of spine synapses in e.g., the cerebellum and cerebral cortex. These 20 nm particles were found in groups reminiscent of polysomes and also were packed against and within the postsynaptic density. The size, distribution and morphology of the particles suggested that they might be ribosomes. Localization of ribosomal proteins was investigated in adult rat brain using two antibodies: a mouse monoclonal antibody to the ribosomal phosphoprotein P2 [Uchiumi et al., 1987. *J. Biol. Chem.* 265:89] and a polyclonal antibody to the ribosomal protein L7a [Ziemiecki et al., 1990. *EMBO J* 9:191]. Preembedding immuno-electron microscopic analysis of the localization of these antibodies indicated that P2 and L7a are associated with 20 nm particles in synapses having the same distribution as seen for ETO. However, L7a was also highly concentrated in cytoplasmic rough ER, but ETO and P2 were not seen in this region. These data suggest a possible role for P2 and ETO in the regulation of synaptic protein synthesis. (Supported by NSF IBN-932 0823).

154.18

EXPRESSION OF ARRESTIN, PHOSDUCIN AND OPSIN IN HIGHLY RESTRICTED SMALL NUMBERS OF CELLS IN THE MOUSE BRAIN. K. Sunayashiki-Kusuzaki[1]*, T. Kikuchi[1], E. Wawrousek[2] and T. Shinohara[1]; [1]The Center for Ophthal. Res., Brigham and Women's Hospital, Harvard Medical School, 221 Longwood Ave. Boston, MA 02115. [2]Section on Molecular and Developmental Biology, NEI, NIH, Bethesda, MD 20892

Arrestin is expressed in the retinal photoreceptor rod cells and pinealocytes. To examine whether it is expressed in the brain tissue, we constructed a fusion gene which has well characterized a mouse or a bovine arrestin promoter and a *Lac* Z reporter gene, and generated three lines of transgenic mice having the fusion gene. (1) Serial sections of the brains were stained with X-Gal after fixation. The positive cells of β-galactosidase were found in the habenula commissura (chb), amygdala, ventral tegmental area (VTA) and superior colliculus in adult transgenic mice brain. (2) Immunohistochemistry on the brain sections with the antibody probes revealed that arrestin and phosducin were expressed at high levels and opsin was expressed at low level in the same brain regions. We found new cell types in brain which express arrestin, phosducin and opsin in these regions. Immunostaining for the 5-hydroxyindole-O-methyltransferase, an enzyme catalyzes a terminal step of melatonin synthesis, was also positive in the same cell types of chb and VTA. (3) The pattern of expression of these proteins is similar to that of pinealocytes but not that of retinal photoreceptor cells. These findings suggest that melatonin is synthesized and secreted from these cells. It is highly probable that a diurnal cycle of the brain cells is controlled by melatonin secreted from those cells. In contrast, melatonin from pineal gland regulates the cycle in the rest of the body through bloodstream.

155.1

PROTEIN INTERACTIONS ASSOCIATED WITH HOMEOBOX PROTEIN MEDIATED SECOND MESSENGER STIMULATION OF THE DOPAMINE β-HYDROXYLASE PROMOTER. DJ Swanson*, E Zellmer, EJ Lewis. Dept. Biochemistry and Mol. Biol., Oregon Health Sci. Univ., Portland OR, 97201

An enhancer element of the rat dopamine β-hydroxylase (DBH) gene that is necessary for tissue specific and second messenger mediated transcriptional activation contains two homeodomain (HD) core sites adjacent to two putative CRE-like domains. We have shown that Arix, an adrenergic-tissue specific homeobox protein which binds to this enhancer, is able to confer adrenergic-like regulation of the DBH promoter in non-adrenergic cells through a synergistic interaction with second messenger stimulation. This synergistic activation suggests an interaction of Arix with other nuclear proteins within the context of this DBH enhancer, termed the HD/CRE.

In an electrophoretic mobility shift assay (EMSA), four distinct protein-DNA complexes are formed using the HD/CRE probe and nuclear extracts from either unstimulated or cAMP/phorbol ester stimulated PC12 cells, suggesting that proteins binding to the enhancer region do so constitutively. Oligonucleotide competition indicate that HD and CRE-like sites contribute to distinct protein-DNA complexes independently and as compound sites. While competition with a consensus CRE indicated that CRE binding proteins contribute to some HD/CRE-protein complexes, antibody supershift analyses demonstrated that neither CREB nor c-fos are constituents of these complexes.

We have also co-purified five distinct proteins with molecular weights ranging from 65-300kD using an Arix affinity assay. To date Western blot and immunoprecipitation analyses have ruled out the second messenger-related transcriptional co-activators CBP and p300 as candidates for the highest molecular weight protein (MR 265-300kD). Further analyses are in progress to identify these candidate HD/CRE and Arix binding proteins as possible co-regulators of tissue-specific DBH transcription.

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155.2

SERUM RESPONSE ELEMENT (SRE)-MEDIATED INDUCTION OF zif268 GENE IN PC12D CELLS IS INHIBITED BY INCREASES IN INTRACELLULAR CALCIUM. T. Ebihara and D. Saffen* Institute for Brain Research, The University of Tokyo, Hongo 7-3-1, Tokyo 113, Japan.

We have previously reported that mRNA encoding the transcription factor Zif268 is rapidly induced following stimulation of m1 muscarinic acetylcholine receptors in PC12D cells, a subline of PC12 that undergoes accelerated neuronal differentiation in the presence of nerve growth factor (NGF). In the present study we examined the molecular mechanism of zif268 induction using expression vectors containing specific segments of the zif268 promoter linked to a luciferase reporter gene. As expected, a 0.5 kb proximal fragment of the zif268 promoter containing 6 SRE-like sequences was found to mediate the induction of the luciferase activity by NGF. Unexpectedly, however, this promoter fragment did not induce the reporter following exposure to carbachol, phorbol ester or calcium ionophore. Carbachol also failed to induce luciferase mRNA, but did rapidly increase levels of endogenous zif2689 mRNA. These results are surprising, because NGF, carbachol, and the other agents are all effective in rapidly activating MAPK, the kinase thought to be required for SRE-dependent induction of the c-fos gene. Also unexpectedly, prior exposure to carbachol or calcium ionophore was found to reduce NGF-mediated induction of the luciferase reporter by 50%. A similar inhibition obtained with membrane-depolarizing concentrations of KCl, was specifically blocked by the L-type calcium channel blocker nifedipine. Taken together, these data indicate that increases in intracellular calcium antagonize NGF-mediated gene induction. This inhibition was not observed for a luciferase expression vector containing only a single SRE, suggesting that calcium does not block the SRE directly, but rather requires additional DNA sequences. (Supported by grants from the Ministry of Education, Science, and Culture of Japan)

155.3

ACTIVATION OF AP-1 TRANSCRIPTION FACTORS AFTER EXTRACELLULAR ATP TREATMENT IN PC12 CELLS. Y.-M. Chen and A.Y. Sun*. Department of Pharmacology, University of Missouri, Columbia, MO 65212

Studies in our laboratory indicate that extracellular ATP may induce cell death by reactive oxygen insults. The ATP-induced cell death is P_{2X} receptor specific and is dependent on Ca^{++}. Lipid peroxidation of cell membranes occurred immediately after the addition of ATP. In addition a delayed cell death was observed. The purpose of this study was to examine how Ca^{++} causes transcription activation of the neuronal-death cascade. Results using a gel shift technique indicated that ATP treatment greatly increases AP-1 DNA binding in nuclear extracts from PC12 cells. Supershift assays using anti-Jun and anti-Fos antibodies demonstrated that Fos protein formed part of the AP-1 heterodimer, but not Jun protein. The changes from the normal Jun/Fos complex may alter the AP-1 DNA binding and transcriptional properties. The activation of AP-1 binding was P_2 receptor specific since suramin blocked the activation. Both cyclohexamide and genistein also blocked the ATP activation of AP-1 binding activity indicating that the activation requires new protein synthesis and protein phosphorylation, especially by tyrosine kinase. This result indicates that the Ca^{++}-induced oxidative stress as elicited by ATP may lead to an activation of a specific AP-1 activity. (Supported in part by NIH Grant #AA02054)

155.4

GLUCOCORTICOIDS ATTENUATE AP-1 AND NFκB DNA BINDING ACTIVITIES IN RAT BRAIN: IMPLICATION OF GLUCOCORTICOIDS IN NEURONAL DEGENERATION. T. Unlap and R.S. Jope*. Dept. of Psychiatry and Behavioral Neurobiology, Univ. of Alabama at Birmingham, Birmingham, AL 35294-0017.

Neuronal degeneration occurs in various diseases including aging and Alzheimer's disease, in part due to oxidative stress, characterized by excess reactive oxygen intermediates. Neurons counteract oxidative stress by increasing antioxidants, DNA and protein repair enzymes, and free radical scavenging enzymes (FRSE). Two transcription factors, AP-1 (heterodimer of Jun/Fos) and NFκB (heterodimer of p50/p65, activate the FRSE system. Aging and Alzheimer's disease are characterized by high circulating glucocorticoid levels which can exacerbate neuronal degeneration. We have previously shown that this involves the attenuation of AP-1 DNA binding activity by glucocorticoids through a mechanism of protein-protein interaction between the glucocorticoid receptor (GR) and c-Jun. To study if this also involved the NFκB transcription factor, we examined NFκB DNA binding activity by gel mobility shift assay in rat cortex and hippocampus after 1, 3, 6 or 24 hr of dexamethasone (DEX; 2mg/kg) treatment. We found that DEX attenuated NFκB activity in both brain regions. It is unlikely that this effect of DEX is mediated through IκB since cytosolic IκB levels were significantly attenuated as well. With a p65 antibody, the GR and p65 coprecipitated. This may suggest that high circulating glucocorticoid levels, as found in aging and Alzheimer's disease, contribute to neuronal degeneration by compromising neurons' ability to counteract oxidative stress by attenuating AP-1 and NFκB DNA binding activities through a mechanism of protein-protein interaction between the GR and constituents of AP-1 and NFκB, and thus partially disabling the FRSE system.

Supported by NIH grant AG06569.

155.5

TNF SIGNALING AND PROTECTION AGAINST Aβ TOXICITY: ROLES FOR NFκB AND MnSOD. G.H. Umberger*, A.J. Bruce, S.W. Barger, F. W. Holtzberg, S. M. Steiner, and M.P. Mattson. Sanders-Brown Center on Aging, Dept. of Anatomy and Neurobiology, and Dept. of Biological Sciences, University of Kentucky, Lexington, KY 40536.

We previously reported that the cytokine tumor necrosis factor-α (TNF), which is released from active microglial cells and has been shown to be increased in Alzheimer's Disease (AD) brains, suppresses reactive oxygen species (ROS) formation and protects primary neurons from Aβ toxicity (*PNAS* 92:9328-9332). We now describe signaling pathways that mediate these neuroprotective actions of TNF. Exposure of cultured hippocampal and cortical neurons to TNF resulted in significant time-dependent increases in MnSOD activity and protein levels; TNF had little or no effect on other antioxidant enzymes, including Cu/Zn-SOD and catalase. One TNF signaling pathway involves ceramide release (from membrane-associated sphingomyelin) and activation of the transcription factor NFκB. We found that C2-ceramide mimics the effect of TNF on MnSOD activity. To determine if a specific increase in MnSOD protein and activity levels is sufficient to protect nerve cells from Aβ toxicity, we stably transfected pheochromocytoma (PC6) cells with human MnSOD. These cells exhibited a 2-3 fold increase in MnSOD protein and activity levels, and no changes in the levels of Cu/Zn-SOD or catalase. Aβ induced time-dependent increases in peroxide formation and subsequent cell death in control cells, while MnSOD over-expressing PC6 cells were protected from both manifestations of Aβ-induced injury. These results suggest that TNF decreases Aβ-induced oxidative stress in neurons through the induction of MnSOD by NFκB, and suggest that TNF associated with plaques could serve a neuroprotective function. (supported by the NIH).

155.6

OXIDATIVE STRESS IMPAIRS CHOLINERGIC SIGNALING. X. Li*, L. Song, and R.S. Jope. Dept. of Psychiatry and Behavioral Neurobiology, Univ. of Alabama at Birmingham, Birmingham, AL 35294-0017.

Oxidative stress appears to contribute to neuronal dysfunction in a number of neurodegenerative conditions, notably Alzheimer's disease in which cholinergic receptor-linked signal transduction activity is severely impaired. To test if oxidative stress could contribute to such deficits in cholinergic signaling, responses to carbachol were measured in human neuroblastoma SH-SY5Y cells exposed to H_2O_2. DNA binding activities of two transcription factors that are respondent to oxidative conditions, AP-1 and NFκB, were measured in nuclear extracts. H_2O_2 and carbachol individually induced dose-and-time-dependent increases in AP-1 and NFκB. In contrast, when given together H_2O_2 concentration-dependently (30 to 300 μM) inhibited the increase after carbachol in AP-1. Carbachol's stimulation of NFκB was not inhibited except with a high concentration (300 μM) of H_2O_2, which was associated with impaired activation of protein kinase C. Lower concentrations of H_2O_2 (30 to 300 μM) inhibited carbachol-induced [^{3}H]phosphoinositide (PI) hydrolysis and this inhibition correlated ($r = 0.95$) with the H_2O_2 concentration-dependent inhibition of carbachol-induced AP-1. Activation of [^{3}H]PI hydrolysis by the calcium ionophore ionomycin was unaffected by H_2O_2, indicating that phospholipase C and phosphoinositides were impervious to this treatment. In contrast, activation with NaF of G-proteins coupled to phospholipase C was concentration-dependently inhibited by H_2O_2, indicating that G-protein function was impaired by H_2O_2. These effects of H_2O_2 are similar to signaling impairments reported in Alzheimer's disease cortical membranes which involve deficits in receptor- and G-protein-stimulated PI hydrolysis, but not phospholipase C activity. Thus, these findings demonstrate that oxidative stress may contribute to impaired PI signaling in Alzheimer's disease and that oxidative stress can differentially influence transcription factors activated by cholinergic stimulation. Supported by NIH grant AG06569.

155.7

INTERACTIONS OF MEMBRANE LIPIDS AND OXIDATIVE STRESS ON AP-1 BINDING IN PC-12 CELLS. I. Cantuti-Castelvetri*, W. Hassan[1], E. Paulson,[1] N.A. Denisova and J.A. Joseph. USDA Human Nutrition Research Ctr. on Aging at Tufts Univ., [1]Department of Biochemistry, Tufts University, School of Medicine, Boston MA, 02111.

Research suggests that interactions between oxidative stress and altered brain membrane lipid composition in senescence (e.g., increased cholesterol/phospholipid ratios, sphingomyelin levels, etc.) may be responsible for the age-related deficits seen Ca^{2+} activity. Studies from our labs have shown that exposure of PC-12 cells to non-lethal H_2O_2 concentrations in growth media for 30 min. increased pre-K^+-stim Ca^{2+} levels and post-stim recovery time (Ca^{2+} clearing), while decreasing the K^+-stimulated Ca^{2+} depolarization levels. Ca^{2+} levels have been proposed to play important roles in AP-1 transcription factor regulation, and events such as long-term potentiation. Furthermore, AP-1 is highly responsive to oxidant stress stimuli. Therefore, we examined the binding and transcription regulation of AP-1 by H_2O_2 and altered lipid composition. Cholesterol was shown to increase overall basal AP-1 binding compared to that of H_2O_2 stimulated cells, but had no effect on H_2O_2 induced AP-1 binding. Interestingly, sphingomyelin was shown to have no effect on basal AP-1, but dramatically increased AP-1 binding in response to H_2O_2 treatment. These results demonstrate that membrane components can dramatically alter DNA-binding properties of the oxidative stress regulated AP-1 protein. (Supported by USDA intramural)

155.8

CYTOKINE INDUCES iNOS AND $sPLA_2$ IN ASTROCYTES. W. Li, J. Xia*, M. Hannink and G.Y. Sun Biochem. Dept and Nutritional Sciences, Univ. Missouri, Columbia, MO 65212.

Astrocyte is known to have multifunctional roles in the brain including conferring various defense mechanisms against neuronal injury. Recent studies indicate the ability of astrocytes to respond to endotoxin (LPS) and pro-inflammatory cytokines such as tumor necrotic factor α (TNFα) and interleukin 1β (IL-1β). This signaling system results in the dissociation of a RelA homodimer from its inhibitory kappa B protein (MAD3) and subsequent binding of RelA to DNA (Diehl et al., J. Biol. Chem. 270: 2703, 1995). Cytokines are known to induce the increase in mRNA encoding the secretory phospholipase A_2 ($sPLA_2$) and nitric oxide synthase (NOS). In this study, we examined the pattern of cytokine induction for $sPLA_2$ and NO in an immortalized astrocyte cell line (DITNC). Although TNFα was the weakest in stimulating $sPLA_2$ release into the culture medium, it could synergistically enhance $sPLA_2$ release when added in combination with IL-1β or LPS. Exposure of cells to staurosporine, a protein kinase C (PKC) inhibitor, abolished completely the cytokine induction of $sPLA_2$ mRNA, suggesting of an involvement of PKC. When these cells were tested for induction of NO, neither TNFα, IL-1β or LPS alone was able to stimulate NO release significantly as compared to interferon γ (IFNγ). In addition, IFNγ together with TNFα and/or IL-1β greatly synergized NO release. Northern blot analysis showed the induction of iNOS mRNA in DITNC cells. Induction of iNOS mRNA could be inhibited by L-N^6-(1-iminoethyl)lysine acetate (NIL), a specific iNOS inhibitor. Interestingly, cytokine induction of $sPLA_2$ mRNA was enhanced by NIL. These results indicate the presence of both commonalities and differences in cytokine induction of iNOS and $sPLA_2$ in astrocytes. (Supported by MU Res. Board & AA 06661 from NIH).

155.9

ANTISENSE RNA IDENTIFIES $G_{\alpha 12}$ AS A MEDIATOR OF AGONIST INHIBITION OF M-CURRENT IN SYMPATHETIC NEURONES, P. Delmas, F. Abogadie, J. Haley, Y. Vallis, and N.J. Buckley and M.C. Caulfield*. Wellcome Laboratory for Molecular Pharmacology, Dept. Pharmacology, University College London, U.K.

We have investigated the role of $G_{\alpha 12}$ in mediation of agonist inhibition of the M-current (K^+_M) in cultured superior cervical ganglia (SCG) neurones. G-protein immunostaining and in situ hybridization in SCGs showed that $G_{\alpha 12}$ is expressed only in neurons whilst $G_{\alpha o}$ is expressed in both neurones and non-neurones. We used a PCR-cloning strategy to make expression plasmids in the pBK-CMV vector containing antisense sequences to the 3'untranslated region of $G_{\alpha 12}$ and the coding region of rat $G_{\alpha o}$. $G_{\alpha 12}$-antisense-injected SCGs show diminished immunostaining for $G_{\alpha 12}$, but not for $G_{\alpha o}$. Conversely, $G_{\alpha o}$-antisense-injected SCGs show diminished staining for $G_{\alpha o}$, but not for $G_{\alpha 12}$. Intranuclear injection of the $G_{\alpha 12}$ antisense construct in rat SCG cells resulted in a reduction of muscarinic inhibition of the K^+_M 48 hours later (300nM Oxo-M inhibition: control, 30 ± 7 %, n=3; injected, 6 ± 1 %, n=8). This inhibition was overcome by increasing the concentration of oxo-M 10 fold. Antisense contructs for $G_{\alpha o}$ did not alter K^+_M inhibition. Thus, muscarinic suppression of the K^+_M can be mediated via 2 different G_α subunits, $G_{\alpha 12}$ and $G_{\alpha q}$ (see accompanying abstract), belonging to 2 different classes of pertussis toxin insensitive G-proteins.

This work is supported by the Wellcome Trust and the Medical Research Council

155.10

ANTISENSE RNA DISTINGUISHES BETWEEN $G_{\alpha q}$ AND $G_{\alpha 11}$ MEDIATED AGONIST INHIBITION OF M-CURRENT IN SYMPATHETIC NEURONES, F. Abogadie*, P. Delmas, J. Haley, Y. Vallis, G. Milligan§, M.C. Caulfield and N.J. Buckley. Wellcome Laboratory for Molecular Pharmacology, Dept. Pharmacology, University College London, U.K. §Dept. Biochemistry, University of Glasgow, Scotland.

We have used an antisense approach in order to determine the involvement of $G_{\alpha q}$ or $G_{\alpha 11}$ in the muscarinic inhibition of the M-current (K^+_M) in cultured superior cervical ganglia (SCG) neurones. A PCR-cloning strategy was used to make pBK-CMV constructs containing antisense sequences to the coding region of rat $G_{\alpha o}$ and the 3'untranslated regions of rat $G_{\alpha q}$ and $G_{\alpha 11}$. SCG neurones were injected with anti-$G_{\alpha q}$, anti-$G_{\alpha 11}$ and anti-$G_{\alpha o}$ (as controls) plasmid expression vectors (400 μg/ml). The ability of Oxo-M (a muscarinic agonist) to inhibit K^+_M was checked 48 h after injection using the perforated patch method. In neurones injected with anti-$G_{\alpha q}$ expression vectors, the inhibition of K^+_M by 1 and 3 μM Oxo-M was significantly ($P<0.01$) less (8% and 19%,respectively; n=9) than in either noninjected or anti-$G_{\alpha o}$ injected neurones (56% and 75%, respectively), whereas in anti-$G_{\alpha 11}$ neurones (n=8) the extent of K^+_M inhibition was similar to that found either in non-injected or anti-$G_{\alpha o}$ injected cells. However, a subpopulation (5/8) anti-$G_{\alpha 11}$ injected cells did show a diminished attenuation of K^+_M. G-protein immunostaining and in situ hybridization in SCGs showed that $G_{\alpha q}$ is expressed in all neurons but not in glia. $G_{\alpha 11}$ is expressed only in a subpopulation of neurons while $G_{\alpha o}$ is expressed in all cells. $G_{\alpha q}$-antisense-injected SCGs show diminished staining for $G_{\alpha q}$, but not $G_{\alpha o}$. These data strongly suggest that the inhibition of K^+_M by muscarinic receptors is primarily mediated via a $G_{\alpha q}$ protein.

This work is supported by the Wellcome Trust and the Medical Research Council

155.11

A NOVEL REGULATION OF EXPRESSION OF THE *α*-SUBUNIT OF THE G STIMULATORY PROTEIN, $G_s\alpha$, BY DOPAMINE VIA D-1 DOPAMINE RECEPTORS. A. Sidhu*, K. Kimura, Z-B. Tong, and D. Pilch Lab. Neurochem., Georgetown Univ. Med. Ctr., Washington D.C. 20007.

Chronic exposure of human neuroblastoma SK-N-MC cells, endogenously expressing D-1 dopamine receptors, to dopamine (DA) produces a progressive loss (upto 70%) of immuno-detectable membrane-bound levels of the *α*-subunit of G_s. The loss in $G_s\alpha$ protein was accompanied by a reduced ability (64.3) of NaF-mediated stimulation of adenylyl cyclase activity, after reconstitution of DA-treated $G_s\alpha$ with cyc$^-$ membranes. This loss of functional $G_s\alpha$ activity was independent of cAMP levels. The reduction in $G_s\alpha$ protein was manifest at the transcriptional level and $G_s\alpha$ mRNA levels were attenuated to 56.5 ± 10% of control. $G_s\alpha$ mRNA levels were also attenuated by the D-1 agonist SKF R-38393 and the attenuation by agonists was blocked by the D-1-selective antagonist, SCH 23390. Accumulated levels of cAMP did not modulate $G_s\alpha$ mRNA levels. These results indicate that in SK-N-MC cells, $G_s\alpha$ is subject to novel regulatory controls by DA, acting specifically via D-1 DA receptors. Supported by grant NS 29685.

155.12

INCREASED EXPRESSION OF PROGESTERONE RECEPTOR mRNA INDUCED BY A D_1 DOPAMINE RECEPTOR AGONIST IN BRAIN. C.M. Isbister* and P.B. Reiner, Kinsmen Laboratory of Neurological Research, University of British Columbia, Vancouver, BC, V6T 1Z3.

Stimulation of the cAMP second messenger cascade has been shown to activate reporter gene transcription by the estrogen receptor in a "ligand-independent" fashion in various cell lines. Since estrogen receptors are known to induce progesterone receptor (PR) gene expression in many tissues including brain, we used PR gene expression as a reporter assay to test the hypothesis that such convergence occurs in the intact mouse brain. Ovariectomized adult female mice were injected (i.p.) once daily for 4 days with either control vehicle, 11μg of estradiol, or 30mg/kg of the D_1 receptor agonist SKF 38393. Semi-quantitative RT-PCR was used to measure expression of PR mRNA, normalized relative to expression of tubulin mRNA. Since both D_1 receptors and PR mRNA are found in high abundance in the prefrontal and cingulate cortex, we focused our investigations on regulation of cortical PR mRNA. As expected based upon previous receptor binding data, we found that estradiol markedly increased cortical PR mRNA. The D_1 agonist SKF 38393 also increased cortical PR mRNA; quantitative densitometry demonstrated that the increase evoked by SKF 38393 was 165% of that induced by estradiol. Our working hypothesis is that dopamine receptor activation results in ligand-independent activation of estrogen receptor transcriptional activity, ultimately resulting in up-regulation of PR gene expression in the brain.

(Supported by the Medical Research Council of Canada)

155.13

INDUCTION OF c-FOS mRNA BY LEAD IN PC 12 CELLS, K. Kim, T. Chakraborti, G.W. Goldstein and J.P. Bressler*, Depts. of Neurology, Pediatrics and Environmental Health Sciences, The Johns Hopkins University and The Kennedy Krieger Research Institute, Baltimore, MD 21205.

In children, subtle defects in behavior, psychomotor development, and learning are associated with exposure to low levels of lead (Pb^{2+}). The mechanisms for these effects are unknown. It has been postulated that long-term neuronal changes such as those that occur during development or learning require the expression of specific genes. Thus, Pb^{2+} may affect neurodevelopment by interfering with the regulation of gene expression. Here, we demonstrate that Pb^{2+} induces expression of the immediate early response gene, c-fos. Induction of c-fos mRNA by Pb^{2+} was concentration- and time-dependent. In the presence of cycloheximide, the c-fos gene was superinduced by addition of Pb^{2+}. This ability of Pb^{2+} to induce c-fos mRNA was not duplicated by adding Cd^{2+} or Zn^{2+}. Induction by Pb^{2+} was inhibited by actinomycin D, a blocker of transcription. Also, c-fos mRNA was less stable than glyceraldehyde phosphate dehydrogenase mRNA in cells exposed to Pb^{2+}. Since the c-fos gene encodes a transcription factor, our data suggest that Pb^{2+} has the potential to regulate the expression of other genes.

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155.14

AMANTADINE INDUCES EXPRESSION OF C-FOS: COMPARISON WITH APOMORPHINE AND MK-801, M. S. Rappaport* and D. P. Yells, Department of Psychiatry, Univ. Nebraska Medical Center, Omaha, NE 68198

Amantadine (AMA) is used therapeutically to ameliorate conditions resulting from diminished dopaminergic neurotransmission (e.g., Parkinson's disease and extrapyramidal side effects of antipsychotics). Effects of AMA include enhanced release of dopamine (DA), inhibition of the reuptake of DA and weak, noncompetitive NMDA receptor antagonism, properties all consistent with its observed therapeutic effects. We recently found that AMA disrupts prepulse inhibition (PPI) of the acoustic startle reflex in rats. Haloperidol pretreatment prevented AMA disruption of PPI. In the same paradigm, MK-801 caused a disruption of PPI which was not prevented by haloperidol. The present study extends our effort to characterize the mechanism of AMA's therapeutic action. We examined the effect of AMA on the expression of immediate early gene product c-Fos in rat forebrain structures. Results were compared to the effects of DA receptor agonist apomorphine (APO) and noncompetitive NMDA receptor antagonist MK-801 on c-Fos induction. Male rats were injected ip with either AMA (20 mg/kg), APO (4.0 mg/kg), MK-801 (1.0 mg/kg) or 0.9% NaCl and were transcardially perfused with 4% paraformaldehyde 2 hr later. Fixed, frozen brains were sectioned coronally and a free floating, fluorescent immunohistochemical technique was used to visualize nuclear c-Fos. AMA induced c-fos in the striatum in a pattern grossly resembling that resulting from treatment with APO. MK-801 did not noticeably induce c-fos in the striatum. These results, along with our finding that haloperidol pretreatment prevents AMA and APO-induced disruption of PPI, but not MK-801-induced disruption of PPI are consistent with a DAergic mediation of AMA's therapeutic actions. Comparative studies of compartmental distributions of c-Fos induced by APO and AMA in rat striatum are in progress.

Funded by a grant from the Nebraska Psychiatric Foundation (MSR) and the Dept of Psychiatry, University of Nebraska Medical Center.

155.15

C-*FOS* GENE EXPRESSION IN GRANULE CELLS DURING CEREBELLAR DEVELOPMENT AND FOLLOWING KAINIC ACID INJECTION. S. Chen* and D.E. Hillman, Department of Otolaryngology and Physiology /Neuroscience, New York University Medical Center, New York, NY 10016.

Immediate early genes (IEG) couple short term external stimuli to long term cellular phenotypic changes by regulating late response target genes. The IEG, c-*fos*, maps to physiologically active neurons corresponding with 2-deoxyglucose. We examined c-*fos* expression in albino rats from birth to postnatal day 60 and after kainic acid administration into adults which normally have low baseline c-*fos* expression. We found that the c-*fos* gene is transiently expressed in granule and basket cells during postnatal development and in granule cells of adult animals following kainic acid administration.

Fos positive cells were found in the internal granular layer of ventral lobules at P5 and increased in all folia by P10. These reactive cells were distributed in clusters across the granular layer reaching their highest intensity at P12. Meanwhile, basket cells, which have reached their destination in the lower part of the molecular layer, also exhibited strong Fos protein staining. At P15, Fos protein labeling gradually declined and was no longer detectable by P21 and later. In adult rats, c-*fos* expression was induced in granular cells within 1 hour after a sub-seizure dosage of kainic acid (8mg/kg), peritoneously. The pattern of Fos protein distribution resembled developing granular cells.

Our results support the hypothesis that the c-*fos* gene plays a role in signaling transcription of target genes related to circuitry establishment as well as neuronal plasticity. Supported by NIH-NINCDS NS-13742 and NIA AG-09480.

155.16

IDENTITY OF THE CHRONIC FRAS INDUCED BY REPEATED COCAINE AND ELECTROCONVULSIVE SEIZURE (ECS) TREATMENTS. J.S. Chen*, M.B. Kelz, B.T. Hope^, Y. Nakabeppu° and E.J. Nestler, Laboratory of Molecular Psychiatry, Yale University School of Medicine, New Haven, CT; ^Molecular and Developmental Neuroscience, Massachusetts General Hospital, Charlestown, MA; °Medical Institute of Bioregulation, Kyushu University, Fukuoka 812, Japan.

Previous studies have shown that repeated administration of cocaine, ECS, or other agents induces several long-lasting Fos-related antigens (Fra), named chronic Fras. These proteins are FosB-related, as indicated by Western blotting with specific anti-FosB antibodies and now confirmed in FosB knock-out mice (see Hiroi et al., this volume). However, the slow induction and persistent expression of the chronic Fras are not consistent with the rapid and transient induction of the mRNAs for FosB and its spice variant ΔFosB. In the present study, we transfected neuronal Cath.a, and C6 glioma, cell lines with FosB or ΔFosB cDNAs, and compared FosB and ΔFosB proteins with the chronic Fras induced by repeated ECS treatment. Four proteins with apparent molecular weights of 29, 33, 35 and 37 kD were generated from the ΔFosB cDNA. Gel shift assays show that the AP-1 complex from the ΔFosB transfected cells migrated at the same position as the chronic AP-1 complex. In 2-D gel Western blotting, the 35 kD protein migrated to the same position as the 35 kD chronic Fra, which suggests that these two proteins are identical. Comparison of the stability of FosB and ΔFosB shows that ΔFosB is more stable than FosB. These results suggest that ΔFosB is an atypical immediate early gene: the mRNA of ΔFosB is induced rapidly and transiently, however, some of the protein products are stable and accumulate with repeated induction. The 37 kD protein from the ΔFosB transfected cells is not as abundant as that induced by chronic ECS or cocaine in brain, which suggests that posttranslational modification in the brain may be required for the accumulation of the 37 kD chronic Fra. We are currently using cell lines in which expression of FosB or ΔFosB can be induced to characterize the unique functional properties of the chronic FRAs. (Supported by DA07359.)

155.17

FOSB "KNOCK-OUT" MICE: LOSS OF CHRONIC FRAs AND ABNORMALITIES IN COCAINE-REGULATED BEHAVIOR. N. Hiroi*[1], J. R. Brown[2], C. Haile[1], M. E. Greenberg[2], E. J. Nestler[1]. [1] Div. of Mol. Psychiatry, Yale Univ. Sch. of Med., New Haven, CT 06508; [2] Children's Hospital, Harvard Med. Sch., Boston, MA 02115.

We have shown that chronic treatment with cocaine or electroconvulsive seizures (ECS) induces chronic FRAs in a region-specific manner in brain. Chronic FRAs are 35-37 kDa proteins recognized by an antiserum raised against a peptide sequence common to Fos family members. The chronic FRAs are immunochemically related to deltaFosB, a truncated splice variant of FosB, but their precise identity has remained elusive (*Mol. Pharmacol.* 48, 880, 1995). We have now used mutant mice, in which the *fos*B gene has been disrupted by homologous recombination, to further study the identity of the chronic FRAs as well as their physiological role in cocaine action.

Chronic FRAs, and their associated AP-1 DNA binding activity, were induced by chronic cocaine and chronic ECS treatments in specific brain regions of wild-type mice, as observed previously in rats. In contrast, induction of the chronic FRAs and the associated AP-1 binding activity was completely absent in *fos*B mutant mice. These findings clearly establish the chronic FRAs as products of the *fos*B gene, perhaps a variant of deltaFosB (see also Chen et al., this volume).

As a first step in assessing the functional role of the chronic FRAs, we studied cocaine-induced locomotor activity and sensitization in the wild-type and *fos*B mutant mice. The wild-type animals exhibited a gradual increase in cocaine-induced locomotor activity (i.e., sensitization) over a 6 day treatment. In contrast, the mutant animals showed a marked increase in locomotor activity in response to the first cocaine exposure, with no further increase seen after repeated exposures. The wild-type and mutant mice, however, exhibited equivalent levels of conditioned locomotor activity.

These findings show that the absence of *fos*B gene products, including the chronic FRAs, is associated with specific abnormal behavioral responses to cocaine and support an important role for these transcription factors in cocaine action. (Supported by DA07359 and DA08227).

155.18

JAK-STAT PATHWAYS IN NEURONAL CELL LINES.
K. Shimoda*, H. Takahashi#, J.W. Commissiona##, T. Oshima, K. Inoue, D. Kaneto and T. Kitagawa.
Div. of Neurology, National Nishitottori Hospital, Tottori 68902, JAPAN. #Lab. Histochem., Mitsubishi-kasei, Inst. of Life Sci. Machida 163, JAPAN. ##NTU, LMB, NINDS, NIH, Bethesda, MD 20892, USA.

In recent studies of the signal transducers and activators of transcription (STATs) of the cytokines in lymphoid cell lines, many STAT proteins were identified. We previously established an S1 cell line prepared by immortalization of cells from the fetal rat diencephalon, using the viral SV40 large T antigen. This S1 cell line expressed several protein tyrosine kinases, that were shown to be coupled to various receptors for neurotrophic factors and cytokiness (Soc. for Neurosci Abst. 446.1, 1994). We also identified the involvement of Janus kinases (Jak) in this cell line, in the signal transduction pathway (Soc. for Neurosci Abst. 415.14, 1995). We have now identified a 100 kDa phosporelated STAT 6, and are investigating the mechanisms of its activation in PC12 and F9 teratocarcinaoma cell lines after exposure to differentiation factors. Understanding and clarifying the mechanisms of signal transduction and activation of transcription pathways would be an important step in our studies of differentiation and may provide clues to help to understand the mechanisms of neuronal degeneration.

155.19

GLUTAMATE ACTIVATES SAPK/JNK IN STRIATAL CULTURES. M.A.Schwarzschild*, R.L.Cole and S.E.Hyman. Mass. Gen. Hosp., Boston, MA 02129.

Stress-activated protein kinase (SAPK) also known as Jun N-terminal kinase (JNK) activates the c-Jun component of the AP-1 transcription factor complex, and can mediate apoptosis and other "stress" responses in cell lines. To explore neurotransmitter regulation of AP-1 mediated gene expression in neurons, we examined the effects of glutamate and dopamine on SAPK/JNK and its targets in primary cultures of striatal neurons from E-18 rats. Glutamate but not dopamine rapidly increases SAPK/JNK activity measured *in vitro* by an immune-complex kinase assay. Pharmacologic analysis suggested primary involvement of an NMDA receptor, as the glutamatergic activation of SAPK/JNK was mimicked and blocked by NMDA agonists and antagonists, respectively. Moreover, the glutamate activation requires the presence of glycine a necessary co-agonist with glutamate at the NMDA receptor. Glutamate was ineffective in glial cultures derived from the same striatal cell preparation, whereas hyperosmolar mannitol exposure led to similar SAPK/JNK activations in both the neuronal and glial cultures. Although the related kinase p38 is typically activated by cellular stresses in parallel with SAPK/JNK, hyperosmolar mannitol but not glutamate increased p38 activity. Western blot analysis demonstrated that glutamate also increases levels of phosphorylated c-Jun. Thus glutamate via NMDA receptors can rapidly and specifically activate SAP/JNK in cultured striatal neurons. The associated increase in phospho-c-Jun and AP-1 mediated gene expression may play a role in the physiologic and neurotoxic effects of glutamate.

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155.20

REGULATION OF AP-1 MEDIATED GENE TRANSCRIPTION BY GLUTAMATE, BUT NOT DOPAMINE, IN RAT PRIMARY STRIATAL CULTURES. R.L. Cole*, M.A. Schwarzschild, M.A. Meyers and S. E. Hyman. Laboratory of Molecular and Developmental Neuroscience, Massachusetts General Hospital, Charlestown, MA 02129.

Corticostriatal glutamatergic projections and mesostriatal dopaminergic projections are the predominant afferents innervating the medium spiny neurons of the striatum. AP-1 transcription complexes have been hypothesized to play an important role in drug-mediated plasticity within these neurons. Here we investigate the differential role of these two major neurotransmitters in the regulation of AP-1 mediated gene expression in primary cultures of rat striatal neurons. Glutamate stimulation of striatal neurons activates Jun kinase, induces c-fos and c-jun mRNA, increases AP-1 protein binding, and activates transcription of a transiently transfected 4X-AP-1-luciferase construct. All of these glutamate effects are blocked by pre-treatment with the non-competitive NMDA-receptor antagonist MK-801. In marked contrast, stimulation by dopamine and forskolin, an activator of cAMP pathways, induces c-fos mRNA and increases AP-1 binding, but fails to activate Jun kinase, induce c-jun mRNA or activate AP-1 mediated gene transcription. Therefore, although glutamate, dopamine and forskolin all increase AP-1 binding in gel mobility shift assays, the activation of c-Jun by glutamate may also be required for AP-1 mediated transcription in rat striatal neurons.

Supported by PHS grant DA07134

NEUROENDOCRINE REGULATION: PARAVENTRICULAR HYPOTHALAMIC NUCLEUS

156.1

PROJECTIONS OF NPY NEURONS IN THE ARCUATE NUCLEUS (ARC) TO THE PARAVENTRICULAR NUCLEUS (PVH): IS THERE REGIONAL SPECIFICITY WITHIN THE ARC? C. Li*, P. Chen and M. S. Smith, Division of Neuroscience, Oregon Regional Primate Research Center; Dept. Physiol./Pharm., Oregon Health Sciences Univ., Beaverton, OR 97006.

We have identified a specific population of NPY neurons in the caudal portion of the ARC that is activated in response to the suckling stimulus or food deprivation. The present studies addressed two questions: 1) do NPY neurons from the caudal portion of the ARC project to the PVH, the site involved in regulation of food intake, and 2) are NPY projections to the PVH evenly distributed throughout the rostral to caudal extent of the ARC? The retrograde tracer, fluorogold (FG), was injected into the PVHp (2% solution, iontophoresed by 5 μA current, pulsed at 7 sec intervals for 20 min) on day 1 postpartum in animals suckling 8 pups. On day 11, the lactating animals were perfused with tissue fixative, and brain tissue (25 μM sections) was subjected to immunocytochemistry to visualize FG, followed by *in situ* hybridization for NPY mRNA. The presence of FG-containing cells in the ventrolateral medulla, an area identified as projecting to the PVH, was used as a positive control for the retrograde labeling technique. FG-labeled NPY cells were identified throughout the rostral to caudal extent of the ARC; however, double-labeled cells were preferentially distributed in the caudal portion of the ARN (27 ± 1% rostral *versus* 73 ± 1 % caudal, p<0.001). There was a similar rostral to caudal distribution of single-labeled NPY cells (as reflected by total NPY mRNA/section). Thus, the relative proportion of NPY neurons projecting to the PVH was similar in the rostral and caudal ARC. However, because the caudal ARC contains a larger number of NPY neurons, their influence on the activity of the PVN would predominate. Thus, the increase in NPY neuronal activity in the caudal portion of the ARC, in conditions such as lactation and food deprivation, would have the greatest effect on altering the activity of PVH neurons. (Support: NIH grants HD14643, RR00163).

156.2

SEQUENTIAL ESTRADIOL (E) AND PROGESTERONE (P) FOLLOWED BY P WITHDRAWAL DOES NOT ALTER THE PLASMA OXYTOCIN (OT) SECRETORY RESPONSE TO CHOLECYSTOKININ (CCK) IN OVARIECTOMIZED (OVX) RATS, N. B. Kim*, D. J. Hollingshead, A. Thomas and J. A. Amico. Div. of Endocrinology, Univ. of Pittsburgh Sch. of Med. and VA Med. Ctr., Pittsburgh, PA 15261.

The hormone OT is important for several pre- and post partum events, including uterine contractions at parturition, the induction of maternal behavior, and milk ejection during nursing. During late pregnancy OT mRNA is increased in the paraventricular nucleus (PVN) due to high E and declining P levels. Administration of sequential E and P to and withdrawal of P from an ovx rat also increases OT mRNA. However, pituitary OT peptide is not affected. In the present experiment, we determined if this steroid exposure alters peripheral OT secretion during a provocative stimulus to OT release, such as CCK. Adult ovx Sprague-Dawley rats were implanted on day 1 with either E or empty silastic capsules, on day 3 with P or empty capsules, and on day 14 P or empty capsules were removed. Forty-eight and 72 hrs after removal of the P capsules, plasma OT was measured before and after i.v. injection of 10 μg/kg of CCK. At the completion of the study, pituitary glands were removed and OT peptide was measured. No significant differences were found between the sham and hormone-treated animals either in their basal or CCK-stimulated plasma OT levels nor their pituitary content of OT peptide. The hormonal pattern of sequential E and P with P withdrawal has no apparent effect on CCK mediated OT secretion or posterior pituitary OT content. This suggests that gonadal steroids can affect OT mRNA independent of pituitary OT peptide content or release.

Supported by Merit Review funds from the Dept. of Veterans Affairs and NIH Grant 5T32-DK-0752.

156.3

TIME COURSE OF INDUCTION OF OXYTOCIN MESSENGER RIBONUCLEIC ACID LEVELS IN GONADAL STEROID-TREATED RATS, B. J. Blyth, D. J. Hollingshead, N. B. Kim and J. A. Amico*. Div. of Endocrinology, Univ. of Pittsburgh Sch. of Med. and VA Med. Ctr., Pittsburgh, PA 15261.

The nonapeptide oxytocin (OT) is important for uterine contractility at parturition, milk ejection during lactation, and the induction of maternal behavior. OT mRNA levels increase in the paraventricular nucleus (PVN) of late pregnant and lactating rats and are modulated by the steroid milieu that accompanies these states. Specifically, exposure to sequential estrogen (E) and progesterone (P) followed by P withdrawal 48 hrs prior to sacrifice increases PVN OT mRNA. To better define the time course of induction of OT mRNA levels following P withdrawal, ovariectomized Sprague-Dawley rats (6-10/group) were treated with empty or steroid-filled capsules. On day 1, animals received an E-filled or empty capsule, followed by P-filled or empty capsules on day 3. On day 14, P-filled or empty capsules were removed and animals were sacrificed 24, 36, or 48 hrs later. The hypothalamic PVN were analyzed for OT mRNA by *in situ* hybridization histochemistry. Significant differences in PVN OT mRNA were found among the groups (P<0.0001, Kruskal-Wallis). Animals in the 48 hr (P=0.007) and 36 hr (P=0.005), but not the 24 hr, steroid-treated groups had significantly increased OT mRNA relative to their respective sham-treated cohorts (Mann-Whitney U test). The relative abundance of PVN OT mRNA differed among the steroid-treated groups (Kruskal-Wallis, P<0.001), with peak OT mRNA at 48 hr. We conclude that increases in PVN OT mRNA occur by 36 hrs, and peak at 48 hrs, after P withdrawal in the E-primed rat. Future studies will determine if the OT-mediated changes in behavior or physiology that surround parturition are related to these changes in OT mRNA.

Supported by Merit Review funds from the Dept. of Veterans Affairs.

156.4

STATIONARY ORGANOTYPIC CULTURES OF RODENT HYPOTHALAMIC OXYTOCIN AND VASOPRESSIN NEURONS. **S.B. House, A. Thomas, K. Kusano* and H. Gainer. Lab. of Neurochemistry, NIH, NINDS, Bethesda, MD 20892-4130.**

In order to perform long-term studies of differentiated magnocellular oxytocin (OT) and vasopressin (VP) neurons in vitro, we previously developed an "organotypic" roller-culture system (Wray et. al., Peptides, 9:1151-1175, 1988) in which many OT, but very few VP cells, survived. Recently, a "stationary" slice culture system has been reported to be very effective in maintaining "organotypic" suprachiasmatic nuclei in vitro (Belenky et. al., Neuroscience, 70:127-143, 1995). In this study we describe the efficacy of this culture system in maintaining magnocellular OT and VP neurons from the mouse and rat hypothalamus. Hypothalamic slices (400uM) from postnatal (PN) day 7-9 rats or PN day 2-6 mice were cultured for 14-16 days on 30mm wide millicell-CM filters (Millipore, Corp.). Following this the cultured slices were fixed in 4% formalin in PBS and evaluated by immunocytochemistry using monoclonal and polyclonal antibodies to OT- or VP-neurophysin. Approximately 15% of the total population of magnocellular OT neurons originally present in the rat hypothalamic slices as well as substantial numbers of VP neurons survived in an "organotypic" fashion in these cultures. Similar results were obtained with mouse hypothalamic slices. Studies in progress which compare various media and procedures used in the slice preparation and culture stages (e.g., the use of serum-containing versus defined media), and the use of these cultures for gene expression (biolistics) and physiological studies will be described. This work is funded by the NINDS, NIH, Intramural Program.

156.5

QUANTITATIVE RT-PCR ANALYSIS OF OXYTOCIN, VASOPRESSIN AND SYNAPTOTAGMIN ISOFORMS IN RAT HYPOTHALAMUS AND PITUITARY. D. Xi, H. Chin, and H. Gainer*. Lab. of Neurochemistry, NINDS, NIH, Bethesda, MD 20892.

Measurement of gene transcripts in small samples of cells or tissues by reverse transcription-polymerase chain reaction (RT-PCR) has become a standard technique due to its high sensitivity. We have developed competition-based RT-PCR methods with DNA or RNA mimics in order to quantify oxytocin (OT), vasopressin (VP) and synaptotagmin (SYT) isoform mRNA as well as other mRNAs present in individual samples of hypothalamus and pituitary during development and various forms of functional activity (e.g., during dehydration and lactation). We found that either mimic could be used to determine the relative changes for a given mRNA, but comparison of mRNA levels among different genes required an RNA mimic. Preliminary RT-PCR analysis shows that SYT isoforms I-IV are present in rat hypothalamus under various experimental conditions. The ratios of SYT I-IV to GAPDH (glyceraldehyde-3-phosphate dehydrogenase was used as an internal control) mRNA appeared changed in postnatal day 7 and lactating female rat hypothalamus when compared to control rats. In addition, we found that the ratio of OT to GAPDH was increased about 5 fold in lactation. These results suggest that gene expression of SYT isoforms undergoes developmental and functional regulation. Further studies of the regulation of OT, VP and SYT isoform mRNA expression in hypothalamus and pituitary will be described. This work was supported by the NINDS, NIH, Intramural Program.

156.6

CELL-SPECIFIC GENE EXPRESSION OF OXYTOCIN AND VASOPRESSIN IN HYPOTHALAMIC MAGNOCELLULAR NEURONS. S.W. Jeong[1], D.M. Witt*[1], H. Chin[1], H. Arnheiter[2] and H. Gainer[1]. Labs. of Neurochemistry[1] and Developmental Neurogenetics[2], NINDS, NIH, Bethesda, MD 20892.

Regulatory elements for restricting expression of oxytocin (OT) and vasopressin (VP) genes to magnocellular neurons of hypothalamus have been hypothesized as being located in an intergenic region (IGR) between the two genes. The IGR of mouse is 3.5 kb in length and shows a 73% identity with a stretch of 3.5 kb found in the 9.9 kb IGR of rats. This stretch of homologous sequence contains several consensus elements for transcription factor binding (Ratty, Jeong et al., in press), suggesting that this region is involved in regulating the expression of the OT and VP genes. To test this hypothesis, we generated 6 OT and VP constructs that contain the mouse IGR and a LacZ reporter gene to be tested in transgenic mice and in mouse hypothalamic slice cultures transfected by gold particle-mediated gene transfer (Biolistics). Of these, two were tested. One was an 11 kb VP transgene construct consisting of 4 kb of 5' upstream region followed by the LacZ reporter and the 3.5 kb mouse IGR. The other was an 8 kb OT transgene construct consisting of 1 kb 5' upstream region, the mouse IGR and LacZ reporter. In transgenic mice, both expressed the LacZ gene throughout the brain of transgenic mice but this expression was not specifically confined to the hypothalamus. This suggested that the exon and/or intron sequences absent in these constructs might be necessary for the cell-specific expression of OT and VP genes. The 4 other OT and VP transgenes contain the exon and intron sequences with the LacZ reporter inserted in either the first or third exon, followed by the IGR. These constructs are presently being tested in transgenic mice and biolistic studies. This work is funded by the NINDS, NIH, Intramural Program.

156.7

GONADAL STEROID-INDUCED INCREASES IN PARAVENTRICULAR NUCLEUS (PVN) OXYTOCIN (OT) MESSENGER RIBONUCLEIC ACID (MRNA) LEVELS: A POSSIBLE ROLE FOR A GAMMA-AMINOBUTYRIC ACID (GABA)- MEDIATED PROCESS. A. Thomas*, N.B. Kim, J.A. Amico. Div. of Endocrinology, Univ. of Pittsburgh Sch. of Med. and VA Med. Ctr., Pittsburgh, PA 15261

Interactions between ovarian steroids and PVN OT neurons have been recognized for sometime but incompletely understood. Changing peripartum levels of ovarian steroids modulate PVN OT mRNA levels. Specifically sequential exposure to estrogen (E) and progesterone (P) followed by withdrawal of P increases OT mRNA in the PVN of the rat. Maintenance of P attenuates OT mRNA. Although gonadal steroids affect OT neurons, they contain few if any, E or P receptors. Within the CNS, P metabolites can bind to the GABA receptor complex. We questioned whether P may act upon OT mRNA via a GABA-mediated mechanism. In the presence of P, its metabolites would increase GABA tone and possibly inhibit OT expression. With removal of P, declining metabolites would lessen GABA tone and possibly increase OT expression. To test this hypothesis, ovariectomized (ovx) Sprague-Dawley rats were administered E-filled implants on day 1 , P-filled implants on day 3, injected with either diazepam (1mg/100g body weight, a GABA agonist) or placebo on days 13-16, with removal of P implants on day 14 and sacrifice on day 16. Ovx rats that received no steroid treatment were used as controls. PVN OT mRNA levels were compared by *in situ* hybridization. Significant increases were found in OT mRNA levels in rats that received steroids and placebo injections (no diazepam) vs. sham hormone-treated or steroid-treated diazepam-injected animals ($p<0.04$,Kruskal-Wallis). Diazepam administration attenuates steroid-induced increases in PVN OT mRNA. The data suggest that the effects of P upon OT mRNA in the E- primed rat may be mediated by GABA. Supported by Merit Review funds from the Dept. of Veteran's Affairs and NIH Grant 5T32-DK-0752.

156.8

PARTIAL COEXISTENCE OF NADPH-DIAPHORASE AND ACETYLCHOLINESTERASE IN THE HYPOTHALAMIC SECRETORY NUCLEI OF THE RAT. F. Sánchez[1], C. Crespo[2], R. Arévalo[2], J. Carretero[1], E. Saldaña*[2],J. Aijón[2], and R. Vázquez[1]. [1]Dept. Human Anatomy and Histology. [2] Dept. Cell Biology and Pathology. Universidad de Salamanca. 37007 Salamanca Spain.

Colocalization of NADPH-diaphorase and acetylcholinesterase (AChE) was explored in the magnocellular secretory nuclei of the rat hypothalamus by means of combined double histochemical staining of the same sections. Partial coexistence was found in all the nuclei studied (supraoptic, paraventricular, fornicals, circular and the hypothalamic area situated between the supraoptic and paraventricular nuclei). All neuronal types considered in the present study (NADPH-diaphorase positive, AChE-positive and neurons expressing both markers), were preferentially located in the magnocellular populations whereas in the parvicellular subdivisions of the paraventricular nucleus only isolated neurons belonging to the same three groups were detected. This partial coexistence extends previous studies on the chemical nature of the neurons producing nitric oxide in the hypothalamic secretory nuclei and suggests that nitric oxide is probably not related to general mechanisms involving AChE but rather in specific functions shared by certain hypothalamic neuronal cell populations.

Supported by a grant of the Junta of Castilla y Leon.

156.9

A PGE_2 RECEPTOR ANTAGONIST (SC19220) SELECTIVE FOR EP_1 DOES NOT ALTER THE RISE IN PLASMA OXYTOCIN (OT) AFTER L-NAME. V. Bui, S. Gestl, M. Kadekaro[1], J. Connor* and J. Summy-Long. Dept. of Pharmacology, M.S. Hershey Medical Center. Pennsylvania State University, Hershey, PA 17033, [1]Div. of Neurosurgery, Univ. Texas Medical Branch, Galveston, TX, 77555-0517.

Recent studies in our laboratory demonstrated that nitric oxide (NO) attenuates the excitatory effect of a prostaglandin(s) on OT release from the magnocellular system. To clarify which prostaglandin(s) stimulates OT release, conscious female rats were injected intracerebroventricularly (icv) with 10µl of vehicle (10% ethanol in CSF), PGE_2 (100 ng) or PGD_2 (20µg) and decapitated 5 min later. Plasma OT was quantified by RIA and differences determined by AOV and Newman Keul's t. Compared with vehicle-treated animals, plasma OT (pg/ml, mean±SEM; n=7,8) increased ($p<0.01$) to a similar magnitude after both prostaglandins (vehicle< PGE_2 & PGD_2; 9±1 < 36±3 & 28±4). In other female rats (n=36) we examined whether 40 min pretreatment (10µl, icv) with a selective antagonist of the EP_1 subtype of the PGE_2 receptor (SC19220; 500ng) could prevent the rise in plasma OT after L-NAME (250µg/5µl, icv) given 5 min before decapitation. Compared with vehicle (4% DMSO in CSF), SC19220 did not alter plasma OT in animals treated with either CSF (9±0.5 vs 9±0.7) or L-NAME (34±4 vs 30±3). Although PGE_2 stimulates release of OT, the EP_1 receptor does not mediate the stimulatory effect of prostaglandins that is attenuated by NO. Funded by RO1-25498 and 2RO1-23055.

157.1

EFFECTS OF INSULAR LESIONS ON THE RAT BARORECEPTOR REFLEX
N Kulshreshtha, ZH Zhang and SM Oppenheimer. Laboratory of Neurocardiology Johns Hopkins University School of Medicine, Baltimore, MD 21287.

Clinical observations and animal experiments indicate that perturbation of the insular cortex's function can induce significant cardiovascular changes. We suggest that inactivation of different insular regions can affect the baroreflex. Pressor or depressor sites were identified by electrical stimulation in 18 male, urethane anesthetised Sprague-Dawley rats. Electrical lesions were made using a twisted stainless steel bipolar electrode with constant current (1 mA DC for 2 minutes). Lesion size ranged from 1.0 mm to 2.0 mm in anteroposterior extent within the insular cortex. Phenylephrine hydrochloride (PE) and sodium nitroprusside (SNP) dose-response curves assessed the baroreflex sensitivity before and after lesion placement. Right posterior insular lesions (n=8) significantly increased the PE dose-response curves (p=0.003), while SNP dose-response curves were unaffected by the lesions. Lesions in other insular locations were without effect on the PE and SNP dose response curves. Inactivation of the right posterior insular cortex can reduce baroreflex sensitivity and resets the threshold of the baroreflex.

SUPPORTED BY THE NIH AND THE EL WIEGAND FOUNDATION

157.2

CHARACTERIZATION, DISTRIBUTION AND LATERALISATION OF BARORECEPTOR-RELATED NEURONS IN THE RAT INSULAR CORTEX.
Z.H. Zhang* and S.M. Oppenheimer. Lab. of Neurocardiology, Johns Hopkins University School of Medicine, Baltimore, MD 21287.

There is controversy as to the lateralization and anteroposterior distribution of insular baroreceptor-related neurons. We recorded the response to baroreceptor challenge of 244 cells within and surrounding the right and left insulae in 34 urethane-anesthetised male Sprague-Dawley rats. Single unit activity (sampled at 20 Kz) , blood pressure, ECG and respiration were digitized and recorded electronically using the BRAINWAVE system. Unit activity was analysed using DATAVIEWER. 1). 169/244 units responded to PE and/or SNP. 127/169 were located within the insula (p<0.0001). Some clustering was seen in the right posterior insula (p=0.036). 2) 60 units responded to both PE and SNP. 49/60 were classified as sympathoexcitatory (SE); 11 units were classified as sympathoinhibitory (SI). 43/49 SE cells were contained within the insula (p<0.0001). These units were also clustered within the right posterior zone (P=0.0081). SI cells showed no insular predominance or lateralisation. 3) 47 units responded only to PE; these cells were also situated mainly within the right posterior insula (p=0.004). 4) 62 units responded only to SNP. There was no demonstrable clustering of these cells within the insula or any lateralisation. Baroreceptor units show a distinct distribution, being predominant in the insula, and particularly within the right posterior granular insular cortex. Responses to SNP baroreceptor challenge lacked insular specificity.

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157.3

HYPOTENSIVE RESPONSES TO THE INJECTION OF ACETYLCHOLINE INTO THE PREFRONTAL CORTEX OF THE RAT.
G.E. Crippa[1], E.M. Krieger[2], V.L. Peres-Polon[3] and F.M.A. Corrêa*. [1]Dept. Pharmacology, FMRP; [2] Unit of Hypertension INCOR-FM; [3]Dept. Physiology, FORP, Univ. of São Paulo, 14040-900, SP, Brazil.

Dose-dependent hypotensive responses were observed after the injection of acetylcholine (ACh) into the prefrontal cortex of anesthetized or conscious rats. The responses were not associated to changes in cardiac or respiratory frequencies. The depressor response was blocked by the intracortical injection of atropine or 4-DAMP, but not pirenzepine, suggesting the involvement of M_3-receptors. Hypotensive responses were observed after injection of ACh throughout the prefrontal cortex. The intensity of the responses were higher when ACh was injected in the lateral or medial prefrontal cortex. No changes in the sympathetic nerve activity (renal nerve) were recorded concomitant with the hypotensive response. Additionally no cardiac debit were simultaneously observed, suggesting that the hypotensive response to ACh does not involve an inhibition of the sympathetic output and is caused only by a fall in the peripheral resistance without a cardiac component. The depressor response to intracortical ACh is blocked by i.v. pretreatment with homatropine methylbromide, suggesting a mediation through a vasodilator peripheral cholinergic mechanism. The present report may be the first evidence of the existence of an centrally controlled cholinergic vasodilator system in the rat.

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157.4

DETECTION OF VASOMOTOR AND RESPIRATORY RHYTHMS IN THE DISCHARGE OF SINGLE THALAMIC NEURONS IN CONSCIOUS CATS.
N. Montano*, T Gnecchi-Ruscone, M. Massimini, C. Cogliati, A. Porta, M. Mariotti, A. Malliani. Centro Ricerche Cardiovascolari, CNR, Medicina Int. II, Osp. L.Sacco & Fisiologia Umana II, University of Milan, Italy.

Spectral analysis of heart rate (HR) and systolic arterial pressure (SAP) variabilities in man as well as in experimental animals detects two major components: a low frequency (LF; 0.04-0.15Hz) and a high frequency (HF; 0.25 Hz) components respectively related to vasomotor and respiratory activity. HF is considered a marker of vagal modulation while LF is a marker of sympathetic modulation. In a previous study (Montano et al, JANS, 57:116-122, 1996) spectral analysis in the range between 0 and 0.5 Hz, of single medullary neurons revealed a predominant LF oscillation, highly correlated with the LF detectable in SAP. The present study was undertaken to evaluate whether LF and HF oscillations could also be present in the discharge variability of single neurons within different thalamic nuclei. We analyzed, by means of autoregressive spectral analysis algorithms, RR interval (RR) and impulse activity of single units recorded extracellularly from different nuclei: reticular thalamic (RE; n=16), mediodorsal (MD; n=16) and ventral posterolateral (VPL; n=5) in unanesthetized, conscious cats. Power spectra of single neuron discharges displayed both LF and HF oscillations, highly coherent (K2= 0.6-0.8) with the same rhythms detectable in RR variability spectrum.

In conclusion the presence of vasomotor and respiratory oscillations in the discharge variability of specific and non-specific thalamic nuclei neurons supports the exsistence of broad codes of information concerning the major variables of neurovegetative regulation.

157.5

EFFECT OF OESTRADIOL (E_2) ON CARDIOVASCULAR RESPONSES TO GLUTAMATE STIMULATION OF BED NUCLEUS OF THE STRIA TERMINALIS (BST).
L. N. Gibbons* and J. Ciriello, Department of Physiology, University of Western Ontario, London, Canada, N6A 5C1.

We have previously shown that circulating levels of E_2 alter the magnitude of the heart rate (HR) response to glutamate (Glu) stimulation of BST. To determine whether E_2 was acting directly at BST neurons to modify the HR response, E_2 (0.5 μM 17β -oestradiol) was microinjected (40 nl) into cardiovascular responsive sites in BST and the sites were re-stimulated with Glu (1 M; 20 nl). The peripheral autonomic components mediating these cardiovascular responses were also investigated using autonomic receptor blockade. All experiments were done in Inactin anesthetized, paralyzed and artificially ventilated female Wistar rats previously ovariectomized and subcutaneously implanted with an empty or E_2 containing silastic capsule that resulted in a constant circulating level of 30 pg/ml of E_2. Microinjection of E_2 into BST resulted in the potentiation of the Glu mean arterial pressure (MAP; 182±21 %) and HR (189±38 %) responses elicited from BST in animals containing circulating levels of E_2 only. Injection of the vehicle (propylene glycol-saline) into BST did not effect the magnitude of the cardiovascular responses. Administration of the muscarinic receptor blocker atropine methyl bromide (1 mg/kg; iv) had no effect on the magnitude of the potentiated responses, whereas injection of the nicotinic receptor blocker hexamethonium bromide (20 mg/kg; iv) abolished the E_2 induced potentiation of the MAP and HR responses to Glu stimulation of BST. Finally, microinjection of the protein synthesis inhibitor anisomycin (2.5-5 mg/ml; 40 nl) into BST 15 min prior to injection of E_2 into the same site, prevented the potentiation of the cardiovascular responses to Glu stimulation of BST. These data suggest that E_2 acts to alter the sensitivity of cardiovascular neurons in BST through a genomic mechanism and that this change in sensitivity results in an increased sympathoinhibition during BST stimulation. (Supported by Heart and Stroke Fdn. of Ontario).

157.6

NUCLEUS OF THE SOLITARY TRACT AND VENTROLATERAL MEDULLARY INPUTS TO NUCLEUS ACCUMBENS.
G. J. Kirouac and J. Ciriello.* Department of Physiology, University of Western Ontario, London, ON, Canada, N6A 5C1.

Nucleus accumbens (NA) is thought to be involved in behavioural and motivational responses. Recently, anatomical studies have shown that nucleus of the solitary tract (NTS) and ventrolateral medulla (VLM), cardiovascular brainstem regions, project directly to NA. Experiments were done in α-chloralose anesthetized rats to investigate the effect of activation of cardiovascular responsive sites in NTS and VLM, and of arterial baroreceptors by the acute rise in arterial pressure to phenylephrine (10 µg/kg, i.v.), on the discharge rate of neurons in NA. Extracellular single unit recordings were made from NA neurons. Electrical stimulation of depressor sites in NTS excited 41/77 (latency, 44.4 ± 4.5 ms) or inhibited 7/77 (latency, 34.8 ± 7.9 ms) of the NA units tested. Similarily, stimulation of the VLM region containing the A1 noradrenergic cell group excited 20/77 (latency, 37.4 ± 7.8 ms) or inhibited 4/77 (latency, 31.5 ± 4.2 ms) of the NA units tested. Fifteen units were found to be excited by stimulation of both NTS and VLM and the discharge rate of 6 of these neurons was also increased by baroreceptor activation. Responsive units in NA were found predominantly within the shell region of the nucleus. These data indicate that NA receives convergent information from sites in NTS and VLM that regulate arterial blood pressure and from arterial baroreceptors. These findings suggest that NA may require information regarding the current status of the cardiovascular system prior to evoking behavioural processes. (Supported by the Heart and Stroke Foundation of Ontario).

157.7

EFFECTS OF NMDA ANTAGONIST MK-801 ON CENTRAL ANGIOTENSIN II ELEVATED BLOOD PRESSURE AND FOS RESPONSES IN RAT FOREBRAIN, Z. Xu, R.L. Thunhorst and A.K. Johnson* Departments of Psychology and Pharmacology and the Cardiovascular Center, University of Iowa, Iowa City, IA 52242-1407

Intracerebroventricular (ICV) administration of angiotensin II (ANG II) elevates arterial blood pressure in rats. The present study examined whether glutamatergic pathways and N-methyl-D-aspartate (NMDA) receptors are involved in mediating the pressor response to ICV ANG II and evaluated the central sites of actions of ANG II after pretreatment of NMDA antagonist, MK-801, by mapping FOS-immunoreactivity in the forebrain. ICV MK-801 (20 nmol for conscious rats, and 60 nmol for anesthetized rats) did not affect mean blood pressure and heart rate, but significantly suppressed ICV ANG II-induced pressor responses in both conscious and anesthetized rats. This suggests the involvement of glutamatergic mechanisms in mediating for the effects of ICV ANG II. FOS-immunoreactivity after ICV ANG II was significantly increased in the subfornical organ (SFO), organum vasculosum of the lamina terminalis (OVLT), median preoptic nuclei (MnPO), supraoptic nuclei (SON) and magnocellular paraventricular nuclei (PVN). Pretreatment with ICV MK-801 had no effects on FOS responses in the SFO, OVLT and magnocellular PVN, but quantitatively decreased FOS in the MnPO, SON and the magnocellular PVN. This suggests the central sites of glutamate system in the forebrain that may relate to ANG II-induced pressor responses. Supported by NHLBI HL 14388 and NASA NAGW-4358.

157.8

NICOTINIC GANGLIONIC AND MUSCARINIC RECEPTOR INVOLVEMENT IN PRESSOR RESPONSE TO CARBACHOL MICROINJECTION INTO POSTERIOR HYPOTHALAMIC NUCLEUS. J.R. Martin*, Dept. of Pharmacol. Kirksville Col. Osteo. Med., Kirksville, MO 63501.

The microinjection of the cholinergic agonist carbachol (CCh) into the posterior hypothalamic nucleus (PHN) of conscious rats evokes an increase in mean arterial pressure (MAP) which can be blocked by pretreatment with the combination of α-adrenergic and V_1-vasopressin receptor antagonists. The present study determined if the CCh induced pressor response could be inhibited by ganglionic and V_1-vasopressin receptor blockade. Male Sprague-Dawley rats (280-300 g) were anesthetized (50 mg/kg of pentobarbital, i.p.) and instrumented with a guide cannula targeted to the left PHN, and catheters in the left femoral artery and vein for direct arterial pressure measurement and intravenous drug administration. Pretreatment with 10 mg/kg of the nicotinic ganglionic receptor antagonist pentolinium (PENT) attenuated the increase in MAP evoked by CCh (3.3 to 13.2 nmol in 50 nl). Adding 2 mg/kg of the muscarinic receptor antagonist methylatropine (MeATR) to PENT resulted in further inhibition of the CCh-evoked increase in MAP. Adding the V_1-vasopressin receptor antagonist [d(CH_2)$_5$tyr(Me)]AVP (20 μg/kg) to PENT and MeATR completely blocked the CCh-evoked increase in MAP. These results suggest that CCh microinjection into the PHN of conscious rats evokes an increase in MAP as a result of sympathoexcitation and increased plasma levels of AVP. The sympathoexcitation appears to involve ganglionic transmission via a nicotinic ganglionic and a muscarinic receptor. (Supported by NIH HL-44531 and Warner/Fermaturo Fund.)

157.9

INJECTION OF MUSCIMOL INTO DORSOMEDIAL OR PARAVENTRICULAR HYPOTHALAMIC NUCLEUS: EFFECT ON STRESS-INDUCED INCREASES IN PLASMA RENIN ACTIVITY AND CARDIOVASCULAR FUNCTION IN RATS. T.W. Bailey, E.H. Stotz-Potter, M.L. Blair and J.A. DiMicco*. Dept. Pharmacol & Toxicol., Indiana Univ. Sch. of Med., Indpls, IN 46202, and Dept of Pharm. & Phys. Univ. of Rochester., Sch. of Med. and Dent., Rochester, NY 14642

Microinjection of muscimol (MUS), a $GABA_A$ receptor agonist, into the dorsomedial hypothalamus (DMH) reduces stress-induced increases in heart rate (HR), blood pressure (BP), and plasma ACTH, while similar treatment in the paraventricular hypothalamus (PVN) attenuates only increases in plasma ACTH. Here, we examined the effect of these treatments on stress-induced increases in plasma renin activity (PRA), another hallmark of the response to acute stress. Rats, with guide cannulae implanted at target coordinates corresponding to the region of the DMH or the PVN, and instrumented for monitoring HR and BP, were tested in either high stress (HS; 10 min air stress) or low stress (LS; associated with microinjection and baseline blood sampling) after bilateral injection of either vehicle (VEH;100 nl saline) or MUS 80 pmol into the DMH (n=4, LS; n=5, HS) or the PVN (n=5, LS; n=4, HS). Injection of MUS into the DMH attenuated the increases in HR seen under HS (mean change from basal HR in beats/min $\pm$ sem over 10 min period: VEH, +96$\pm$18; MUS, +30$\pm$11; p=0.015) and reduced HR below baseline under LS, (VEH, +62$\pm$23; MUS, -28$\pm$7 beats/min; p=0.002), while injection of MUS into the PVN had no effect in either setting. Mean basal PRA for all rats was 4.0$\pm$0.5 ngAngI/ml/hr. HS but not LS increased PRA by an average of 9.8$\pm$ 2.1 (p=0.0008) in all treatment groups, and the increases were not affected by microinjection of MUS into the DMH or PVN. Thus, while the DMH is a key site for integration of cardiovascular and ACTH responses to stress, neural activity in this region may not play a role in stress-induced increases in PRA. (Supported by NIH grant NS 19883, American Heart A ssoc. Grant 9300794, and a Fellowship to E.H.S.-P. from American Heart Assoc., Indiana Affiliate)

157.10

EFFECT OF CHEMICAL STIMULATION OF THE DORSOMEDIAL HYPOTHALAMIC NUCLEUS ON BLOOD PLASMA GLUCOSE, TRIGLYCERIDES AND FREE FATTY ACIDS IN RATS. C. T. B.V. Zaia*[1], L. C. I. Gaziri[1], D. A. M. Zaia[2], M. S. Dolnikoff[3], C. Timo-Iaria[4]. [1]Depto. de Ciências Fisiológicas and [2]Depto. de Química, Universidade Estadual de Londrina, Londrina, PR 86051-970; [3]Depto. de Fisiologia, EPM-UNIFESP; [4]Faculdade de Medicina, USP, São Paulo, SP, Brasil.

The effects of chemical stimulation of the dorsomedial hypothalamic nucleus (DMH) on blood plasma concentration of glucose, triglycerides, insulin and free fatty acids (FFA) were investigated in anesthetized adult Wistar rats. Microinjection of 12.5 nmoles (0.5 µl/min) of norepinephrine into the DMH increased blood plasma concentration of glucose (136.0 ± 1.5 mg/dl) and FFA (104.5 ± 6.2 µmoles/dl), decreased triglycerides (58.7 ± 3.5 mg/dl), and did not change plasma insulin within 5 minutes compared with control group (113.2 ± 1.2 mg/dl; 76.4 ± 6.6 µmoles/dl e 86.9 ± 6.6 mg/dl, respectively); after 20 minutes, blood glucose and FFA reached control values. Microinjection of epinephrine (12.5 nmoles; 0.5 µl/min) into the DMH also increased blood plasma glucose concentration (134.3 ± 3.7 mg/dl) and decreased triglycerides (53.3 ± 6.6 mg/dl) after 5 minutes. These effects are probably mediated by beta-adrenergic mechanisms, since they were prevented by beta-adrenergic antagonist propranolol, but not by alpha-adrenergic antagonist prazosin. Microinjection into the DMH of glutamate, dopamine, or acetylcholine failed to cause any change in those metabolic parameters, corroborating the hypothesis that the DMH is part of a beta-adrenergic pathway involved in short-term modulation of the availability of glucose and FFA.

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157.11

Effects of GABA-A Receptor Blockade on Inhibition of Autonomic Neurons in the Hypothalamic Paraventricular Nucleus (PVN) Evoked by Cardiopulmonary Vagal Inputs. G.M. Toney* and S.W. Mifflin Department of Pharmacology, University of Texas, HSC, San Antonio, TX 78284-7764

Parvocellular PVN (pPVN) neurons influence autonomic activity through pontine, medullary and spinal projections. Evidence that lesions of pPVN neurons attenuate the renal vasodilation and sympathoinhibition that accompany saline infusions, implicates these neurons in the control of plasma volume. In this study, changes in pPVN cell discharge were recorded extracellularly following rapid (1-2 s), bolus (200 µl) injections of saline or saline containing 5 µg of phenylbiguanide (PBG) into the right cardiac atrium of chloralose/urethane anesthetized Sprague-Dawley rats. Spontaneously active cells (n=21) discharged either continuously (n=6) or with bursts of discharge (peak interspike intervals; 3-46 ms) (n=15). Eight cells responded to PBG - 6 inhibited, 2 excited. Inhibition reduced discharge 87 ± 13 % (mean ± SD) from 6.3 ± 3.7 to 0.5 ± 0.4 Hz. Saline injections alone (n=7) were without effect. Averages of renal sympathetic activity triggered from spontaneous spikes of PBG-inhibited cells showed a prominent peak at 162 ± 36 ms. Similar averages from PBG-excited cells had reduced amplitude at a similar latency. Two cells inhibited by PBG were antidromically activated by spinal cord (T2-3) stimulation. Antidromic spike latencies (42&76 ms) suggest unmyelinated axons. Iontophoresis of bicuculline methiodide (BMI)(10 mM, 1-2 min) did not change spontaneous discharge (baseline: 6.3 ± 3.7 Hz, BMI: 6.6 ± 1.8 Hz) and failed to prevent PBG-induced unit inhibition (n=6). The same "dose" of BMI (8.0 ± 1.2 nA), however, reversed GABA (2 mM,~12 nA)-mediated inhibition of unit activity. In 3 PBG-inhibited cells, vagus nerve stimulation (3 pulses-500 Hz, 500 µA) inhibited cell firing for 140 ± 34 ms, with a latency of 92 ± 32 ms. Like PBG, vagal inhibition was BMI insensitive. Results indicate that autonomic pPVN neurons are inhibited by activation of unmyelinated vagal afferents-probably of cardiopulmonary origin. These inputs appear insensitive to rapid, atrial saline injections. Failure of somatic application of BMI to alter tonic activity or to attenuate evoked afferent inhibition, indicates that if inhibition is GABA-A receptor mediated, inhibitory synapses are likely to be located distant from the presumed somatic recording site. (support: HL36080)

157.12

HEMODYNAMIC RESPONSES TO HYPOTHALAMIC PARAVENTRICULAR NUCLEUS (PVN) INJECTIONS OF SPECIFIC OPIOID ANTAGONISTS IN CONSCIOUS RATS. Andrée Lessard and Hélène Bachelard*. Unité de Recherche sur l'Hypertension, Centre de Recherche du CHUL, Université Laval, Québec, G1V 4G2

The present study was designed to investigate the influence of the endogenous PVN opioid innervation on cardiovascular system. Therefore, the cardiovascular changes elicited by bilateral PVN injections (0.2µl) of naloxone (0.1-5.0 nmol), ICI-174,864 (0.1-1.0 nmol, a δ-opioid antagonist), Nor-binaltorphimine (0.1-1.0 nmol, a κ-opioid antagonist), or ß-funaltrexamine (0.05-0.5 nmol, a µ-opioid antagonist) were characterized in conscious male Wistar rats. The rats were instrumented with intracerebral cannulae, intra-vascular catheters and pulsed Doppler flow probes implanted around specific arteries in a three step surgery. PVN injection of naloxone and ß-funaltrexamine produced significant cardiovascular changes characterized by quick and transient increases in blood pressure, heart rate, and hindquarter vascular conductance and falls in renal and superior mesenteric vascular conductances. Thus, PVN injection of naloxone (5.0 nmol) produced increases in mean blood pressure (+12 ± 2mm Hg) and heart rate (+80 ± 18bpm), falls in renal (-17 ± 5%) and mesenteric (-29 ± 7%) vascular conductances and increases in hindquarter (+38 ± 18%) vascular conductance. However, PVN injections of ICI-174,864 or Nor-binaltorphimine had no cardiovascular effects at the doses we used. Together these results suggest that the endogenous PVN opioid innervation exert a µ-opioid receptor-mediated tonic inhibitory influence on the PVN neurons involved in cardiovascular regulation.

The work was supported by the FMCQ and the NSERCC.

157.13

HYPOTHALAMIC DISTRIBUTION OF FOS-LIKE IMMUNOREACTIVITY FOLLOWING HEMORRHAGE IN CYCLING AND PREGNANT FEMALE RATS. R.L. Parman*, M.L. Blair and D. Piekut. Univ. of Rochester Sch. of Medicine and Dentistry, Rochester, NY 14642.

In male rats, blood loss causes increased Fos expression in the supraoptic nucleus (SON), magnocellular paraventricular hypothalamic nucleus (PVH), and parvicellular PVH (*Clin. Exptl. Pharm. Phys. 23:161, 1996*). The goal of this study was to determine if hypothalamic neurons are similarly activated by decreased blood volume in cycling virgin females and pregnant females. Conscious unrestrained female Sprague-Dawley rats were subjected to a slow hemorrhage (HEM) on the morning of proestrus (n=3), metestrus (n=3) or mid-pregnancy (pregnancy day 11-13, n=3). HEM was performed by gradual withdrawal of 16 ml/kg blood over 24 min via an arterial catheter.The rats were anesthetized and perfused (4% paraformaldehyde) 100 min after initiation of HEM. Following HEM, blood pressure decreased similarly (to <55 mmHg) in cycling and pregnant female rats, in association with a significant bradycardia. Plasma renin activity (PRA) increased 4-fold in both cycling and pregnant rats. Immunocytochemical localization demonstrated numerous Fos-positive cell nuclei in the SON, magnocellular PVH, and parvicellular PVH, with no apparent difference in the distribution of Fos-immunoreactivity in females studied during proestrus, metestrus, or pregnancy. Non-hemorrhaged control rats (n=6 cycling, n=5 pregnant) showed few Fos-positive neurons within PVH or SON. In conclusion, HEM increases hypothalamic Fos expression in female rats in a pattern of distribution which is similar to that of males, and is not substantially altered by estrous cycle phase or mid-pregnancy. *(Sup. by American Heart Assoc. #9300794)*

157.14

HYPOTHALAMIC DISTRIBUTION OF FOS-LIKE IMMUNOREACTIVITY AND NADPH-DIAPHORASE (NADPH-d) FOLLOWING GRADED HEMORRHAGE. M.L. Blair*, D. Mickelsen and D. Piekut. Univ. of Rochester Sch. of Medicine and Dentistry, Rochester, NY 14642.

The goal of this study was to determine if hypothalamic neurons containing nitric oxide synthase (NADPH-d) are selectively activated by decreased blood volume. A graded hemorrhage (HEM) was performed in conscious male Sprague-Dawley rats by gradual withdrawal of 9-16 ml/kg blood over 24 min via an arterial catheter. The rats were anesthetized and perfused (4% paraformaldehyde) at 85 min after initiation of HEM. Histochemical localization demonstrated intense NADPH-d staining in the median preoptic nucleus, dorsal supraoptic nucleus (SON), perifornical region, lateral hypothalamus, anterior paraventricular nucleus (PVH), ventrolateral parvicellular PVH, and posterior PVH. In rats subjected to a 14-16 ml/kg HEM which decreased blood pressure to <50 mmHg (n=6), immunocytochemical localization demonstrated numerous Fos-positive cell nuclei in ventral SON, magnocellular PVH, and ventrolateral parvicellular PVH. Only faint NADPH-d was seen in Fos-positive neurons in the SON and magnocellular PVH. However, >60% of NADPH-d-positive cells in the ventrolateral parvicellular PVH were also Fos-positive. A similar distribution of Fos-positive and double-labelled neurons was observed in rats subjected to a smaller HEM of 9-11 ml/kg which did not significantly decrease arterial pressure (n=4). Non-hemorrhaged control rats (n=6) showed few Fos-positive neurons within PVH or SON. In conclusion, NADPH-d-positive neurons within the parvicellular PVH are selectively activated by blood loss, even in the absence of hypotension. *(Sup. by Am. Heart Assoc. #9300794)*

157.15

NEUROTRANSMITTERS IN THE LATERAL HYPOTHALAMIC AREA (LHA) MEDIATING CARDIOVASCULAR RESPONSES FROM THE INFRALIMBIC CORTEX (ILC). M. Way and D.F. Cechetto*. University of Western Ontario, London, Ontario, Canada, N6A 5K8.

Stimulation of the ILC elicits a number of autonomic responses and previous neuroanatomical studies have suggested that there is an efferent pathway from the ILC to the LHA. To determine the role of the LHA in mediating ILC cardio-vascular responses the ILC was stimulated before and after injection of a synaptic blocker or glutamate antagonists into the LHA. Male, Wistar rats (21) were anesthetized with inactin and prepared for monitoring mean arterial pressure (MAP), heart rate, and renal nerve activity (RNA). A five second train of electrical pulses (200-500 μA, 80 Hz, 2 ms duration) administered to the ILC elicits a decrease in MAP of 17.5 $\pm$ 2.0 mmHg. Injection of a general synaptic blocker (10 mM cobaltous chloride; 300 nl) into the LHA attenuates the depressor response to 53.7 $\pm$ 3.23 % of the initial response (range 40 to 72.7 %). Injection of a general excitatory amino-acid antagonist (250 mM kynurenic acid; 240 nl) into the LHA attenuates the depressor response to 56.3 $\pm$ 12.18 % of initial (range 0 to 79.2 %). Injection of a specific NMDA receptor antagonist (250 μM AP-5; 240 nl) into the LHA attenuates the depressor response to 53.7 $\pm$ 4.52 % of initial (range 43.5 to 61.7 %). Injection of Non-NMDA receptor antagonists (200 μM GDE, 2 mM NBQX and 200 μM NBQX; 240 nl) into the LHA attenuates the depressor response by 39.8 $\pm$ 10.46 %, 30.4 $\pm$ 21.52 % and 38.2 $\pm$ 6.49 % respectively. These results suggest that there is a synapse in the LHA that mediates the cardiovascular responses from the ILC. In addition, glutamatergic synapses, primarily non-NMDA, in the LHA mediate these responses. (Supported by the Heart and Stroke Foundation of Ontario).

157.16

CARDIAC-RELATED PARASYMPATHETIC NEURONS IN THE CENTRAL NUCLEUS OF THE AMYGDALA IN GUANETHIDINE TREATED RATS. A. Standish* and J.S. Schwaber. E. I. DuPont Co., Neural Computation Group, The Experimental Station, Wilmington, DE 19880.

The distribution of labeled neurons in the central nucleus of the amygdala (CNA) following injection of pseudorabies virus (PRV) into the heart was investigated in guanethidine-treated rats. There is a large body of evidence to implicate the CNA in cardiac control. However, in our previous viral tracing studies from the heart, neurons in the amygdala regions were not or were only sparsely labeled. In an effort to solely study the parasympathetic cardiac circuit, rat pups were treated with guanethidine which resulted in permanent destruction of the peripheral sympathetic nervous system. Following a 5 week treatment period, we injected the rat heart with PRV. Since only the parasympathetic system was infected with PRV, these guanethidine treated rats lived significantly longer following PRV cardiac injection than non-treated rats. The additional survival time enabled PRV, which is transsynaptically transported in a temporal fashion, to infect neurons further along the hierarchical chain of synaptically linked neurons involved in cardiac control. We found a significant number of labeled neurons in the CNA in guanethidine treated rats sacrificed more than 100 hours after cardiac injection. The labeling of CNA was confined to neurons of the medial subdivision, a subdivision we have previously described as projecting to the vagal complex of the dorsal medulla. Thus, CNA neurons are presumed to be retrogradely labeled from neurons located in the cardiorespiratory (dorsal) nucleus of the tractus solitarius. These results suggest that neurons in the CNA are at least three perhaps four synapses from the vagal preganglionic cardiac motor neurons. Supported by grants from the ONR, NSF and by the E.I. DuPont Co.

157.17

BRAINSTEM PATHWAYS ACTIVATED BY THE SUCKLING STIMULUS: A COMPARISON OF NEURAL STIMULI DERIVED FROM SUCKLING VERSUS PUP EXPOSURE. P. Chen, C. Li and M. S. Smith*, Division of Neuroscience, Oregon Regional Primate Research Center; Dept. Physiol./Pharm., Oregon Health Sciences Univ., Beaverton, OR 97006.

We have identified a number of brainstem areas that are activated by the suckling stimulus. These studies in rats compare patterns of cFos expression induced by the suckling stimulus with those induced by sensory stimuli of pup exposure. The 8-pup litters were removed for 48 hrs, beginning on day 9 postpartum. On day 11, animals were divided into 3 groups: no resuckling (0 Pups), pup exposure for 90 min (PE), and resuckling 8 pups for 90 min (+8 Pups). Double-label immunocytochemistry was performed for cFos and tyrosine hydroxylase (TH) to identify catecholamine neurons. Data are reported for those areas with major projections to the hypothalamus. In the ventrolateral medulla [VLM/A1 (% cFos-positive TH cells): 0 Pups, 0.5 $\pm$ 0.4; PE, 1.4 $\pm$ 0.7; +8 Pups, 37.4 $\pm$ 4.1], suckling caused a significant increase in cFos expression ($p<0.001$), whereas PE had no effect. There were no differences among the 3 groups in the VLM/C1 area. In the Nucleus of the Solitary Tract (NTS/A2), cFos expression (15-25% cFos-positive TH cells) was similar in the three groups. In the locus coeruleus [LC/A6 (# of cFos positive TH cells/section): 0 Pups, 3 $\pm$ 1; PE, 3 $\pm$ 1; +8 Pups, 24 $\pm$ 4], PE had no effect, whereas suckling increased cFos expression ($p<0.001$). In non-catecholaminergic areas, such as the periaqueductal grey [PAG (# of cFos positive cells/section): 0 Pups, 39 $\pm$ 7; PE, 140 $\pm$ 32; +8 Pups, 210 $\pm$ 14], PE increased cFos expression ($p<0.01$), and suckling caused an even greater level of cFos than did PE ($p<0.05$). Therefore, the neural impulses arising from the suckling stimulus, itself, result in activation of specific brainstem areas (VLM, LC, PAG), suggesting that projections from these areas may relay signals arising from suckling to the hypothalamus. (Support: NIH grants HD14643, RR00163).

157.18

SYSTEMIC NITROGLYCERIN ACTIVATES PEPTIDERGIC AND CATECHOLAMINERGIC PATHWAYS IN RAT BRAIN

Cristina Tassorelli*[1], Giuseppe Nappi[1] and Shirley A. Joseph[2]

[2] *Division of Neurological Surgery, Strong Memorial Hospital Medical Center, Rochester, 14642 New York (USA) -[1]*Neurological Institute "C. Mondino", University of Pavia, 27100 Pavia (I).*

Nitroglycerin (NTG) is a nitric oxide donor which induces sustained expression of Fos protein, a marker of neuronal activation, in specific neuronal groups in the central nervous system. The mechanisms which underlie nitroglycerin-induced neuronal activation are elusive at this moment. The aim of this study was to provide, by means of a dual immunohistochemical technique, a neurochemical characterization of the neurons which express Fos following systemic adminstration of nitroglycerin, in order to shed light on the pathways and neurotransmitters which are possibly involved in the central effect of the drug. Adult Sprague-Dawley rats were injected, subcutaneously, with NTG (10mg/kg b.w.). Animals were sacrifed and brain sections were processed for immunohistochemical detection of Fos using a rabbit polyclonal antiserum directed against an in vitro translated product of the *c-fos* gene; sections were subsequently processed according to an immunocytochemical staining protocol for the demonstration of two antigens with contrasting colors in the same tissue section, previously developed. In the brainstem, a significant percentage of activated neurons contained noradrenaline as a neurotransmitter, while only a few of them contained serotonin. In the paraventricular and supraoptic nuclei of the hypothalamus, numerous Fos-immunoreactive neurons were also positive for vasopressin, oxytocin and corticotropin releasing factor. Co-distribution with corticotropin releasing factor was also observed in the central nucleus of the amygdala. These data are consistent with and expand on those previously reported by our group; they provide a neurochemical map of the brain areas which respond to nitroglycerin administration, which include catecholaminergic, peptidergic and, to a lesser extent, serotonergic structures.

Supported by NIH grant NS21323 (S.A.J.) and CNR grant AI94.00462.04 (C.T.).

158.1

NK_1 RECEPTOR IN SYMPATHETIC PREGANGLIONIC NEURONS (SPN): DIFFERENTIAL EXPRESSION AND ULTRASTRUCTURAL LOCALIZATION. IJ Llewellyn-Smith[1]*, JB Minson[1], PM Pilowsky[1], LF Arnolda[1], AI Basbaum[2], SR Vigna[3] and JP Chalmers[1]. [1]Dept of Medicine, Flinders University, Bedford Park, SA AUSTRALIA; [2]Keck Center for Integrative Neuroscience, UCSF, San Francisco, CA, and [3]Dept of Cell Biology, Duke University Medical Center, Durham, NC.

Substance P is involved in cardiovascular control at the spinal cord level, acting through neurokinin-1 receptors (NK_1R). We used immunocytochemistry and retrograde tracing with cholera toxin B (CTB) to investigate the expression and ultrastructural localization of the NK_1 receptor in rat SPN that project to the superior cervical ganglion (SCG) or the adrenal medulla. NK_1R -immunofluorescence outlined the somatic and dendritic surfaces of autonomic neurons in spinal cord segments T1-T12; immunofluorescence for CTB filled the retrogradely-labelled cells. There was a significant difference in the proportion of NK_1R-positive SPN supplying the SCG and the adrenal medulla: 38% of SPN to the SCG contained NK_1R vs. 70% of sympathoadrenal neurons. Of neurons projecting to the SCG, 15% were NK_1R-immunoreactive in segment T1 vs. 45% in segments T2-6. Ultrastructurally, NK_1R was localized mainly at the cell membrane, both at synaptic and non-synaptic sites. Label was also seen in rough endoplasmic reticulum and Golgi. When sections were double-stained for both substance P and NK_1R, only 37% of substance P inputs were on NK_1R-positive neurons. Thus, the expression of the NK_1 receptor by SPN is related to their target. Furthermore, SPN may be affected not only by substance P released from conventional synapses but also by substance P that diffuses from distant sites. Supported by National Health & Medical Research Council of Australia

158.2

DETECTION AND POSSIBLE FUNCTION OF PITUITARY ADENYLATE CYCLASE ACTIVATING POLYPEPTIDE IN THE RAT SPINAL SYMPATHETIC NUCLEI. H. H. Lin*, C. C. Lai, S. L. Dun and N. J. Dun. Dept. of Anatomy & Neurobiology, Medical College of Ohio, Toledo, OH 43614.

Pituitary adenylate cyclase activating polypeptide (PACAP)-like immunoreactivity was detected by immunohistochemical methods in nerve fibers projecting to the intermediolateral cell column and other sympathetic nuclei of rat thoracolumbar spinal cords. In urethane-anesthetized rats, the right femoral artery and left femoral vein were cannulated for blood pressure recording and intravenous administration of drugs. Peptides and glutamate were administered intrathecally via an implanted cannula to the T2-T3 segments. Intrathecal injection of PACAP (0.1, 0.3 and 1 nmol) dose-dependently increased the mean arterial blood pressure and heart rate from minutes to over 1 hr. The pressor effect of PACAP (0.1 nmol) was small and not statistically significant as compared to saline control. Intrathecal injection of glutamate (1 μmol) and substance P (5 nmol) caused a short- and long-lasting pressor response, whereas vasoactive intestinal polypeptide (1 nmol) had little or no significant pressor effect. In contrast to a pressor effect, intravenous injection of PACAP (0.3 nmol/kg) consistently lowered the blood pressure. The pressor effect of PACAP was not blocked by prior intrathecal injection of the type II PACAP receptor antagonist PACAP (6-38, 0.5 nmol). Intravenous injection of the α-adrenergic receptor blocker phentolamine (1 mg/kg), which caused a fall in blood pressure, attenuated the pressor response of a second intrathecal injection of PACAP. The result shows that PACAP administered intravenously lowers and intrathecally increases blood pressure, respectively. The pressor effect caused by intrathecal injection of PACAP is probably exerted on spinal sympathetic preganglionic neurons. This study provides preliminary evidence of a novel peptidergic pathway to the intermediolateral cell column, the activation of which may increase spinal sympathetic outflow. (Supported by NS18710 & HL51314).

158.3

THE ACTIVITY OF A SEGMENTALLY-RESTRICTED POPULATION OF DORSAL HORN NEURONS IS CORRELATED WITH RENAL SYMPATHETIC NERVE DISCHARGES IN SPINALLY TRANSECTED RATS. D. Chau, and L.P. Schramm*. Departments. of Biomedical Engineering and Neuroscience, The Johns Hopkins University School of Medicine, Baltimore, MD 21205.

We used the spike-triggered averaging technique to identify dorsal horn interneurons whose spontaneous activities were synchronized with renal sympathetic nerve discharges. Experiments were conducted on 52 male, C1-transected, chloralose-anesthetized, paralyzed, artificially-respired, Sprague-Dawley rats. We recorded from renal nerves and from dorsal horn neurons at levels T2, T8, T10, T13, and L2 ipsilateral to the renal nerve recording and also from neurons at T10 contralateral to the renal nerve recording. Based on responses to noxious and innocuous stimuli, all neurons were classified as low-threshold (LT), high-threshold (HT), or wide-dynamic range (WDR). None of the T2 (0/22), T13 (0/25), or L2 (0/26) ipsilateral neurons, nor the T10 (0/26) contralateral neurons were correlated with RSA. Twenty percent (5/25) of T8, and 40.0% (14/35) of T10 ipsilateral neurons were correlated with RSA. Correlated T10 neurons were found only in laminae III-V. Uncorrelated neurons were located in laminae I, III-V. Correlated neurons were most likely to be WDR (of 13 tested: 12=WDR, 1=HT). Uncorrelated neurons, however, were heterogeneous with respect to modality (of 17 tested: 9=WDR, 6=HT, 2=LT). Action potentials of correlated T10 neurons preceded the major peak in the spike-triggered average by 50ms (±10ms). This latency was consistent with the measured conduction time from the spinal cord to the recording electrodes. Excitatory and inhibitory somatic fields of correlated neurons overlapped somatic fields which, when stimulated, excited and inhibited RSA, respectively. Further, somatic stimulation evoked responses in dorsal horn neurons similar to those simultaneously elicited in RSA. We suggest that correlated dorsal horn neurons may mediate the generation of sympathetic tone and of somato-sympathetic reflexes in the spinally transected rat. (Supported by NIH grant HL16315).

158.4

LATERAL FUNICULUS AND LATERAL SPINAL NUCLEUS NEURONS PROJECT TO THE INTERMEDIOLATERAL CELL COLUMN, A.S.P. Jansen* and A.D. Loewy, Dept. Anat. & Neurobiol., Wash. Univ. Sch. Med., St. Louis, MO 63110.

In two earlier studies (Brain Res., 616 (1993) 251; ibid. 683 (1995) 1), we found that neurons lying in the lateral spinal nucleus and lateral funiculus of the upper cervical spinal cord became transneuronally labeled following pseudorabies virus injections into the kidney or stellate ganglion. In the present study, we have extended these findings and now report that similar results occur following pseudorabies virus injections into the superior cervical ganglion, celiac ganglion or adrenal gland. These findings were confirmed with an anterograde tracing method. Iontophoretic injections of the plant lectin *Phaseolus vulgaris* leuco-agglutinin were made in the lateral funiculus or lateral spinal nucleus of the C_3 spinal segment, resulting in bilateral axonal labeling in the intermediolateral cell column, laminae VII and X. Sparse axonal labeling was observed in the dorsal horn (lamina V) and ventral horn of the C_5-C_8 segments as well as the thoracic spinal cord. A few labeled fibers were found in the lower lumbar or sacral spinal cord, but their termination sites were not identified.

In summary, this report provides the first evidence that two descending projection systems arise from neurons lying within the white matter of the cervical spinal cord and terminate in close apposition to sympathetic preganglionic neurons.

This research was supported by the National Institute of Heart, Lung, and Blood (HL-25449) of the NIH.

158.5

IMMEDIATE-EARLY GENE RESPONSES TO SPINAL CORD TRANSECTION. D.A. Ruggiero[1]*, A.L. Sica[2], M. Anwar[1], N. Gootman[2] and P.M. Gootman[2]. [1]Dept. of Neurol. & Neurosci., Cornell Univ. Med. Coll., NY, NY 10021; [2]Dept. of Physiol., SUNY Health Sci. Ctr., Brooklyn, NY 11203.

Sympathetic nerve discharge (SND) is maintained and regulated at the spinal level after high spinal cord transection (SCT). The identities of the spinal circuits were explored by monitoring induction patterns of c-fos gene expression produced by high thoracic SCT (n=4). Isofluorane-anesthetized adult male rats were spinalized at T3. Spinal cords were exposed but not transected in four age-matched controls. After 2.5h rats were perfused for immunocytochemistry. Dorsal root ganglia (DRG) and spinal segments C3-C5 and T1-L3 were cut at 35 μm, incubated in sheep or rabbit anti-Fos and processed using the avidin-biotin immunoperoxidase technique. SCT induced neurons to express Fos in thoracolumbar laminae I, $V_{ret.}$, VII, X and the intermediolateral cell column. DRG and cervical segments revealed no evidence of c-fos gene induction. Neuronal responses to SCT were localized to laminae that contain sympathetic interneurons and preganglionic motoneurons identified by retrograde transneuronal transport of Pseudorabies virus Bartha ($1x10^9$ pfu/ml) from the superior cervical ganglion or adrenal medulla. Peripheral inputs processed by spinal-sympathetic reflex circuits may regulate SND in the absence of supraspinal drive. (Supported by NIH grants HL18974; NS28200 [DAR]; HL20864; HD28931 [PMG])

158.6

THE PRESENCE OF SYMPATHETIC (SYMP) ACTIVITY FOLLOWING SPINAL CORD TRANSECTION (SCT) IN DEVELOPING SWINE. A.L.Sica[1], B.W Hundley[2], D.A. Ruggiero[3], M. Anwar[3], and P.M. Gootman[3]*. [1]Long Island Jewish Med. Ctr., New Hyde Park, NY 11040. [2]SUNY Hlth. Sci. Ctr. at Bklyn., NY 11203. [3]Cornell University College of Medicine, NY., NY 10021.

SYMP activity is not abolished by high spinal transection. It is possible that sensory inputs transmitted through dorsal root ganglia can retain activity via intraspinal reflex circuits. In Saffan anesthetized, vagotomized, paralyzed and artificially ventilated (100% O_2) piglets, recordings were obtained from phrenic and lumbar SYMP nerves before and after SCT. Intact animals displayed modulation of SYMP activity by both baroreceptor and central respiratory-related inputs. Following SCT, modulation of SYMP activity related to lung inflation emerged; SYMP activity was maximum during inflation and at a minimum during deflation. The relationship of SYMP to lung inflation was quantified by autopower and coherence spectra. Prominent peaks (ca.1 Hz), were highly correlated in coherence spectra (>0.1). These results suggest pulmonary afferent modulation of SYMP discharge may account for the observed vasomotor changes occurring in SCT mammals. *C-fos* gene expression was used to monitor functional responses of spinal cord neurons below SCT. Age-matched controls were subjected to identical surgical procedures but not to spinal cord injury. After 2.5-3 hrs, animals were prepared for immunocytochemistry. After SCT, larger numbers of intensely labeled nuclei were concentrated in C3-C5 (laminae V, VIII and X) and thoracic spinal gray (laminae I, V, VII, X, including intermediolateral cell column). Neurons expressing Fos after SCT occupy laminae that harbor SYMP interneurons and motoneurons; interneurons may regulate SYMP in response to spinal cord injury. It appears that the activity present in efferent preganglionic discharge in developing animals is less dependent on supraspinal mechanisms, possibly because functional synaptic contacts with brain stem SYMP neurons are not yet fully expressed. (Supported by NIH grants HL-20864, HD-28931 [P.M.G., A.L.S] and HL18974, NS-28200 [D.A.R.]).

158.7

POTENTIAL SUPERSENSITIVITY TO CATECHOLAMINES IN A CHRONIC RAT MODEL OF AUTONOMIC DYSREFLEXIA. L.M. Landrum, G.M. Thompson, and R.W. Blair*. Dept Physiol, Univ OK Hlth Sci Ctr, Oklahoma City, OK, 73190.

Episodic hypertension, known as autonomic dysreflexia, can be induced by visceral and somatic stimuli following spinal cord injury above T6. Catecholamine supersensitivity was evaluated as a potential mechanism for autonomic dysreflexia by comparing dose-response curves to phenylephrine in chronic lesioned and intact control rats. Spinal cords were completely transected at T4 in two rats and partially transected in three rats. Thirty five days were allowed for the lesioned rats to recover. One day prior to the experiment, arterial and venous catheters were implanted in both lesioned and control rats to monitor blood pressure and infuse drugs, respectively. Dose-response functions were assessed on the next day in conscious lesioned or age- and weight-matched control rats. Pressor responses to phenylephrine were enhanced at all doses (0.25, 0.50, 0.75, 1.0, 2.0 ug/kg) in rats with complete transections compared to intact controls. Animals with incomplete lesions, as determined by presenting motor skills, reflected dose-response curves similar to intact controls. These results suggest that chronic lesioned rats with complete spinal cord transections are more sensitive to catecholamines than partially transected animals or intact controls. This study provides initial support for supersensitivity to catecholamines as a potential mechanism for autonomic dysreflexia. Supported by Provost Research Award.

158.8

CONFOCAL MICROSCOPY FOR MONITORING THE SURVIVAL OF SYMPATHETIC PREGANGLIONIC NEURONS IN LIVE EXPLANTS OF THE SPINAL CORD.

A. Krassioukov[1]* S. McDonell[1] & K. Rogers[2]. [1]John P. Robarts Research Inst. & Dept. of Physiol. Univ. of Western Ontario., & [2]Dept. of Anatomy and Cell Biology. Univ. of Western Ontario, London, Ont., Canada, N6A 5K8.

It is well documented that after spinal cord injury sympathetic preganglionic neurons (SPNs) caudal to the site of injury first atrophy, and then regenerate, forming new connections within the spinal cord. The purpose of this study was to develop an *in vitro* slice preparation of a neonate rat spinal cord as a model of spinal cord injury, and to study the responses of SPNs to various concentrations of ciliary neurotrophic factor (CNTF). CNTF has previously been shown to promote motor neuron survival *in vivo* following axotomy, and has known trophic effects on SPNs as well. In order to investigate the survival and morphology of SPNs *in vitro*, a retrograde tracer was injected into the adrenal glands of 7 day old rat pups three days before they were sacrificed, following which their spinal cord tissues were put into culture. Explants were maintained in culture for a period of 21 days in media containing 50 ng/ml CNTF. Samples were taken periodically for analysis using a confocal microscope: SPNs were identified and their integrity monitored at each time period. When compared to age-matched pups, their morphology appeared to be normal. Physiological integrity of SPNs was determined by examining for the presence of enzymes that are typically expressed in these neurons, such as acetylcholinesterase and NADPH diaphorase. SPNs in explants cultured without CNTF did not survive for a long period of time and showed signs of degeneration within 24h of culturing. (Supported by the Heart and Stroke Foundation of Canada).

158.9

CONTRIBUTION OF SPINAL CORD NMDA AND AMPA RECEPTORS TO EPISODIC HYPERTENSION IN CONSCIOUS SPINAL RATS.

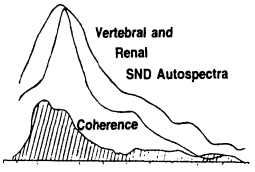
D.N. Maiorov*, N.R. Krenz, A.V. Krassioukov and L.C. Weaver. Neurodegeneration Research Group, John P. Robarts Research Institute and Dept. of Physiology, University of Western Ontario, London, ON, Canada.

Spinal viscero-sympathetic reflexes play a major role in the onset of episodic hypertension after spinal cord injury. The importance of glutamatergic transmission in mediating these spinal reflexes is not established. Therefore, we tested effects of intrathecally injected antagonists of the NMDA receptor (AP-5; 5 nmol) or the AMPA receptor (NBQX; 1.25 nmol) on the reflex hypertension caused by colon distension. Experiments were done using conscious rats 1-2 days (acute) or 14-15 days (chronic) after cord transection at the 5th thoracic segment. Before AP-5 injection, colon distension increased mean arterial pressure by 25±2 mmHg (from 93±3 mmHg) and by 33±3 mmHg (from 122±5 mmHg) in the acute (n=6) and chronic (n=6) groups, respectively. Intrathecal pretreatment with AP-5 attenuated the pressor responses by 45% and by 40% in the acute and chronic groups, respectively. Before NBQX injection, colon distension caused pressor responses of 19±3 mmHg (from 104±5 mmHg; n=4) and 30±3 mmHg from (108±8 mmHg; n=5) in the acute and chronic groups, respectively. Intrathecal pretreatment with NBQX attenuated the pressor responses by 39% and by 49% in the acute and chronic groups, respectively. At the doses used, AP-5 and NBQX blocked the pressor responses caused by intrathecal injection of NMDA (0.1 nmol) and AMPA (1 nmol), respectively. These data suggest that both NMDA and AMPA receptors contribute to spinal viscero-sympathetic transmission and initiation of episodic hypertension in conscious spinal rats. Supported by the Ontario Heart and Stroke Foundation.

158.10

EFFECT OF SPINAL CORD TRANSECTION ON THE 2-6 Hz COHERENT RHYTHMIC ACTIVITY IN DIFFERENT SYMPATHETIC (SY) NERVES. B. Kocsis* and K. Gyimesi-Pelczer. National Institute of Neurosurgery, Budapest, Hungary.

Periodically occurring slow waves between 2 and 6 Hz dominate basal discharge in all SY efferents. In the cat with an intact neuraxis, the 2-6 Hz rhythm is highly coherent between different nerves and also with similar oscillations in the population activity of brainstem SY networks. Although basal sympathetic nerve discharge (SND) is heavily dependent on the activity of these same networks, there is some evidence that some rhythmic synchronization is possible in the absence of rhythmic bulbospinal input. In order to examine the spinal SY oscillators and primarily the possibility of their coupling, we analyzed the SND simultaneously recorded in three nerves of 19 acute spinal cats after intrathecal injection of glutamate (i/t-GLU). We found that the response to i/t-GLU was highly variable but usually included activation of rhythmically modulated SND (still with relatively high power above 6 Hz) which could indeed be correlated in different (even distant) nerves (see Figure), suggesting that they were generated by multiple oscillators capable of synchronization at low frequencies. (Supported by OTKA Grant T-020351)



158.11

RESPIRATORY MODULATION AND CHEMORECEPTOR REFLEX RESPONSES OF ADRENAL SYMPATHETIC PREGANGLIONIC NEURONS. S.F. Morrison* Dept. of Physiology, Northwestern Univ. Med. Sch., Chicago, IL 60611.

Two populations of adrenal sympathetic preganglionic neurons (SPNs), putatively effecting selective control of the secretion from epinephrine- and norepinephrine-storing chromaffin cells in the adrenal medulla, have been differentiated by their responses to the blockade of cellular glucose utilization with 2-Deoxy-D-glucose and by their responses to activation of the RVLM. To examine how these SPNs are influenced by the central respiratory networks and by activation of the peripheral chemoreceptor reflex, the relationship of their spontaneous discharge to the phrenic nerve cycle was monitored and their responses to 10s periods of breathing 100% N_2 were tested in urethane/chloralose-anesthetized, vagotomized, and artificially-ventilated rats. Adrenal SPNs potentially regulating norepinephrine release displayed a prominent respiratory modulation, usually with an increase in discharge probability during the late I-early E phase, and were also potently excited during the period of increased respiratory frequency arising from chemoreceptor stimulation. The discharge probability of adrenal SPNs potentially regulating epinephrine release was not modulated over the phrenic nerve cycle and was changed little during chemoreceptor activation. These data suggest a further functional differentiation among sympathetic premotor neurons innervating SPNs regulating different target tissues. Supported by NIH HL47196.

158.12

CHARACTERISTICS OF THE RENAL SYMPATHETIC NERVE ACTIVITY RECORDED IN CONSCIOUS DAHL SALT-SENSITIVE RATS. K. Kato, T. Kunitake, T. Hanamori and H. Kannan*. Dept. Physiol., Miyazaki Medical College, Miyazaki, Japan 889-16.

Dahl salt-sensitive (DS) rats develop hypertension when fed a high-salt diet, but remain normotensive on a low-salt diet. The mechanisms underlying salt-dependent hypertension are not entirely known, although several abnormalities in DS have been proposed to be of pathogenic importance ; involvement of the kidneys, including abnormal renal excretory and humoral mechanisms, is one such suggested contributory factor. Renal sympathetic nerve activity (RSNA) has a direct effect on the control of renal blood flow, rennin release and urinary sodium excretion. We have recorded RSNA in free-moving Dahl-rats on a low NaCl diet in order to characterize the RSNA discharge pattern and evaluate the RSNA response to intracerebroventricular (i.c.v.) administration of hypertonic sodium chloride. Under resting conditions, two types of discharge pattern were seen, a grouped cardiac-related discharge (GD) and a non-grouped irregular discharge (NGD). The GD was inhibited by i.v. administration of phenylephrine chloride, while the NGD was not. Both GD and NGD were seen in DS and Dahl-salt-resistant (DR) rats and both were completely abolished by i.v. administration of the ganglionic blocker, hexamethonium chloride, indicating postganglionic efferent nerve involvement. I.c.v. infusion of NaCl (0.3-1.0 M, 20 min, 1 µl/min) produced decreased RSNA in DS and DR rats, but more long-lasting decreased RSNA in DS rats was observed, compared to DR rats. The result does not support the hypothesis that salt-sensitive hypertension is due to a failure to suppress RSNA in response to salt loading. The experimental results also emphasize the importance of recording with a high signal-to-noise ratio to evaluate the true activity of the autonomic nervous system.

158.13

THE INFLUENCE OF THE PARAVENTRICULAR NUCLEUS ON SYMPATHETIC ACTIVITY TO THE HEART AND KIDNEY: MEASUREMENT BY CLUSTER ANALYSIS

Jane Gardner* and John H. Coote. Department of Physiology, University of Birmingham, Birmingham B15 2TT. U.K.

Following our previous studies (Gardner et al, 1995) a pattern of response in sympathetic nerve activity produced by stimulation in the paraventricular nucleus (PVN) was investigated using a computerised program of cluster analysis. Experiments were carried out on rabbits anaesthetised with urethane. Recordings were made from the left renal and cardiac sympathetic nerves whilst stimulating neuronal perikarya in PVN by microinjection of 200nl 0.2M D,L-homocysteic acid containing 1% Pontamine Sky Blue. Stimulation in PVN always produced an inhibition in renal nerve activity (RNA, 32±7.1%), accompanied by an increase in cardiac nerve activity (CNA, 41±8%), and an increase in mean arterial blood pressure (9±3.4%). There was no significant change in heart rate ($p>0.8$). The filtered and rectified nerve activity was subsequently analysed using a peak detection program, providing data on the peak to peak (p-p) intervals and peak heights. RNA showed a mean decrease in p-p interval of 24.6±14% whilst CNA increased by 19.7±5.5% suggesting a reduction in the number of active neurones in RNA and an increase of active neurones in CNA. Similarly the peak height, which is indicative of changes in firing frequency of the same neurones, was decreased in RNA (17±6.1%) and increased in CNA (9.7 ±2.4%). This analysis demonstrates that the PVN induced differential response involves an alteration in the number of active neurons and their firing rate, thus providing a more comprehensive understanding of the influence of PVN on the cardiovascular system.

Supported by the Wellcome Trust.

158.14

CALCIUM-BINDING PROTEIN IMMUNOREACTIVITY MARKS SPECIFIC AUTONOMIC PATHWAYS IN THE RAT SUPERIOR CERVICAL AND STELLATE GANGLIA.

C.R. Anderson* and I. Grkovic. Department of Anatomy and Cell Biology, University of Melbourne, Parkville, 3052, Australia.

The superior cervical ganglion (SCG) and the stellate ganglion from Sprague Dawley rats were stained for the immunohistochemical demonstration of the calcium-binding proteins, calbindin and calretinin. Calbindin and calretinin were present separately, or in some cases in combination, in subpopulations of nerve terminals surrounding postganglionic nerve cell bodies. Calbindin, but not calretinin was also present in a subpopulation of postganglionic neurons in both ganglia. The postganglionic targets of some of the calbindin and calretinin terminals were determined in a series of retrograde tracing studies using Fast Blue or Fluorogold in combination with immunohistochemistry. Terminals containing calbindin but not calretinin were present around a subset of neurons projecting to the hairy skin. The vast majority of these neurons lacked NPY-immunoreactivity, suggesting they were pilomotor in function. Many large neurons lacking NPY-immunoreactivity, projecting to the submandibular salivary gland and predominantly in the SCG, were surrounded by terminals containing both calretinin and calbindin. Postganglionic neurons projecting to the skeletal muscle of the forelimb and iris were never surrounded by calbindin or calretinin terminals. Postganglionic neurons projecting to brown fat were not surrounded by calretinin or calbindin terminals but nearly all postganglionic neurons lacking NPY immunoreactivity were calbindin immunoreactive. Thus in the rat SCG and stellate ganglion, calbindin and calretinin are restricted to a range of functionally defined autonomic pathways.

This work was funded in part by the Australian National Heart Foundation.

158.15

GENE TRANSFER INTO SYMPATHETIC PREGANGLIONIC NEURONS IN VIVO BY THYMIDINE KINASE DEFICIENT OR MULTIMUTANT HERPES SIMPLEX VIRUS TYPE 1.

M.A. LeVatte, G.A. Dekaban and L.C. Weaver*. Neurodeg. Research Group, Robarts Research Institute, London, Ontario, N6A 5K8.

Long term improvements in faulty control of discrete functional groups of spinal sympathetic preganglionic neurons (SPNs) might be accomplished by introducing corrective genes into these cells using replication-defective herpes simplex virus type 1 (HSV-1). Thymidine kinase deficient $HSV\text{-}1_{LWDC}$ ($TK^-HSV\text{-}1$) expressing the *E. coli* β-galactosidase (β-gal) from the HSV ICP6 promoter, was previously assessed as a transfer vehicle into SPNs *in vivo*. $TK^-HSV\text{-}1$ ($1\text{-}6x10^6$ plaque forming units, PFU) infected many sympathoadrenal SPNs (353±97; n=5) 2 days post-inoculation into to the left adrenal gland of hamsters. After 5 days, fewer SPNs (98±78; n=7) were detected and many showed abnormal morphology. Inflammatory infiltrates were abundant throughout the white and grey matter. $TK^-HSV\text{-}1$ expresses all immediate early transcriptional activators, many of which are known to contribute to the cytotoxicity of HSV-1. Another recombinant HSV, 14HΔ3vhsZ, has mutations in the transactivator vp16, the immediate early transactivator ICP4 and virus host shutoff, expresses β-gal from a cytomegalovirus promoter and was noncytotoxic for cells in culture (Johnson *et al., J. Virol.* **68**: 6347, 1994). Three days after adrenal inoculation, 14HΔ3vhsZ ($3\text{-}5x10^6$ PFU) was retrogradely transported to many SPNs (212±97;n=3). These SPNs had normal morphology and elaborate dendritic processes. After 5 days, 112±76 (n=2) SPNs were detected and fewer SPNs demonstrated abnormal morphology than had occurred after infection with $TK^-HSV\text{-}1$. However, inflammatory infiltrates were also detected 5 days after inoculation with 14HΔ3vhsZ. 14HΔ3vhsZ was detected in few oligodendrocytes, microglia or astrocytes in contrast to the extensive extrasynaptic spread of $TK^-HSV\text{-}1$ from infected SPNs. In conclusion, the multimutant 14HΔ3vhsZ is highly neurotropic *in vivo*, can be transported from the periphery to spinal neurons, appears to produce less neurocytotoxicity than $TK^-HSV\text{-}1$ and may be a favorable gene transfer vehicle. Support: MRC Canada.

GASTROINTESTINAL REGULATION: CNS CONTROL

159.1

PSEUDORABIES VIRUS (PRV) LABELING OF BOMBESIN-LI NEURONS IN THE PARAVENTRICULAR NUCLEUS (PVN) OF THE HYPOTHALAMUS CONTROLLING GASTRIC FUNCTION.

R.B. Lynn*[1], G.-Y. Cao[1], M. Yang[2], R.R. Miselis[2]. [1]Dept. of Med., Jefferson Medical College, Thomas Jefferson Univ., Phila. PA 19107; [2]Dept. of Animal Biol., Univ. of Penn., Phila. PA, 19104;

Bombesin injected centrally in rats inhibits gastric acid secretion and motility. Bombesin-like immunoreactive (LI) neurons in the PVN of the hypothalamus project to the dorsal vagal complex, consistent with a potential influence on vagal pathways. The aim of this study is to locate the bombesin-LI neurons that are synaptically connected to gastric pathways in rats. PRV, a neurotropic virus, was used as a retrograde transsynaptic tracer to label circuits of neurons in the brain that project to the stomach. PRV (6 µl) was injected subserosally into the rat stomach. 60-65 hrs later, a time at which PRV has already reached PVN neurons, colchicine (50 µg/100 gm body wt) was injected into the lateral ventricle. After an additional 28-34 hrs the animal was perfused and the entire brain was frozen sectioned. Bombesin was labeled with a primary antiserum raised in rabbits (Incstar) and a secondary antibody conjugated to Texas Red. PRV was labeled with a primary antibody raised in goat and a secondary antibody conjugated to FITC. Bombesin-LI neurons double labeled with PRV were mostly located in the medial parvocellular portion of the PVN of the hypothalamus. These neurons, labeled both for their phenotype and connectivity, likely play a role in the central inhibitory effect of bombesin on gastric function. This study provides further evidence that bombesin containing neurons in the brain synapse directly onto gastric vagal motoneurons since vagotomy eliminates most medial parvocellular PVN labeling. Support: DK02094(RL); GM27739(RM).

159.2

DORSAL MOTOR NUCLEUS CELL TYPES DISTINGUISHED WITH MULTIVARIATE ANALYSES.

M.K. Jarvinen*, T.L. Powley. Purdue U., West Lafayette, IN 47907.

A full classification of the neuronal cytoarchitecture of the dorsal motor nucleus of the vagus (dmnX) is still not available. Previous quantitative groupings of dmnX neurons, including univariate analyses of Lucifer Yellow-fill neurons projecting to the abdomen (Fox and Powley, 1992) and descriptions of HRP-filled neurons responsive to intestinal distension (Zhang et al. 1992) would not have detected interneurons or all preganglionics projecting to all targets, because of the sampling strategies employed. Golgi staining should randomly sample all subpopulations, but such material has not been analyzed by multivariate quantitative methods. To provide this analysis, male SD rats were perfused, and their brainstems stained *en bloc* with a Golgi-Cox protocol. Prior to perfusion, each animal received a Fluoro-gold injection (3mg i.p.) to label the dmnX. Separate stained blocks were sectioned at 170 µm in horizontal, sagittal, and coronal planes. All darkly stained and non-truncated neurons were digitized (Eutectics Neuron Tracing System). Three-dimensional morphometric measures included dendritic length, number of total segments, spine density, number of primary dendrites, dendritic orientation, and soma form factor. Samples of 100+ neurons from a given plane of section were then subjected to cluster analysis (Ward's algorithm) and principle components analysis (PCA). Consistent with previous suggestions, neurons of the dmnX tended to be most elongated, i.e. to have their major axes running longitudinally. Cluster analyses identified four major categories of neurons in the horizontal sample and a similar set of homologous classes in the sagittal sample. PCA also identified a number of principle variables consistent with the defining characteristics of each cluster. As a further validity check on the cell categories identified in both horizontal and sagittal sections, a replicate sample of an additional 104 neurons from horizontal sections was analyzed. This replicate identified a similar set of four groupings. We are currently mapping the distributions of these four multivariate types within the dmnX, analyzing their dendritic branching outside of the nucleus, and relating them to previous descriptions of subsets of dmnX neurons. NIH DK27627 and NIMH MH01023.

159.3

VAGAL AFFERENT AND EFFERENT INNERVATION OF THE GI TRACT COMPARED. J. Kelly*, F. B. Wang, E. A. Baronowsky, R. J. Phillips, and T.L. Powley. Purdue University, West Lafayette, IN 47907.

The number of axons and the ratio of sensory to motor fibers in the vagus nerve have been the basis for inferences about the innervation of the gut. Specifically, the number of axons in the abdominal vagus (~22000) has reinforced the assumption that the GI tract is sparsely innervated, and the disproportionate percentage of afferent fibers (e.g. 73%; Prechtl and Powley, '90) has generated the extrapolation that vagal innervation of the GI tract is largely sensory. These assumptions do not consider the divergence of the peripheral projections, hence we re-examined the issue by comparing terminal distributions. Male SD rats received unilateral injections of WGA-HRP in either the nodose ganglion or the dorsal motor nucleus of the vagus. Before injections, animals received unilateral intracranial vagal sensory rhizotomies (for efferent injections) or motor rhizotomies (for afferent injections) to eliminate inappropriate labeling. Control animals received WGA-HRP injections without prior rhizotomies or injections of DiI. The nodose ganglia, stomach, and the first 4 cm of duodenum were prepared as wholemounts and processed with TMB. Injections of either efferents or afferents caused heavily labeled vagal bundles and myenteric connectives, although, overall, motor label was denser than sensory label. This pattern was more pronounced as axons coursed from the connectives to targets: Efferent label delineated all myenteric ganglia, whereas afferent ganglionic label (i.e., intraganglionic laminar endings) was absent or barely detectable in many ganglia. In different gut locations, the relative densities of the motor and sensory terminal label (motor > sensory) were generally correlated, but in some regions this covariance was weak. In the antrum, for example, motor label was particularly dense whereas sensory label was relatively sparse. Conspicuous motor label is at odds with traditional expectations derived from counts of axons, but it is consistent with recent reconstructions of collateralization and termination in single efferent axons. More generally, these data indicate that axon counts--which do not consider divergence and terminal ramifications--are unreliable estimators of innervation densities. NIH DK27627 and NIMH MH01023.

159.4

REINNERVATION OF THE STOMACH BY VAGAL AFFERENTS AFTER SELECTIVE VAGOTOMY: AXONS & BUNDLES. T. L. Powley*, E. A. Baronowsky, & R. J. Phillips. Purdue University, West Lafayette, IN 47907.

Vagotomies are used extensively to study GI function, but little attention is usually given to the possibility that the vagus might regenerate or reorganize after vagotomy. Extrapolations from robust remodeling of VIIth and IXth nerve innervation of the tongue after axotomy as well as clinical observations after palliative vagal surgery, however, suggest that the vagus might exhibit plasticity. To investigate such potential plasticity, we gave male SD rats partial subdiaphragmatic vagotomies by cauterization, sparing only the hepatic branch. Animals were perfused 2 weeks (2wk; n=26), 6 weeks (6wk; n=12), and 18 weeks (18wk; n=21), post-vagotomy. Each rat received injections of Fluoro-gold (1-2 mg, i.p.), and WGA-HRP (3 µl, in the left nodose ganglion) 6 and 3 days before sacrifice, respectively. The medulla was sectioned (56 µm) and used to verify the vagotomy (Fluoro-gold). The nodose ganglia and stomach were processed (TMB) as wholemounts. Vagal bundles (2 or more axons) and individual axons in the stomach were counted and mapped with a sampling grid. At 2wk post-vagotomy, vagal afferents were limited to the sparse projections of the hepatic branch: innervation of the ventral stomach consisted of 1-2 bundles which branched into relatively small terminal fields; the only fibers on the dorsal stomach were mainly distal axons that wrapped around from the ventral side. By 6wk post-vagotomy, both sides were innervated by several bundles and numerous axons. Most axons in these projections, however ended in growth cone profiles or free endings, not differentiated terminals. By 18wk, both sides of the stomach were densely (albeit incompletely) innervated by bundles and axons. At this later stage, afferent axons terminated in elaborate, differentiated types of endings (see Phillips et al., this meeting). Ingrowing vagal bundles and axons appeared to follow the normal organization of branches and connectives, although some axons separated from the bundles and followed torturous, looping, or recurrent paths. The finding of vagal plasticity after damage to its peripheral axons, and the ability to quantify this phenomenon opens up a new arena in which to study regeneration and reorganization. NIH DK27627 and NIMH MH01023.

159.5

REINNERVATION OF THE STOMACH BY VAGAL AFFERENTS AFTER SELECTIVE VAGOTOMY: TERMINALS. R. J. Phillips*, E. A. Baronowsky, & T. L. Powley. Purdue University, West Lafayette, IN 47907.

The vagus has been shown to supply two distinct types of endings in the gastric muscle wall: intramuscular arrays (IMAs) and intraganglionic laminar endings (IGLEs). We have previously reported that after selective subdiaphragmatic vagotomy, the vagal bundles and axons normally innervating the stomach are able to undergo dramatic plasticity in response to the injury (Powley et al., this meeting). To further investigate the plasticity of vagal endings, inventories of terminals were taken in stomach specimens from male SD rats that had been given partial subdiaphragmatic vagotomies by cauterization, sparing only the hepatic branch. Animals were perfused 2 weeks (2wk; n=26), 6 weeks (6wk; n=12), and 18 weeks (18wk; n=21), post-vagotomy. Each rat received injections of Fluoro-gold (1-2 mg, i.p.) and WGA-HRP (3 µl, in the left nodose ganglion) 6 and 3 days before perfusion, respectively. The medulla was sectioned (56 µm) and used to verify the vagotomy (Fluoro-gold). The nodose ganglia and stomach were prepared (TMB) as wholemounts. Terminals in the stomach were counted and mapped with a sampling grid. At 2wk post-vagotomy, afferent endings (IGLEs and IMAs) were limited to the sparse terminal fields of the spared hepatic branch. At 6wk post-vagotomy, few new differentiated endings were present, although bundles and individual axons had entered the denervated regions of the stomach. By 18wk, both gastric walls were innervated by well developed endings. IGLEs were diffusely distributed throughout the stomach, whereas IMAs were found to be focused in the region of the fundus on both sides of the stomach. Although these restored distributions were similar overall to the spatial patterns of endings found in intact animals, some blurring and imperfections of the putative receptor maps occurred in the reorganized tissue. The formation of well developed IMAs and IGLEs by 18wk suggests that some afferent function(s) may be restored to the stomach. Additional analyses are required to determine the individual contributions of reorganization (of the hepatic branch) and regeneration (of the transected branches) to this plasticity. NIH DK27627 and NIMH MH01023.

159.6

RECIPROCAL CONTROL OF GASTRIC MOTILITY BY Y1 and Y2 RECEPTORS IN THE DVC. CH Chen,[A] IL Taylor,[B] and RC Rogers[A]*
Ohio State Univ.: Department of Physiology, Columbus, OH 43221[A]; Medical Univ. of South Carolina: Department of Medicine, Charleston, SC 29425[B]

The pancreatic polypeptide family of hormones and neurotransmitters including PP, PYY and NPY are emerging as potent central regulators of gastric function. There is, however, considerable debate concerning the mechanisms and even the direction of effects mediated by these peptides. Evidence shows that PYY is the "enterogastrone" released by the ileum into the circulation after feeding which acts on vagal reflex control circuits to *reduce* gastric motility [i.e., the "ileal brake"]. However, PYY and its close structural relative NPY may also act in the dorsal vagal complex [DVC] to *increase* gastric motility.

We hypothesize that the confounding observations are due to agonist effects on two different receptor types, Y1 and Y2. Both receptors are present in the DVC but may be accessed differentially by peripheral humoral [PYY] versus central [NPY] pathways. In our initial approach to this problem, we studied the effects of NPY, PYY, Y1 and Y2 agonists microinjected directly into the DVC on gastric motility in both basal and stimulated [by central TRH] conditions. Our results show that Y2 agonists applied to the DVC during conditions of TRH - stimulated gastric motility mimic the suppressive effects of PYY applied under the same conditions. Under basal conditions, Y2 agonists have no effect on motility. The DVC effects of the Y1 agonist are the opposite; Y1 agonist has no further effect to stimulate gastric motility in the TRH stimulated condition while Y1 agonist strongly stimulates motility in the basal condition. NPY effects depend upon the prevailing gastric motility. Under TRH stimulation [maximal motility] NPY in the DVC reduces, but does not completely suppress, gastric motility. In the basal state, NPY is a strong activator of motility. Our results are discussed in terms of the possible differential localization of Y1 versus Y2 receptors within the DVC and in terms of recent findings suggesting that PYY is rapidly converted to a Y2 agonist by a ubiquitous diamino peptidase [DAP IV]. Supported by grants from the NINDS and NIDDK

159.7

ACTIVATION OF RECEPTORS OF THE 5-HT$_1$ AND 5-HT$_2$ FAMILY AUGMENTS TRH ANALOG INDUCED GASTRIC ACID SECRETION AT THE DORSAL VAGAL COMPLEX. **S.Varanasi, J.Chi, R.L.Stephens, Jr*., Dept. of Physiology, The Ohio State University, Columbus, OH 43210**

Neurons from the caudal raphe (nucleus raphe obscurus and nucleus raphe pallidus) nuclei project to the dorsal vagal complex and contain colocalized thyrotrophin releasing hormone (TRH), and serotonin (5-HT). Previous work has established that 5-HT enhances TRH- induced stimulation of gastric acid secretion at the DVC and that receptors of the 5-HT$_2$ family mediate this augmented response. However in the present study, using more caudal co-ordinates for DVC injection, ketanserin or ritanserin pretreatment did not attenuate the 5-HT induced augmentation response. To investigate this discrepancy , 5-HT receptor selective agonists were coinjected with RX [12 pmole] into the DVC at a 5-HT agonist/RX molar ratio of 12. The selective agonists used were 5-CT (5-HT$_1$), DOI (5-HT$_2$) and 2-methyl-5-HT (5-HT$_3$). All injection volumes were 30 nl. The DVC co-ordinates were 0.2 mm anterior, 0.2 mm right, 0.6 mm ventral with respect to the calamus scriptorius. Acid responses (mean ± SEM, * p < 0.05 compared with RX))

Cum. acid,	Saline	RX	5-CT/RX	DOI/RX	2-M-5HT/RX
µmole/90 min	(n= 3)	(n=7)	(n=5)	(n= 3)	(n= 7)
± SEM	11 ±4	42 ± 8	156±23*	131±37*	42 ± 12

The results suggest that 5-HT$_1$ and 5-HT$_2$, but not 5-HT$_3$ receptors are involved in the augmentation response. Examination of RX/5-HT agonist response in more rostral DVC confirms involvement of 5-HT$_1$ receptor in the augmentation response. The results reveal that receptors of both the 5-HT$_1$ and 5-HT$_2$ family mediate enhanced acid response to DVC TRH-analog. Supported by NIH-DK 730037.

159.8

DIRECT EVIDENCE THAT NEURONS IN THE DORSAL MOTOR NUCLEUS OF THE VAGUS (DMNV) ARE INHIBITED BY NEURONS IN THE NUCLEUS OF THE SOLITARY TRACT (NST). X. Zhang*, R. Fogel and W.E. Renehan. Div. Gastro., Henry Ford Hosp., Detroit, MI

It is known that mechanical or chemical stimulation of the gut activates a circuit that inhibits the activity of neurons in the DMNV. Though it is presumed that this reflex involves inhibitory neurons in the NST, direct evidence of such a relationship is lacking. The purpose of this study was to directly evaluate the effect of NST activation on DMNV neuronal activity. A multibarrel glass micropipette array was used to record from and label individual neurons in the NST and DMNV while releasing small volumes of 0.1 M glutamate or 0.9% NaCl in the vicinity of the recorded cell. A separate microsyringe filled with 0.1 M glutamate was lowered into the ipsilateral NST. The stomach and proximal small intestine were cannulated to allow delivery of distention and chemical stimuli to the gut. Ten neurons in the vagal complex were successfully characterized and labeled in this preliminary study. Each of the two NST neurons was excited by gastrointestinal stimulation, glutamate released from the multibarrel array, and glutamate injected into the NST via the separate microsyringe. The 8 DMNV neurons were either excited or inhibited by gastric and/or intestinal distention. Some of the DMNV neurons were also excited by injection of glutamate in the region of the recorded neuron, but all were inhibited by injection of glutamate into the NST. We suggest that these results provide direct evidence that gut-sensitive DMNV neurons are directly inhibited by neurons in the NST. Supported by NS 30083.

159.9

MODULATION OF NEURONAL ACTIVITY IN THE NUCLEUS OF THE SOLITARY TRACT BY THE PARABRACHIAL NUCLEI Z. Liu, R. Fogel*, X. Zhang and W.E. Renehan Division of Gastroenterology, Henry Ford Health Sciences Center, Detroit, MI 48202.

There is anatomical evidence that the parabrachial nuclei (PB) project to, and receive projections from, the nucleus of the solitary tract (NST). We know relatively little, however, about the role of the descending inputs from the PB to the NST. The principal aim of the present investigation was to evaluate the influence of the descending PB input on gut-sensitive neurons in the NST. Glass micropipettes filled with 2.0% Neurobiotin were used to record the response of individual NST neurons to an array of gastrointestinal stimuli (distention, 0.1 M HCl, 20% glucose and 3.2% NaCl) and electrical current stimulation of the ipsilateral PB. A total of 13 NST neurons were successfully characterized and labeled. Four of the 13 neurons were antidromically driven at 200 Hz by the PB electrode (presumably these four neurons projected to the PB). Each of the four neurons was excited by gastric and/or intestinal distention, with 2 of the 4 neurons responding to HCl as well. The remaining 9 NST neurons exhibited response properties that suggested the presence of descending modulatory inputs from the PB. The 9 NST neurons receiving PB input exhibited an increase (n = 4) or a decrease (n = 5) in activity during PB stimulation. These preliminary data suggest that the NST transmits information regarding a variety of mechanical and chemical gastrointestinal stimuli to the PB. In addition, the NST receive both excitatory and inhibitory inputs from the PB. Supported by NS 30083.

159.10

POSTNATAL DEVELOPMENT OF VAGAL-HYPOTHALAMIC CIRCUITS IN RATS. L. Rinaman*. Dept. of Neurosci., Univ. of Pittsburgh, Pittsburgh, PA 15260.

In adult rats, ascending and descending connections between neurons in the dorsal vagal complex (DVC) and paraventricular nucleus of the hypothalamus (PVN) help integrate interoceptive stimuli and generate appropriate centrally-mediated autonomic, neuroendocrine, and behavioral responses. There is evidence suggesting that vagal-PVN connections are functionally immature in newborn rats. The present study was designed to further analyze the structural ontogeny of these connections. Methods: The anterograde and retrograde neural tracer cholera toxin (CTβ; 100 nl, 0.25%; List) was microinjected into the DVC in anesthetized adults and pups at postnatal (P) days 1 to 6. Rats were anesthetized 24-48 hrs later and perfused transcardially with fixative. Fixed brains were sectioned coronally and treated for the immunocytochemical localization of tracer using goat anti-CTβ (1:50K; List) and the Vectastain ABC immunoperoxidase procedure (Vector). Results: The membrane binding properties of CTβ limited its diffusion from DVC injection sites. In adult rats, the PVN contained many retrogradely labeled neurons (491±32; n=3) and a network of anterogradely labeled fibers and terminals. In contrast to the adult labeling pattern, in neonates anterograde labeling in the PVN appeared sparse or absent through P6. Retrogradely labeled neurons were present in the PVN in pups injected with CTβ as early as P1, but their number (205±28; n=3) was approximately half that observed at P6 (437±41; n=2). Summary: 1) Ascending projections from the DVC to the PVN appear sparse or absent during the first postnatal week. These data support an earlier report (*Dev. Brain Res.* 77:140) that PVN neurons are not activated by gastric vagal stimulation in newborn rats as they are in adults. 2) Descending projections from the PVN to the DVC may increase significantly during the first postnatal week. It remains to be determined whether retrogradely labeled PVN neurons form functional synapses within the DVC at these early ages. Ongoing work will further characterize the ontogeny of these and other CNS circuits that mediate homeostatic responses to interoceptive stimuli. Supported by NIH grant MH01208.

159.11

INTRACISTERNAL ANTISENSE OLIGONUCLEOTIDES TO TRH RECEPTOR ABOLISHES TRH-MEDIATED GASTRIC MOTOR EXCITATION IN RATS. D.V.Sivarao, Z.K.Krowicki and P.J.Hornby*. Dept. Pharmacology, LSUMC, New Orleans, LA 70112.

TRH in the dorsal vagal complex (DVC) potently stimulates gastric motor activity. The nucleus raphe obscurus (nROb) is a major source of TRH afferents to the DVC. Characterization of TRH reflex pathways controlling gastric function has been hampered by the lack of TRH receptor antagonists. Thus, we administered antisense oligonucleotides against the first 18 base pairs of TRH receptor mRNA intracisternally (100μg per day for four days). The change in intragastric pressure (peak IGP and total AUC) after microinjection into the DVC of TRH (1 and 10 pmol; N=6), L-glutamate (positive control) and saline was assessed. Controls received intracisternal mismatch oligonucleotide (N=4) or saline (N=4). Mean ±SEM; *P<0.05 compared to saline

Microinj.	Antisense		Mismatch		Saline	
agent	Peak IGP	AUC	Peak IGP	AUC	Peak IGP	AUC
Saline	-0.1±0.1	0±0	0.1±0.1	0±0	0.4±0.2	0.3±0.2
L-Glu	2.7±0.8*	0.6±0.1*	3.0±1.0*	1.2±0.4*	7.6±1.7*	1.8±0.2*
TRH 1	1.2±0.9	0.6±0.4	3.3±1.0*	0.9±0.2	4.7±1.0*	2.5±1.1*
TRH 10	1.9±0.8	1.1±0.6	5.2±1.8*	1.4±0.2	5.0±0.9*	3.2±0.6*

In the same animals, L-glutamate microinjected into the nROb to release endogenous TRH in the DVC resulted in much diminished increases in peak IGP and AUC in antisense-treated compared to saline- and mismatch-treated animals. This illustrates the utility of *in vivo* antisense oligonucleotides for characterization of central TRH pathways controlling gastric function. Support: DK42714 and Sloan Foundation.

159.12

SYNAPTIC INPUTS IN NEURONS OF RAT DORSAL MOTOR NUCLEUS OF THE VAGUS REGULATING GASTROINTESTINAL ACTIVITY. M. Bertolino*, R. Houghtling, S. Vicini† and R. Gillis Department of Pharmacology, †Department of Physiology and Biophysics Georgetown University School of Medicine, Washington DC 20007.

Evoked synaptic currents were studied in thin (200 μm) coronal slices of rat brainstem using the whole-cell configuration of the patch clamp technique. The synaptic currents were evoked by focal electrical stimulation either of the tractus solitarius (TS), or the commissural area of the nucleus TS (ComNTS). Stimulation evoked two types of synaptic currents in DMV neurons: an inward excitatory postsynaptic current (EPSC) at a holding potential (HP) of -70 mV blocked by 20 μM CPP and 5 μM NBQX (suggesting that the EPSC was elicited by glutamate receptors); and an outward inhibitory postsynaptic current (IPSC) at a HP of -40 mV blocked by 20 μM bicuculline (suggesting that the current was elicited by $GABA_A$ receptors). This dual innervation was recorded in neurons located either in the medial as well as in the lateral portion of the DMV. To investigate whether both types of ionotropic glutamate receptors were involved in the EPSCs evoked from the ComNTS, the decay phase of the EPSCs was studied with and without Mg^{++} in the perfusing solution. In both conditions the decay phase was fitted by a double exponential function showing the existence of a fast and a slow component. NBQX and CPP (in Mg^{++}-free solution containing 100 μM picrotoxin to block $GABA_A$ receptors) blocked the fast and slow component, respectively. These data suggest that $GABA_A$ and glutamate receptors may regulate the neurons controlling the GI tract function. Supported by NIH grant AM29975.

159.13

THE NITRIC OXIDE SYNTHASE INHIBITORS N^G-NITRO-L-ARGININE METHYL ESTER AND N^G-NITRO-L-ARGININE ARE ANTIEMETIC IN FERRETS.
R.W. Dunn*, R.L. Wynn and R.L. Gregory, NOS/CETS, Old Lyme, CT and University of Maryland, Baltimore, MD.

N^G-nitro-L-arginine methyl ester (L-NAME) and N^G-nitro-L-arginine (L-NOARG) inhibited cerebellar rat neuronal nitric oxide synthase (NOS) activity with IC_{50} values of 0.90 and 0.48 uM, respectively. The effects of these NOS inhibitors were assessed in the morphine- and cisplatin-induced emesis models in ferrets. Non-fasted male ferrets (1-2 kg) were intravenously (iv) administered L-NAME or L-NOARG either 30 minutes prior to subcutaneous morphine (0.3 mg/kg) or 30 minutes after iv cisplatin (10 mg/kg). Animals were observed for emetic episodes (expulsion of solids or liquids) for either 30 or 180 minutes following morphine or cisplatin administration, respectively. Both L-NAME and L-NOARG dose-dependently antagonized the emetigenic effects of morphine (ED_{50} values of 0.004 and 0.44 mg/kg, respectively) and cisplatin (ED_{50} values of 0.019 and 0.008 mg/kg, respectively). For comparison, the ED_{50} values for the $5\text{-}HT_3$ antagonist ondansetron in the morphine and cisplatin assays were 2.58 and 0.41 mg/kg respectively. D-NAME (the stereoisomer of L-NAME which is devoid of NOS activity) had no protective effect in either the morphine or cisplatin models, suggesting that the antiemetic activity of L-NAME and L-NOARG was due to NOS inhibition. Therefore, NOS inhibitors may represent a novel class of antiemetic agents.

159.14

GASTRIC EXCITATORY MOTOR RESPONSES TO ENDOTHELIN-1 IN THE DORSAL VAGAL COMPLEX ARE MEDIATED THROUGH ET_A RECEPTORS. Z.K. Krowicki* and P.J. Hornby, Dept. of Pharmacology, Louisiana State University Medical Center, New Orleans, LA 70112.

Intracisternal administration of endothelin-1 (ET-1) and ET-3 in anesthetized rats evokes increases in gastric motor function (Inflammopharmacology 1996, in press). The aim of the present study was to investigate whether the dorsal vagal complex (DVC) is a medullary site for the gastric motor effects of ET and to identify the ET receptor subtype through which these effects are mediated. ET-1 (0.1-10 pmol per site) and ET-3 (1 and 100 pmol per site) were microinjected into the right DVC (30 nl) of α-chloralose/xylazine anesthetized and artificially ventilated rats, while monitoring intragastric pressure and contractility of greater curvature longitudinal and pyloric circular smooth muscle. Endothelin-1 dose-dependently increased intragastric pressure and gastric contractility. The gastric excitatory motor effects of ET-1 (10 pmol) in the DVC were abolished by bilateral vagotomy. Since a 100-times higher dose of ET-3 (100 pmol) was required to produce changes in intragastric pressure of comparable magnitude to those elicited by ET-1 (1 pmol), we hypothesized that the gastric motor responses to ET in the DVC could be mediated via ET_A receptors. This was supported by the observation that a specific ET_A receptor antagonist, cyclo(-D-Trp-D-Asp-Pro-D-Val-Leu) (BQ-123; 400 pmol per site), microinjected into the DVC 15 min before ET-1 (10 pmol), completely blocked the gastric motor responses to ET-1. Therefore, we conclude that ET-1 acts in the brainstem at the level of the DVC to increase gastric tone and contractility via a vagally-mediated pathway and that these effects are mediated through ET_A receptors. This maybe important in mediating gastric motility alterations such as these occurring in diabetes, where the plasma levels of ET-1 are elevated. Supported by the LSU Neuroscience Center incentive grant (ZKK) and PHS grant DK42714 (PJH).

159.15

GASTRIC ACID SECRETION (GAS) AND RESISTANCE OF THE MUCOSA TO ETHANOL INJURY IS MODULATED BY CENTRAL PYY THROUGH VAGAL CHOLINERGIC DEPENDENT PROSTAGLANDIN (PG) AND CALCITONIN GENE RELATED PEPTIDE (CGRP) MECHANISMS. **H. Yang* and Y. Taché. CURE:DDRC, VA Medical Center, Brain Research Institute and Dept. of Medicine, UCLA. Los Angeles, CA 90073, U.S.A.**

We previously reported that PYY injected into the dorsal motor nucleus of the vagus induced vagal stimulation of GAS (AJP 268:G943, 1995). There is evidence that central vagal activation induced PG and CGRP dependent modulation of gastric secretion and resistance of the mucosa to injury (J Gastro Hepatol 9:S29, 1994). We explored whether intracisternal (ic) PYY induces vagal mediated, PG/CGRP dependent changes in gastric function and resistance to ethanol lesions. Male rats weighing 250-310 g were fasted for 24 h and anesthetized with urethane, GAS was measured by flushing technique and gastric erosions were induced by intragastric administration of 45% ethanol. PYY 100, 200 or 500 ng (ic) did not influence basal GAS in vehicle-pretreated rats but increased GAS in indomethacin (5 mg/kg, -60 min, ip) pretreated rats by 18, 23 ($P<0.05$) and 23 ($P<0.05$) μmol/90 min respectively. This effect was completely prevented by cervical vagotomy. Gastric erosions induced by intragastric 45% ethanol, which covered 22 ± 3% of the mucosa surface in 1 h, were significantly decreased by ic PYY 100, 200 and 500 ng (-30 min) by 37%, 62% ($P<0.05$) and 58% ($P<0.05$) respectively. Pretreatment with indomethacin (5 mg/kg, -60 min, ip), atropine (2 mg/kg, -30 min, sc) or CGRP antagonist, $CGRP_{8-37}$ (100 μg/kg, -15 min, iv) did not significantly alter the erosion induced by 45% ethanol but reversed the cytoprotective effect of ic PYY (200 ng) by 66% (indomethacin) or completely (atropine and $CGRP_{8-37}$). PYY (200 ng, ic) completely prevented the 133% increase of ethanol erosions induced by L-NAME (6 mg/kg, iv) pretreatment. These results suggest that ic PYY induced vagal dependent gastric PG release which inhibits GAS. In addition, central administered PYY increased the resistance of the gastric mucosa against ethanol injury through PG and CGRP mechanisms independent from nitric oxide.

159.16

MICROINJECTION OF BICUCULLINE METHIODIDE INTO DORSOMEDIAL HYPOTHALAMIC NUCLEUS STIMULATES GASTRIC ACID SECRETION IN RATS. S.L. Kendall-Eagleson*, M.H. Bender, A.J. Monroe, and J.A. DiMicco. Department of Pharmacology and Toxicology, and Program in Medical Neurobiology, Indiana University School of Medicine, Indianapolis, IN 46202

Acute stress evokes characteristic increases in heart rate (HR)and blood pressure (BP), and stimulates both colonic motility and gastric acid secretion (GAS). We have shown that microinjection of the $GABA_A$ receptor antagonist bicuculline methiodide (BMI) into the dorsomedial hypothalamus (DMH) mimics the cardiovascular and colonic motor changes seen in stress, while inhibition of neurons in the same region suppresses stress-induced tachycardia. Here, we examined the effect of microinjection of BMI into the DMH on GAS. In 10 fasted urethane-anesthetized rats, a femoral artery was cannulated for continuous monitoring of HR and BP. The gastro-esophageal junction and pylorus were ligated and the stomach was cannulated. The gastric lumen was lavaged and then flushed with two 4 ml aliquots of 0.9% saline at 10 minute intervals. GAS was assessed by calculating $[H^+]$ from pH of each pooled 10 minute sample. After attainment of a stable baseline, BMI 10 pmol/50 nl was microinjected into the DMH and GAS and cardiovascular function were monitored for an additional 50 minutes. All injection loci were verified by post-mortem histology. At t_0, the mean (±SE) baseline $[H^+] \times 10^{-6}$ was 62 ± 17, and HR was 390 ± 12 beats/min. In every rat, microinjection of BMI into the DMH was followed by increases in GAS (mean ± SE at t_{20} = +52 ± 14; 222% with respect to original baseline) and HR (+60 ± 7 beats/min). In two additional rats in which BMI was injected in identical fashion into the ventromedial hypothalamic nucleus, no increases in GAS were seen and HR increased by 20 beats/min or less. Thus, blockade of inhibitory $GABA_A$ receptors in the DMH increases GAS in rats. In light of our previous findings, these results suggest that activation of the DMH may be responsible for stress-induced increases in GAS. (Supported by USPHS Grant NS 19883)

159.17

PITUITARY GALANIN (GAL) AND VASOPRESSIN (AVP) IN RELATION TO HIGH-FAT DIET IN FEMALE RATS. H.J. Yu, J. Wang, H.J. Chae, Z. Bedrin and S.F. Leibowitz*, Rockefeller Univ, NY, NY 10021

Previous biochemical and pharmacological studies have linked the peptide, GAL, in the hypothalamic paraventricular nucleus (PVN), to the overeating of fat. This relationship is further demonstrated by results, reported at this meeting (Wang et al.), showing higher GAL mRNA and peptide immunoreactivity (ir), in the anterior PVN (aPVN) and median eminence (ME), of animals on a high-fat diet. Since the anterior pituitary (AP) is also known to synthesize GAL and PVN projections pass through the ME to the posterior pituitary (PP), we examined whether this peptide in both pituitary lobes may be affected by a high-fat diet. We also studied AVP (n=4), which coexists with GAL in certain PVN neurons. Female rats, maintained for 2 weeks on either a single high-fat diet (60% fat + 10% carbohydrate, n=6) or high-carbohydrate diet (63% carbohydrate + 10 % fat, n=6), were intracardially perfused and their pituitaries examined, using in situ hybridization with a digoxigenin labeled cRNA probe for GAL mRNA and immunocytochemistry for GAL peptide. Results show that, in rats on the high-fat diet, there was a significant ($p<0.05$) increase in AP GAL mRNA (+413%, 1725±71 vs 418±68 cell density/cm^2), and GAL peptide (+270%, 409 ±68 vs 152 ± 12 cell density/cm^2). This increase in GAL fiber density was also seen in the PP (+24%, $p<0.05$). While GAL-ir was similarly increased in the aPVN ($p<0.05$), no effect of high-fat diet on AVP fiber density was seen in the PVN (79±19 ys 116±77 cell density/mm^2) or PP (164±35 vs 169±23 fiber density/mm^2). These results show that AP cells synthesizing GAL are similar to PVN GAL neurons in their response to a high-fat diet, whereas PVN or PP AVP-ir shows little change.

159.18

INVOLVEMENT OF MEDULLARY CATECHOLAMINE CELLS IN NEUROENDOCRINE RESPONSES TO CHOLECYSTOKININ. K.M. Buller* and T. A. Day, Dept. of Physiology & Pharmacology, University of Queensland, AUSTRALIA 4072.

Systemic administration of cholecystokinin (CCK) stimulates neurosecretory oxytocin (OT) and tuberoinfundibular corticotrophin releasing factor (CRF) cells of the hypothalamus. Previous work suggests that A2 noradrenergic neurons of the dorsal medulla contribute to the OT cell response, but the role of other medullary catecholamine cells remains unclear. Using c-*fos* expression as a marker for cellular activity, we have found that CCK (100 μg/kg, ip) activates overlapped noradrenergic and adrenergic cell populations in both the ventrolateral and dorsomedial medulla. In the ventrolateral medulla (VLM) there was a particularly prominent activation of C1 adrenergic neurons at the level of the obex. To directly test the contribution of VLM catecholamine cells to hypothalamic neuroendocrine cell responses to CCK, animals were prepared with unilateral VLM lesions corresponding to those areas that had displayed the most marked response to CCK. VLM lesioned animals treated with CCK displayed a significant although small reduction in paraventricular nucleus (PVN) OT cell c-*fos* expression ipsilateral to the lesion, but no change in the responses of supraoptic nucleus OT cells or in cells of the medial parvocellular PVN, many of which are CRF cells. These findings indicate that VLM catecholamine cells contribute little to hypothalamic neuroendocrine cell responses to CCK and thus serve to further highlight the role of dorsal medulla catecholamine cells. However, it is clear that in addition to A2 cells, CCK treatment also recruits C2 adrenergic cells of the dorsal medulla, many of which project to the PVN. (Supported by NHMRC and NHF.)

159.19

CHARACTERIZATION OF LEPTIN RECEPTOR. R.R.C. Huang, M. Tota, K. Mazina, C. Rosenblum, D. Cully, H. Chen, T. Smith, A. Vongs, S. Qureshi, F. Chen, R. Smith, L.H.T. Van der Ploeg, and T.M. Fong* Merck Research Laboratories, Rahway, NJ 07065

The recently identified OB receptor (OBR) belongs to the cytokine receptor family, and the structure of the OB protein (leptin) is believed to resemble that of helical cytokines. Since many cytokine receptors consist of two or three subunits, it is important to determine what constitutes a functional leptin receptor. Two strategies will be discussed. One is to co-express the OBR with one of several common cytokine receptor subunits and measure the binding affinity of leptin. The common cytokine receptor subunits tested were gp130, KH97 and IL2R-gamma. The present study indicated that these subunits, when expressed in COS cells individually with OBR, did not lead to an increased leptin binding affinity for the OBR. The second strategy is to identify cell lines expressing endogenous OBR or responding to leptin with exogenous OBR transfection. Endogenous OBR has been detected in hypothalamic cell lines by RT-PCR, binding and intracellular activity. The availability of cell lines, recombinant leptin and novel functional assays will facilitate the characterization of the leptin receptor.

159.20

THE INTERACTION OF TWO ORIXIGENIC PEPTIDES, NPY AND MCH (MELANIN CONCENTRATING HORMONE), AND LEPTIN IN DIFFERENT MODELS OF OBESITY. D. Qu, C. Mantzoros, R. Ahima, J. Elmquist, D. Vicent, D. Hoersch, J. S. Flier, C. Saper*, E. Maratos-Flier. Joslin Diabetes Center and Beth Israel Hospital, Boston, MA, 02215

The regulation of eating behavior results from a complex interaction of signals from the periphery and the central nervous system. NPY acts to stimulate eating behavior. We have found that MCH also stimulates eating behavior (Nature **380**:243-247). In view of the potential importance of the fat hormone leptin in regulating eating behavior, we studied the interaction of leptin and MCH and NPY in different models of rodent obesity. *ob/ob* mice are absolutely leptin deficient and have increased hypothalamic mRNA levels of both MCH and NPY (both by at least two-fold). Despite high levels of expression, mRNA levels are increased further after fasting (as seen in normal controls). Two other models of rodent obesity demonstrate very high circulating leptin levels (100 fold higher than control animals) and are therefore leptin "resistant." Surprisingly, in brown adipose tissue deficient mice, NPY mRNA is low (50% of control) and the response to fasting is blunted. MCH mRNA levels appear to be unchanged. In the agouti mouse NPY and MCH mRNA levels are unchanged compared to control mice; however, neither peptide appears to respond to fasting. These data indicate that most models of rodent obesity are leptin resistant. We also examine the effect of leptin on NPY and MCH mRNA expression in response to fasting. Leptin treatment blunts the increase in NPY seen with fasting, but has no effect on MCH expression. Eating behavior is regulated by multiple peptides, understanding the mechanism of leptin action and resistance requires defining the multiple pathways regulated by leptin and other possible signals from the periphery.

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American Diabetes Association

160.1

EDGE SELECTIVE CELLS CODE FIGURE-GROUND IN AREA V2 OF MONKEY VISUAL CORTEX. Hong Zhou*, Howard Friedman, Rüdiger von der Heydt. Krieger Mind/Brain Inst. and Depts. of Neurosci. and Biomed. Eng., Johns Hopkins Univ., Baltimore, MD.

Visual perception organizes 2-D images into figure and ground, assigning the borders to the figure. We have studied the neural basis of this phenomenon.

We recorded from orientation selective cells of V1 and V2 in the awake, fixating monkey. A square (typically 4°) of uniform color or gray was displayed in an uniform surround field (11°) of different color or gray. The square was much larger than the response fields of the cells studied. Its orientation and color were optimized for each cell. In interleaved tests, we centered two opposite edges of the square in the RF, and also reversed the colors of square and surround. Flipping edges and colors produced pairs of displays with an identical edge in the response field, but the figure on opposite sides.

In V2 we found cells that were highly discriminative for the edge assignment, e.g., responding only to the left edge of a gray square with white surround, but not to the right edge of a white square with gray surround. In some cells, this discrimination was nearly independent of the figure size. The response could either be independent of local edge polarity (general edge assignment), or conditional on figure color (joint assignment of edge and color). We have observed direction-of-figure preference also in V1, but with smaller discrimination ratios.

In conclusion, figural edge assignment is part of early cortical processing.

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160.2

MODULAR ORGANIZATION OF AXONAL PROJECTIONS FROM FUNCTIONALLY IDENTIFIED V2 COMPARTMENTS TO V4 IN MACAQUES. Y. Xiao and D.J. Felleman*. Dept. of Neurobiology and Anatomy, Univ. Texas Houston Medical School, Houston, TX 77225.

Area V4 receives a dense projection from V2 thin stripes and interstripes, but the degree to which their axonal terminations remain segregated from each other and contribute to the functional architecture of V4 remains unclear. In the current study, V2 functional compartments were first identified, *in vivo*, by optical recording of intrinsic cortical signals in response to different visual stimuli. Multiple distinguishable anterograde and retrograde tracers were then injected into specific V2 compartments. The patterns of axonal terminations were examined in horizontal and tangential sections. Axonal projections to V4 from V2 thin stripes and interstripes are characterized by both global segregation and locally, restricted integration. Terminals from V2 interstripe injections form multiple dense clusters (~500 μm) which aggregate to form a ~2.5x3 mm primary terminal field and provide weaker projections to smaller satellite foci. Terminals from thin stripe injections are distributed more diffusely to form ~2x2.5 mm primary terminal fields and smaller satellite foci. The thin stripe primary terminal field is separated from the interstripe primary terminal field. However, within each of these primary terminal fields, smaller satellite foci from the other V2 compartment are observed and thus provide a mechanism for functional integration between compartments. Optical recording in V4 reveals chromatically activated foci comparable in size to these thin stripe satellite foci. Finally, projections from the thin/interstripe border region is more diffusely distributed in V4 than those from either stripe compartment. Supported by NIH EY-08372, Texas ARP 011618-025, and by a grant-in-aid in honor of Bob Hope of the Fight For Sight research division of Prevent Blindness America.

160.3

RESPONSE PROPERTIES OF AXIS-ORIENTATION SELECTIVE NEURONS OF THE PARIETAL ASSOCIATION CORTEX OF THE MONKEY STUDIED BY 3D COMPUTER GRAPHICS. M. Kusunoki*, Y. Tanaka, E. Shikata, H. Nakamura[1], and H. Sakata. Nihon University, School of Medicine and PRESTO, JRDC[1], 30-1 Oyaguchi-kamicho, Itabashi, Tokyo 173, Japan.

Clinical studies of the patients with parietal cortex lesion showed deficit in the perception of line orientation as one of the symptoms of visuospatial disturbances. Recently we found a group of visual neurons in the monkey parietal cortex that were sensitive to the orientation of the axis of an elongated object in 3-dimentional space. Many of these neurons were binocular and their response to a bar of preferred orientation in binocular viewing condition was better than that in monocular viewing condition. However, in our previous study using real object as a stimulus, it was not clear what kinds of visual cues were effective to activate them. In the present experiment, we displayed visual stimuli on a stereoscopic display of 3D computer graphics and changed various parameters of the stimulus such as the length, thickness, distance and shape. Most of the axis-orientation selective (AOS) neurons preferred a longer and thinner stimulus to a shorter and thicker one. Thus the length response curve and thickness response curve were both monotonically decreasing function in most AOS neurons. However, we also found several AOS neurons which showed maximum response to the stimulus of intermediate thickness. Some of these neurons showed preference in the shape of the stimulus, preferring either cylinder or square column. Almost all the AOS neurons were strongly binocular and their response was much less to the monocular stimulus than to the binocular one. We also examined the orientation-selectivity of AOS neurons in both eyes to see the difference in preferred orientations between two eyes (orientation disparity) which is one of the important cues for the tilt in depth. (Supported by Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture No.05267105, No.07244103)

160.4

CONNECTIONS OF VISUAL AREA VIP WITH SOMATOSENSORY AND MOTOR AREAS OF THE MACAQUE MONKEY. J. W. Lewis*, D. C. Van Essen. Dept. Anatomy & Neurobiology, Washington University School of Medicine, St. Louis, MO 63110.

The intraparietal sulcus (IPS) of the macaque monkey contains multiple areas involved in motor control and in visual, somatosensory, or multimodal sensory processing. In this study, we examine the anatomical connections of area VIP (ventral intraparietal) with somatosensory, motor and premotor cortical regions, in an extension of our previous report on the connections of area VIP with other visual areas (Soc. Neurosci. Abstr. 20:324.11., 1994).

We pressure-injected retrograde tracers into the IPS using multibarreled glass electrodes that permitted simultaneous electrophysiological recording. Restricted injections of three different tracers were placed into a combination of cortical sites including area VIP. In several cases, we physiologically mapped the face and/or digit representations of areas 1 and 2. Areal borders were determined using a combination of Nissl, myelin, SMI-32 antibody and CAT-301 antibody stains. Labeled cells, section contours and architectonic boundaries were digitized and computationally reconstructed in 3D. These data were also computationally flattened to assist with quantitative analyses and to enhance the visualization of labeled field patterns across large expanses of cortex.

Area VIP received projections from the hand and/or face representations of several topographically organized somatosensory and motor areas. The strongest connections were from subdivisions of area 6 (mostly along the posterior bank of the arcuate sulcus), and moderate to light label from SMA, 7b, area 2, and primary motor cortex (area 4). We found consistently strong projections from STP, moderate to light projections from a ventral subdivision of area 5, S2 and surrounding cortex, AIP, area 23 and area 24. Projections from MDP (7m) were very strong in some cases, but weak in others. These results support the notion that area VIP contributes to the sensorimotor integration of eye-hand and mouth coordination. NIH EY02091.

160.5

A COMPARISON OF BIMODAL, VISUAL-TACTILE NEURONS IN PARIETAL AREA 7B AND VENTRAL PREMOTOR CORTEX OF THE MONKEY BRAIN. Michael S. A. Graziano*, Charles G. Gross and Tom Fernandez. Department of Psychology, Princeton University, Princeton, NJ 08544.

Most neurons in the ventral premotor cortex (PMv) of the monkey brain respond to tactile stimuli and about 30% also respond to visual stimuli. These bimodal, visual-tactile cells have tactile receptive fields (RFs) on the face or limbs, and corresponding visual RFs, which extend outward from the tactile RFs into the space surrounding the body. For most cells with a tactile RF on the arm, the visual RF is anchored to the arm, moving as the arm is moved. These neurons appear to code the locations of visual stimuli with respect to the arm, in "arm centered" coordinates. (Graziano, Yap and Gross, *Science*, vol. 266, pp. 1054-1057, 1994).

PMv receives a strong projection from parietal area 7b, which also contains bimodal, visual-tactile neurons. We studied 349 neurons in area 7b of anesthetized monkeys and found that 33% were bimodal. Fifty-five bimodal neurons that had a tactile response on the arm were tested by placing the arm in different positions. In all cases, the visual RF did not move when the arm moved. Instead, the visual RF remained at the same location in space, independent of the position of the arm. In addition, for 23 neurons tested, the spontaneous activity and the magnitude of the visual response were unaffected by arm position. We could find no evidence that the static position of the arm had any affect on bimodal neurons in 7b. We conclude that area 7b may be an important source of visual and tactile information to PMv, but that the arm-position signal in PMv must come from a different source.

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160.6

OVERLAPPING AND NON-OVERLAPPING PREFRONTAL, POSTERIOR PARIETAL, AND SUPERIOR TEMPORAL PROJECTIONS TO CINGULATE AND PARAHIPPOCAMPAL CORTEX IN THE MONKEY. J. Padberg*, C.G. Cusick, and B. Seltzer. Depts. of Psychiatry & Neurology and Anatomy, Tulane Univ. School of Medicine; V.A. Medical Center, New Orleans, LA 70112.

Networks of cortical areas, including dorsal prearcuate cortex, the caudal inferior parietal lobule (IPL), and the superior temporal gyrus (STG), have been implicated in attentional mechanisms. Other potential components of these networks include: superior temporal polysensory (STP) cortex, the cingulate gyrus, and parahippocampal gyrus (PHG). We have previously shown that cortical areas that are themselves interconnected send overlapping connections to the STP region whereas non-interconnected areas send spatially segregated projections to the target zone (Seltzer et al., 1996). To examine potential overlap in two other component areas of these networks, in three cases we combined in the same hemisphere a radioisotope injection into dorsal prearcuate cortex with WGA-HRP injections into the caudal IPL or STG, and then examined alternate or identical coronal sections for labelled terminals within the cingulate and PHG. Although many afferents from the two different sources were segregated, there were also columns within caudal area 23 where there was overlap between frontal and parietal and frontal and temporal fibers. Overlap was also found between frontal and parietal afferents within the presubiculum and areas TF and TL of the PHG. Supported by NIH grant EY08906, New Orleans VAMC, and Tulane University.

160.7

PATTERN OF THALAMIC STAINING WITH THE LECTIN WISTERIA FLORIBUNDA AGGLUTININ IN NON-HUMAN PRIMATES. C.G. Cusick,* D. Gray, and T.M. Preuss. Dept. of Anat. and Neurosci. Training Program, Tulane Medical School, New Orleans, LA 70112 and Div. of Behav. Biology, Univ. of Southwestern Louisiana-New Iberia Research Center, New Iberia, LA 70560.

Wisteria floribunda agglutinin (WFA), like the monoclonal antibody Cat-301, binds to GalNac-containing proteoglycans concentrated in pericellular glial nets and neuropil. Using biotinylated WFA, we examined the dorsal thalamus of 3 macaques and 2 squirrel monkeys, and found a regionally specific pattern of staining similar to that reported for Cat-301 (Hendry et al., 1988, J. Neurosci. 8:518). In the anterior nuclei, WFA staining was very weak in AM and AV, while AD was more prominently stained. In MD, the parvocellular subnucleus (MDpc) was lightly stained, but laterally, patches of stained neuropil and large cells marked the multiform division (MDmf). The intralaminar paracentral (Pc) and central lateral (CL) nuclei were well stained, but the parafascicular (Pf) and centromedian (CM) nuclei were very light. In the motor thalamus, VA and dorsal VL were light, but the remainder of VL was well stained. In somatosensory nuclei, strong WFA staining overlapped cytochrome oxidase (CO) rich compartments in VPL and VPM and was found throughout VPS; the CO-poor zones in VPL and VPM were lightly stained, as were the VPI and anterior pulvinar (Pa) nuclei. In the LGN, the magnocellular layers were more darkly stained than parvocellular layers. Isolated patches of label were found in the lateral posterior nucleus (LP). The medial pulvinar was lightly stained, and label occupied specific subdivisions of the inferior pulvinar, as described in a separate abstract. Supported by NIH grant EY08906 and by USL-NIRC.

160.8

INFERIOR PULVINAR SUBDIVISIONS IN SQUIRREL MONKEYS AND MACAQUES REVEALED BY AChE HISTOCHEMISTRY, CALBINDIN-D_{28k} CAT-301 IMMUNOSTAINING, AND *WISTERIA FLORIBUNDA* AGGLUTININ BINDING. D. Gray,* C. Gutierrez, and C.G. Cusick. Dept. of Anat. and Neurosci. Training Prog., Tulane Univ., New Orleans, LA 70112.

The inferior pulvinar (PI) of macaque monkeys, defined as classical PI and ventral PL, contains multiple chemoarchitectonic subdivisions, medial (PI_M), central (PI_C), lateral (PI_L), lateral-shell region ($PI_{L\text{-}S}$) and posterior (PIp; Gutierrez et al., 1995, JCN 363:545). These subdivisions in macaques differed from those described in squirrel monkeys, in which the PI_C division was relatively broader, and a lateral-shell zone was not described (Cusick et al., 1993, JCN 336:1). The present study reassessed the evidence for common patterns of PI subdivisions in monkeys. Similar results were obtained in squirrel and macaque monkeys using a variety of neurochemical markers. Acetylcholinesterase (AChE) staining was intense and patchy in PI_M; intense in PI_L; moderate in $PI_{L\text{-}S}$; and light in PI_C and PIp. Calbindin-D_{28k} immunostaining was poor in PI_M; intense in PI_C and PIp; and moderate in $PI_{L/L\text{-}S}$, which was characterized by a dispersed population of intensely stained cells. *Wisteria floribunda* agglutinin (WFA) binding, studied with biotinylated WFA, showed perineuronal nets and neuropil staining localized within PI_M and throughout $P_{IL/L\text{-}S}$, but little staining in PIp and PI_C. In macaque monkeys, perineuronal nets immunostained for Cat-301 were also found around clusters of neurons in PI_M, supporting the interpretation that PI_M shares neurochemical features with magnocellular layers of the LGN. Scattered Cat-301 positive neurons were also found in $PI_{L/L\text{-}S}$ of macaques. The PI_C subdivision in both species was narrow compared to PI_L, and the lateral shell in squirrel monkeys appears to correspond to the previous PI_L zone. The findings suggest similar PI subdivisions exist in New and Old World monkeys and thus probably in all primates. Supported by NIH grant EY08906.

160.9

POSTERIOR PARIETAL, SUPERIOR TEMPORAL, AND PREFRONTAL PROJECTIONS TO HISTOCHEMICALLY DEFINED COMPARTMENTS OF THE DORSAL PULVINAR IN MACAQUE MONKEYS. C. Gutierrez,* D. Gray, B. Seltzer, and C.G. Cusick. Dept. of Anat. and Neurosci. Training Prog., Tulane Univ., New Orleans, LA 70112.

The posterodorsal thalamus of macaques has traditionally included the medial and lateral pulvinar (PM and PL) and the lateral posterior nucleus (LP). PM has widespread connections with diverse functional regions of the cerebral cortex. To better understand the connectional organization of the dorsal pulvinar complex, we placed separate injections of WGA-HRP and radiolabeled amino acids in 2 different cortical sites in the same hemisphere. The distribution of label was then compared with the neurochemical architecture of the dorsal pulvinar revealed by a variety of markers (parvalbumin, calbindin, AChE, Cat-301 and binding of the lectin *Wisteria floribunda* agglutinin (WFA)). A distinct parvalbumin- and AChE-dense, calbindin-poor wedge crossed the traditional dorsal PL and PM rostrally and was continuous with LP. This zone contained numerous neuronal profiles stained for Cat-301 and WFA. PM was generally light to moderate for AChE and parvalbumin, but contained an oval region ventromedially (PMvm) that stained more intensely than the surrounding region. Patchy neurochemical staining was also seen dorsal and lateral to PMvm. Injections in the inferior parietal lobule (IPL), superior temporal gyrus (STG), and prefrontal cortex labeled LP as well as the dorsal pulvinar between PMvm and LP. The oval PMvm was only labeled by STG injections; IPL and STG projections were found surrounding PMvm. Overlapping connections were found following IPL or STG and prefrontal injections in the same hemisphere but not after IPL and STG injections. The results suggest the neurochemical architecture of the dorsal pulvinar correlates with its connectional heterogeneity. Supported by NIH grant EY08906.

160.10

MULTI-LEVEL SYNCHRONIZATION OF FAST (20-60 Hz) OSCILLATIONS IN A PROTOTYPICAL MODEL OF THE THALAMOCORTICAL SYSTEM. E. D. Lumer*, G.M. Edelman, and G. Tononi. The Neurosciences Institute, 10640 John Jay Hopkins Drive, La Jolla, CA 92121

Neural activity in the mammalian thalamocortical system is often characterized by the presence of fast synchronized rhythms involving neurons distributed over multiple cortical and thalamic sites. What role do network mechanisms play in the generation of these widespread coherent oscillations? We investigated this question by using a large-scale computer model based on the anatomy and physiology of the cat thalamocortical visual system. The model consisted of 65,000 spiking neurons organized topographically to represent multi-layered sectors of a primary and secondary area of visual cortex, as well as two associated regions of the dorsal thalamus and of the reticular thalamic (RT) nucleus. A dense plexus of reciprocal intra- and interlaminar, interareal, corticothalamic, and thalamoreticular connections was established. Simulations of the neural responses to visual input revealed sporadic epochs of fast rhythmic activity involving all levels of the model, similar to the fast rhythms recorded *in vivo*. Selective manipulations of structural and physiological parameters demonstrated that, in addition to the known oscillatory influences imposed by mutual inhibition in local circuits, high frequency oscillations could arise in population-averaged activities as an emergent property of the dynamics in polysynaptic loops. An interlaminar cortical loop provided a high-gain amplification mechanism that drove local cortical networks into an oscillatory regime, even in the absence of thalamic rhythmicity. In addition, a corticothalamic loop involving the RT complex induced synchronous oscillations at thalamic and cortical levels. Horizontal networks within each cortical layer, as well as forward and backward interareal projections, contributed to the coherence of these oscillations between different parts of the cortex. These results suggest that specific polysynaptic loops have distinct roles in organizing distributed, multi-level, activity patterns in thalamocortical systems. (*Supported by Neurosciences Research Foundation*)

160.11

DISTINCT REACTIVITY OF CORTICAL EVOKED RESPONSES AND RHYTHMS TO LUMINANCE AND PATTERN STIMULI

K. Portin*, S. Salenius, R. Salmelin and R. Hari, Brain Research Unit, Low Temperature Lab., Helsinki Univ. Techn., FIN-02150 Espoo, Finland

We recorded neuromagnetic signals from 10 subjects with a Neuromag-122™ whole-scalp neuromagnetometer to visual luminance and pattern stimuli, which were semicircles (Ø 16-17 deg), either white on a black background (luminance) or black-and-white 50'x50' checks on an equiluminant gray background (pattern). The stimuli were shown for 2 s once every 5 s, alternately to the right and left visual hemifield. Both stimuli evoked clear responses over the occipital lobes at 70-170 ms; luminance stimuli elicited additional strong signals over the parieto-occipital region at 180-350 ms. Cortical activation was modelled with a time-varying 3-dipole model, with 2 dipoles bilaterally in the occipital visual cortex and 1 in the medial parieto-occipital cortex. The occipital activation was contralaterally dominant both for pattern (contra/ipsi ratio 18) and luminance (ratio 2) stimuli. Parietal activation was 66% stronger for luminance than pattern stimuli. The posterior 10-Hz rhythm was suppressed more strongly by luminance than pattern stimuli, particularly in the parietal cortex. The contralateral dominance of the occipital suppression was stronger for pattern than luminance stimuli. Thus, the occipital activation is more lateralized for pattern than luminance stimuli, and the parietal cortex is predominantly responsive to luminance stimuli.
This study was financially supported by The Academy of Finland, the Magnus Ehrnrooth foundation and the Sigrid Jusélius foundation.

160.12

HUMAN PATTERN ELECTRORETINOGRAMS AFTER COMPLETE CEREBRAL HEMISPHERECTOMY. P. Azzopardi, S.M. King & A. Cowey (SPON: European Brain and Behaviour Society), Dept. of Exptl. Psychology, University of Oxford, Oxford OX1 3UD, UK.

Subjects with damaged striate cortex can nevertheless detect and discriminate stimuli presented in their scotomas. Recently we showed that, unlike such subjects, hemidecorticate subjects have no detectable blindsight in their scotomas (King et al. 1996 *Vis. Neurosci.* **13**, 1-13). Given that lesions restricted to striate cortex cause degeneration of 80% of Pβ ganglion cells, and that degeneration following complete hemidecortication might be even greater, we assessed ganglion cell function in the retinae of a hemidecorticate subject by means of pattern electroretinography.

The subject, a 24 year old male, had had a complete right cerebral hemispherectomy 11 years previously. His visual acuity was 20/17. The stimuli, presented in 12°x10° fields at 6°-18° horizontal eccentricity, were 95% contrast vertical sinusoidal luminance gratings (mean 103 cd/m^2) of 2.0 cpd and 12.0 cpd, and photometrically isoluminant chromatic (RG) gratings (mean 20.25 cd/m^2) of 2.0 cpd, modulated temporally at 7.95 Hz. ERG signals were recorded using gold foil electrodes, amplified 20,000x. A 3-way ANOVA revealed significant differences in the amplitude of the second harmonic of the averaged signal with respect to eyes ($p=0.012$), intact and blind fields ($p=0.048$) and stimuli ($p=0.031$), but no significant interactions. Most importantly, the results showed that the pattern-evoked signals in the blind hemiretinae, though of slightly lower amplitude than the sighted hemiretinae, were otherwise indistinguishable from normal.

Our results suggest that the absence of residual vision in the scotomata of hemidecorticate subjects is not explicable in terms of degeneration of retinal ganglion cells. This lends support to our proposal that intact extrastriate cortex is required for mediating voluntary responses to visual stimuli in the scotoma.
Supported by grants from the MRC and The Royal Society.

160.13

HERE'S NOT LOOKING AT YOU KID: AN ELECTROPHYSIOLOGICAL STUDY OF A REGION OF HUMAN EXTRASTRIATE CORTEX SENSITIVE TO HEAD AND EYE AVERSION. T. Allison*, D. Lieberman, and G. McCarthy. Neuropsychology Lab., VA Medical Center, West Haven CT 06516, and Dept. of Neurology and Section of Neurosurgery, Yale University School of Medicine, New Haven CT 06510.

In intracranial recordings faces evoke a field potential at about 200 msec (N200) in small regions of the fusiform and inferior temporal gyri (Allison et al J Neurophysiol 1994); eyes alone evoke a smaller potential. However, in scalp recordings in normal subjects a similar potential (NI70), best recorded from left (T5) and right (T6) temporal scalp, is larger to eyes alone than to a full face, suggesting the operation of an "eye detector" (Bentin et al J Cog Neurosci in press). Here we tested the responsiveness of the putative eye detector while normal subjects viewed faces and eyes in varying orientations. The assumption that N170 would be largest when the eyes are looking at the viewer was incorrect for all manipulations of eye and head position. At T5 N170 was largest when the head was directed away from the viewer regardless of eye direction. At T6 N170 was largest when the eyes were directed away from the viewer regardless of head direction. These results suggest that regions of cortex, perhaps located in the occipitotemporal sulcus, are sensitive to head and eye aversion, similar to cells in monkey superior temporal sulcus that respond best to head and eye aversion (Hasselmo et al Exp Brain Res 1989; Perrett et al Int J Comp Psych 1990). Such cells may allow analysis of social interactions, specifically of the direction of attention and of threat and submission.

160.14

ORIENTATION-SPECIFIC TRAINING EFFECT IN VISUAL ORIENTATION DISCRIMINATION, A PET STUDY. C. Schiltz, J.M. Bodart, S. Dubois, S. Dejardin, C. Michel[1], G.A. Orban[2], M. Crommelinck and A. Roucoux*. Lab. of Neurophysiology, U.C.L., Brussels; [1]PET Unit, U.C.L., Louvain-la-Neuve; [2]Lab. of Neuro- en Psychofysiologie, KULeuven, Leuven, Belgium.

This study aims to assess changes in cerebral activity induced by training in visual orientation discrimination. In a previous experiment (Schiltz et al., 1995), we observed changes of activity in striate and extrastriate regions. The present study was designed to test which changes were specifically related to the orientation trained. Three tasks were tested: two orientation discriminations (using two different references) and a baseline task. During the orientation discrimination, subjects had to identify the orientation presented by pressing a button. During the baseline condition, subjects had to respond to the presentation of an uniforme noise stimulus by alternative left/right button press. Six subjects were trained (10.000 trials) for the discrimination task at one reference only. rCBF was measured before and after training using ^{15}O-water PETscan (ECAT 961 HR). Statistical analysis on the rCBF brain images was performed by Statistical Parametric Mapping (Friston et al., 1995). Regions activated during orientation discrimination were located in left and right inferior occipital/fusiform gyrus (BA 18), in right posterior fusiform gyrus (BA 19), in left hippocampal gyrus (BA 27), in cerebellum (vermis and left nuclei) and in superior parietal lobule (BA 7). Orientation-specific training decreased activation of the right extrastriate regions (right posterior fusiform gyrus (BA 19)) and cerebellar vermis. A tendancy towards decrease existed in the right inferior occipital/fusiform gyrus (BA 18). Training decreased the deactivations situated on the left middle temporal/fusiform gyrus (BA 20, 37) and on left superior frontal gyrus (BA 8).
Supported by FMRE, ARC n°95/00-189 and PAI n°22

160.15

HIGH SPATIAL RESOLUTION FUNCTIONAL MAGNETIC RESONANCE IMAGING OF THE HUMAN VISUAL SYSTEM J.T. Voyvodic*, G.X. Shen, S.Y. Chang and K.R. Thulborn, Magnetic Resonance Research Center, University of Pittsburgh Medical Center, Pittsburgh, PA 15213

Neuroimaging studies of the functional organization of specific brain regions requires optimization of the spatial resolution of the imaging technique. Although functional magnetic resonance imaging (fMRI) has the best spatial resolution of the human neuroimaging techniques, current approaches do not have adequate spatial resolution for detailed functional mapping of specific brain regions. We have developed methods for achieving higher resolution fMRI images of human brain activity using a combination of strategies, including: (1) optimized signal sensitivity by using a custom-designed 3 Tesla (GE Signa) scanner, (2) a custom-built 7" proton surface coil, (3) optimized pulse sequences sensitize to the microvasculature rather than the venous drainage, (4) a control system for accurate stimulus presentation and temporal synchronization of paradigm and image acquisition and (5) optimized statistical processing. The hardware combination provides improved signal stability (<1% over 30 minutes) and a 2-fold increase in signal to noise ratio (SNR) compared to a standard 1.5 T system. The increased SNR allows voxel size to be decreased by a factor of greater than 2 within reasonable acquisition times (15 minutes) with adequate signal sensitivity for near real-time generation of high resolution functional activation maps.
This strategy has been used in the human visual system to explore aspects of within-region functional organization with enhanced spatial resolution.
Support: Radiology and Psychiatry Depts., Univ. Pittsburgh Med. Ctr.

160.16

ARE THERE SPECIFIC ANATOMICAL CORRELATES OF "BIOLOGICAL MOTION" PERCEPTION IN THE HUMAN VISUAL SYSTEM? C.A.Racine[1], L.M. Vaina[1,2], J.M. Diaz[1], A. Zamani[2]& C.G. Gross[3]*, Dept. of Biomedical Eng. & Neurology, Boston University, Boston, MA[1]; Brigham & Women's Hospital, Boston, MA[2]; and Department of Psychology, Princeton University, N.J.[3]

"Biological motion" was defined by Johansson (1973) as the pattern of movement of a small number of lights attached to the major joints of a human performing simple actions. Normal observers watching such displays immediately recognize a person and their actions. In the present study we investigated the effect of focal brain lesions on the ability to recognize biological motion displays of varying complexity and how this ability relates to both the performance on psychophysical tasks which address different stages of motion analysis and to tasks of object and action recognition. We report results from 19 patients, 15 with unilateral brain lesions and 4 patients with bilateral lesions. The criterion for inclusion in the study was impaired performance on biological motion tasks. According to the anatomical locus of their lesion and the pattern of their spared and impaired visual abilities these patients were categorized into three groups: (1) patients with anterior temporal lobe lesions, similar to the cases reported by Vaina & LeMay (1992) and Vaina & Gross (1996), with normal performance on low level motion tasks but severe impairment on the recognition of objects and actions under conditions of limited information and inability to establish motion correspondence in texture-defined stimuli; (2) patients with occipital-parietal lesions whose visual deficits were consistent with "simultagnosia"; (3) patients with lesions involving the higher stages of the dorsal or ventral system, with intact early motion mechanisms but with deficits on tasks of motion integration or object recognition. We relate our results to the electrophysiological findings that neurons in the rostral part of the superior temporal lobe (area STP) respond selectively to biological motion and to the idea proposed by Boussaud et. al (1990) and Gross (1995), that STP integrates the late stages of the dorsal and ventral cortical visual streams. *Supported by NIH grant EY-2RO-1-07861-06*

160.17

HUMAN CORTICAL REGION WHICH RESPONDS TO THE COLOR CHANGE. Y. Kaneoke*, S. Koyama, R. Kakigi. Dept. of Integrative Physiology, National Institute for Physiological Sciences, Myodaiji, Okazaki 444, Japan.

To investigate the precise region of human color center, we have developed the visual stimulus method by which one can observe the electrical response from the human cortical region which responds to the abrupt color change without the obvious response from the primary visual cortex. We studied the location of the human cortex which responds to this stimulus using 37 channels' BTi Neuromagnetometer. The subjects gazed the small light (0.2x0.2°) at the center of their visual field. The light was induced into the shielded room by fibroptics from the two-color light emitting diode. The light changed its color from red to green or vise versa at 2 to 3 seconds' random interval. The subjective brightness of the two colors was adjusted to be identical by the flicker method. The responses from 200-225 repetitions of each color change were averaged to obtain the evoked magnetic fields. The latency of the first deflection and its dipole location was investigated with several normal subjects and compared with the results of motion visual stimulus. The onset latency of the first deflection evoked by the color change was about 130 ms and the peak latency was about 190 ms for the subject YK. Its electrical source was well estimated by the single dipole model (Correlation between the measured and estimated values >0.97) and determined to be in the temporo-occipital cortex. On the other hand, the onset and peak latency of the first deflection evoked by the motion stimulus for the same subject were about 110 ms and 155 ms, respectively. They were significantly faster than those for the wave form evoked by the color change. The electrical source was estimated to be different from that for the color change. The results indicate our method enables the precise localization of the individual color center and add the evidence of the functional segregation of the human visual system.

160.18

BILATERAL FOCAL TRANSCRANIAL MAGNETIC STIMULATION (TMS) OF EXTRASTRIATE CORTEX DEGRADES THE EXTRACTION OF FORM FROM NOISE. S. Anand*, J. D. Olson, J. Hotson. Stanford Univ. Sch. of Med., Stanford, CA 94305; Calif. Inst. for Med. Res., San Jose, CA 95128.

Unilateral TMS of a large area encompassing human temporo-parieto-occipital cortex (TPO) degrades the perception of motion-defined form (MDF) in a time window that begins approximately 40 ms later than the effect of TMS of striate cortex (ARVO, 1995). We asked whether bilateral TMS of a discrete TPO region could selectively degrade the perception of MDF. Three subjects discriminated the orientation of a MDF and isoluminant color-defined form (CDF), both consisting of a random dot block letter C presented for 66 ms. The MDF was presented within dynamic noise and the CDF within static noise. Subjects correctly discriminated between four possible orientations of the MDF and CDF in 80-95% of control trials. Control trials were randomly intermixed with TMS trials. TPO was mapped in multiple locations in both hemispheres using 7x14 cm butterfly coils. In each subject, a TPO location was identified where bilateral focal TMS delivered 140 or 160 ms from the onset of the MDF degraded performance to 30% correct discriminations. TMS delivered 60-120 ms or 180-240 ms following stimulus onset had little effect on performance. TMS at the same TPO location degraded the perception of CDF in a similar time window. One of the subjects was also tested with a task in which he discriminated whether a uniformly colored square presented for 66 ms was reddish or greenish. The color difference between the reddish and greenish isoluminant stimuli was adjusted such that correct discriminations were made in 90% of control trials. Bilateral focal TMS delivered 120-180 ms from the onset of this color stimulus did not degrade performance. In sum, bilateral focal TMS of an extrastriate visual area degrades the extraction of form from noise in a discrete time window in which color discrimination may be spared.
Supported by NIH EY03387.

160.19

POSTERIOR CORTICAL AREAS ACTIVATED DURING VISUALLY-GUIDED GO/NO-GO TASK: A PET STUDY. A. Mikami[1*], I. Ando[2], K. Kubota[1], T. Sawaguchi[1], E. Yoshikawa[2], T. Kakiuchi[2], K. Nakamura[1] and H. Tsukada[2]. [1]Dept. of Behavioral and Brain Sciences, Primate Res. Inst., Kyoto Univ., Inuyama, 484 JAPAN, [2]Central Research Lab., Hamamatsu Photonics K.K., Hamakita, 434 Japan

To determine the temporal and occipital areas working when the animal is choosing one of two behavioral conditions (GO or NO-GO), the regional cerebral blood flow (rCBF) was measured with ^{15}O-labeled H_2O and positron emission tomography (PET) in two rhesus monkeys. In the GO/NO-GO task, with pressing a hold lever, a white square was presented for 1-3 s as a warning signal. Then a yellow or a blue square was presented surrounding the white square for 0.5 s as a cue to choose behavior. After the end of this period, the color of the central square was changed to red. When the color of the cue was blue, the monkey was required to release the lever within 0.8 s (GO trial). When the color of the cue was yellow, the monkey was required to hold the lever for 1.8 s (NO-GO trial). The monkey, with their upper head being surrounded by the gantry, looked down the 21 in. monitor display 69.6 cm away. The rCBF measurement was conducted for 120 s at 3 different task conditions; GO/NO-GO, GO only or NO-GO only. We found activation in the hippocampus, the superior temporal sulcus, the ventral V4 and the posterior cingulate during choice behavior (GO/NO-GO) compared with the non-choice condition (GO only or NO-GO only). Thus, these areas are involved to use the visual cues to select behavioral conditions.

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160.20

DIAZEPAM DECREASES PERFORMANCE IN A VISUAL LONG-TERM MEMORY TASK: A PET STUDY IN HUMANS. A. Rosier*[1,2], L.Cornette[1,2], P.Dupont[2], R. Vandenberghe[1,2], L. Mortelmans[2] and G.A. Orban[1]. [1]Lab. Neuro- en Psychofysiologie, Medical School, KULeuven, GHB. [2]PET Centre, Dept. of Nuclear Medicine, UZ, GHB, B-3000 Leuven, Belgium.

In this PET-rCBF ($H_2{}^{15}O$) study, we compared activation patterns during delayed recognition of abstract visual shapes (Fourier) following a diazepam- or placebo-challenged acquisition. Twelve right-handed male subjects received placebo (n=6) or 15 mg diazepam (n=6) 1 hr before the acquisition of a list of visual shapes. In the PET-study for delayed recognition, performed 3 days later in diazepam-free conditions, the following tasks were scanned twice: (1) delayed recognition, (2) delayed recognition with a shortened stimulus presentation, and (3) fixation as control task. A diazepam-challenged acquisition resulted in a mean delayed recognition performance of 63%, compared to 84% as control value. Performance in the 'shortened' delayed recognition was 66% in placebo and 59% in the diazepam group. In both control and diazepam group, a significant increase in rCBF during delayed recognition as compared to fixation was observed bilaterally in fusiform gyrus, lateral occipital region, superior parietal cortex, precentral gyrus, and right cingulate and lateral cerebellum. When comparing the control with the diazepam group for [delayed recognition minus fixation], the left fusiform gyrus displayed a significant higher activation in controls, suggesting a role in recognition. The same fusiform region was significantly less activated in the recognition with shortened presentation than in the standard recognition, both in the placebo and diazepam group. This suggests that the left fusiform gyrus plays a role in the matching of incoming and stored visual information during recognition. (Supported by the IUAP grant nr. 22, and the NFWO, Belgium)

AUDITORY SYSTEMS: CENTRAL PHYSIOLOGY—BIRDS AND BATS

161.1

VOLTAGE-GATED OUTWARD CURRENTS IN THE CHICK NUCLEUS MAGNOCELLULARIS (NM) M.M. Rathouz* & L.O. Trussell. Dept. of Neurophysiology, U. Wisconsin, Madison, 53706.

A combination of strong voltage-gated outward currents and fast-kinetic transmitter-gated channels allows neurons of the NM to phase-lock to high frequency acoustic stimuli. Previous studies (Reyes et al., 1994) showed that two K currents contribute to the firing properties in NM: a low threshold 4-AP-sensitive current, and a high threshold TEA-sensitive current. We have further explored the types of K currents in NM neurons by voltage clamping E17-19 cells in brain slices with the whole-cell patch technique. Currents were isolated by subtraction of records +/- hyperpolarizing pre-pulses or pharmacological blockers. In addition to an inward rectifying, "H"-like current, four different outward currents were apparent. Two were enhanced by strong (-100mV) prepulses and were resistant to 0.2-1 mM 4-AP. Of these, one had slow activation and deactivation kinetics and was sensitive to 1 mM TEA. Two additional currents were less sensitive to prepulse, had rapid (0.5-2 ms) deactivation kinetics and were blocked by 4-AP. Due to the large size of these currents (>20 nA), we explored ways to improve the voltage clamp. Reduction of internal [K] to 1/5 normal reduced peak outward currents by half and revealed stronger inactivation than was apparent in control [K]i. Replacement of all [K]i with Cs blocked the pre-pulse sensitive currents, leaving the 4-AP sensitive currents which were carried by Cs ions. Under these conditions, we have characterized the kinetics of activation and deactivation, and the relative permeabilities of these two currents. The mixture of inactivating and non-inactivating currents observed in NM may shape neuronal firing properties at different stimulus rates. Supported by NIH DC02004.

161.2

ROLE OF PROTEIN KINASES A AND C IN ACTIVITY-DEPENDENT REGULATION OF $[Ca^{2+}]_i$ IN AVIAN COCHLEAR NUCLEUS NEURONS. *L. Zirpel* and E. W Rubel*, Virginia Merrill Bloedel Hearing Research Center, University of Washington School of Medicine, Seattle, WA 98195.

Neurons of the cochlear nucleus, nucleus magnocellularis (NM), of young chicks require excitatory afferent input from the eighth nerve for maintenance and survival. One of the earliest changes seen in NM neurons following deafferentation is an increase in intracellular calcium concentration ($[Ca^{2+}]_i$). This increase in $[Ca^{2+}]_i$ is due to loss of activation of one or more subtypes of metabotropic glutamate receptors (mGluR) that activate second messenger cascades involved in $[Ca^{2+}]_i$ regulation. It is prevented by orthodromic activation of NM neurons, or bath application of 1 mM ACPD, but not by antidromic stimulation. The goal of this study was to determine the direct roles of PKA and PKC activities in the regulation of NM neuron $[Ca^{2+}]_i$ by eighth nerve stimulation.

Brain stem slices from E17 to E19 chicks were loaded with fura-2/AM. NM field potentials were monitored in all experiments in which the eighth nerve was stimulated to achieve orthodromic activation of NM. $[Ca^{2+}]_i$ of individual NM neurons was monitored using ratiometric fluorescence imaging. Five Hz orthodromic stimulation maintained NM neuron $[Ca^{2+}]_i$ at approximately 110 nM for 180 minutes. In the absence of stimulation, NM neuron $[Ca^{2+}]_i$ increased steadily to 265 nM by 120 minutes. This increase was prevented by superfusion of either phorbol-12,13-myristate acetate (PMA; 100 nM), an activator of PKC, or by 1 mM 8-Br-cAMP, an activator of PKA. Inhibition of PKA (100 µM Rp-cAMPS) or PKC (50 nM bisindolylmaleimide) during continuous orthodromic stimulation resulted in an increase in NM neuron $[Ca^{2+}]_i$ that reached 202 nM and 170 nM, respectively, by 120 minutes. These results suggest that eighth nerve activity maintains $[Ca^{2+}]_i$ of NM neurons at physiologic levels via mGluR-medited activation of PKA and PKC. (Supported by NIH grant DC00520.)

161.3

IONIC CURRENTS IN NUCLEUS LAMINARIS NEURONS OF THE CHICK. Larry Proctor* & Gilles. Laurent. Division of Biology, 139-74, California Institute of Technology, Pasadena, CA 91125.

In avian species, nucleus laminaris (NL) is the first site of binaural convergence in the time pathway of the auditory system and where interaural phase-difference tuning arises. When phase-locked inputs from each nucleus magnocellularis (NM) coincide at a particular neuron in NL, that neuron is driven maximally. Should the inputs from each NM arrive 180° out-of-phase, the activity of the NL neuron is less than when it is stimulated from one side alone. We have investigated the role of NL neurons' intrinsic membrane currents to assess their role in the formation of phase-difference tuning.

Neurons from NL were obtained in 250 µm thick coronal brainstem slices from E16 to E20 day chicks and voltage clamped on their soma with whole-cell patch-clamp electrodes. Neurons were recorded in a submersion chamber at 32-34° C. K^+ currents were isolated by adding 1 µM TTX and 1 µM Ω-conotoxin GVIA to the perfusion medium. Under these conditions, the outward currents were very large (60-80 nA at +10 mV) and consisted of a small rapidly inactivating component and a slowly inactivating component. The K^+ current activated at -60 to -50 mV, i.e. in a range of potentials very close to the resting potential of NL neurons. Na^+ currents were isolated by applying 20 mM TEA and 1 µM Ω-conotoxin GVIA to the perfusion medium and 10 mM TEA and 10 mM CsCl to the recording pipette. Inward currents under these conditions consisted of one component with a peak of ~20 nA at -15 mV. Ca^{++} currents were isolated using the same solutions as for isolation of Na^+ currents but substituting 1 µM TTX for the Ω-conotoxin. The Ca^{++} current appeared to consist of at least two components; a rapidly activating and inactivating component and a slowly activating component. The maximum Ca^{++} current was approximately 5 nA. Supported by NIMH NRSA F32MH10783-01 to LP and NSF-PFF award and NIMH Center grant to GL.

161.4

FUNCTIONAL ORGANIZATION OF TIME-CODING CIRCUITRY IN THE NUCLEUS LAMINARIS OF BUDGERIGARS. S Amagai*[1], MF Kubke[2], C Blanco[2], EA Shelden[3], RJ Dooling[1] and CE Carr[2]. [1]Dept. of Psychology and [2]Dept. of Zoology, University of Maryland, College Park, MD 20742. [3]Dept. of Anatomy & Cell Biology, University of Michigan, Ann Arbor, MI 48109.

In the avian auditory brainstem, auditory nerve afferents synapse on the neurons of the nucleus magnocellularis (NM), which project bilaterally to the nucleus laminaris (NL), where they form maps of interaural time differences (ITDs). The organization of the ITD circuit differs among different species of birds. In the chicken, NL is composed of a monolayer of bipolar neurons forming a single map of ITDs in the medio-lateral direction. This is in contrast to the barn owl, where NL is hypertrophied to a multicellular structure with multiple ITD maps arranged in the dorso-ventral axis. We propose that this variation is correlated with time coding ability. Budgerigars can localize sound well, and recordings from NL show sharply tuned responses to ITDs. We investigated the level of organization of the budgerigar NL and the direction in which ITD maps are arranged.

We have used immunohistochemical and Golgi techniques to examine the morphology of NL in the budgerigar. Anti-calbindin staining reveals that, like in other birds, budgerigar NL neurons in the lateral-caudal low best frequency region have complex long dendritic arbors, while those in the medio-rostral high best frequency region have many shorter dendrites. In the high frequency region, NL forms a multilayered structure like the barn owl, but in the low frequency region, NL is more reminiscent of the chicken monolayer structure. The cell count of NL reveals that the budgerigar NL is more developed than the chicken and less so than the barn owl. Organizations of NM afferent delay-lines in NL is also under investigation both by small injections of biotinylated dextrans amine in NM and by physiological mapping of ITD sensitivity.

Supported by NIH grant DCD 00436 to CEC, MH 00982 and DCD 00198 to RJD.

161.5

ELECTRICAL STIMULATION OF THE BARN OWL'S NUCLEUS LAMINARIS NEURONS IN VIVO. J.L. Peña, S. Viete and M. Konishi*. Division of Biology 216-76, California Institute of Technology, Pasadena, CA 91125.

Nucleus laminaris neurons serve as coincidence detectors in the neural circuit that measures interaural time differences. The discharge rates of these neurons could be manipulated in vivo by current injection under "loose patch" recording conditions. Positive current excited the neurons and negative current reduced or extinguished their spontaneous and evoked discharge. The firing rate increased monotonically as a function of positive current. The neurons fired tonically to prolonged current steps, after an initial period of adaptation. Electrical stimulation could drive the neurons to maximal discharge rates much higher than the saturation levels reached by sound stimulation suggesting that gain control mechanisms operate during acoustic stimulation.

Injection of positive current during sound stimulation showed that the sensitivity for interaural time difference (ITD) could persist over a range of discharges rates which does not normally occur with sound stimulation. However, some of the neurons lost their ITD-tuning with large positive current. Negative current also had graded effects on sound evoked responses. ITD tuning persisted for all current levels used. In some of the neurons, stimulation with their least favorable ITD caused their discharge rates to decrease below the level maintained by current alone.

Supported by NIH grant R01 DC00134, and fellowships from the Pew Foundation (JLP) and the Deutsche Forschungsgemeinschaft (SV).

161.6

MAPPING OF ITD IN THE NUCLEUS LAMINARIS OF THE BARN OWL. C.E. Carr* and M.F. Kubke. Univ. Maryland, Dept. of Zoology, College Park, MD 20742.

Barn owls use interaural time differences (ITDs) to localize sound in azimuth. ITDs are computed in the nucleus laminaris (NL) by a circuit composed of delay line inputs and coincidence detectors. A previous study (Sullivan and Konishi, 1986) had used multiunit recording of the tone-induced evoked potential, or neurophonic, to map NL, and had demonstrated an orderly map of ITD within NL, such that from dorsal to ventral, the best ITD shifts from the far contralateral hemifield through 0 ITD to the frontal ipsilateral hemifield (-130 to 50μs). Iso-ITD contours run along the mediolateral direction of NL, forming multiple maps of ITD within a single isofrequency slab.

The redundant nature of this representation has been questioned. Since the conclusions were reached after reconstruction of recordings not confined to an isofrequency slab or parallel to the delay line inputs, we repeated the experiments with a head angle of 70°, and recorded parallel to the delay line inputs. In 2 owls, we found essentially identical results to Sullivan and Konishi. A large neurophonic was encountered over a 700μm depth in the dorsal brainstem, corresponding to the position of NL. Recordings from 4.5-6.1 kHz regions showed steady changes in best ITD from -120/-90μs (dorsal) to +60μs (ventral), with 0 ITD represented about 500μm below the dorsal surface of NL. Multiple recordings along an isofrequency slab showed small variations in the position of 0 ITD; these changes did not appear to represent a systematic shift in iso-ITD along the mediolateral axis of NL.

Supported by NIH DCD00436 to CEC.

161.7

A SIMPLE BIOPHYSICAL MODEL FOR ANALYZING PHASE-LOCKING AND COINCIDENCE DETECTION IN AUDITORY CELLS. H. Agmon-Snir*, C. E. Carr+ and J. Rinzel. Math Research Branch, NIH, Bethesda, MD 20892, +Dept. Zoology, Univ. Maryland, College Park, MD 20742.

Using electrophysiological data gathered on cells in the auditory ITD (interaural time difference) pathway in birds, we developed a simple biophysical model for relay cells (in the N. magnocellularis) and coincidence detectors (in the N. laminaris). The simplest version of this Hodgkin-Huxley-like model assumes just two voltage-dependent currents, having just two dynamic variables and few parameters. The inward current produces the action potential's fast depolarization and the outward current repolarizes the cell back to rest. Input trains to the model mimic auditory nerve (AN) fiber activity for pure tone stimuli.

Our analysis shows that the Poissonian property of the AN input train lowers the cell's phase-locking ability: the phase of the output spike depends on the interspike interval that precedes this spike (due to the refractory period after the previous output spike). This reduction in phase-locking can be avoided by using very large synaptic inputs. We also find that convergence of a few AN fibers onto a single relay cell may improve the phase-locking of the output of this cell (see also Joris et al., 1994). This improvement is found regardless of synaptic input amplitude (sub- or suprathreshold), and can be explained using concepts from order statistics. The requirements for an improvement are analyzed and our results are compared to previous models (e.g., Rothman et al., J. Neurophysiol., 1993). Our model is also used for coincidence detection and several main results are shown.

Generally, the present model fails to phase-lock and perform coincidence detection at high frequencies (>3kHz). However, the model gives insights on the mechanisms of phase locking and coincidence detection in these cells and on the problems found in the high frequency domain.

This work was supported by HFSPO (H.A-S) and by NIHDCD00436 (C.E.C.).

161.8

SPIKING PATTERNS IN THE OWL'S INFERIOR COLLICULUS PRESERVE TEMPORAL ACOUSTICAL STRUCTURE. T.T. Takahashi* & C.H. Keller, Inst. of Neurosci., Univ. of Oregon, Eugene, OR 97403.

Neurons in the owl's inferior colliculus (IC) have well-defined spatial receptive fields. We recently observed that the temporal pattern of their spikes, elicited by the repeated presentation of a noise burst, was remarkably consistent from repetition to repetition. We report the results of two experiments suggesting that this pattern is related to the temporal structure of the stimulus noise. In one experiment, we recorded spike trains from isolated IC neurons (n=45) responding to a series of partially-correlated noise bursts (100-ms; 5 ms on & offsets). Correlation levels were controlled by adding to a reference noise, known proportions of an uncorrelated noise. PSTHs were constructed from spike trains obtained with each noise (20 reps/stimulus) and cross-correlated with the PSTH obtained with the reference noise alone. Typically, the cross-correlation coefficients for the PSTHs, when plotted as a function of the noise-bursts' correlation coefficients, showed a systematic relationship, suggesting that the spiking pattern changed in a manner related to the noise bursts' temporal structure. In the second experiment, a series of 100-ms noises were synthesized by inserting a 40-ms noise segment, N1, at successively later points along the time course of another noise burst, N2. PSTHs from IC neurons (n=44) were aligned with stimulus onsets and, in most neurons, found to have a consistent pattern of peaks and troughs whose positions changed in synchrony with the position of N1 within N2. These results suggest that neurons in IC, in addition to place-coding space, can also convey information about the temporal structure of complex stimuli. (Supported by NIH grant DC02050.)

161.9

Anatomical basis for the adjustment of interaural time difference (ITD) tuning in the external nucleus of the inferior colliculus (ICX) of juvenile barn owls: selective pruning or synaptogenesis? DE Feldman* and EI Knudsen. Neurobiology Dept., Stanford University Medical School, Stanford CA 94305.

Neural tuning for ITD in the barn owl's ICX is calibrated by visual experience during development. Normally, ICX neurons in adult owls are narrowly tuned to an ITD value systematically related to location in the ICX. In adult owls reared wearing laterally displacing prismatic spectacles, however, ICX neurons exhibit ITD tuning that is offset from the normal value by an amount corresponding to the prismatic displacement. Similarly, afferents providing the principal auditory input to the ICX from the central nucleus of the inferior colliculus (ICC) have a characteristic point-to-point topography in normal adults, but display an altered topography in prism-reared owls that corresponds to the shifted ITD tuning. How are these distinct physiological and anatomical adult states arrived at during development? Possibilities include differential sculpting of initially broad anatomical and physiological states, or the modification by prism rearing of an initial state that is already adult-like, perhaps by formation of novel synaptic connections. To distinguish these possibilities, we are assaying the initial state of the anatomy and physiology in juvenile owls before prism attachment.

Owls were reared with normal vision until 60 d of age, when the head has reached adult size. Preliminary data indicate that at this age, ICX neurons already have adult-like ITD tuning. After subsequent prism attachment, ICX neurons undergo robust ITD tuning modification, including the acquisition of responses to previously ineffective ITDs. Thus, prism rearing can cause the acquisition of novel auditory responses. We are currently determining the initial topography of the ICC-ICX projection in 60 d old owls to see how it, too, is modified by subsequent prism-rearing.

Supported by NIH R01 DC00155-16.

161.10

Can Hebbian pairing of a binaural noise stimulus and electrical microstimulation alter binaural tuning in the inferior colliculus of barn owls? J. J. Gold* and E. I. Knudsen. Dept. of Neurobiology, Stanford University School of Medicine, Stanford, CA 94305.

The binaural tuning properties of neurons in the auditory space map of the optic tectum (OT) of barn owls adaptively adjust to changes in auditory and/or visual experience during development. A site of neuronal plasticity underlying such adjustments is the external nucleus of the inferior colliculus (ICX), in which tuning to interaural time difference (ITD) adaptively changes as a result of rearing with either prism-induced displacement of visual space or ear plug-induced, frequency-dependent displacement of auditory cue values. Although little is known about the cellular mechanisms of plasticity in either case, the existence of prism-induced changes in the auditory map suggests the presence of a vision-based "instructive signal" to guide changes in tuning. Such a signal could work via phasic excitation of ICX neurons by a structure containing visual information, causing potentiation of coincident auditory-driven input. To test this model, the effects of a Hebbian pairing of a binaural stimulus and direct electrical activation of ICX neurons were evaluated.

Juvenile owls were raised with either an ear plug-like device in one ear or optical displacing prisms. In these birds the deep layers of the OT, which receive input directly from neurons in the ICX, were surveyed for sites at which tuning to ITD differed significantly from normal (based on the site's visual receptive field) but responses to normal values remained. Microstimulation (150 Hz biphasic pulses of 50 - 200 μA) at these sites, which antidromically activated ICX neurons, was paired with dichotically presented, broadband noise using the site's normal ITD. Responses to the microstimulation-paired ITD were evaluated relative to both pre-training and post-training, non-paired values and the time course of change, if any, determined. Preliminary results suggest that this manipulation increases neural responsiveness to the microstimulation-paired ITD values. Supported by NIH: R01 DC00155-16.

161.11

EFFECTS OF UNILATERAL COCHLEAR REMOVAL AND FREQUENCY-SPECIFIC LESIONS ON LOCALIZATION AND DISCRIMINATION OF SOUNDS BY THE BARN OWL (*Tyto alba*). R. Egnor*Biology Division 216-76, California Institute of Technology, Pasadena, CA 91125

The barn owl is a nocturnal predator capable of using sound cues alone to localize prey. The anatomy, physiology and psychophysics of barn owl sound localization have been extensively studied. How barn owls process auditory information for sound recognition has not been studied, however. In order to investigate this capacity, three barn owls were trained to perform a sound localization task and a simple sound recognition task: frequency discrimination. Previous work has identified an anatomical segregation of the information from binaural cues used in auditory localization into two parallel pathways. Both bilateral pathways carry information about stimulus frequency. One pathway is specialized to preserve information about time and the other to preserve information about intensity. The effect of the removal of both of these pathways unilaterally (cochlear removal) and the complete removal of time information in a frequency band (local, frequency-specific inactivation of the time pathway) on frequency discrimination is discussed. In addition, although much is known about the processing of binaural cues for sound localization in the barn owl, the use of monaural cues has never been tested. Cochlear removal addresses whether owls can localize using only monaural cues.

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161.12

HEAD-RELATED TRANSFER FUNCTIONS OF THE BARN OWL. Keller*, C.H., K. Hartung, & T.T. Takahashi., Institute of Neuroscience., Univ. of Oregon, Eugene, OR 97403 USA & Lehrstuhl für allgemeine Elektrotechnik und Akustik. Ruhr-Universtät Bochum D-44780 FRG.

In an effort to develop a headphone-based simulation of various complex acoustical environments, we have measured the head-related transfer function (HRTF) of the barn owl. HRTFs were computed from microphone recordings obtained in the ear canal of 4 freshly-euthanized adult barn owls. Stimuli were Gaussian enveloped broadband noise bursts (68 ms) broadcast from a speaker mounted on a vertical hoop. Samples were obtained in the frontal hemisphere with 5°-10° resolution on a double-pole co-ordinate system. Recordings were made with microphones (Knowles EM4046) placed in the ear canals with the ports facing outward at a distance 4 mm from the eardrum in 3 birds. In 1 bird, probe tubes (7mm x 1mm OD) attached to the microphone were inserted to within 2 mm of the eardrum. Microphone output was averaged over 100 stimulus repetitions at each location and digitized at 30,000 points/sec. Binaural phase and amplitude spectra, corrected for speaker and microphones, agreed well with earlier reports (Brainard et al. *J Acoust Soc Am* 91:1015 '92). At low frequencies (3-4kHz), binaural phase and amplitude differences co-varied with the source's azimuth but not its elevation. At higher frequencies (4-8kHz), the spatial axes along which the two cues changed were more orthogonal. Removal of the preaural flap decreased the magnitude of binaural differences at high frequencies, but had little effect on the directional dependency of these cues. By contrast, removal of the facial ruff affected the directional dependency and the magnitudes of the cues, particularly at higher frequencies, in agreement with earlier reports (Knudsen et al. *J Neurophysiol* '94). (Supported by grants from the NIDCD (DC02050) & NATO (CRG 951075).)

161.13

A LATENCY MAP IN THE MUSTACHED BAT VENTRAL NUCLEUS OF THE LATERAL LEMNISCUS. W. E. O'Neill*, Dept. of Neurobiology and Anatomy, Univ. of Rochester School of Medicine and Dentistry, Rochester, NY 14642

The ventral nucleus of the lateral lemniscus (VL) in echolocating bats is highly organized and differentiated into "multipolar" and "columnar" subdivisions. The columnar division (VLc) is composed of rows and columns of tightly-packed, glycinergic spherical cells. Excitatory inputs to VLc arise in the contralateral ventral cochlear nucleus and project in an orderly tonotopic fashion to the cell rows. VLc neurons in turn project tonotopically to the central nucleus of the inferior colliculus (IC). Presumably inhibitory inputs arise in the ipsilateral medial and lateral nuclei of the trapezoid body. VLc cells reliably fire only one or two spikes to the onset of best-frequency tone bursts. Latency variation is extraordinarily low (<40 to 250 μs s.d.), and with level changes over 60 dB, latency shifts are less than 1 ms. These features are particularly useful for precise encoding of stimulus timing.

Afferents enter the VLc laterally and make their way across the nucleus, synapsing with cells lying in only one or two horizontal laminae. Therefore, lateral VLc cells should have the shortest latencies, and medial VLc cells should have the longest latencies. We reconstructed single-unit best frequencies (BF) and latencies from a series of parallel penetrations passing through the IC and crossing the columns of VLc cells diagonally in the dorsomedial to ventrolateral direction. In the VLc, a weak, dorsoventral, low-to-high frequency tonotopic gradient was found along cell columns, but not along cell rows. By contrast, a significant short-to-long latency gradient was found in the lateral-to-medial direction along cell rows, but not along cell columns. This latency gradient spanned a narrow range of latencies between 2 and 6 ms. However, the latency representation remained quite stable over wide changes in stimulus level, unlike that for an expanded latency gradient previously found in the IC. The level-tolerant representation in VLc can be considered a "map" for latency, although for what purpose such a map is intended is not yet clear.

161.14

THE INFLUENCE OF GABAERGIC INHIBITION ON THE RECOVERY CYCLES OF BAT INFERIOR COLLICULAR NEURONS. Y. Lu*, Q.Y. Zheng and P. H.-S. Jen. Division of Biological Sciences, University of Missouri-Columbia, MO 65211

In echolocation, bats use the time lag between the outgoing orientation signal and the returning echo for measurement of the target distance. This echo ranging process depends heavily upon the recovery cycles of auditory neurons. To understand the dynamic aspect of the echo ranging, we examined the influence of GABAergic inhibition on the recovery cycles of inferior collicular neurons of the big brown bat, *Eptesicus fuscus*.

Recovery cycles of 64 inferior collicular neurons (in which 55 were phasic responders and 9 were tonic responders) were studied by means of two identical frequency-modulated (51 neurons) or best frequency pure tone (13 neurons) pulses. Their recovery cycles were classified into three types: (1) undelayed long inhibition (29 neurons, 45%) in which a neuron did not reach 50% recovery until the interpulse interval was more than 20ms; (2) short suppression (30 neurons, 47%) in which a neuron reached 50% recovery in 10 to 20ms; and (3) fast recovery (5 neurons, 8%) in which a neuron recovered to 50% within 10ms. The influence of GABAergic inhibition on the recovery cycles of 62 neurons was examined by ionophoretically injecting bicuculline methiodide (10mM, pH3.0, Sigma) into their recording sites. This application shortened the 50% recovery time of 47 (76%) neurons by more than 10% but it lengthened the 50% recovery time of 4 (6%) neurons by more than 10%. However, the 50% recovery time of the remaining 11 (18%) neurons was not affected by more than 10%. These data demonstrated that GABAergic inhibition contributes significantly to the recovery cycle of inferior colliculuar neurons. (work supported by NIH DC 247 to P Jen).

161.15

A MIDBRAIN TEGMENTAL AUDITORY AREA (SAGULUM?) SPECIALIZED FOR PROCESSING FREQUENCY MODULATED SOUNDS IN THE MUSTACHED BAT M. Gordon*, W.E. O'Neill. Neuroscience Program and Dept. of Neurobiology and Anatomy, Univ. of Rochester Sch. of Med. and Dent., Rochester, NY 14642

Frequency modulations are an important component of the communication vocalizations of many species. For bats, they are also an important and often the only component of echolocation calls. In the mustached bat, we have found a region beneath the inferior colliculus and just lateral to the dorsal nucleus of the lateral lemniscus where every neuron so far recorded showed strong directional preference for linear and sinusoidal FM stimuli.

Units in this region responded with phasic discharge patterns to tone bursts and had best frequencies between 62 and 63 kHz (dominant biosonar component). In response to FM stimuli, these units were consistent in preferring upward sweeps over downward, by nearly 100% for certain FM rates (ΔF/T). Upward preference was maximal at rapid FM rates (0.8-2.4 kHz/ms) for most units, and at slow rates (0.18-0.34 kHz/ms) for 1 unit. Evoked field potentials were also consistently larger to upward FM than to downward FM over multiple electrode penetrations through the area. Such consistency of the evoked field potential is a further indication of the uniformity of unit response type in this area. All units had inhibitory sidebands, some of which were temporally asymmetric. These sidebands have been previously implicated as a likely mechanism for creating FM selectivity (Gordon and O'Neill '96, Assoc. for Res. in Otolaryngology Abst. 19: 482).

HRP labeling of our recording site revealed it to be just beneath the anterolateral division of ICC in a region of small cells and light cytochrome oxidase staining. This is consistent with descriptions of the sagulum in cat (Henkel and Shneiderman '88, J. Comp.Neurol. 271:577-588).

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161.16

INHIBITION CREATES FILTERS FOR FREQUENCY MODULATED SOUNDS IN THE IC OF THE BIG BROWN BAT (*EPTESICUS FUSCUS*) U. Koch and B. Grothe*, Zoologisches Inst., Univ. of Munich, 80333 Munich, Germany

Most natural occurring sounds are modulated in amplitude and frequency. Going from lower to higher auditory nuclei the range of modulation rates a neuron responds to becomes narrower and the optimal rate lower. This suggests active neuronal filtering. We have recently shown that binaural interaction influences the filter properties of neurons in the inferior colliculus (IC). Here, we investigated the role of GABAergic and glycinergic inhibition on the monaural and binaural filtering of frequency modulated sounds in the IC.

Action potentials were recorded from IC neurons while presenting sinusoidal frequency modulated sounds monaurally and binaurally (same intensity at each ear) via earphones. Neurons were tested at their best center frequency and best modulation depth with different modulation rates ranging from 10 Hz to 800 Hz. Based on spike counts the monaural and binaural modulation transfer function (MTF) for modulation rates was calculated and the upper and lower 50%-cut-off was determined. The same tests were repeated while iontophoretically applying the $GABA_A$-receptor antagonist bicuculline or the glycine receptor antagonist strychnine.

Blocking GABAergig inhibition increased the upper 50%-cut-off in 50% of the neurons. Across the population of neurons this change was more pronounced for binaural than for monaural sound presentation. Bicuculline decreased the upper 50%-cut-off of some neurons. In 25% of the neurons the lower 50%-cut-off decreased after applying bicuculline for the monaural and binaural presentation. Strychnine had comparable effects. However, the difference of filter changes between monaural and binaural stimulation was less pronounced.

The results suggest that binaurally and monaurally induced GABAergic as well as glycinergic inhibition participate in creating filter properties for frequency modulated sounds of neurons in the IC.

Supported by SFB 204

161.17

COMBINATION-SENSITIVE NEURONS IN THE INFERIOR COLLICULUS OF THE MUSTACHED BAT: POSSIBLE ANALYSIS OF SOCIAL COMMUNICATION SIGNALS. S. A. Leroy* and J. J. Wenstrup. Dept. of Neurobiology, Northeastern Ohio Univs. Coll. of Med., Rootstown, OH 44272

Neurons of the inferior colliculus (IC) were tested for responses to combinations of tone or noise bursts. We focused on neurons in two tonotopic regions: 1) the 24-31 kHz region, representing frequencies in the fundamental component of the biosonar signal, and 2) the 33-46 kHz region, representing frequencies outside the biosonar range, between the fundamental and second harmonic components of the biosonar signal. We examined whether single units in these parts of the IC are combination-sensitive, as are neurons in other IC regions.

It was difficult to record single units with best frequencies in the 24-31 kHz range. Penetrations through the anterolateral division of the IC often revealed a gap in this frequency range. Of the neurons tuned to 24-31 kHz, none showed additional sensitivity (whether alone or in combination) to frequencies in higher sonar harmonics, even when tested over a range of delays between the two stimuli. In contrast, about half of the neurons tuned in the 33-46 kHz range responded to the combination of two signals. These neurons, with best frequencies in the 37-46 kHz range, also responded to signals in 18-22 kHz range. Both facilitatory and inhibitory effects of the low frequency stimulus were observed, and these effects were strongest at or near 0 msec delay between the two components.

This latter group of neurons may encode information in social communication signals. Social vocalizations often include energy in both the 10-22 kHz and 33-46 kHz ranges (Kanwal et al., J. Acoust. Soc. Am. 96:1229-1254, 1994). These findings suggest that neurons at auditory levels as low as the IC may be specialized to respond to complex, multi-harmonic communication signals. (Supported by the National Institute on Deafness and other Communication Disorders.)

161.18

PERCEPTUAL ORGANIZATION OF SOUND FOR SPATIALLY-GUIDED BEHAVIOR IN THE ECHOLOCATING BAT. M. Morris and C.F. Moss*, Departments of Psychology, Harvard University, Cambridge, MA 02138 and University of Maryland, College Park, MD 20742

Auditory perception involves the organization of sound, or the analysis of auditory scenes. Auditory scene analysis draws upon many dimensions of sound perception, including that of auditory space. Here we present data on the perceptual organization of sound in the echolocating bat, *Eptesicus fuscus*, an animal that emits brief FM sonar vocalizations and extracts spatial information about the environment from the features of returning echoes. The particular focus of our work is on the perceptual organization of sound along the axis of echo delay, the bat's cue for target distance. We have conducted a series of experiments on the bat's perception of sonar targets with changing echo delay to explore the extent to which the animal integrates and segregates spatial information from a dynamic auditory scene. In these experiments, the bat was trained in a 2-AFC task to discriminate between sets of echoes that differed in their patterns of delay-change over time. Targets were electronically simulated by digitizing, delaying and playing-back the bat's sonar emissions. Each stimulus set contained six different echo delays, and the bat received only one echo playback for each sonar emission. In one echo discrimination set, for example, the delays systematically increased or decreased (set A), and in the other echo set, the sequence of delays was random (set Z). The delay step-size of echoes in stimulus set A was unequal in some experiments to ensure that the bat was discriminating the direction of echo delay change, and not simply between variable and fixed delay-steps in echo sets A and Z. Our data show that the bat can integrate echo sequences along the dimension of delay, and these findings lay the foundation for behavioral experiments that explore the bat's segregation of echoes into separate streams along the range axis. Supported by an NSF Young Investigator Award to C.F.M.

161.19

SPATIAL TRACKING OF MOVING TARGETS BY THE ECHOLOCATING BAT, *EPTESICUS FUSCUS*: PERCEPTUAL CONSEQUENCES OF VOCAL-MOTOR BEHAVIOR. C. F. Moss, R. Iannucci, and W.W. Wilson*, Department of Psychology, University of Maryland, College Park, MD 20742

Field data show that an echolocating bat modifies its outgoing sonar emissions, relying on incoming echo information to shape the characteristics of its subsequent sonar cries. In *Eptesicus fuscus*, a bat using FM vocalizations for echolocation, there are systematic changes in sound repetition rate, duration and bandwidth with closing target distance. We hypothesize that the temporal patterns of sound production during insect pursuit may provide the bat with acoustic information that is used in its perception of dynamic auditory scenes.

Here, we report on studies of insect capture by bats in a large laboratory flight room, where animals were trained to intercept tethered moving insects. The animal's flight behavior was recorded on video tape at high speed (500 frames/sec), and its sonar vocalizations were recorded on audio tape (30 inches/sec, Racal Store 4D). Our video analyses show that the bat typically positions its head 3-4 cm above the tethered insect, locking its head-aim with an accuracy of approximately 5 deg. Our analysis of sonar sounds produced by the bat in this insect capture task reveals that the repetition rate during target pursuit does not change continuously over time. Rather, the repetition rate remains stable for fixed intervals before increasing: During the approach phase of insect capture, for example, the sound repetition rate may plateau at around 30 Hz for time periods as long as 150 ms. We hypothesize that the stable periods of sound repetition rate produced by *Eptesicus* may be used by the bat in the processing of spatial acoustic information about a dynamic environment. Thus, the bat's perception of dynamic auditory scenes may be shaped by its own vocal production patterns.
Supported by an NSF Young Investigator Award and a Whitehall Foundation Grant to C.F.M. W.W.W is supported by the NIDCD Comparative and Evolutionary Biology of Hearing Training Program at the University of Maryland.

161.20

CORTICOFUGAL CONTROL OF CENTRAL AUDITORY SENSITIVITY. P. H.-S. Jen*, X. D. Sun and Q. C. Chen. Division of Biological Sciences, University of Missouri-Columbia, Missouri 65211

During sensory signal processing, the brain has the built-in ability to edit and adjust the flow of information that reaches it. Our previous study on bats showed that electrical stimulation in the auditory cortex inhibited acoustically evoked responses of inferior collicular neurons (Sun et al., Brain Research 495:1-8, 1989). We have extended this study by examining the corticofugal control of rate-intensity function, auditory spatial sensitivity and sharpness of frequency tuning of inferior collicular neurons of the big brown bat, *Eptesicus fuscus*.

Electrical stimulation at 40 cortical sites (depths: 488-690 μm) reduced acoustically evoked responses of 84 recorded inferior collicular neurons (depth: 270 and1962 μm). At the optimal interstimulus interval, electrical stimulation in the cortical site lowered the intensity-rate function, reduced the auditory spatial response area and narrowed the frequency tuning curve of recorded collicular neurons. Ionophoretical injection of Lidocaine into the cortical site significantly raised the intensity-rate function, expanded the auditory spatial response area and broadened the frequency tuning curve in these neurons. This suggests that the corticofugal pathway continuously supresses acoustic signal processing in the central inferior colliculus. Ionophoretical application of bicuculline into the collicular recording sites greatly raised the intensity-rate function, expanded the auditory spatial response area and broadened the frequency tuning curves of these neurons. Application of GABA into the collicular recording sites produced the opposite effects. These findings suggest that corticofugal control of inferior collicular auditory sensitivity is likely mediated through GABAergic inhibition (work supported by NIH).

BASAL GANGLIA: STRIATUM I

162.1

Developmental Expression of Plateau Potentials in Rat Striatal Neurons. E. Ghansah*, T. DeFazio, P. Quan and J. P. Walsh, Andrus Gerontology Center, University of Southern California, Los Angeles, CA 90089-0191.

Previous studies using microelectrode techniques have shown that an age-related decrease in the duration of calcium-mediated plateau potentials (PP) was correlated with a decrease in striatal neuron dendritic length. Our goal was to use development as a tool to further examine the relationship between dendrites and PPs. Whole-cell recording techniques were used on 400 μm coronal sections obtained from P1 - P16 rats. All slices were perfused with oxygenated ACSF containing 30 mM TEA. Recordings were obtained using 4 - 8 MΩ patch electrodes filled with a K^+-based internal containing biocytin. PPs were generated by 40, 80, 200 or 400 ms depolarizing current pulses, and biocytin-filled cells were examined for correlated morphology. In general, PP durations (PPD) increased with age while the threshold for evoking PPs decreased. P1 - P5 neurons showed increased action potential duration (APD), but no PPs even at supramaximal current injections. Long duration PPs were produced in neurons > P14, but removal of dendrites through acute isolation eliminated PP expression. Developmental differences could not be accounted for by changes in seal or input resistance. These data were supported by a dendritic compartment model developed through *NEURON* software. Our results suggest that developmental changes in dendritic length contribute to the expression of PPD. *Supported by NIA grants AG09793 and AG00093.*

162.2

Minimal stimulation of excitatory striatal afferents reveals age-dependent changes in quantal parameters. R. Nori and J. P. Walsh*, Andrus Gerontology Center & Dept. of Biomedical Engineering., University of Southern California, Los Angeles, CA 90089-0191.

The present study determined whether quantal analysis of minimally evoked EPSPs would reveal age-dependent differences in quantal parameters. Each cell was tested for its "paired-pulse" response to activation of a large population of afferents (strong stimulation), and then the stimulation intensity was reduced to activate a small number or single afferent. Quantal content (m) was determined through the method of failures using a stimulation intensity set to produce failures ≈ 50% of the time. Release probability (p) was determined from the EPSP amplitude variance and m, and the number of release sites were determined from the binomial equation (m=np). Paired, high intensity stimulation of the corpus callosum generally produced facilitation at a pairing interval of 50 msec in young rats. In the same cells, minimal stimulation revealed a paring induced increase in m, p, and n. Pairing caused a reduction in failures and increased the likelihood of multiple release events. By contrast, aged cells generally responded to high intensity stimulation with paired-pulse depression and minimal stimulation revealed a pairing induced decrease in m and p. Pairing caused an increase in the number of failures and decreased the number of multiple release events in aged animals.

Addition of carbachol (1 μM) reduced the amplitude of the EPSP produced in response to strong stimulation and changed paired-pulse depression into paired-pulse facilitation. By contrast, carbachol did not change the amplitude of the minimally evoked EPSP. These data indicate that aging affects the quantal characteristics of synapses and that carbachol sensitive and insensitive excitatory synapses exist in the striatum. Supported by NIA grant AG09793.

162.3

INTERACTION BETWEEN AGONISTS OF CHOLECYSTOKININ AND ACETYLCHOLINE AT NEOSTRIATAL NEURONS IN RATS. H. Davidowa, G. Vierig, K. Wetzel and D. Albrecht* Institute of Physiology, Charité, Humboldt University Berlin, Tucholsky Str. 2, D - 10117 Berlin, Germany

Cholecystokinin (CCK) mainly excites striatal neurons via CCK-A or CCK-B receptors. Acetylcholine is the transmitter of one type of the interneurons within the neostriatum. Now we studied the interaction between iontophoretically administered agonists of CCK and acetylcholine (Ach) in rats anaesthetized with urethane. Action potentials of single units were recorded extracellularly by means of glass micropipettes filled with trypan blue solution. A seven-barrel micropipette was affixed to the recording electrode. It contained one of the CCK-A agonists A-71623 (Boc-Trp-Lys-(N-methylphenylaminocarbonyl)-Asp-[NMe]-Phe-NH2, 0.25 mM, pH 7.8) or A-71378 (Des-amino-Tyr-Nle2-Nle5-NMe-Asp-CCK7, 0.25 mM, pH 7.8) the CCK-B agonist Suc-CCK4 (Suc-Trp-NMe-Nle-Asp-Phe-NH2, 0.25 mM, pH 7.8), KI 1001 (Suc-Tyr(SE)-Met-Gly-Trp-Met-PEA, 0.25 mM, pH 7.8), a CCK-A antagonist (all synthetized by P. Henklein, Charité), PD 135,158 (N-methyl-D-glucamine salt, 0.25 mM, pH 7.8) (RBI). All these drugs were dissolved in solution of phosphate buffered saline (60mM). These substances were ejected with negative currents between 5 to 90 nA. Furtheron, acetylcholine chloride (0.7 M, pH 4) or atropine sulfate (0.1 M, pH 4,5) were administered with positive currents. Retention currents of opposite polarity (2-5 nA) were applied between the ejection periods. The agonists had mainly excitatory effects on neuronal discharge rates. These changed from 1.75 $\pm$ 2.4 impulses/s (mean $\pm$ STD; control, n = 45) to 2.4 $\pm$ 2.7 imp./s during administration of the CCK-A agonists and to 2.3 $\pm$ 2.7 imp./s during administration of the CCK-B agonist (Wilcoxon Signed Rank Test (Wt) $p < 0.0001$). Acetylcholine mainly reduced these effects or converted them to a suppression (Wt $p < 0.001$). During coadministration of Ach and one of the agonists the discharge rates of the neurons were 1.8 $\pm$ 2.6 (CCK-A) and 1.6 $\pm$ 2.3 (CCK-B). The coadministration of atropine did not significantly change the responses to the CCK receptor agonists and reduced the suppressive action of Ach. It can be concluded that cholinergic interneurons may reduce the activating action of the neuropeptide CCK within the neostriatum. Supported by the BMFT, F.R.G.

162.4

PRESYNAPTIC STRIATAL INHIBITORY ACTION OF GABA. M. Garcia-Munoz*, P. Patino, S.J. Young, and P.M. Groves, Dept. Psychiatry, Sch. of Medicine, UCSD, La Jolla, CA 92093-0603.

Published evidence supports the presence of presynaptic GABA-B receptors on cortical and dopaminergic axons. Increased stimulation of these receptors appears to attenuate cortical evoked postsynaptic responses and also to decrease the evoked striatal release of glutamate and dopamine. We used an in vivo electrophysiological technique, the measurement of electrical excitability, to examine presynaptic effects of GABA-B receptor activation on corticostriatal and nigrostriatal axons. Antidromic action potentials elicited by striatal stimulation were recorded extracellularly either from a substantia nigra pars compacta neuron or a medial prefrontal cortical cell in different experiments in urethane anesthetized, male Sprague-Dawley rats. Excitability was assessed in terms of the current required to elicit an antidromic response. Drugs in normal saline were delivered (0.3 µl over 5 min) to the dorsal striatum via cannulae placed alongside the stimulating electrode. Striatal administration of the GABA-B receptor agonist baclophen (10µM) produced a significant decrease in nigral and cortical excitability relative to pre-drug levels (-17.2 ± 3.5 %; -16.1 ± 2.1 %, respectively) which was reversed by subsequent intrastriatal infusion of the GABA-B receptor antagonist saclophen (100nM) (-1.7 ± 2.7 % for nigral and -4.1 ± 0.8 % for cortical afferents). Prior administration of saclophen (100nM) significantly increased nigral (+8.7 ± 0.5 %) and cortical (+10.8 ± 0.9 %) excitability, consistent with a tonic inhibitory action of GABA on these axons, and blocked the ability of baclophen to decrease nigral (+3.2 ± 0.5 %) and cortical (+3.6 ± 0.8 %) presynaptic excitability. These results are consistent with previous findings that GABA presynaptically inhibits nigral and cortical transmission. Evidence for a tonic action of GABA mediated by GABA-B heteroreceptors on nigral and cortical striatal afferents suggest that presynaptic interactions are a route through which GABA can influence striatal neurotransmission. This research was supported in part by DA02854 from the National Institute on Drug Abuse and Research Scientist Award DA00079 to P.M.G.

162.5

DIRECT AND INDIRECT EFFECTS OF SUBSTANCE P ON STRIATAL PRESYNAPTIC EXCITABILITY. P. Patino*, S.J. Young, P.M. Groves and M. Garcia-Munoz, Dept. Psychiatry, Sch. of Medicine, UCSD, La Jolla, CA 92093-0603

Evidence suggests that striatal presynaptic Substance P (SP) heteroreceptors modulate neurotransmitter release from cortical-glutamate and nigral-dopamine (DA) afferents. We used the measurement of terminal excitability, an in vivo electrophysiological method, to study the activation of SP receptors on nigrostriatal and corticostriatal axons. Stimulating electrodes were placed in the dorsal striatum of urethane anesthetized male Sprague-Dawley rats to elicit antidromic action potentials recorded extracellularly from a substantia nigra pars compacta or medial prefrontal cortex neuron in different experiments. Drugs in saline were delivered (0.3 µl over 5 min) via cannulae placed alongside the stimulating electrode. Excitability was assessed by determining the stimulating current required to elicit an antidromic response. Striatal administration of SP (2 nM) produced a significant increase in excitability (+11 ± 0.6 %) in nigrostriatal axons which was reversed (+2.2 ± 0.6 %) by intrastriatal infusion of the specific SP antagonist Spantide II (10nM), confirming neurochemical reports that the SP-induced increase in DA release is due to a direct action of SP on DA axons. Infusion of SP (2 nM) significantly decreased corticostriatal excitability (-11 ± 1.9 %) in intact rats and in animals with kainic acid lesions (1.5 µg/0.3µl) that eliminated most classes of intrinsic neurons (-19.6 ± 2.1 %). This suggests an indirect effect due to a SP-induced increase in DA and stimulation of corticostriatal DA receptors since, in animals pretreated with the DA antagonist sulpiride (10mg/kg, i.p.), SP significantly increased corticostriatal presynaptic excitability (+6.5 ± 0.8 % in animals with kainate lesions) presumably by a direct effect on corticostrial SP heteroreceptors . Experiments are underway to study the participation of glutamate and DA autoreceptors in these SP-induced changes in presynaptic excitability. These results provide electrophysiological evidence consistent with neurochemical studies that SP exerts a powerful presynaptic effect on DA release. Futher, these studies suggest that direct and indirect activation of pre and postsynaptic receptors are important considerations in SP neurotransmission as well as in the interpretation of its effects following intracerebral administration. This research was supported in part by grant DA02854 from the National Institute on Drug Abuse and Research Scientist Award DA00079 to P.M.G.

162.6

STRIATAL GLUTAMATE ANTAGONISM PRODUCES CONTRALATERAL NEGLECT J.J. Schuller* and J.F. Marshall, Dept. of Psychobiology, University of California, Irvine, CA 92717

Unilateral ablation of the medial agranular cortex (AGm) in rats produces an initial impairment in behavioral orientation toward contralateral sensory stimuli, followed by recovery to control performance. Following this lesion, regions of the striatum that receive input from AGm show diminished glucose utilization, immediate-early gene expression, and glutamate receptor ligand binding. Because these striatal physiological changes are temporally correlated with the pattern of behavioral impairment and recovery after the AGm lesion, it is hypothesized that the loss of glutamatergic projections from AGm to the striatum may contribute to the subsequent deficit in responding to contralateral sensory stimuli. To test that hypothesis, we administered glutamate receptor antagonists into the left striatum of awake rats and quantified behavioral orientation to contralateral and ipsilateral stimuli of the visual, tactile, and auditory modalities. The AMPA-kainate antagonist DNQX (3.0 µg/1 µl) and the NMDA antagonist CPP (2.0 µg/1 µl) both induced a large asymmetry in responses, such that the rats oriented less to contralaterally-presented stimuli than to ipsilaterally-presented stimuli. These effects were significant for all sensory modalities tested. To determine the volume of tissue affected by the DNQX and CPP infusions, the ability of these antagonists to block kainate (10 mg/kg, i.p.)-induced Fos immunoreactivity was measured. DNQX blocked Fos through much of the anterior caudate-putamen, while not affecting cortical Fos. These data support the hypothesis that AGm lesion-induced neglect may be at least partially due to striatal glutamatergic denervation. *Funded by NS 22698 and NS 33670.*

162.7

GAP JUNCTION INACTIVATION IN THE STRIATUM PREVENTS ORAL STEREOTYPY INDUCED BY APOMORPHINE *A.A. Grace* and H. Moore, Depts. Neurosci. and Psychiatry, Univ. Pittsburgh, Pittsburgh, PA 15260*

In the striatum, electrotonic coupling via gap junctions occurs between medium spiny projection neurons and is modulated by corticostriatal activity and dopamine (DA) receptor activation. In vivo intracellular studies have shown that systemic administration of apomorphine (APO) dramatically increases the extent of electrotonic coupling in the caudate-putamen (Onn & Grace, 1994). In the present study, we examined whether this dramatic increase in gap junction conductance mediates a subset of the effects of APO. To test this, we determined the effects of the gap junction inactivator, carbenoxolone, on APO-induced behaviors. The dose- and time-dependent behavioral responses to APO (0.2-2.0 mg/kg, i.p.) began with hyperlocomotion accompanied by stereotypical sniffing and progressed to spatially-confined stereotypical licking and biting. Carbenoxolone (35.0 mg/kg, i.p.) injected 30 min prior to APO blocked the oral stereotypy without affecting the increase in locomotion or sniffing. Similarly, infusion of carbenoxolone into the ventral caudate blocked oral stereotypy without affecting the locomotion and sniffing produced by systemic APO. These results suggest that the APO-induced increase in electrotonic coupling in the caudate may selectively mediate the oral stereotypy produced by this drug. One interpretation is that gap junctions between striatal medium spiny neurons mediate specific components of motor programs, and that the increase in gap junctions produced by APO may lead to oral stereotypy by allowing these components to be repeatedly initiated independent of stimuli that normally regulate the motor program. By contrast, the increased intensity of locomotion and sniffing is likely a result of DA receptor activation that is not dependent on gap junctions. Supported by USPHS MH 45159 and MH 42217 and an NRSA Fellowship to H.M..

162.8

DEPOLARIZATION ACTIVATED POTASSIUM CURRENTS IN CHOLINERGIC INTERNEURONS OF RAT NEOSTRIATUM. W.-J. Song* and D. J. Surmeier, Dept. of Anatomy and Neurobiology, College of Medicine, University of Tennessee, Memphis, TN 38163.

Cholinergic interneurons comprise only 1-2% of the total striatal neuronal population. Because of their scarcity, physiological study of these neurons has been difficult. In those recordings that have been made (e.g. Wilson et al., 1990), interneurons appear to reside at relatively depolarized potentials (ca. -60 mV) for long periods of time. In an attempt to understand the ionic mechanisms underlying this behavior, depolarization activated K^+ currents in acutely isolated cholinergic interneurons were characterized.

Large cholinergic interneurons were readily visualized after dissociation and their identification verified after recording by single cell RT-PCR detection of choline acetyltransferase mRNA. Depolarizing steps to -20 mV evoked outward currents that were dominated by a rapidly inactivating component. Depolarizing pre-pulses inactivated this transient, A-like component of the current, revealing a persistent, delayed rectifier-like current. This separation was confirmed by the observation that the transient component was blocked in a voltage-dependent manner by 4-aminopyridine whereas tetraethylammonium blocked most of the persistent current. While the voltage-dependence of A-current activation (V_h=5.5 mV, n=6) was similar to that reported in other neurons, the half inactivation voltage was considerably more depolarized than seen in many other cell types (V_h=-36 mV, n=5).

Our results show that as a consequence of its inactivation voltage-dependence, the A current in cholinergic interneurons is capable of participating in spike repolarization and the regulation of repetitive activity at relatively depolarized resting potentials.

This work was supported by USPHS grants NS 26473 and NS 34696.

162.9

SUBSTANCE P MODULATES CA^{2+} CURRENTS IN CHOLINERGIC INTERNEURONS OF RAT NEOSTRIATUM. D. J. Surmeier*, J. Flores-Hernandez and W.-J. Song. Dept. of Anatomy and Neurobiology, College of Medicine, University of Tennessee, Memphis, TN, 38163.

Neostriatal medium spiny neurons projecting to the substantia nigra co-release GABA and the neurokinin substance P (SP). Within the neostriatum, SP released from axon collateral terminals appear to selectively target cholinergic interneurons. The functional consequences of SP on this clinically important set of interneurons are largely undefined, however.

In a variety of other cell types, SP has been shown to modulate voltage-dependent Ca^{2+} currents. To determine whether similar mechanisms were at work in the striatum, whole cell voltage clamp recordings of acutely-isolated cholinergic interneurons were obtained. Application of nanomolar concentrations of SP reduced peak Ca^{2+} currents. The NK_1 receptor specific agonist SAR ([SAR^9,$Met(O_2)^{11}$]-SP) reproduced the effects of SP with an IC_{50} of 20 nM, suggesting that NK_1 receptors were responsible for the modulation. The NK_1 antagonist WIN-51,708 also blocked the SP effect. Single cell RT-PCR experiments were performed to confirm NK_1 receptor mediation.

The SP modulation was G protein mediated. Dialysis with GDP-β-S blocked development of the modulation. However, unlike the M2 muscarinic modulation of Ca^{2+} currents, the SP effect was not blocked by exposure to NEM (50 μM, 2 min), suggesting that a G_i/G_o class protein was not involved. In other cell types, SP couples to PLCβ through a G_q-class G protein. However, release of Ca^{2+} from intracellular stores did not appear to be involved as dialysis with 20 mM BAPTA did not block the SP modulation. Activation of protein kinase C also failed to mimic the modulation suggesting that an alternative signaling pathway was involved. This work was supported by USPHS grants NS 26473 and NS 34696.

162.10

POSTNATAL DEVELOPMENT OF POTASSIUM CURRENTS IN RAT STRIATAL NEURONS. E.S. Nisenbaum*. Department of Psychology, University of Connecticut, Storrs, CT 06269.

Adult striatal neurons possess at least three types of depolarization-activated potassium (K) currents. These include 4-aminopyridine (4-AP)-sensitive fast (I_{Af}) and slowly (I_{As}) inactivating A-currents and a 4-AP-resistant, persistent K current (I_{Krp}). These K currents are responsible for much of the outward rectification in the depolarizing voltage responses of striatal neruons. Indeed, in adult neurons, application of 10 mM 4-AP reveals a transient overshoot and subsequent polarizing sag in the depolarizing voltage transients near spike threshold, which reflects blockade of I_{Af} and the flow of I_{Krp}, respectively. A similar transient overshoot and polarizing sag is evident in the responses of neonatal striatal cells, but at more depolarized membrane potentials and in the absence of 4-AP. These results suggest that the voltage-dependence of I_{Af} and I_{Krp} may be more depolarized early in development. This hypothesis was tested using whole-cell voltage-clamp recording from acutely isolated striatal neurons at several developmental time points.

The whole-cell K current recorded from striatal cells taken from animals in the first postnatal week (P0-7) was composed of I_{Af} and I_{Krp}. Application of 10 mM 4-AP isolated I_{Krp} and also permitted isolation of I_{Af} by current subtraction. The voltage-dependence of activation of I_{Af} in P0-7 cells (half-activation voltage, V_h = 16.3 mV; slope factor, V_c = 9.5 mV) was more depolarized than in adult neurons (V_h = 4.1 mV, V_c = 8.9 mV). Similarly, the voltage-dependence of I_{Krp} in P0-7 neurons (V_h = 10.8 mV, V_c = 11.3 mV) was considerably more depolarized than in adult neurons (V_h = -12.8 mV, V_c = 11.0 mV). In P8-14 cells, the voltage-dependence of I_{Krp} (V_h = -1.2 mV, V_c = 11.4 mV) was intermediate to that observed for P1-7 and adult neurons.

These results demonstrate a hyperpolarizing shift in the voltage-dependence of I_{Af} and I_{Krp} during development. These data suggest that early in development, the capacity of I_{Af} to dampen the initial response to strong depolarizations is diminished, revealing a transient overshoot. Likewise, the depolarized shift in I_{Krp} accounts for the polarizing sag at more depolarized membrane potentials. *Support: NS34254*

162.11

SELECTIVE SEQUESTRATION OF EXOGENOUS NEUROTENSIN IN LARGE DIAMETER STRIATAL NEURONS IN THE RAT, M.A. Chapman* and D.S. Zahm. Department of Anatomy and Neurobiology, St. Louis University School of Medicine, St. Louis, MO 63104.

Numerous studies have demonstrated that dopamine D-2 antagonists increase the number of neurotensin-immunoreactive (IR) neurons detected in the rat striatum. Increased synthesis, decreased release, and/or decreased degradation of the peptide may contribute to this effect. Another possibility is that striatal cells sequester neurotensin from the extracellular fluid following its release from nearby neurons. In order to test this hypothesis, neurotensin (.32nmol/400nl) was infused directly into the rat striatum and brains were processed for neurotensin-IR using standard immunoperoxidase methods. Although neurotensin-IR was rarely observed in striatal medium spiny neurons, which are evident following administration of dopamine D-2 antagonists, the cell bodies and dendrites of large diameter striatal neurons consistently exhibited neurotensin-IR at 10 minutes, but not 1 hour or 2 hours, post-infusion. These neurons probably correspond to striatal cholinergic cells. It is possible that neurotensin is sequestered in these cells via receptor-mediated endocytosis, as indirect evidence indicates that striatal cholinergic neurons possess neurotensin receptors (Lapchak et al., J. Neurochem. 56: 651-657, 1991). This observation raises questions regarding the possible sequestration of endogenous neurotensin under normal cellular conditions and its subsequent fate. Supported by NS-23805 and NS-07254.

162.12

PRONEUROTENSIN mRNA EXPRESSION DISTINGUISHES SUBSETS OF RAT STRIATAL NEURONS FOLLOWING HALOPERIDOL ADMINISTRATION: TEMPORAL DISSOCIATION AND DESENSITIZATION. D.S. Zahm*, E.S. Williams, D. Poulad and J.E. Krause[1]. Depts. of Anat. and Neurobiol., St. Louis Univ. & [1]Washington Univ. Schs. of Med., St. Louis, MO, 63104 & [1]63110.

The hypothesis that neurotensin (NT) acts as a functional antagonist of DA neurotransmission within the ventral mesencephalon and its mesotelencephalic dopamine (DA) projections (C.B. Nemeroff, *Biol. Psychiat.* 15:283-302, 1980) has intrigued basic scientists and clinicians. While it's clear that exogenous NT affects DA transmission, [1] whether endogenous NT does and, if so, [2] the neuroanatomical substrates and mechanisms responsible remain at issue. Here, subsets of striatal neurons that respond to blockade of dopamine receptors with altered expression of proneurotensin mRNA were examined. Injections of haloperidol (hal, 2 mg/kg, s.c.) were given at four *or* twenty-four hours and both twenty-four *and* four hours prior to sacrifice (3 rats/group). Hal rats were paired with age- and weight-matched controls injected with equivalent volumes of vehicle. Sections of striatum were processed non-isotopically with a cRNA probe against neurotensin/neuromedin N. As shown previously by others, massive numbers of neurons exhibited hybridization in the dorsolateral caudate at four hours. At twenty-four hours, hybrids were nearly absent dorsolaterally, but were more numerous in the dorsomedial and ventrolateral caudate than in controls. A second injection further enhanced the dorsomedial/ventrolateral response, but failed to elicit substantial numbers of dorsolateral hybrids, as are observed four hours after one injection. This resistance of NT expression to a second injection was selective for the dorsolateral quadrant and may reflect residual blockade by hal or altered DA receptors or second messengers. Support: NIH NS-23805 & 21937.

162.13

DISTRIBUTION OF NEUROTENSIN NEURONS AND FIBERS IN THE RAT BASAL GANLGIA FOLLOWING ADMINISTRATION OF d-AMPHETAMINE ALONE OR WITH HALOPERIDOL M.A. Welch, Y. Tan, J.S. Brog* and D.S. Zahm. Department of Anatomy and Neurobiology, St. Louis Univiversity School of Medicine, St. Louis, MO, 63104.

Haloperidol (hal), an antagonist with preference for dopamine $D_{2/3}$ receptors, and amphetamines elicit increases in striatal concentrations of neurotensin (NT), expression of striatal proNT mRNA and numbers of striatal NT immunoreactive (IR) neurons, suggesting that NT expression is suppressed by $D_{2/3}$ receptor agonists and facilitated by agonists acting at $D_{1/5}$ receptors. It is possible that striatal NT expression is co-regulated by $D_{2/3}$ *and* $D_{1/5}$ receptors, either co-localized or otherwise interacting. Here, the distributions of striatal NT-IR neurons and fibers, demonstrated with conventional immunoperoxidase methods, were compared following adminstrations of hal (0.25 mg/kg), d-amphetamine (amph, 4 mg/kg), hal&amph (same doses combined) and vehicle given thrice at 24, 8 and 4 hours before sacrifice. Numbers of NT-IR neurons were recorded in olfactory tubercle, nucleus accumbens core and shell and rostral, dorsomedial, dorsolateral, ventrolateral, central and caudal parts of the caudate-putamen. Patterns of immunostained fibers in the pallidum and substantia nigra were also evaluated. In most districts this sequence was observed: vehicle < amph < hal&amph < hal. The greatest production of NT-IR neurons, however, was in the olfactory tubercle following amph and was antagonized by hal. Amph and hal&amph elicited fiber staining in parts of substantia nigra reticulata and globus pallidus exceeding that seen after hal alone. The data suggest significant amph-hal interactions in the regulation of striatal perikaryal and fiber NT-IR. Supporting D_1 antagonist (SCH-39166) co-administration data are planned, but unavailable at this writing. Support: NS-23805.

162.14

MEASUREMENT BY SEMI-QUANTITATIVE RT-PCR OF C-FOS mRNA TRANSCRIPTS INDUCED BY DEXFENFLURAMINE IN RAT STRIATUM. B. Guibert[1], C. Jacquot[1], H. Lebrec[2] and A.M. Gardier*[1]. [1]Lab. Neuropharmacol. JE MESR 92-372; [2]Lab. Toxicol. CJF INSERM 9301, Fac. Pharmacie, Univ. Paris-Sud, F92296 Chatenay-Malabry, FRANCE.

A single dexfenfluramine (d-fen) administration increases the extracellular serotonin (5-HT) levels in various brain regions as measured by *in vivo* microdialysis in rats. This presynaptic effect reflects the drug's ability to enhance central serotoninergic transmission by both increasing the release of 5-HT and inhibiting its reuptake from nerve endings. However, little is known about the cascade of molecular events that follows d-fen's activation of postsynaptic receptors. Studies focusing on the expression of immediate early genes (IEGs) in nuclei of postsynaptic neurons might help to identify the selective neuroanatomical loci targeted by the drug. IEGs encode transcription factors that may be involved in the control of genomic events following neuronal stimulation. To better understand the rapid transcriptional activation of IEGs induced by d-fen, we studied the time course of c-*fos* induction by this drug in the caudoputamen (CPu) and whether this c-*fos* induction occurs in a dose-dependent manner. In a first experiment, rats were injected with a single dose of d-fen (10 mg/kg, i.p.) or saline (2 ml/kg). Then, we extracted total RNA from the CPu 0.5, 1, 2 and 6 hr after d-fen injection, converted extracted mRNA to cDNA by reverse-transcription and amplified cDNA by polymerase chain reaction (RT-PCR) for 30 cycles. Each level of c-*fos* mRNA is expressed as percentage of ß actin mRNA expression. The time course study of c-*fos* mRNA induction demonstrated that d-fen induced a transient increase in c-*fos* mRNA that reached its maximum at 0.5-1 hr after d-fen injection and had returned to control levels after 6 hrs. In a second experiment, rats received a range of d-fen doses (0, 5, 10, 20, 40 mg/kg, i.p.) and RT-PCR analysis was performed on RNA isolated from the CPu 1 hour after the single drug injection. mRNA expression of c-*fos* and ß actin was quantified by using successive fractions of cDNA. A dose-related c-*fos* gene expression induced by d-fen was observed in the CPu. These results confirm our previous immunohistochemical findings showing a dose-related increase in Fos protein levels in rat CPu 2 hrs after a single d-fen administration.

162.15

Epibatidine induces c-fos expression selectively in the limbic striatum. M. Cola and A. Jayaraman *, Dept. of Neurology, LSU Sch. of Med. New Orleans, LA 70112.

Epibatidine is a powerful antinociceptive agent that mediates its analgesic properties through nicotinic acetylcholinergic receptors. Epibatidine is also a potent releaser of dopamine in striatal slices. To study the interactions between epibatidine and dopamine, we have studied the pattern of expression Fos in the mesolimbic and mesostriatal systems. Acute injections of epibatidine resulted in the expression of Fos-like immunoreactivity in the nucleus accumbens and medial caudate nucleus and the prefrontal cortex. In the midbrain neurons with Fos-Li were noted in the medial and the lateral terminal nuclei of the accessory optic system, the superficial layers of the superior colliculus and the interpeduncular nucleus. Only very few neurons of the nucleus parabrachialis pigmentosus and caudal linear subdivisions of the ventral tegmental dopamine neurons were labeled with Fos-Li. Fos reactive cells were conspicuously absent in substantia nigra pars compacta. The pattern of expression of Fos-Li in the midbrain and the striatum shows significant similarities to the pattern of Fos-Li induced by acute injections of nicotine. Supported by the Dept. of Defense.

162.16

COMPARISON OF NEURONAL GENE INDUCTION BY DIFFERENT DRUGS OF ADDICTION USING IMMUNOCYTOCHEMISTRY AND TWO-DIMENSIONAL PROTEIN ELECTROPHORESIS.
M.W. Sganga, B. Bontempi, K. Baner, S. Massa, S. Sagar* and F. Sharp.
Dept. of Neurology, Veterans Administration Medical Center and University of California, San Francisco 94121.

Induction of immediate early genes has been shown to play an important role in the activation of neuronal pathways associated with addiction. Little is known about the genes targeted by the activation of these transcription factors and how they may differ between various drugs. The induction of c-fos in the rat striatum was studied immunocytochemically following systemic administration of morphine, cocaine, nicotine, and the caffeine analogue IBMX. While induction of Fos was common to morphine, cocaine and IBMX, each had its own specific regional pattern. Morphine induced Fos primarily in the dorsomedial striatum, while IBMX induced Fos mainly in the lateral striatum. Cocaine induced Fos over the entire striatum. Also, while nicotine has been shown to induce Fos expression in other brain regions, we were unable to demonstrate any such activity in the striatum. The implications of these different patterns of IEG induction were analysed by large format two-dimensional electrophoresis of proteins from primary cultured neurons treated with addictive drugs. The time course of gene induction in cerebellar granule neurons revealed a very similar pattern of IEG induction after short treatments, but vastly different patterns of protein expression after longer treatments with these drugs. These in vivo and in vitro models of the neuronal plasticity associated with adaptation to addictive drugs will help delineate the course of molecular events which underlie the transition from acute to chronic effects of these substances.
Supported by grants from the NIH and the VA.

162.17

SUPPRESSION OF AMPHETAMINE-INDUCED c-fos AND ngfi-a EXPRESSION IN THE STRIATUM USING END-CAPPED ANTISENSE OLIGONUCLEOTIDES: FUNCTIONAL AND BEHAVIORAL ANALYSES
M.O. Hebb*, M.Hong and H.A. Robertson. Lab. Mol. Neurobiol., Dept. of Pharmacology, Dalhousie University , Halifax,N.S., Canada B3H 4H7.

We have studied the intrastriatal application of phosphothioate end-capped antisense oligonucleotides directed at c-fos or ngfi-a mRNA transcripts to determine if sulfur substitution solely at terminal nucleotides will be sufficient to produce nuclease-resistant oligonucleotides. Additionally, we wish to know if these antisense oligodeoxynucleotides are effective at both knocking down expression of the targeted protein and minimising toxicity. The end-capped oligonucleotides directed at c-fos and ngfi-a used in the present study were effective at reducing the expression of their targeted proteins for a maximal period of between 2-4 hours following infusion. Immunohistochemistry showed that while antisense oligonucleotides directed at c-fos mRNA had no effect on amphetamine-induced ngfi-a expression, those directed at ngfi-a transcripts reduced both the targeted protein as well as c-fos expression. Also, both oligonucleotides produced negligible toxicity. Unilateral administration of either antisense oligodeoxynucleotide produced an ipsiversive rotational bias when the animals were challenged with D-amphetamine. Infusions of random oligodeoxynucleotides or vehicle produced no decrease in c-fos or ngfi-a expression nor a significant rotational bias following amphetamine injection. These results suggest that while end-capped oligonucleotides may be degraded relatively rapidly, they are effective in suppressing expression of specific proteins for short time periods (2-4 hours). Also, the previously reported correlation between Fos protein knockdown and rotational bias is further supported and a possible link between ngfi-a and c-fos expression is suggested. [Supported by the MRC, SmithKline Beecham, and the Huntington Society of Canada.]

162.18

INTRASTRIATAL CHOLINERGIC RECEPTORS MEDIATE AMPHETAMINE-INDUCED BEHAVIOR AND NEUROPEPTIDE GENE EXPRESSION.
J. F. McGinty* and J. Q. Wang. Dept. Anatomy & Cell Biology., East Carolina Univ. School of Medicine., Greenville, NC 27858-4354.

Systemic administration of the muscarinic receptor antagonist, scopolamine, augments, whereas the muscarinic receptor agonist, oxotremorine attenuates, stereotypical behaviors and striatonigral gene expression induced by amphetamine. In contrast, scopolamine blocks, and oxotremorine augments, amphetamine-induced preproenkephalin (PPE) mRNA induction in striatopallidal neurons (Wang & McGinty in press). We investigated the site of action of these effects by administering scopolamine and oxotremorine directly into the striatum. Three h. after a systemic saline or amphetamine (2.5 mg/kg, i.p.) injection, the rats were euthanized and the brains processed for quantitative in situ hybridization. Unilateral intrastriatal scopolamine augmented, whereas oxotremorine attenuated, amphetamine-induced behaviors. Intrastriatal infusion of 75, but not 7.5, mM scopolamine increased basal substance P (SP) and preprodynorphin (PPD) mRNA in the dorsal striatum. In addition, both 7.5 and 75 mM scopolamine significantly augmented amphetamine-induced SP and PPD mRNA levels. Intrastriatal infusion of 3 and 15 mM oxotremorine did not alter basal levels but both doses completely blocked amphetamine-induced SP and PPD mRNA. In contrast, a small but significant increase in PPE induced by amphetamine was completely blocked by 75, but not 7.5, mM scopolamine whereas both doses of oxotremorine slightly, but non-significantly, augmented basal and amphetamine-stimulated PPE mRNA levels in the dorsal striatum. These data indicate that cholinergic interneurons in the dorsal striatum exert a potent influence on amphetamine-induced stereotypies and striatal neuropeptide gene expression. Supported by DA03982.

162.19

THE EFFECT OF CHRONIC HALOPERIDOL TREATMENT ON DENDRITIC SPINES IN THE RAT STRIATUM.
J.J. Kelley, X.M. Gao, C.A. Tamminga, and R.C. Roberts*. Maryland Psychiatric Research Center, Department of Psychiatry, University of Maryland School of Medicine, Baltimore, MD 21228.

Previous ultrastructural studies have shown that schizophrenics in comparison to controls have reduced spine density in the cortex and smaller spine size in the striatum. The current study in rat was conducted to determine whether such differences could result from neuroleptic treatment and whether they correlated with oral dyskinesias. Sprague-Dawley rats were administered 1.5 mg/kg/day of haloperidol (HA) (n=28) or water (n=10) for 6 months and tested for vacuous chewing movements (VCMs). After 6 months, rats were divided into low (<10VCMs/5min, n=15) and high (>10VCMs/5min, n=13) VCM groups; all but 7 high VCM rats (withdrawn group) were sacrificed. These rats were withdrawn from HA for 4 weeks before sacrificing. Random electron micrographs of the striatum were analyzed for spine changes. Criteria for spine identification consisted of a dendritic protrusion or a solitary spine receiving a synapse. Spine size was not significantly effected by HA (0.193 vs 0.174 (control) nor correlated with oral dyskinesias (0.191 vs 0.196, low and high VCM groups). These results suggest that the difference in spine size seen in schizophrenic striatum may be correlated with the disease rather than caused by neuroleptic treatment. Spine density decreased in the HA treated group (32.7 ± 9.5) in comparison to controls (53.7 ± 7.3, $p < .001$) and remained low in the withdrawn group (35.0 ± 4.2, $p < .01$). Spine density decreased in both the low (37.3 ± 9.9, $p < .01$) and the high (28.0 ± 7.0, $p < .000$) VCM rats in comparison to controls; however there was no significant difference between high and low VCM rats. The observed decrease in spine density within the striatum of chronic HA treated rats suggests a drug effect; this may be correlated with the decreased synaptic density that we have previously observed. The results suggest that decreased spine density observed in schizophrenics may be a result of chronic neuroleptics. Supported by the Stanley Foundation.

163.1

Regulation of dopamine fibre ingrowth into the striatum by metabotropic glutamate receptors. B. Teng*, D. Plenz and S.T. Kitai, Department of Anatomy and Neurobiology, University of Tennessee, College of Medicine, Memphis, TN 38163.

Glutamatergic inputs from the cortex and dopaminergic inputs from the substantia nigra converge in the striatum. We therefore studied the interaction of these two pathways during development using a triple culture preparation.

Cortical, striatal and nigral slices from rats at postnatal day 0 - 1 were cultured for 16 *days in vitro* (DIV) using a modified roller-tube technique. After 8 DIV glutamate receptor antagonists and/or agonist were added to the culture medium for 8 days, after which the cultures were fixed and processed for tyrosine hydroxylase (TH) immunoreactivity. The TH-fibre density was analyzed using confocal microscopy and correlated with total number of TH-positive neurons present in the nigral tissue.

The metabotropic glutamate recptor antagonist L-AP3 (100 µM) decreased TH-fibre density by 70%, whereas the blockade of non-NMDA and NMDA neuronal transmission using 50 µM DNQX and 50 µM APV had no effect on striatal TH-fibre density, the morphology and numbers of TH-positive neurons. Furthermore, the addition of the metabotropic agonist 1S,3R-ACPD (100 µM) increased the TH-fibre density in the striatum by 180% without affecting the total number of neurons in the mesencephalic tissue. This increase in TH-fibre density was antagonized by L-AP3.

These results indicate that metabotropic glutamate receptors activation increases the density of TH-positive fibres in the striatum during development. We propose that glutamatergic transmission in the striatum can regulate the development of the nigro-striatal pathway.

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163.2

ROLE OF NMDA RECEPTORS OF THE STRIATUM IN THE INITIATION AND MAINTENANCE OF APPETITIVE ACTIONS. M. Pisa* and S. Shangardass, Dept. Biomed. Sci., McMaster Univ., Hamilton, Ont., Canada, L8N 3Z5.

In support of the hypothesis that NMDA receptors of the rat's dorsal striatum are involved in a response-selection mechanism, we previously reported that injections of the NMDA receptor blocker CPP into the lateral, "sensorimotor"subregion of the striatum increased a *consummatory*-type of oral movement, i.e. chewing in vacuo, while decreasing body grooming, a behavior that involves *appetitive*, i.e. reaching, movements of the mouth. Here, we extended these observations to examining the effects of CPP injections into the lateral striatum on appetitive movements required to feed on relatively large (2x3 cm) Purina Chow food pellets. Food-restricted rats with chronic intracerebral guide cannulae were examined for food consumption and feeding movements after injections of either vehicle or CPP (either 0.08 or 0.4 or 2 nmole in 0.2 µl) into the lateral striatum, with dose as between-factor, N=8. Compared with vehicle, the effects of CPP were as follows: 1) food consumption significantly changed with increasing dose, with virtually no change at the 0.08 nmole dose and a decrease to 44% and 7% of control with 0.4 nmole and 2 nmole, respectively; 2) the 0.4 nmole dose increased the latencies to first contact with food and to first food bite, and also increased the duration of the intervals spent not eating between food-chewing bouts; 3) the 2 nmole dose virtually blocked the initiation of any feeding movements, including pellet picking, holding and biting, even though the number of snout approaches to the food pellets increased 4.5 times over baseline. The results indicate that activation of NMDA receptors of the lateral striatum is crucial for the initiation and maintenance of appetitive, contact-manipulatory actions, although not of actions reflecting motivational sensitivity (e.g. snout approach to food). Together with our previous results, they further support the hypothesis of a NMDA-receptor mediated mechanism in the striatum that controls the switching between appetitive and consummatory motor components of goal oriented actions. (Supported by NSERC).

163.3

NMDA RECEPTOR DEVELOPMENT IN NEOSTRIATUM: II. WHOLE-CELL VOLTAGE CLAMP ANALYSIS OF CURRENTS IN VISUALLY IDENTIFIED CELLS. C. Cepeda*, L. Shumate, C.S. Colwell, M.S. Levine. Mental Retardation Research Center, University of California, Los Angeles, CA 90024.

These experiments were designed to examine the development of NMDA-induced membrane currents and compare their maturation with that of currents induced by application of kainate (KA) in neostriatal (NS) cells. Whole-cell patch clamp recordings were obtained from visually identified NS neurons using infrared differential interference contrast videomicroscopy. Data were obtained from slices from rat pups of 4-18 postnatal days (PNDs). Induced currents were analyzed using two paradigms. In the first, NMDA and KA were applied iontophoretically 15-30 μm from the recorded cell and inward currents were examined in standard artificial cerebrospinal fluid. In the second, NMDA or KA were bath-applied (10-30 μM concentrations) and changes in currents induced by ramp voltage commands were analyzed also in standard artificial cerebrospinal fluid. At PNDs 4-7, NMDA-induced currents were small regardless of holding potential whereas KA-induced currents were typically larger. By PNDs 12-14, NMDA currents increased considerably. KA-induced currents also displayed further increases in amplitude over the first two postnatal weeks of age. At all ages examined, NMDA-induced currents were always smaller than KA-induced currents. The present findings indicate that NMDA- and KA-induced currents in NS neurons develop over the first two postnatal weeks in the rat.

Supported by USPHS Grant HD 05958.

163.4

NMDA RECEPTOR DEVELOPMENT IN NEOSTRIATUM: I. CELL SWELLING AND RECEPTOR BINDING. M.S. Levine*, C.S. Colwell, C.A. Crawford, C.Cepeda. Mental Retardation Research Center, University of California, Los Angeles, CA 90024.

NMDA receptors serve many crucial functions in the maturing nervous system. The present studies were designed to examine functional development of NMDA receptors in the rat neostriatum (NS) using two experimental approaches. In the first approach, infrared differential interference contrast video microscopy was used to measure the development of cell swelling induced by NMDA and kainate (KA). This technique permits viewing live cells in brain slices without using dyes or stains. Swelling of single cells was assessed in response to bath application of NMDA or KA (1-1000μM). At postnatal day (PND) 3, there was no measurable response to NMDA while cells swelled in response to KA. By PND 7, NMDA caused swelling although responses were smaller than those found in older tissue. By PNDs 14-28, NMDA and KA produced concentration-dependent swelling which did not vary further with age. These results form the basis of a developmental time-course and suggest that NMDA receptor function develops later than KA receptor function in the NS.

The second approach examined the possibility that the developmental changes in NMDA responses may be due to age-related variation in receptor number. [^{3}H] MK-801 was used in receptor binding assays with homogenates of NS tissue. The number of binding sites was significantly lower at PNDs 3-14 than at later ages. The affinity of the receptor for the ligand did not change over the age period examined. Thus, variation in receptor binding can only partially account for developmental changes in NMDA receptor function.

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163.5

MODULATION OF NMDA-INDUCED CURRENTS BY METABOTROPIC GLUTAMATE RECEPTORS IN VISUALLY IDENTIFIED NEOSTRIATAL NEURONS. C.S. Colwell*, C. Cepeda, M.S. Levine. Mental Retardation Research Center, University of California, Los Angeles, CA 90024.

Previous data indicate that activation of metabotropic glutamate receptors (mGluRs) selectively inhibits NMDA-evoked responses in neostriatal (NS) slices. The present study was designed to examine mechanisms underlying this regulation. Whole cell patch clamp recordings were obtained from NS neurons visualized in slices using infrared differential interference contrast video microscopy. Iontophoretic application of NMDA and Kainate (KA) induced currents which were blocked by their appropriate antagonists AP5 and CNQX, respectively. NMDA currents were voltage-dependent and were enhanced in low magnesium. Bath or iontophoretic application of the mGluR agonist tACPD inhibited NMDA-induced currents but produced variable effects on KA-induced currents. mGluR inhibition of NMDA currents occurred at membrane potentials ranging between -70 and -40 mV and appeared to be mediated by regulation of voltage-sensitive calcium channels (VSCC). Inhibitory effects of tACPD were prevented by application of cadmium (50μM), a blocker of VSCCs. Furthermore, the addition of fluoride (125mM) into the internal solution of the electrode also prevented the tACPD-induced inhibition. Among other actions, fluoride blocks VSCCs. Together, these findings indicate that mGluRs postsynaptically regulate NS NMDA-induced currents via mechanisms involving calcium conductances.

Supported by USPHS Grants MN10735 to CSC and HD05958 to MSL.

163.6

EFFECTS OF NMDA RECEPTOR BLOCKADE ON DEVELOPMENT OF RAT CORTICOSTRIATAL SYNAPTIC INPUT N.A. Sharpe*, and J.M. Tepper, Aidekman Research Center, Center for Molecular and Behavioral Neuroscience, Rutgers University, Newark, NJ USA 07102.

Glutamate containing corticostriatal afferents form asymmetric synapses onto spine heads of medium spiny neurons. These synapses develop primarily during the 3rd post-natal week. Glutamate, and NMDA receptors, have been suggested to play a role in this development. To evaluate the role of NMDA receptors, MK-801 was administered once daily (0.25 mg/kg, i.p.) from P15 to P21. On P22, biocytin was iontophoretically injected into the frontal cortex. Rats were sacrificed 4-6 hours later and the striatum prepared for biocytin visualization and sequential light and electron microscopy.

Light microscopy revealed that the striatum of MK-801-treated rats appeared similar to controls based on the level of myelination. Biocytin labeled corticostriatal afferents were found to terminate almost exclusively on dendritic spines, as in control rats. No difference was noted in the distribution of asymmetric synapses with 87% of asymmetric synapses in MK-801 rats being axospinous compared to 83% in controls. In several cases, however, asymmetric synapses terminated on spine shafts near the base of the spine, which appeared unusually large. This type of synaptic contact was not seen in control rats. The mean number of vesicles per synapse, and mean active zone length for asymmetric axospinous synapses were similar for both MK-801 (33.1 $\pm$ 5.1 ves/syn, 256 $\pm$ 14 nm, respectively) and control (31.5 $\pm$ 3.3 ves/syn, 256 $\pm$ 18 nm, respectively) rats.

These data indicate that chronic blockade of NMDA synaptic input during the 3rd postnatal week had a minimal effect on corticostriatal asymmetric synapses. This may be because NMDA receptors have a minimal role in the development of corticostriatal synapses or because the NMDA blockade may need to be maintained longer or begun earlier in development. The latter appears more likely since following MK-801 treatment corticostriatal afferents are found to terminate more often at the base of large spines. This type of re-organization could give individual excitatory inputs a larger influence on the electrophysiological status of the spiny neuron. Additional MK-801 studies of longer duration are needed to conclusively determine the role of the NMDA receptor in corticostriatal synapse development. Supported by NS 30679.

163.7

FLUOXETINE AND DOI REDUCE MRNA LEVELS OF NEUROPEPTIDES AND GLUTAMIC ACID DECARBOXYLASE IN THE STRIATUM OF THE RAT.

M.J. Mijnster, T. van Haeften* and P.Voorn
Research Institute Neurosciences, Department of Anatomy, Vrije Universiteit, Amsterdam, the Netherlands.

In order to investigate serotoninergic effects on striatal gene regulation, the serotonin reuptake blocker Fluoxetine (FLU, 10 mg/kg) and the serotonin 5-HT2 agonist DOI (7 mg/kg) were administered daily i.p. for 5 and 9 days respectively. Quantitative *in situ* hybridization was used in the caudate-putamen to study changes in mRNA levels of preproenkephalin (ppEnk), preprodynorphin (ppDyn), preprotachykinin (ppT) and glutamic acid decarboxylases (GAD) 65 and 67. The indirect agonist FLU was found to reduce ppEnk mRNA levels with 14% over the entire rostro-caudal extent of the caudate-putamen, whereas DOI had no effect. Both the direct and the indirect agonist tended to reduce ppDyn (-16% and -6% respectively) and GAD 65 mRNA. In contrast, ppT and GAD 67 mRNA levels were not altered after FLU and only slightly increased after DOI administration. The preferential regulation of GAD 65 mRNA was unexpected in view that GAD67 and not GAD65 is thought to be regulated at the transcriptional level. Considering that two different striatal output-pathways exist, one marked by ppDyn/ppT/GAD65 and 67 and the other containing ppEnk/GAD65 and 67, we conclude that the activity in both output-pathways of the striatum appears to be inhibited by the indirect serotonin agonist FLU and that the serotonin 5-HT2 agonist DOI reduces activity in the direct route. However, since GAD65 is produced in both routes, it cannot be excluded that DOI administration also affects the indirect route.

163.8

REGULATION OF STRIATAL PREPROENKEPHALIN AND PREPROTACHYKININ mRNAS FOLLOWING SEROTONIN STIMULATION. J.M. Sall*, T.J. Cummings & P.D. Walker, Depts of Anatomy & Cell Biology, Psychiatry & Behavioral Neurosciences (Cellular & Clinical Neurobiology Program), and Neurosurgery, Wayne State University School of Medicine, Detroit, MI 48201

Serotonin neurotransmission is hypothesized to provide a positive regulation of striatal tachykinin and enkephalin biosynthesis. In this study, we determined the effects of 5-HT_1 receptor stimulation on preproenkephalin (PPE) and preprotachykinin (PPT) mRNA levels within the rodent striatum. Adult male SD rats (175-200g) received single intraperitoneal injections of saline or the 5-HT_1 agonist RU24969 (5 mg/kg) followed by sacrifice 4 hours later. HPLC analysis with electrochemical detection revealed significant increases in tissue serotonin (5-HT) content accompanied by decreased levels of 5-hydroxyindoleacetic acid (5-HIAA) within the striatum as well as tissues containing the substantia nigra and midbrain raphe. The ratio of 5-HT to 5-HIAA was also significantly heightened in these tissues indicating that RU24969 causes increased storage of 5-HT in raphe neurons and terminal fields. Northern analysis revealed significant increases (>200%, $p<0.003$) in striatal PPE mRNA levels in the RU24969 group as compared to saline-injected controls. Striatal PPT mRNA amounts were only mildly raised in the RU24969 group. These results indicate that striatal enkephalinergic neurons may be more sensitive to 5-HT_1 receptor stimulation with RU24969 as compared to striatal tachykinin neurons. Additional *in situ* hybridization experiments will examine whether mRNA expression in specific striatal subregions is differentially sensitive to 5-HT_1 stimulation. Supported by NIH NS30550.

163.9

SEROTONIN DEPLETION EXACERBATES STRIATAL GENE EXPRESSION CHANGES DURING THE NMDA RECEPTOR-MEDIATED EXCITOTOXIC CASCADE. T.J. Cummings* & P.D. Walker, Depts of Anatomy & Cell Biology, Psychiatry & Behavioral Neurosciences (Cellular & Clinical Neurobiology Program), and Neurosurgery, Wayne State Univ. School of Medicine, Detroit, MI 48201.

Controversy exists as to whether serotonin (5-HT) plays a neuroprotective role when the brain is subjected to excitotoxic damage. We sought to test the hypothesis that neuronal loss in the striatum would be greater in the serotonin-depleted rat following induction of the excitotoxic cascade. Adult male SD rats (175-200g) received intraperitoneal injection of saline or *p*-chlorophenylalanine (*p*CPA, 300mg/kg) to block 5-HT synthesis. After 3 days, these rats received intrastriatal injection of saline or quinolinic acid (QA, 40µg in 1µl saline). All rats were sacrificed 6 or 48 hours later. Striatal tissue containing the saline or QA injection site was subjected to Northern mRNA analysis and HPLC-EC detection of monoamines. Contralateral uninjected striata served as intra-animal control tissue. Within all *p*CPA/saline or *p*CPA/QA groups, 5-HT levels were depleted greater than 90% as compared to saline/saline controls. At 48 hours post-QA injection, preproenkephalin (PPE) and preprotachykinin (PPT) mRNAs were markedly reduced within the striatal lesion site of saline/QA and *p*CPA/QA groups. In the *p*CPA/QA group, striatal PPE and PPT mRNA levels were further reduced as compared to the saline/QA group with PPE mRNAs reaching statistical significance at 95% (ANOVA with Fisher PLSD). Exacerbation of the excitotoxic lesion in the 5-HT-depleted rat was further exemplified by observations of a larger, prolonged zif/268 transcriptional response at 6 hours in the *p*CPA/QA compared to saline/QA groups. These results suggest that 5-HT depletion may adversely affect neuronal survival following intrastriatal excitotoxic damage. Supported by NIH NS 30550.

163.10

REGULATION OF BASAL GANGLIA NEUROPEPTIDE mRNAs BY SEROTONIN: ROLE OF SEROTONIN-2 RECEPTOR. P.J. Gresch*, T.J. Cummings, J.M. Sall & P.D. Walker, Departments of Psychiatry & Behavioral Neuroscience (Cellular and Clinical Neurobiology Program), Neurosurgery, and Anatomy & Cell Biology, Wayne State University School of Medicine, Detroit, MI. 48201

Decreased serotonin (5-HT) neurotransmission lowers preprotachykinin (PPT) and preproenkephalin (PPE) mRNA levels in the rat striatum (STR). In this study, we examined the effects of 5-HT_2 receptor stimulation on PPT and PPE mRNA levels in striatal subregions from control or 5,7-dihydroxytryptamine (5,7-DHT) lesioned rats. Adult male SD rats received 20µl icv injections of 5,7-DHT (10µg/µl) or vehicle (0.1% ascorbic acid). After 21 days, saline or the 5-HT_2 receptor agonist, DOI (1 mg/kg), was administered i.p. q.d. for 7 days. Whole STR or ventromedial (VM), ventrolateral (VL), and posterior (P) STR subregions were assayed for monoamines and metabolite levels by HPLC-EC and mRNA levels by Northern analysis. Striatal 5-HT levels were reduced >90% by 5,7-DHT as compared to controls. Striatal 5-HT levels were unchanged by DOI. Whole STR PPT and PPE mRNA levels were decreased in 5,7-DHT lesioned rats. DOI increased PPT and PPE mRNA levels to control levels in 5,7-DHT lesioned rats.

	5,7-DHT		DOI		5,7-DHT/DOI	
	PPT mRNA	PPE mRNA	PPT mRNA	PPE mRNA	PPT mRNA	PPE mRNA
VM-STR	118	105	162	137	133	106
VL-STR	48	37	89	72	83	76
P-STR	51*	38	156*	165*	132†	133

Expressed as % of veh/sal (n=3-5; *$p<0.05$ of veh/sal; † $p<0.05$ of 5,7-DHT/sal).

These results suggest that 5-HT_2 receptor stimulation reverses the reduction in striatal PPT and PPE mRNA expression in 5-HT-depleted animals. Supported by NIH NS 30550.

163.11

NMDA AND AMPA GLUTAMATE RECEPTORS IN STRIATAL IMMEDIATE EARLY GENE INDUCTION BY DOPAMINE D2 RECEPTOR BLOCKADE. J.D. Wagstaff * and C.R. Gerfen. Lab of Neurophysiology, NIMH, Bethesda, MD 20892.

In the striatum, removal of the tonic inhibitory influence of dopamine by blockade of D2 receptors results in the induction of immediate early genes in striatopallidal neurons. One possible mechanism responsible for such activation involves the unmasking of underlying glutamatergic excitatory input to these neurons. To examine this we used quantitative in situ hybridization histochemical localization of the mRNAs encoding the immediate early genes (IEGs) cfos and zif268 to mark changes in the function of striatopallidal neurons in response to D2 receptor blockade in the presence of NMDA or AMPA glutamate receptor antagonists. NBQX (20, 40 or 60 mg/kg) or MK 801 (1.0 mg/kg) was administered 15 minutes prior to treatment with the D2 antagonist eticlopride (1.0 mg/kg). Neither NMDA or AMPA antagonists alone affected the basal levels of immediate early gene expression in striatopallidal neurons. When combined with D2 receptor antagonist treatment (eticlopride, 1.0 mg/kg) the NMDA antagonist MK801 was without affect on the D2 antagonist induction of cfos or zif268, whereas the AMPA antagonist NBQX resulted in a potentiation of this response. These results suggest that systemic administration of AMPA and NMDA antagonists do not significantly reduce the induction of immediate early genes in the striatum following D2 dopamine receptor blockade. Moreover, these data suggest that blockade of AMPA receptors reduces the activity of local inhibitory circuits resulting in further disinhibition of D2 expressing striatopallidal neurons. (supported by NIMH)

163.12

DELTA AND MU OPIOID RECEPTOR REGULATION OF IMMEDIATE-EARLY GENE EXPRESSION IN STRIATOPALLIDAL NEURONS. H. Steiner* and C. R. Gerfen. Dept. of Anatomy and Neurobiology, Univ. of Tennessee, College of Medicine, Memphis, TN 38163, and Laboratory of Neurophysiology, NIMH, Bethesda, MD 20892.

We have shown that, in the striatum, dynorphin, an endogenous kappa opioid receptor ligand, regulates D1 dopamine receptor responses in striatonigral neurons. Striatopallidal neurons contain D2 dopamine receptors and the opioid peptide enkephalin, an endogenous ligand of delta and mu opioid receptors. In the present study, we investigated effects of enkephalin receptor activation on striatopallidal neurons, using D2 receptor-mediated immediate-early gene (IEG) induction as a functional marker. Expression of IEGs (*c-fos, zif 268*) was measured with *in situ* hybridization histochemistry. Intrastriatal infusion of the delta receptor-preferring agonist DADLE blocked IEG induction by the D2 antagonist eticlopride and induced contraversive turning in a dose-dependent manner. Both DADLE-induced blockade of IEG induction and turning were inhibited by systemic and intrastriatal administration of the nonselective opioid receptor antagonist naloxone. Blockade of IEG induction was also produced by intrastriatal infusion of the more selective delta agonist deltorphin, or by the mu agonist DAMGO, but not by the kappa agonist U-50488. However, in contrast to delta agonist-induced turning, mu agonist-induced turning was inhibited by the D2 antagonist. These results show that, in the striatum, both enkephalin receptors (mu and delta), but not the dynorphin receptor (kappa), inhibit IEG expression in striatopallidal neurons. The different effects of the D2 antagonist on mu and delta agonist-induced turning indicate that other striatal functions are differentially affected by these two receptors. (Supported by NIMH/DIRP and USPHS Grants NS26473 and NS20702).

163.13

EFFECTS OF DIFFERENT CLASSES OF NMDA RECEPTOR ANTAGONISTS ON D1 DOPAMINE RECEPTOR-MEDIATED CHANGES IN STRIATAL NEURON FUNCTION. K.A. Keefe* and A. Ganguly. Dept. of Pharmacology & Toxicology, Univ. of Utah, Salt Lake City, UT 84112.

Previous work has shown that intrastriatal administration of the non-competitive NMDA receptor antagonist MK-801 potentiates immediate early gene expression in lateral striatum induced by D1 dopamine receptor stimulation, whereas the competitive antagonists CPP and APV do not. We therefore hypothesized that NMDA receptor antagonists interacting preferentially with different NMDA receptor subtypes exert different effects on striatal function. To test this hypothesis, we are examining the effects of different classes of NMDA receptor antagonists on immediate early gene expression induced by stimulation of D1 dopamine receptors. In intact rats, systemic administration of CPP (0.1-10 mg/kg, i.p.) attenuated *zif268* induction by the D1 dopamine receptor agonist SKF 82958, although this effect was not dose-dependent, and CPP alone at the highest dose attenuated basal *zif268* expression. Like CPP, the polyamine-site antagonist ifenprodil (5 mg/kg, i.p.) also attenuated basal *zif268* expression. However, ifenprodil (0.05-5 mg/kg, i.p.) potentiated *zif268* induction by SKF 82958. Like ifenprodil, MK-801 potentiated the effects of SKF 82958 at lower doses (0.01-0.1 mg/kg, i.p.). At higher doses (1-5 mg/kg, i.p.), however, regional differences in the effects of MK-801 were observed. *Zif268* expression in medial striatum was attenuated relative to that seen with SKF 82958 alone, whereas it was maintained in lateral striatum. These findings provide further evidence that different classes of NMDA receptor antagonists interact differently with D1 dopamine receptor-mediated processes in striatum. (Supported by University of Utah Health Sciences Center and College of Pharmacy)

163.14

CORTICALLY-DRIVEN IMMEDIATE-EARLY GENE EXPRESSION IN STRIATAL NEURONS SHOWS SELECTIVITY FOR ENKEPHALINERGIC NEURONS OF THE RAT AND MONKEY STRIATUM A.M. Graybiel, H.B. Parthasarathy, A.W. Flaherty* and S. Berretta Dept. of Brain & Cognitive Sciences, M.I.T., Cambridge, MA 02139

The direct and indirect pathways of the basal ganglia have their primary origins in different populations of striatal projection neurons. Both cell types are GABAergic, but most indirect pathway neurons coexpress enkephalin, whereas most direct pathway neurons coexpress dynorphin and substance P. To examine activation of striatal projection neurons by the sensorimotor cortex, we electrically microstimulated physiologically identified sites in primary somatic sensory and motor cortex (SI, MI) in anesthetized squirrel monkeys (250 Hz, 40 msec pulses, Parthasarathy et al., Soc. Neurosci. Abstr 20:987, 1994) and used epidural picrotoxin (0.3 mM) to stimulate sensorimotor cortex in freely moving rats (Berretta et al., Soc Neurosci. Abstr. 1994). Both modes of cortical stimulation induced immunohistochemically detectable Fos- and JunB-like immediate-early gene proteins in the striatum within 2 hr after the start of stimulation. The induction followed known corticostriatal topography. In the monkey, the induced proteins were in cell clusters in the putamen that matched input fiber patches (matrisomes) labeled anterogradely from corresponding cortical sites. Dual antigen immunostaining demonstrated that most of the striatal neurons expressing Fos were enkephalin-positive (70-80%, rat; >70%, monkey). In rat, in which dynorphin immunostaining was possible, only 7% of the Fos-positive neurons were dynorphin-immunoreactive. Similar results were obtained in rat for JunB. These findings suggest that the sensorimotor cortex can selectively influence the indirect pathway of the basal ganglia and thus basal ganglia release functions. Supported by NIH Javits Award R01 NS25529.

163.15

CIRCUIT-LEVEL CHANGES IN bZip GENE EXPRESSION INDUCED IN THE STRIATUM DURING CHRONIC COCAINE TREATMENT AND WITHDRAWAL R. Moratalla* B. Elibol M. Vallejo[1] and A.M. Graybiel Dept. of Brain & Cognitive Sciences, M.I.T., Cambridge, MA 02139; [1]Reproductive Endocrine Unit, MGH, Harvard Medical School, Boston MA 02114

Repeated exposure to psychostimulants induces long-term changes in behavior characterized by addiction and sensitization. The neural mechanisms responsible for these long-term changes are not well understood, but pre- and postsynaptic changes in mesostriatal dopamine systems have been implicated. To examine the striatal changes, we administered cocaine to rats in a paradigm that produces behavioral sensitization and studied the changes in inducibility of Fos-, JunB- and Fra-like proteins in the striatum of rats treated with cocaine 25 mg/kg i.p., b.i.d. for 7 days and challenged with cocaine after 18 hrs or 3, 7 or 14 days of withdrawal. To detect changes during the chronic treatment, other rats were challenged after 4 days. After a week of chronic cocaine treatment, the numbers of neurons that expressed Fos 2 hr after cocaine challenge decreased 90% after 7 days, whereas JunB expression was reduced by half and Fra expression remained the same. Levels gradually returned to control values during withdrawal. A cluster of 35-37 kDa Fras with extended half-lives was detectable without challenge for up to 7 days of withdrawal by Western blot analysis. As we have previously shown, acute cocaine induced Fos/JunB proteins in both striosome and matrix compartments of the striatum. However, after the chronic cocaine treatment, the anatomical distributions of induced Fos/JunB expression shifted towards striosomal predominance in the anterolateral striatum. The pattern change was evident during the chronic treatment (day 5), persisted throughout the 2-week withdrawal period, and was observed for Fos and JunB as well as for persistently expressed Fras. This system-level change thus had a time course paralleling behavioral sensitization. Supported by NIDA 5R01 DA08037.

163.16

EFFECTS OF CHRONIC COCAINE EXPOSURE ON CORTICOSTRIATAL TRANSMISSION IN THE RAT V. Hillegaart*[1], S. Berretta and A.M. Graybiel Dept. of Brain & Cognitive Sciences, M.I.T., Cambridge, MA 02139

A single shot of cocaine (25 mg/kg, i.p.) rapidly induces expression of Fos and Fos-family immediate-early gene proteins in striatal neurons. Repeated exposure to cocaine (25 mg/kg, b.i.d.) leads to a sharp decline in inducibility of Fos that is nearly complete after 7 days. In the present experiments we asked whether this depression of Fos inducibility would affect the capacity for cortical stimulation to induce Fos-like protein in the caudoputamen. The motor cortex of freely moving rats was stimulated with epidural local application with the $GABA_A$ receptor antagonist, picrotoxin (18 mg/100 ml). Evoked motor tics were noted and the expression of nuclear Fos-like immunoreactivity (Fos-LI) in striatal neurons was monitored postmortem by immunohistochemistry (Berretta *et al.*, Soc. Neurosci. Abstr. 20:987, 1994). The induction of Fos-LI was analyzed by counting Fos-positive nuclei with a quantitative computer-assisted method. Values were subjected to a log transformation and expressed as means±S.D. (nuclei/striatal section). The levels of Fos induction were studied in four experimental groups: (1,2), rats pretreated for 7 days with cocaine (25 mg/kg i.p., b.i.d.) and given picrotoxin epidurally (1.90±0.40) or saline epidurally (0.82±0.44); (3) rats pretreated for 7 days with saline (i.p.) and given picrotoxin epidurally (2.28±0.44); (4) control rats pretreated for 7 days with cocaine (25 mg/kg i.p., b.i.d.) and given a final challenge of cocaine (25 mg/kg i.p.) (1.35±0.13). As predicted, chronic cocaine strongly down-regulated induction of Fos-LI by subsequent cocaine challenge. Two-tailed Student's *t*-tests between groups showed that in rats chronically treated with cocaine, cortical stimulation can still significantly increase Fos induction if compared to the down-regulation shown with cocaine challenge $t_{(18)}=3.46$ ($p<0.01$). However, the Fos levels induced by cortical stimulation were significantly lower than those in rats pretreated with saline $t_{(23)}=2.24$, ($p<0.05$). We suggest that chronic exposure to cocaine induces network-level changes that affect the efficacy of corticostriatal transmission. Supported by NIDA 5R01 DA08037 and [1]Swedish MRC K96-12P-11008-03A.

163.17

DEVELOPMENTAL CHANGES IN BASAL GANGLIA RESPONSIVENESS TO AMPHETAMINE AND COCAINE. E. Fusco* and A.M. Graybiel Dept. of Brain & Cognitive Sciences, M.I.T., Cambridge, MA 02139

The induction of immediate-early genes (IEGs) provides a sensitive cellular assay to test functional responsiveness of neurons to stimulation. We have used an immunohistochemical IEG expression assay to test, in the rat, the functional maturation of striatal responsiveness to dopaminergic stimulation. We used polyclonal antisera against FOS, FRA and NGFI-A..

Cocaine (25mg/kg) and amphetamine (5mg/kg) both induced FOS expression predominantly in the striosomal compartment during the first postnatal week, confirming Johnson K. et al., 1991. Induction of FRA and NGFI-A also was confined to the striosomal compartment during this time. FOS induction was dependent on D1-class dopamine receptors, as pretreatment with the selective D1-class antagonist SCH23390 at doses of 0.2mg/kg blocked FOS expression. FRA and NGFI-A exhibited basal expression in striosomes but this was not completely blocked by pretreatment with SCH23390. Remarkably, although cocaine and amphetamine treatments led to a robust IEG induction in the striatum at P6/P7 and at P21, they did not at P15. Conversely, there was strong IEG induction in the globus pallidus (GP) at P15, but induction in GP was low at P6/P7 and P21. We tested whether the IEG induction in the GP required D1- or D2-class dopamine receptors by using pretreatments with SCH23390 or the D2-class antagonist, etilclopride (0.5mg/kg). Pretreatment with the D1 antagonist did not block the GP expression. The D2 antagonist suppressed the IEG induction in the GP and induced IEG expression in the striatum. By P21, the IEG induction acquired mature patterns of expression.

These results suggest that significant modifications occur in the effects of psychomotor stimulants on the basal ganglia through a prolonged period of postnatal development. We thank Drs. M.. Iadarola and J. Milbrandt for antisera. Supported by NIDA grant 5R01 DA08037.

163.18

CHOLINERGIC PRESYNAPTIC MODULATION OF GLUTAMATERGIC AFFERENTS TO THE NEOSTRIATUM AS SEEN WITH PAIRED PULSE FACILITATION AND 4-AP-INDUCED RELEASE E. Hernández, E. Galarraga and J. Bargas* Instituto de Fisiología Celular, UNAM, POBox: 70-253, México City DF 04510 México.

Both 4-AP-induced release of transmitters and paired pulse facilitation have been validated as a way to evaluate presynaptic modulation of transmitter release (e.g., Tibbs et al., J Neurochem 53: 1693; Dunwiddie and Haas, J Physiol 369: 365; Flores-Hernández et al., J Neurophysiol 72: 2246; Soc Neurosci Abs 1994: 563). Here we use both methods to evaluate cholinergic modulation of neostriatal afferents. In the presence of 10 μM bicuculline, cholinergic agonists 1 μM carbachol or 20 nM muscarine, significantly enhanced (μ ± SD: 53 ± 2 and 94 ± 20 %, respectively) the second synaptic potential of a pair of responses separated by 25-50 ms, evoked by field stimulation, and recorded intracellularly in a slice preparation. Facilitation of the second response with respect to the first one occurred in spite of a reduction in both responses by the cholinergic agonists. Paired pulse facilitation by cholinergic agents was blocked by 1 μM atropine and 100 nM gallamine, but not by 500 nM pirenzepine, suggesting that cholinergic-muscarinic effects are not mediated by $M_{1\ or\ 4}$-receptors. Cholinergic agonists decreased transmitter release induced by 100 μM 4-AP in about 50% of experiments, suggesting that not all afferents may be modulated. This effect was reversed by atropine and occurred with no significant changes in synaptic potentials' mean amplitude or distribution. At the concentrations used, no cholinergic agent caused a change in postsynaptic input resistance, membrane potential or firing threshold. Financed by DGAPA-UNAM and CONACyT.

163.19

ION MECHANISM FOR DOPAMINERGIC EXCITATION IN NEOSTRIATAL NEURONS. S. Hernández, J. Bargas, A. Reyes and E. Galarraga* Instituto de Fisiología Celular, UNAM, POBox: 70-253, México City DF 04510 México.

As reported previously (e.g., Hernández et al., 1995; Soc. Neurosci: 913), dopaminergic D_1-receptor agonists inhibit firing in neostriatal neurons when stimulated at resting membrane potential (ca., -80 mV). Here we report that, at depolarized membrane potentials (> - 60 mV), D_1-agonists turn out to be excitatory on all neostriatal neurons tested. Thus, dopaminergic action is voltage-depependent. Slow maintained depolarizations were elicited in neostriatal neurons by brief intracellular current steps given at depolarized membrane potentials. Slow depolarizations could evoke firing after the stimulus (delayed firing), were facilitated by K^+-blockers (2 mM TEA, 2 mM Ba) and dihydropyridines (e.g., 1 μM BayK 8644), and were blocked by 50 μM Cd^{2+} or 200 nM calciseptine, which also blocked the dihydropyridines' agonist effects. This suggests that slow depolarizations are generated by the L-type of inward Ca^{2+}-currents. Dopaminergic D_1-, but not D_2-receptor agonists, enhance the slow depolarizations and facilitate delayed firing (1 μM Cl-APB or 6-Cl-PB vs. 1-5 μM quinpirole). Effects are blocked by 1 μM SCH23390 but not by 5 μM sulpiride. D_1-actions were mimicked by cAMP analogs and occluded by dihydropyridines. Since the only inward current enhanced by D_1-agonists (through the PKA pathway) is the one carried by L-channels (Surmeier et al., 1995, Neuron 14: 385), it is suggested that this current generates the D_1-enhanced slow depolarizations and delayed firing. In conclusion, facilitation of Ca^{2+}-mediated slow depolarizations and firing is the way dopamine uses to excite neostriatal neurons.

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BASAL GANGLIA: ANATOMY I

164.1

EFFERENT PROJECTIONS OF DIFFERENT FUNCTIONAL TERRITORIES OF THE INTERNAL PALLIDUM IN MONKEYS. E. Shink*, M. Sidibé, J.-F. Bouffard and Y. Smith. Ctre Recherche Neurobiol., Univ. Laval, Québec, Canada.

The functional segregation imposed upon the striatum by the cortical afferents is maintained at the level of the output structures of the basal ganglia, hence in the internal pallidum (GPi), the dorsal third is related to associative functions, the caudo-ventro-lateral two-thirds process sensorimotor information and the rostromedial pole receives limbic-related inputs. The objective of this study was to verify whether this segregation is maintained in the targets of the GPi, namely the ventrolateral and intralaminar thalamic nuclear groups, the pedunculopontine nucleus (PPN) and the lateral habenular nucleus (LHb). This was achieved by using the anterograde transport of biotinylated-dextran amine (BDA) alone, or combined with the anterograde transport of *Phaseolus vulgaris*-Leucoagglutinin.

In a first series of experiments, we placed small injections of BDA in the different functional territories of the GPi. Following these injections, a common pattern disclosed in the different targets was the overlap of the field of anterogradely labelled terminals arising from the associative and limbic territories; which were largely segregated from the sensorimotor-related afferents. A second series of experiments is currently in progress to verify whether the overlap of terminal fields is reflected at the single cell level by the convergence of synaptic inputs. To do so, we use a double anterograde labelling method suitable with light and electron microscopic observations. So far, combined injections in the limbic and sensorimotor territories confirmed the segregation of afferents from those territories at the level of the thalamus, the LHb and the PPN. The possibility of convergence of synaptic inputs from associative and limbic territories in currently under investigation at the electron microscopic level.

In conclusion, these findings suggest that the cognitive and limbic information that remain segregated in the striatopallidal complex may be processed by common neurones in the different targets of the GPi. *Supported by the Medical Research Council of Canada and the Fonds de la Recherche en Santé du Québec.*

164.2

SYNAPTIC INTERACTIONS BETWEEN THALAMIC AFFERENTS AND STRIATAL INTERNEURONES IN MONKEYS. M. Sidibé* and Y. Smith. Centre de Recherche en Neurobiologie, Univ. Laval, Québec, Canada.

Four major populations of interneurones are chemically characterized in the striatum, namely those immunoreactive for parvalbumin (PV), somatostatin (SS), calretinin (CR) and choline acetyltransferase (Chat). The objective of the present study was to analyse the synaptic relationships between the striatal interneurones and the thalamic afferents arising from the centre median (CM) nucleus in squirrel monkeys. This was achieved by combining the anterograde transport of biotinylated-dextran amine (BDA) with the immunostaining specific for the different populations of interneurones at the electron microscopic level.

BDA injections in the CM led to dense bands of anterograde labelling in the post-commissural region of the putamen. Double labelling at the light microscopic level showed that the thalamic fibres and interneurones largely overlapped in different regions. So far, analysis of those zones of overlap in the electron microscope revealed the existence of asymmetric synapses between BDA-containing terminals from the CM and the dendrites of PV and SS interneurones. In contrast, synapses between CR-positive elements and BDA-containing terminals were not found. The possibility of CM inputs to Chat-positive interneurones is currently under investigation. Double-labelled terminals for BDA and PV or CR were observed, which indicates that these calcium binding proteins may be used as neuromediators by thalamostriatal neurones. This was confirmed by the labelling of PV- and CR-immunoreactive neurones in the CM after injections of a retrograde marker in the putamen. In contrast, none of the BDA-containing terminals displayed immunoreactivity for SS and Chat. Some of the double-labelled terminals formed synapses with PV- or CR-positive dendrites.

In conclusion, our data indicate: (1) that striatal interneurones immunoreactive for PV or SS, but not those that contain CR, receive strong thalamic input from the CM and (2) that PV and CR are used as neuromediators by thalamostriatal neurones in monkeys. *Supported by the canadian MRC and the FRSQ.*

164.3

LOCALIZATION OF METABOTROPIC GLUTAMATE RECEPTOR SUBUNITS IN THE BASAL GANGLIA OF MONKEYS. Y. Smith*, M. Paquet and J.-F. Bouffard. Centre Recherche Neurobiologie, Univ. Laval, Québec, Canada.

In order to further our knowledge on the role of metabotropic receptors in the basal ganglia (BG), we analysed the distribution of the mGluR1α and mGluR2/3 subunits in the BG of squirrel monkeys by means of immunocytochemistry.

In the light microscope, the neuropil in the different structures of the BG displayed strong mGluR1α immunoreactivity. The immunostaining was particularly dense along the membranes of dendrites and perikarya. No particular regionalization of labelling was found except in the caudate nucleus, where zones that probably correspond to the "patches or striosomes" were slightly less immunoreactive than the "matrix". At the electron microscopic level, a common feature was the association of dense reaction product with post-synaptic densities at the level of asymmetric synapses on dendrites, spines and perikarya. Occasionally, aggregates of reaction product were also found at non-synaptic sites along the dendrites. With the exception of the STR, thin glial processes were immunostained in the BG, but abounded particularly in the internal pallidal segment (GPi). In the subthalamic nucleus (STN) and the substantia nigra pars reticulata (SNr), a few terminals displayed immunoreactivity. Overall, the BG were devoid of mGluR2/3-immunoreactive neuronal elements except for lightly labelled perikarya in the STR and the STN. However, the neuropil in the different structures contained thin immunoreactive processes, presumably of glial nature. This needs to be confirmed in the electron microscope. In contrast, neurones in the nucleus basalis of Meynert displayed strong pericellular mGluR2/3 immunoreactivity.

In conclusion, our findings indicate that the mGluR1α subunit of the metabotropic receptors may participate in the modulation of glutamatergic transmission in the BG in different ways including post- and pre-synaptic mechanisms as well as glial cell recapture of neurotransmitter. *Supported by MRC and FRSQ.*

164.4

LOCALIZATION OF AMPA AND NMDA GLUTAMATE RECEPTOR SUBUNITS IN STRIATAL INTERNEURONES IN MONKEYS. M. Paquet* and Y. Smith. Ctre Recherche Neurobiologie, Univ. Laval, Québec, Canada.

Four major populations of interneurones were chemically characterized in the striatum by their immunoreactivity for somatostatin (SS), parvalbumin (PV), calretinin (CR) and choline acetyltransferease (Chat). In order to understand better the nature and composition of the receptors that mediate the excitatory effects induced in these neurones by glutamatergic afferents, we compared the expression of NMDA (NMDAR1) and AMPA (GluR1, GluR2/3 and GluR4) receptor subunits between the different populations of interneurones. This was achieved by using a double immunohistochemical technique at the light microscopic level.

Although the intensity of immunostaining for GluR2/3 and NMDAR1 subunits was quite homogeneous throughout the striatum, such was not the case for the other subunits. The GluR1 immunoreactivity was light throughout the striatum except for the strong labelling associated with a sparse subpopulation of medium-sized neurones. Three populations of striatal neurones were distinguished on the basis of their GluR4 immunoreactivity; the majority were medium-sized and lightly immunoreactive whereas subpopulations of medium- and large-sized perikarya were much strongly labelled. A common feature to the four populations of interneurones was the expression of the GluR2/3 subunits. The SS-positive neurones were lightly immunoreactive for GluR1 and GluR4 and displayed NMDAR1 immunoreactivity. On the other hand, the majority of PV- and CR-positive neurones were strongly labelled for GluR1 and GluR4. However, the PV-positive cells displayed NMDAR1 immunoreactivity whereas such was not the case for most of the CR-containing neurones. The Chat-immunoreactive neurones were lightly labelled for GluR1 but displayed strong GluR4 immunoreactivity and contained NMDAR1.

In conclusion, our findings indicate that AMPA and NMDA receptor subunits are differentially distributed in the subpopulations of striatal interneurones in monkeys. *Supported by MRC and FRSQ.*

164.5

DUAL REPRESENTATION OF STRIATUM IN THE SUBSTANTIA NIGRA PARS RETICULATA: RELATIONSHIP TO SACCADE ZONES IN SUPERIOR COLLICULUS.
AB Kelly* and C R Gerfen. Lab of Neurophysiology, NIMH, Bethesda, MD.

Processing of cortical information by the striatum is determined by several input-output organizational schemes. In one, striatal patch-matrix compartments provide parallel pathways for cortical neurons in different laminae to selectively target dopamine or GABA neurons in the substantia nigra. In another, different populations of striatal neurons provide either indirect, via the globus pallidus, or direct projections to the substantia nigra. In another scheme, individual striatopallidal (Chang *et al.*, 1981, Science 213:915) and striatonigral neurons provide inputs to two separate zones in each of the target nuclei. Thus there exist dual maps of the striatum in the globus pallidus and substantia nigra (Gerfen, 1985, J. Comp. Neurol., 236: 454). We examined the output organization to the superior colliculus (SC) of these dual striatal map zones of the substantia nigra, one located roughly dorsally, subjacent to the pars compacta and another located ventrally. The SC displays a map of eye movement saccades, from long saccades caudally to short saccades rostrally, ending in a rostral zone of the SC of active visual fixation (Munoz & Wurtz, 1992, J. Neurophysiol., 67:1000). Retrograde tracers, Fluoro-Gold and Fast Blue, were injected into rostral and caudal SC, respectively. Results showed that nigral neurons projecting to the caudal long-saccade zones of the SC are located in the ventral nigral map of the striatum, whereas those projecting to the anterior short-saccade zones of the SC are located in the dorsal nigral map of the striatum. Individual neurons do not appear to project to both zones of the SC. These results indicate that each of the dual striatal map zones of the substantia nigra provide inputs to different functional areas in the superior colliculus. (supported by NIMH)

164.6

INFEROTEMPORAL CORTEX IS THE TARGET OF BASAL GANGLIA OUTPUT.
F.A. Middleton*[1] and P.L. Strick[2]. [2]Research Service, VA Medical Center and Departments of [2]Neurosurgery and [1,2]Physiology, SUNY Health Science Center, Syracuse, NY 13210.

The basal ganglia of primates are known to receive inputs from widespread regions of the cerebral cortex, such as the frontal, parietal, and temporal lobes. Of these cortical areas, only the frontal lobe is generally regarded as the target of output from the basal ganglia. One of the temporal lobe regions which is a source of input to the "visual" striatum is area TE, in inferotemporal cortex. This cortical region is thought to be critically involved in the recognition and discrimination of visual objects. We used retrograde transneuronal transport of the McIntrye-B strain of herpes simplex virus type 1 (HSV1) to examine whether TE is the target of basal ganglia output. Multiple small injections of HSV1 (0.05 - 0.1 µl) were placed into area TE in Cebus monkeys (*Cebus apella*, n=3). Five days after these injections, HSV1 was detected in several thalamic nuclei that are known to project to TE, including the nucleus ventralis anterior pars magnocellularis (VAmc). In addition, neurons labeled by retrograde transneuronal transport of HSV1 were also found in the substantia nigra pars reticulata (SNpr). The region of SNpr that contained labeled neurons after TE injections lies within the region that receives input from the visual striatum. In addition, this region of SNpr was different from the regions labeled by HSV1 injections into motor and prefrontal areas of cortex. These results indicate that TE is not only a source of input to the basal ganglia, but also is the target of basal ganglia output via the thalamus. Thus, basal ganglia loops with the cerebral cortex appear to be involved in higher order aspects of visual processing, as well as motor and cognitive behavior. Support: NARSAD Established Investigator Award (PLS), VA Medical Research Service (PLS), USPHS 24328 (PLS), and NIMH Predoctoral Fellowship MH11262 (FAM).

164.7

ORGANIZATION OF TEMPORAL POLAR AND TEMPORAL OPERCULAR CORTICAL PROJECTIONS TO THE STRIATUM IN PRIMATES.
M. Chikama* and S.N. Haber. Department of Neurobiology & Anatomy, Univ. of Rochester School of Medicine and Dentistry, Rochester, NY 14642.

On the basis of connections with cortices in primates, the striatum can be divided into the limbic-related ventral striatum and the sensorimotor-related dorsolateral striatum. Previously, we have shown that the cingulate, orbitofrontal and insular cortical projections to the striatum are topographically organized according to cytoarchitectonic cortical and functional striatal differentiations. For example, agranular cortical regions project primarily to the ventromedial striatum, while granular cortical regions project to the dorsolateral striatum.

Temporal pole and temporal opercular regions surrounding the piriform olfactory cortex, also contain consecutive cytoarchitectonic differentiations from agranular to granular cortex. We investigated the organization of projections from the different cytoarchitectonic temporal cortical inputs to the striatum. Agranular and dysgranular portions of the temporal polar cortex strongly project to the medial and ventral parts of the ventral striatum. In contrast, the dorsolateral striatum receives inputs primarily from the granular portion of the temporal opercular cortex.

According to cortical cytoarchitectonic differentiations, functionally segregated cortical areas project to a similar functional division of the striatum. (Supported by NS2251 and the Lucille Markey Charitable Trust)

164.8

THE THALAMOSTRIATAL PROJECTION AND ITS RELATION TO CORTICOSTRIATAL AND CORTICOTHALAMIC INPUTS.
N.R. McFarland* and S.N. Haber. Department of Neurobiology & Anatomy, University of Rochester School of Medicine and Dentistry, Rochester, NY 14642.

The thalamus plays a central role in basal ganglia circuitry. It receives output from the basal ganglia and relays this information to the cerebral cortex. The thalamus also provides a major source of input to striatum. Previously, we have demonstrated massive thalamostriatal projections from the midline, intralaminar and ventral thalamic nuclei. These thalamostriatal projections are topographically organized such that the medial thalamic nuclei project to the ventral 'limbic' striatum, while lateral nuclei (i.e. ventral anterior and ventral lateral nuclei) project to the dorsolateral 'sensorimotor' striatum. Retrograde tracer injections into discrete striatal regions show corticostriatal projections from areas which receive thalamic input from nuclei that also project to the striatum. These findings suggest that interconnected thalamic and cortical areas project to similar striatal regions. Our current study examines whether the thalamostriatal projection is influenced by cortical inputs to both its thalamic origin and its terminals in the striatum.

Anterograde tracer injections were placed into motor, prefrontal (area 9), and anterior and posterior cingulate cortices. Fiber and terminal labeling was examined using darkfield/light microscopy and charted with a drawing tube. After tracer injection into the medial motor cortex, corticothalamic fibers were observed primarily in the ventral lateral pars oralis, ventral posterior lateral par oralis, and dorsolateral centre médian nuclei, while corticostriatal projections terminated in the dorsolateral putamen. Cortical projections from area 9 terminated in the ventral anterior and mediodorsal (MD) thalamic nuclei and in the rostral head of the caudate and central putamen. Anterior cingulate projections targeted mainly the ventral anterior, MD, and dorsal intralaminar thalamic nuclei and the rostral striatum and ventral body of the caudate. The posterior cingulate cortex projected to the ventral lateral pars caudalis, rhomboid and caudal MD nuclei.

These observations, when combined with our previous studies on the organization of thalamic projections to the striatum, support the idea that thalamostriatal projections may be influenced by cortical inputs to both the striatum and the thalamus. (Supported by NS2251 and the Lucille Markey Charitable Trust)

164.9

THE RELATIONSHIP OF AMYGDALOID AND STRIATAL INPUTS TO THE VENTRAL MIDBRAIN: A RETROGRADE STUDY.
J.L. Fudge* and S.N. Haber. Department of Neurobiology and Anatomy, Univ. of Rochester School of Medicine and Dentistry, Rochester, NY 14642.

The role of the amygdala in psychotic illness has not been well-studied. Nevertheless, evidence to date suggests that the amygdala is involved in the regulation of affect, and in the emotional "coloring" of environmental stimuli. Because dysregulation of dopamine is thought to play a role in psychotic syndromes, the connection of the amygdala to the ventral midbrain may be important. A previous study using anterograde tracing methods documented a projection from the central nucleus of the amygdala to the ventral tegmental area (VTA) and dorsal tier cells of the substantia nigra (SN) in primates (Price and Amaral, 1981). We analyzed the amygdalonigral projection in macaques by placing retrograde tracers in the SN and VTA at several dorsoventral and mediolateral levels. In addition, the projection was analyzed in relation to striatal inputs. An injection in the VTA and medial SN revealed cell bodies in the central and medial nuclei of the amygdala. Striatal inputs to this region of midbrain are predominantly from the rostral, pre-commissural striatum. Cell density was greatest in the ventromedial to central striatum, areas associated with limbic and association cortex input, respectively. An injection in the lateral SN also revealed a projection from the central and medial nuclei of the amygdala. The striatal innervation of this area arose primarily from the caudal limbic-related striatum. A central, ventral SN injection in the region of the reticulata and cell columns and densocellular area of the compacta failed to reveal cell bodies in the amygdala. Striatal cells innervating this area were broadly distributed rostrocaudally, with greatest cell density in central and dorsolateral regions. In summary, the amygdala projects to cells of the VTA and medial and lateral thirds of the SN. Striatal inputs to these areas arise from areas considered "limbic-related" on the basis of cortical innervation. The central, ventral SN, which did not have an amygdaloid innervation, had striatal input predominantly from association and sensorimotor regions. (Supported by NIMH T32MH18911, NS22511 and the Lucille P. Markey Charitable Trust)

164.10

ANATOMICAL AND FUNCTIONAL RELATIONSHIPS BETWEEN THE PREFRONTAL CORTEX AND THE BASAL GANGLIA IN THE RAT.
A.M Thierry*, N. Maurice, J.M Deniau, A. Menetrey and J. Glowinski. U114, Collège de France; URA1488, Université Paris VI; Paris, France.

Recently, we have described, a prefrontal cortex-striato-thalamo-cortical circuit which involves the substantia nigra pars reticulata (SNR) *(Montaron et al., Neuroscience: 71,371-382,1996).* This circuit originates from the ventral prelimbic (PL) and medial orbital (MO) areas of the prefrontal cortex and involves successively: a subterritory of the core of nucleus accumbens (NAcc), the dorsomedial part of the SNR and thalamic nuclei related to the prefrontal cortex. Since the ventral pallidum (VP) has been considered as a main output stucture of the prefrontal cortex-basal ganglia circuits, instead of/or in addition to the SNR, the aim of the present study was to determine the position of the VP in the PL/MO-basal ganglia circuit. Following injection of biocytin into the close vicinity of NAcc neurons presenting an excitatory response to the stimulation of PL/MO areas, anterogradely labelled fibers were visualized in a delineated area of the VP. When biocytin injection was performed into this VP area, labelled fibers were observed in the medial part of SNR and in the medial region of subthalamic nucleus (STN) but not in thalamic nuclei. The existence of a functional link between these structures was further established using an electrophysiological approach. Electrical stimulation of the core of NAcc induced an inhibitory response (D=39.6±15.4ms) in 61% of the VP cells identified as projecting to the STN by the antidromic activation method and stimulation of the VP evoked an inhibition (D=19.8±7.3ms) in 55% of the STN neurons identified as projecting to the medial SNR.The present data show that the VP area involved in the PL/MO-basal ganglia circuit, cannot be considered as an output structure of the basal ganglia but participates with the STN to an indirect NAcc-SNR pathway. Thus, it can be proposed that the VP occupies in this prefrontal-basal ganglia circuit a position analogous to that of the external segment of the globus pallidus in the sensorimotor circuits of the basal ganglia. *Supported by INSERM.*

164.11

SPECIFIC ANATOMICAL RELATIONSHIPS BETWEEN HIPPOCAMPAL AND BASAL AMYGDALOID AFFERENTS AND DIFFERENT POPULATIONS OF PROJECTION NEURONS IN THE NUCLEUS ACCUMBENS OF RATS. A.V.J. Beijer and H.J. Groenewegen. (SPON: European Neuroscience Association). Dept. Anat. & Embryol., Vrije Univ. Amsterdam, The Netherlands.

The nucleus accumbens (Acb) is considered to be an interface between limbic structures and several effector regions in the diencephalon and mesencephalon. Within the Acb, afferents from the basal amygdaloid complex (BAC) and the subiculum of the hippocampus (Sub) as well as projection neurons, are inhomogeneously distributed. This raises the question whether these limbic structures specifically contact different populations of projection neurons within the Acb. To address this question, double anterograde tracer injections in parts of the BAC and the Sub were combined with a retrograde tracer injection in an Acb target. Double anterograde tracing revealed a complex pattern of overlap and segregation of afferents from different Sub and BAC subregions within the Acb. Combined anterograde/retrograde studies showed striking relationships between limbic inputs and specific populations of projection neurons. For example, ventral subicular efferents selectively innervate a neuronal population in the dorsomedial Acb that projects to the ventral tegmental area. This population is largely avoided by BAC efferents. Efferents from the dorsal Sub and the rostral BAC converge on a population of substantia nigra projecting cells in the rostrolateral Acb. Strong relationships were also found between efferents from the BAC and Acb cells that project to different parts of the ventral pallidum. Different BAC subnuclei appear to selectively innervate or avoid subpopulations of VP projecting neurons. These results indicate that distinct populations of Acb projection neurons receive input from specific parts of BAC and/or Sub. It may thus be postulated that different amygdaloid and hippocampal subregions, via the Acb, exert differential influences on behavior.

164.12

BASAL AMYGDALOID RELATIONSHIPS WITH THE PREFRONTAL CORTEX AND THE VENTRAL STRIATUM IN RATS: CORTICAL LAMINATION AND STRIATAL COMPARTMENTATION.

H.J. Groenewegen* and C.I. Wright. Department of Anatomy and Embryology, Vrije Universiteit, 1081 BT Amsterdam, The Netherlands.

The basal amygdaloid complex (BAC) has specific anatomical relationships with both the prefrontal cortex (PFC) and the nucleus accumbens (Acb). In general, specific subnuclei of the BAC project to regions of the PFC and the Acb that are connected through corticostriatal projections. Recently, the relationships of the amygdalostriatal projections with the striatal compartments have been determined (Wright et al., 1996). For example, the mid-rostrocaudal BAC projects to the matrix of the core of the Acb and the adjacent ventral caudate-putamen, whereas the rostral and caudal parts of the BAC project to the patches of the same striatal region. It has previously been demonstrated that the superficial layers of the PFC project to the matrix whereas the deep layers reach the striatal patches (Gerfen, 1989). Amygdalocortical fibers terminate in a bilaminar pattern in both the deep and the superficial PFC layers. Our present aim was to determine whether and how this termination pattern relates to the amygdalostriatal compartmental organization. We used small injections of anterograde tracers in various BAC subnuclei. The results show that BAC subnuclei that target striatal patches have predominant terminations in the deep layers of the PFC, whereas those that project to the striatal matrix primarily project to the superficial layers. Therefore, these results indicate that amygdalocortical and amygdalostriatal projections both consists of subsystems and further stress the close anatomical association of the BAC with the prefrontal-ventral striatal system.

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164.13

PRE- AND POSTNATAL DEVELOPMENT OF THE CORTICOSTRIATAL PATHWAY IN THE MOUSE. L.K. Nisenbaum* and J.J. LoTurco. Dept. of Physiology & Neurobiology, University of Connecticut, Storrs, CT 06269

The striatum receives excitatory glutamatergic input from the cerebral cortex. These cortical afferents are segregated into the functionally distinct patch and matrix regions of the striatum. Neurons from the prelimbic cortex, particularly those from the deeper layers, project predominantly to the striatal patch neurons whereas neurons from the somatosensory cortex project exclusively to the striatal matrix neurons. Although the corticostriatal innervation has been characterized in adults, the developmental timecourse and segregation of this pathway have not been investigated.

To determine the timecourse of corticostriatal innervation, the fluorescent dye, DiI, was injected into various regions of cortex in mice ranging in ages from embryonic day (E) 16 through postnatal day (P) 15. Injections into frontal cortex at E16 labeled corticofugal axons within the internal capsule fiber bundles. Relatively few collateral branches extended from the fiber bundles into the striatum at this age. In contrast, by E21 a plexus of labeled collateral branches extending from the internal capsule fibers into the striatum was clearly detectable. The density of corticostriatal axon collaterals continued to increase from P0 through P15. Whole-cell recordings from striatal neurons in corticostriatal slices were used to assess the functional innervation of the striatum during the first postnatal week. Stimulation of the corpus callosum produced a synaptic response that reversed at +10 mV, suggesting an excitatory glutamatergic innervation of the striatal neurons.

Injections into the prelimbic cortex of P1 and P9 mice revealed the initiation of cortical afferent segregation. At both ages, corticostriatal axon collaterals formed stripes and patches of apparent terminal fields within the medial aspects of the striatum. No such pattern formation was detected following injections into the frontal or occipital cortex in P1 and P9 mice. Together, these data suggest that cortical neurons innervate the striatum prenatally. In addition, corticostriatal afferent segregation may begin during late prenatal or early postnatal development.

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164.14

SYNAPTOLOGY OF THE NIGROSTRIATAL PROJECTION IN RELATION TO THE COMPARTMENTAL ORGANISATION OF THE RAT NEOSTRIATUM.

J. J. Hanley & J. P. Bolam. SPON: Brain Research Association. MRC Anatomical Neuropharmacology Unit, Mansfield Road, Oxford, OX1 3TH, U.K.

The heterogeneous organisation of the neostriatum, the patch (striosome)/matrix system, is defined on the basis of neurochemical heterogeneities and differential inputs and outputs. Sub-populations of midbrain dopamine neurons differentially innervate the patch and matrix compartments[1,2]. In view of these differences and the recognised structural differences between patch and matrix, the objective of the present study was to determine whether there are differences in the morphology and synaptology of dopaminergic axons innervating patch or matrix. Two approaches were used. 1) Rats received iontophoretic deposits of the anterograde tracer, biotinylated dextran amine (BDA) in different regions of the substantia nigra (SN). They were perfuse-fixed and sections of the neostriatum were processed to reveal the BDA and calbindin D28k, a marker for the patch/matrix system. 2) Perfuse-fixed sections of rat neostriatum were immunostained to reveal tyrosine hydroxylase (TH), a marker for dopaminergic structures, and calbindin D28k. The deposits of anterograde tracer in the SN led to two types of labelled afferents in the neostriatum. The anterogradely labelled fibres and the TH-positive fibres formed mainly symmetrical synapses in both the patch and matrix. Their synaptic targets were similar in both compartments and included spines, dendritic shafts and perikarya. The results so far demonstrate that deposits of BDA lead to the labelling of heterogeneous populations of fibres in the neostriatum, but that dopaminergic structures appear homogeneous. Although the nigrostriatal projection arises from sub-populations of dopaminergic neurons there appears to be no differences in their morphology and synaptology in relation to the patch and matrix. This implies that the dopaminergic input to the neostriatum modulates the flow of cortical information in a similar manner in the patch and matrix.

1) Gerfen, C.R. et. al . (1987) *J Neurosci* , **7**, 3915-3934.
2) Jimenez-Castellanos, J. & Graybiel, A.M. *Neuroscience* **23**, 223-242 (1987).

Supported by the Medical Research Council

164.15

EVIDENCE OF MORPHOLOGICAL HETEROGENEITY IN THE CAT THALAMOSTRIATAL PROJECTIONS TO THE CAUDATE NUCLEUS. S. de las Heras*, E. Mengual, J.L. Velayos and J.M. Giménez-Amaya. Departamento de Morfología, Facultad de Medicina, U.A.M., 28029 Madrid, Spain.

The morphological heterogeneity of the cat thalamostriatal projections to the caudate nucleus (CN) and their relationships with the nigrothalamic connectivity were studied by means of retrograde and anterograde tracer techniques. In a first group of experiments, HRP-WGA, and fluorescent retrograde tracers such as Fast Blue and Diamidino Yellow were injected in different regions of CN, and then the distribution of single and double retrogradely labeled neurons in the thalamus was analyzed. Adjacent sections processed for acetylcholinesterase (AChE) were used as histochemical markers for the thalamic localization of labeled thalamostriatal neurons. In a second group of experiments, both fluorescent tracers Fast Blue and Diamidino Yellow were injected in different regions of CN whereas HRP-WGA was placed in the substantia nigra. The thalamic distribution of retrogradely labeled thalamostriatal neurons and anterogradely labeled nigrothalamic terminals was analyzed, in order to determine whether there are overlapping territories between the thalamostriatal and nigrothalamic projections. The main findings of this study were: (a) the thalamostriatal neurons displayed different patterns of organization depending on the thalamic group considered; (b) no double labeled neurons were observed when the two fluorescent tracers were injected in different dorsoventral and rostrocaudal sectors of the rostral CN whereas some double labeled cells were detected when larger injections in CN were localized far apart from each other in the rostrocaudal coordinate; (c) the thalamostriatal cells were heterogeneously distributed in reference to the thalamic distribution of AChE activity; and, (d) large overlapping thalamic territories between the thalamostriatal neurons projecting to CN and the nigrothalamic connections were observed in the rostral nuclei of the ventral thalamic group and, partially, in the rostral and caudal intralaminar nuclei. These results might help to understand the complex architecture of thalamic neurons projecting to CN in carnivores.

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164.16

THE NIGROSTRIATAL PROJECTIONS TO THE CAT CAUDATE NUCLEUS: A TOPOGRAPHICAL RE-EVALUATION WITH MULTIPLE RETROGRADE TRACERS. B. Hontanilla, S. de las Heras and J.M. Giménez-Amaya*. Departamento de Morfología, Facultad de Medicina, U.A.M., 28029 Madrid, Spain.

The anatomical organization of the cat nigrostriatal projections to the caudate nucleus (CN) was studied by retrograde tracers such as HRP-WGA, Fast Blue and Diamidino Yellow. These tracers were injected concomitantly in different regions of CN. The distribution of single and double retrogradely labeled neurons was analyzed in the substantia nigra (SN) compacta (SNC), substantia nigra lateralis (SNL), retrorubral area (RR) and ventral tegmental area (VTA). Adjacent sections processed for acetylcholinesterase were used as histochemical markers for the densocellular zone of SN. The main findings of this study are: 1) The rostral CN receives projections mainly from the caudal SN while the caudal CN receives projections from all rostrocaudal levels of SN. 2) SNL projects very specifically to the caudal CN. 3) The ventral RR close to the medial lemniscus projects to all rostrocaudal levels of CN. 4) The rostral CN receives projections mainly from the medial SN while more caudal sectors of CN receive projections from the medial and lateral SN. 5) A dorsoventral inversion of nigrostriatal projections from the medial SNC and VTA to CN was established. In contrast, we found zones within RR projecting both to the dorsal and ventral CN. 6) Distant injections of two different fluorescent tracers regarding both the dorsoventral and the rostrocaudal coordinates, yielded double-labeled neurons that were mainly located in the medial and caudal portions of SN and in the ventral RR. However the number of double-labeled neurons was higher after separate injections in the dorsoventral axis, suggesting that the collateralization to CN occurs mainly in the dorsoventral plane. 7) A clustering organization of nigrostriatal cells projecting to CN was detected mainly in the intermediate rostrocaudal part of SNC and in RR. The results of this comprehensive study on the cat nigrostriatal pathway to CN show novel findings on the anatomical organization of the nigrostriatal projections which might help to understand the complex architecture of nigral neurons projecting to CN in carnivores. Supported by DGICYT PB88-0170, PB90-0220 and FIS 93/0337.

164.17

EFFERENT CONNECTIONS OF THE STRIATUM AND THE NUCLEUS ACCUMBENS IN ANURAN AND URODELE AMPHIBIANS. W.J.A.J. Smeets[a], O. Marín[b], M. Muñoz[b], H. Vara[b], C. Sánchez-Camacho[b] and A. González[b]. [a]Graduate Sch. Neurosci., Dept. Anatomy & Embryology, Vrije Univ., Amsterdam, The Netherlands. [b]Dept. Cell Biology, Univ. Complutense, Madrid, Spain.

Recently it has been shown that, on the basis of position, chemoarchitecture and afferent connections, the basal forebrain of anuran and urodele amphibians can be subdivided into a nucleus accumbens and a striatum. As a further step in unraveling the organization of the basal ganglia of amphibians, we have studied the efferent connections of these basal forebrain subdivisions in the anurans *Rana perezi* and *Xenopus laevis*, and the urodele *Pleurodeles waltl*, using biotinylated or fluorescent dextran amines as anterograde tracers. A common pattern of efferent connections was observed in both groups of amphibians, but those in anurans were more elaborated. *Striatal efferent fibers* were found to reach the lateral and medial amygdala, the anterior and posterior entopeduncular nuclei, several thalamic nuclei, the dorsomedial posterior tubercle, the pretectum, the optic tectum, the torus semicircularis, the pontomesencephalic reticular formation and the caudal brainstem. *Efferent fibers of the nucleus accumbens* projected to the medial amygdala, the preoptic area, the ventral hypothalamic nucleus, the dorsomedial posterior tubercle, the medial tegmental area, the pontomesencephalic reticular formation and the raphe. In addition, the study has revealed the existence of intrinsic connections within the ventral telencephalic wall, suggesting a possible further compartimentalization of the amphibian basal forebrain. In conclusion, the results of the present study corroberate the notion that the basal ganglia of amphibians share many features with their presumed homologue in amniotes.

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BASAL GANGLIA: FUNCTION I

165.1

ROLE OF ANTERIOR STRIATUM IN MOTOR INITIATION AND POSTERIOR STRIATUM IN MOTOR EXECUTION IN HUMAN AND NON-HUMAN PRIMATES. B. Leis[1], E. B. Montgomery[1]* and W. C. Koller[2]. Human Motor Control Lab., Neurology, U. of Arizona[1], Tucson, AZ 85724; Neurology, U. of Kansas Med. Center[2].

We investigated motor initiation and execution using wrist flexion-extension movements in ten neurologically intact controls, ten patients with probable idiopathic Parkinson's disease (PDi), and four patients with Progressive Supranuclear Palsy (PSP). Patient groups were tested off medications. One monkey was studied before and after MPTP-induced Parkinsonism.

Montgomery et al. (1991) propose two separate physiologic mechanisms in the basal ganglia underlying motor initiation and execution, represented by reaction time (RT) and movement velocity (MV), respectively. The anterior striatum is advanced as programming motor initiation whereas the posterior striatum as programming motor execution. Parkinson's disease is characterized by greater dopamine loss in the putanum whereas in PSP and MPTP induced PD, more severe dopamine loss occurs in the caudate. We hypothesized lengthened RTs in PSP compared to PDi and equivalent MVs in PSP and PDi.

PSP RT exceeded PDi RT in pooled median RT across tasks, approaching statistical significance [Independent t-test, t (12)=2.0; p=0.068]. Median RT among groups were significantly different for the bounded flexion condition [One way analysis of variance (Anova), F (2,21)=13.6; p<0.001]. A Bonferroni t-test indicated the PSP RT (472ms) was significantly longer than the PDi RT (328ms). There were significantly prolonged median RTs in the monkey after MPTP using pooled RT between tasks [Mann-Whitney Rank Sum, p<0.01]. No significant differences in median MV occurred among groups [Kruskal-Wallis one way Anova, p>0.05] and no significant differences in MV occurred in the monkey before and after MPTP pooling MV between tasks, after Bonferroni correction [Independent t-test, t(40)=2.1]. There was no association between RT and MV in the PSP or PDi patient groups [Spearman rank order correlation, p>0.05]. Preliminary analysis suggests movement initiation is separate from execution and supports the role of the anterior striatum in motor initiation and the posterior striatum in execution.

Support: Jane K. Pelton Fund for Movement Disorders Research; Southern Arizona Chapter, APDA.

165.2

A PET STUDY OF THE EFFECTS OF THALAMIC AND GLOBUS PALLIDUS STIMULATION IN PARKINSON'S DISEASE. K.D. Davis*[1], E. Taub[1], J.O. Dostrovsky[2], S. Houle[4], A.E. Lang[3], R.R. Tasker[1] and A.M. Lozano[1]. [1]Dept of Surgery, [2]Physiology, [3]Neurology, and [4]The PET Centre, Clark Institute of Psychiatry, University of Toronto, Toronto, Ontario, Canada.

Chronic stimulation of the ventrointermediate (Vim) thalamus and internal segment of the globus pallidus (GPi) attenuates respectively, parkinsonian tremor and akinesia/rigidity. To better understand the neural mechanisms underlying these effects, we have obtained positron emission tomography (PET) images of the rCBF changes associated with Vim (n=6) and GPi stimulation (n=9) in patients with PD.

A Scanditronix GEMS-2048 scanner was used to image emissions from each i.v. injection of 40mCi $H_2{}^{15}O$. Six one minute scans were obtained for each patient: without stimulation (scans 1&4) and during stimulation at clinically ineffective (scans 2&5) or effective (scans 3&6) intensities. Scans consisted of 15, 6.5mm axial slices spanning the cerebrum and cerebellum. Right stimulation data were left-to-right flipped to pool with left stimulation data. Images were smoothed (15mm filter) and normalized to fit the Talairach and Tournoux atlas and analyzed with SPM software.

Vim stimulation at a clinically effective intensity increased rCBF bilaterally in frontal association areas and decreased rCBF ($p<0.0001$) in the cerebellum, supplemental/cingulate motor cortex contralateral to Vim stimulation (ipsilateral to tremor). GPi stimulation at a clinically effective intensity increased rCBF ($p<0.0001$) in the premotor cortex and post-commissural putamen/GPe region ipsilateral to GPi stimulation and decreased rCBF ($p<0.001$) in the contralateral premotor and frontal association cortex.

These data suggest that Vim and GPi act differently through motor loops including the cerebellum, pallidum, thalamus, putamen and premotor cortical areas to alleviate parkinsonian symptoms. (Supported by Medtronics Corp.)

165.3

COGNITIVE MODULATION OF HUMAN GLOBUS PALLIDUS (GP) NEURONS W.D. Hutchison*, A.M. Lozano, E. Taub, R.R. Tasker, A.E.Lang, J. Saint-Cyr, J.O. Dostrovsky, Departments of Surgery, Medicine, Psychology, Physiology, University of Toronto, Ontario, Canada M5S 1A8.

Recent studies have suggested that the GP is not only involved in the planning and execution of movements, but may also process cortical information subserving higher cognitive function (Middleton and Strick, *Science* **266**: 458, 1994; Mushiake and Strick, *J.Neurophysiol.* **74**: 2754, 1995). During 8 stereotactic pallidotomies for Parkinson's disease, the activity of single units in GP was studied during visually cued sequential pointing movements. Patients were requested to press a variable sequence of 3 of 5 buttons over 5 trials in each of 2 tasks: an internally cued task with a memorized sequence (MEM) and an externally cued task with the sequence given during the task performance (FOLLOW). EMG activity was measured in wrist flexors and extensors, biceps and shoulder, and hand movement was monitored with accelerometry. Average firing rate histograms of neurons were constructed for each task and aligned on the go cue, movement onset, and start of each button-press. Trajectories were reconstructed based on stereotactic co-ordinates and the location of the optic tract and internal capsule provided by visual or motor responses to microstimulation (1-100 μA; 300 Hz, 0.2ms p.w.).

A total of 60 units was tested including 13 in GPe , 42 in GPi and 5 'Border cells'. Twelve of the 42 GPi cells (29%) showed MEM-specific inhibition or greater inhibition during MEM than FOLLOW, either during the entire sequence or at the end of one or more button press(es). The remaining 30 GPi cells did not respond to the task or showed no task-specificity. One of the 13 GPe cells showed excitatory MEM-specific activity. Two 'Border cells' were excited during the tasks but showed no specificity. Averaged EMG traces were the same on both tasks. MEM neurons were located in the dorsal portion of GPi in sagittal planes about 20 mm from the midline. The demonstration of cognitive modulation of neurons in dorsal GPi supports a role of basal ganglia in processing mnemonic function.

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165.4

NEURONAL CORRELATIONS IN THE BASAL GANGLIA OF NORMAL AND MPTP-TREATED MONKEYS A. Feingold, A. Nini, A. Raz, H. Slovin, V. Zelenskaya, E. Vaadia, M. Abeles and H. Bergman*, Dept. Physiol., Hebrew Univ.-Hadassah Med. Sch., Jerusalem, ISRAEL 91120.

To test whether information processing in the basal ganglia is carried by parallel or integrative circuits, we simultaneously recorded the activity of 4-10 single pallidal neurons or striatal TANs of two rhesus and two vervet monkeys before and after MPTP treatment.

The regular cross-correlation histograms of >93% of pallidal pairs were flat for most behavioral epochs. Even when the neuronal pairs were coactivated, the normalized correlograms were flat. Similar analysis of thalamic and cortical pairs showed significant correlated activity in more than 25% of the studied pairs, whereas 60% of TAN pairs demonstrated correlated activity.

Following MPTP treatment the monkeys developed typical parkinsonian symptoms. The rhesus monkeys developed infrequent short episodes of 7-11Hz tremor, whereas the vervet monkeys had many prolonged episodes of 5-7Hz tremor. Crosscorrelation of the pallidal and TAN pairs revealed significant synchronous oscillations, with bimodal distribution of oscillation frequencies (around 5 and 10-15Hz). All possible phase shifts were found in the oscillatory correlograms of the rhesus pallidal pairs.

The results support the view that in the normally-behaving monkeys pallidal neurons are engaged in "parallel processing," and a patho-physiological consequence of dopamine depletion in Parkinson's disease is increased oscillatory correlated neural activity in the basal ganglia.

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165.5

COMPARISON OF THE EFFECTS OF EXPERIMENTAL PARKINSONISM ON NEURONAL DISCHARGE IN MOTOR AND NON-MOTOR PORTIONS OF THE BASAL GANGLIA OUTPUT NUCLEI IN PRIMATES. T. Wichmann*, H.Bergman, and M. R. DeLong. Dept. Neurology, Emory Univ. Sch. of Med., Atlanta, GA 30322, and Dept. Physiol., Hadassah Med. Sch., Jerusalem, Israel.

These experiments tested the hypothesis that parkinsonian motor signs are primarily related to altered output from areas of the basal ganglia that are involved in "motor" rather than "non-motor" functions. Changes in neuronal discharge were compared between the predominantly "motor" internal pallidal segment (GPi), and the "non-motor" dorsal and central pars reticulata of the substantia nigra (SNr) in an African green monkey before and after treatment with MPTP. Neuronal discharge was evaluated with autocorrelograms and with a burst/ inhibition detecting algorithm.

With MPTP-treatment, the discharge rate of neurons in GPi rose from 41 ± 18 spikes/s (n = 20; mean ± SD) before, to 49 ± 27 spikes/s (n = 59; $p < 0.05$, t-test). The fraction of neurons discharging in bursts increased from 78% to 84%. Periodic bursts were virtually not seen before MPTP, but seen in 40% of cells thereafter. The contribution of increased phasic activity to the change in mean discharge rate was 79%. In the sampled regions of SNr, average neuronal discharge rates were unchanged: 50 ± 26 spikes/s (n = 29) before, and 46 ± 25 spikes/s (n = 31) after MPTP. Before MPTP, 48% of SNr cells discharged in bursts, and 44% thereafter (periodic bursts in 15%).

These findings support the notion that the non-motor areas of the basal ganglia output nuclei are not as severely involved in parkinsonism as are the motor areas. Moreover, much of the MPTP-induced change in average neuronal discharge in the motor portions of these structures appears to be due to increased phasic activity. Further experiments exploring the effects of MPTP in motor and non-motor areas of SNr are under way.

This study was supported by NIH grant 5-ROI-NS15417-14

165.6

LOCAL BLOCKADE OF GABAERGIC INHIBITION IN THE PUTAMEN INDUCES BRIEF CHANGES IN PALLIDAL DISCHARGE AT RESTRICTED TIMES. M.E. Anderson*, M. Dubach, and J.A. Buford. Depts of Rehab. Med. and Physiol. & Biophys. and Reg. Primate Res. Cntr, U. of Wash., Seattle, WA 98195.

Striatal projection neurons receive GABAergic inhibition both from recurrent axon collaterals and from striatal interneurons. What is the effect of intrastriatal inhibition on the output of the striatum in awake, behaving animals?

We recorded from striatal target cells in internal (GPi) and external (GPe) globus pallidus while bicuculline was administered locally in the putamen. The drug was allowed to dialyze across a 6-7 mm extent of implanted permselective tubing in each experiment for a period of 0.5 to 1.5 hr. Pallidal cells were studied before and after placement of drug in the tubing while the monkey performed hand movements to visually-displayed or remembered sequences of target positions.

Within 15 min of bicuculline introduction, pallidal cells with either the regular discharge characteristic of GPi or the less regular activity characteristic of GPe showed brief, very high frequency bursts of activity. In some cells these bursts were preceded by abrupt pauses that lasted 35—70 msec. These unusual bursts or pause/bursts did not occur at random. Instead, they clustered at specific task-related epochs, e.g., around the initiation or termination of arm movement.

These data support the hypothesis that GABAergic inhibition is not the primary reason for the "resting" low discharge of striatal output cells. Instead, functional GABAergic inhibition is activated when input from extrastriatal sources excites both striatal output cells and GABAergic interneurons. Feedback or feedforward inhibition normally would act to limit the task-related activation of striatal output cells. When their discharge is not limited, pause-burst episodes in GPi cells could result from the interaction of striatal inhibition and intrinsic properties of target pallidal cells and/or by synaptic actions via the direct and indirect paths.

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165.7

SLOWING OF WRIST MOVEMENT AND IMPAIRED ABILITY TO TURN OFF ACTIVE MUSCLES FOLLOWING INACTIVATION OF GLOBUS PALLIDUS PARS INTERNA (GPi): LOCALIZATION OF EFFECTIVE SITES. J.W. Mink.* Dept. of Neurology, Washington. Univ. School of Medicine, St. Louis, MO 63110.

It has been shown previously that injection of of musicmol into GPi slows wrist movement to the greatest degree when the movement is made by relaxing a previously active muscle group (Mink and Thach, 1991). The purpose of the present study was to inject small volumes of muscimol into different GPi sites to determine 1) whether this effect is localized to a particular region of GPi and 2) whether inactivation of different sites in GPi affects aspects of the task differently (e.g. maintained position, flexion, extension, end-point position).

A rhesus monkey was trained to sit in a primate chair with its hand inserted into a wedge-shaped manipulandum. A visual target and cursor were displayed on an oscilloscope in front of the monkey. The monkey was trained to flex and extend the wrist to move the cursor and track the visual target in a hold-step-hold paradigm. The wrist movements were made with and without constant torque loads (0.15 Nm) opposing or assisting the movement. After the boundaries of GPi were mapped with single unit recording, muscimol (0.5 µl of a 1 µg/µl solution) was injected into sites in GPi. Injections were done once each week at sites 1 mm apart. Wrist position, velocity, force and EMG were recorded before and after injection of muscimol.

Wrist movement was slowed after injection into 9 of 10 sites studied to date in the posterior third of GPi. The impairment was qualitatively similar across sites, but the largest deficits were seen after injection into the more ventral and medial sites. The greatest impairment was seen in movements requiring relaxation ('turning off') the loaded flexors or extensors. There was a slight flexor positional bias. In the no-load condition extension was slow but flexion was near normal. After all GPi injections reaction time was normal.

These results confirm previous findings of relative inability to 'turn off' previously active muscles after GPi inactivation.

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165.8

SUBTHALAMIC 5-HT$_{2C}$ RECEPTORS: BEHAVIORAL SUPERSENSITIVITY AND INCREASED EXPRESSION AFTER SEROTONIN DEPLETION. K. Eberle-Wang*, A. Mehta, R.J.A. Banks and M-F Chesselet. Dept. of Pharmacology, U. Pennsylvania, Philadelphia, PA 19104.

Acute stimulation of 5-HT$_{2C}$ receptors within the subthalamic nucleus (STh) by peripheral or local administration of the 5-HT$_{2C}$ agonist m-cholophenyl-piperazine (m-CPP) produced abnormal orofacial movements in rats (Eberle-Wang et al. '95, 96). The present studies examined the regulation of this effect after serotonin depletion to determine whether removal of stimulation by endogenous serotonin affected the behavioral response and the 5-HT$_{2C}$ receptors. Rats received an intraventricular infusion of 5,7 dihydroxytryptamine (100mg/10ml) or vehicle after desimipramine (25 mg/kg, i.p.) pretreatment. Efficacy of serotonin depletion was confirmed by the presence of hyper reactive behaviors 3 days post-surgery and a decrease in serotonin uptake sites measured by autoradiography post-mortem. Repeated administration of m-CPP (1.0 mg/kg, i.p.) caused reproducible increases in orofacial movements throughout the 20 day testing period. In contrast, m-CPP-induced orofacial movements were markedly increased 4 (+91%) and 8 (77%) days post-surgery in the lesioned animals. In a separate group of rats, similar lesions induced a significant increase in both 5-HT2c receptor mRNA (+28%) and binding sites (+17%) in the STh 10 days post-surgery. The behavioral sensitivity and compensatory changes in receptor expression observed after serotonin depletion suggest that the subthalamic 5-HT$_{2C}$ receptors involved in the control of movements are normally stimulated by endogenous serotonin. Functional and molecular adaptation of 5-HT$_{2C}$ receptors in the STh may be involved in hyperkinetic disorders. Supp. by PHS grant MH-48125 and the Tourette Syndrome Association.

165.9

INTRACOLLICULAR MICROINJECTIONS OF MUSCIMOL BLOCK MOTOR AND DEFENSIVE RESPONSES INDUCED BY KAINIC ACID LESIONS OF THE SUBTHALAMIC NUCLEUS IN THE RAT.

M.A. Crumling, R.J.A. Banks, L. Naudon and M-F. Chesselet*. Dept. of Pharmacology, and Inst. Neurological. Sciences, Univ. of Pennsylvania, Philadelphia, PA 19104.

Pharmacological manipulation of the subthalamic nucleus (STh) in rats elicits abnormal movements (Parry et al., 1994; Eberle-Wang et al., 1995, 1996). However, the behavioral effects of STh lesion are poorly understood. Unilateral lesions of the STh with kainic acid (KA; 200 ng/200 nl) in rats reliably induced transient locomotor activation, vacuous mouth movements, and a long lasting increase in defensive responses. These effects, however, were inconsistently observed after lesions induced by ibotenic acid. Although KA infusion into the STh induced a delayed neuronal loss in the reticular nucleus of the thalamus, all behaviors were observed prior to this secondary effect of the lesion. To identify the neuronal circuits involved in the behavioral effects of KA-induced lesions of the STh, anesthetized male Sprague-Dawley rats were stereotaxically infused with 0.9% saline or KA into the STh and implanted with a unilateral guide cannula into the ventromedial superior colliculus on the side of the lesion. Intracollicular administration of the GABA agonist muscimol (100µg/500nl) 1 hour prior to testing abolished the lesion-induced increase in locomotion, sniffing, and mouth movements observed 4 days post-surgery, as well as the lasting (up to 31 days) increase in vocalization to handling. Despite their different time-courses, the blockade by muscimol of both motor and defensive responses suggests the involvement of the subthalamo-nigro-collicular pathway in both types of behavioral effects of the KA-induced lesion. Supported by MH-44894 and MH-48125.

165.10

LESION OF THE SUBTHALAMIC NUCLEUS (STN) IMPAIRS COMPENSATION FOR MECHANICAL LOADS DUE TO INABILITY TO SUPPRESS ONGOING ANTAGONIST MUSCLE ACTIVITY. D. Flament*, I. Neyman, J.J. Nicholas, D.M. Corcos. Dept. of Phys. Med. & Rehab., Rush Medical College, and School of Kinesiology, Univ. Illinois, Chicago, IL 60612.

Flexions of the wrist were studied in a patient who developed unilateral hemiballismus following a stroke localized to the STN. Three load conditions were tested: 1) a torque loading flexors (agonist), 2) a torque loading extensors (antagonist), and 3) a no torque condition. As it has been suggested that one function of the basal ganglia is to inhibit unwanted motor actions, these 3 loads were applied to test whether antagonist activity can be appropriately suppressed when initiating a movement. Movements of the affected limb were compared with those made by the contralateral limb.

Movements on the affected side were discontinuous, with multiple peaks in acceleration and deceleration. Extensor loads tended to produce discontinuities earlier in the movement, and were associated with an EMG pattern where agonist onset followed movement onset. This was found to be a consequence of the inability to rapidly inhibit tonic antagonist activity at movement onset when this muscle was loaded, and to simultaneously activate the agonist muscle.

The results suggest that the STN is necessary to compensate for mechanical loads and to suppress ongoing antagonist activity when a movement is initiated. The data support the hypothesis that one function of the basal ganglia is to enable selected motor programs and to inhibit potentially competing programs.

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165.11

LESIONS OF THE SUBTHALAMIC NUCLEUS DO NOT BLOCK REACTION TIME IMPAIRMENTS FOLLOWING SYSTEMIC ADMINISTRATION OF D1 OR D2 RECEPTOR ANTAGONISTS.

V.J. Brown and J.M. Phillips (SPON: Brain Research Association) School of Psychology, Univ. St. Andrews, St. Andrews KY16 9JU, Scotland, UK

Blockade of dopamine (DA) in the striatum results in impairments in reaction time performance of humans and animals. This akinesia appears to benefit from lesions, at the level of the pallidum or subthalamic nucleus (STN), of the overactive striatal outflow pathways via pallidum to thalamus and cortex. We examined the effects of systemic administration of the D1, SCH 23390 and the D2 receptor antagonist, Raclopride, on reaction time in rats with bilateral lesions of the STN.

We found a tendency for STN lesioned rats to show an increase in anticipatory responses, consistent with previous reports using this conditioned reaction time task (Baunez et al., 1995, *J. Neurosci.* 15:6531). However, employing a modified procedure in which error trials were repeated until successfully completed, we found that mean reaction time was not speeded following the lesion. Furthermore, the lengthened reaction time induced by systemic administration of either D1 (SCH23390 20, 40 and 80 μg/kg) or D2 (Raclopride 50, 100 and 200 μg/kg) receptor antagonists, was not blocked in the lesioned rats. However, both lesioned and control rats made few or no responses at the beginning of the drug sessions (particularly following higher drug doses) but the STN lesioned rats started responding earlier in the sessions than controls, indicating partial blockade of the effects of the drugs, particularly the D2 antagonist, Raclopride.

The lesions of the STN did not result in general hyperactivity, which might have accounted for the efficacy of this lesion in improving akinetic state induced by striatal dopamine depletion, but they were effective in reducing the cataleptic effects of DA antagonists. *(The Wellcome Trust, Project Grant # 040551/z/94)*

165.12

RESPONSES OF VENTRAL PALLIDAL NEURONS TO ELECTRICAL ACTIVATION OF THE SUBTHALAMIC NUCLEUS. M.S. Turner and T.C. Napier Dept. of Pharmacol., Loyola Univ. Chicago, Sch. of Med., Maywood, IL 60153.

An efferent pathway from the subthalamic nucleus terminates in the ventral pallidum (VP). Neurons in the subthalamic nucleus use glutamate as a neurotransmitter, and the VP contains excitatory amino acid receptors. Microiontophoretic application of glutamate rapidly increases firing in the majority of VP neurons examined. These observations suggest that the VP may be under an excitatory amino acid control that originates in the subthalamic nucleus. The objective of this study was to characterize the response of VP neurons to electrical activation of the subthalamic nucleus and to determine the proportion of VP neurons that exhibit evoked responses to this stimulation. To do this, the subthalamic nucleus of chloral hydrate-anesthetized rats was electrically activated using a concentric bipolar stimulating electrode, and neuronal activity in the VP was monitored using extracellular electrophysiological recording techniques. Of the 67 neurons studied, 60 neurons (90%) demonstrated evoked responses to subthalamic nucleus stimulation. Of these, 39 exhibited an excitatory response (neuronal firing frequency increased to 249% above control) whose onset was 4.3±0.4ms following stimulation, with a duration of 14.5±1.9ms. Ongoing studies are evaluating the excitatory amino acid receptor-subtypes responsible for this response. Some sensitive neurons displayed other responses, including short latency (7.9±1.4ms; n=20) inhibitions, long latency (30.6±5.7ms; n=13) excitations and long latency (47.4±12.0ms; n=7) inhibitions. Complex responses occurred in 30% of sensitive neurons, with the most common (13% of sensitive neurons) being a short latency excitation followed by a long latency inhibition. Long latency evoked responses probably represent multisynaptic pathways and short latency responses are consistent with a monosynaptic pathway from the subthalamic nucleus to the VP. The large number of neurons responding to stimulation of the subthalamic nucleus indicates that the subthalamic nucleus has a significant influence on the VP. Thus, the response of the VP to the increased release of excitatory amino acid from the subthalamic nucleus that occurs in Parkinson's Disease could contribute to the symptoms of this neuropathology. This work is supported by USPHSG #DA05255 to TCN.

165.13

EFFECT OF SUBTHALAMIC NUCLEUS (STN) STIMULATION ON MOTOR FUNCTION IN PARKINSON'S DISEASE (PD). I: CLINICAL AND PHYSIOLOGICAL MEASURES. P Limousin, R Brown, P Brown, M Jahanshahi, J Rothwell*. MRC Human Movement and Balance Unit, Institute of Neurology, Queen Square, London, WC1N3BG, UK

High frequency electrical stimulation of STN is an experimental neurosurgical treatment in disabling PD which is reversible when the stimulation is switched off. Five PD patients (mean age (± sem) 53 years ± 2 and mean duration of the disease 16 years ± 2) with bilateral STN stimulation (Grenoble University Hospital, Pr Benabid) were studied after an overnight withdrawal of medication. Motor function was compared with both stimulators turned either off or on in a randomized sequence. Strength and bereitschaftpotential (BP) were assessed for the worst clinical side, stretch reflexes were assessed on both sides and the results averaged. The mean clinical evaluation score (motor UPDRS) decreased from 53 (/108) ± 8 to 21 ± 4 (60%, $p < 0.05$) when the stimulation was turned on. The upper limb akinesia subscore decreased from 8 (/12) ± 1 to 3 ± 1 (63%, $p < 0.005$), the rigidity item decreased from 3 (/4) ± 0.3 to 1 ± 0.3 (76%, $p < 0.0005$).

The maximal strength of the forearm extensors, during an isometric contraction against a strain gauge, was increased by a mean of 195% ± 29 in the stimulated condition. The BP, preceding self-initiated free direction joystick movement was slightly increased (peak amplitude at Cz: 4.78 μV ± 1.27 to 5.78 μV ± 0.88, NS), with the major effect being on the second (NS') component. The latency, the duration and the amplitude of the short and long latency component of the forearm flexor stretch reflex were not modified by the stimulation.

The increase in strength by STN stimulation and the increase in premovement BP may contribute to the decrease in bradykinesia. The effect of STN stimulation in rigidity does not seem to be related to a change in the stretch reflex, other mechanisms might be involved. (Funded by MRC UK, Wellcome Trust and EU)

165.14

EFFECTS OF SUBTHALAMIC NUCLEUS (STN) STIMULATION ON MOTOR FUNCTION IN PARKINSON'S DISEASE (PD) II: BEHAVIOURAL MEASURES OF SIMPLE AND COMPLEX HAND-ARM MOVEMENT. R.G. Brown, M. Jahanshahi, P. Limousin, J.C. Rothwell, S. Channon*. MRC HMBU, Institute of Neurology, Queen Square, London WC1N 3BG, UK.

The times taken to initiate and execute a variety of voluntary movements were assessed in 5 patients with PD, with and without STN stimulation in counter-balanced order. The tasks were: time to make a self-initiated 30° forearm flexion either alone (FLEX), or with a simultaneous 20 N 'squeeze' of the hand (FLEX+); the time to initiate (IT) and execute (MT) a multi-joint 15 cm movement in the sagittal plane in response to a visual reaction stimulus; the rate of repetitive finger tapping over a 30 s period (TAP), and the rate of picking up and placing pegs in a row of 3 mm holes over a 30 s period (PEG). Both right and left sides were assessed and the results averaged. Without stimulation, subjects showed marked slowing on all tasks. STN stimulation had a significant (1-tailed paired t-tests, $p<0.05$), positive effect on several measures. Most marked and consistent across subjects were the improvement in the speed of executing arm-hand movements. Simple FLEX time decreased by 154.3 ms±28.6 (39.2%), while FLEX+ improved by 213.6 ms±31.4 (45.8%). MT decreased by 283 ms±153 (mean±sem) (29.5%±9.9). The ability to perform the PEG task was also reliably improved, with an additional 2.3±0.87 pegs (24.9%±8.6). Smaller mean improvement, with greater between-subject variability, was seen in the other measures. IT improved by 101.8 ms±77.8 ($p>0.13$) (12.3%±9.5), while TAP increased by only 5.3 taps±10.1 over 30 s ($p>0.32$) (4.9%±12.3).

Thus stimulation of the STN in PD increases the speed of simple and complex movements indicating a direct facilitation of motor execution. In contrast, the effects on movement preparation (IT) and the ability to sustain repetitive movement seem less sensitive to the effects of STN stimulation. (Grant funded by MRC UK, Wellcome Trust and EU).

165.15

EXCITOTOXIC LESIONS OF THE SUBTHALAMIC NUCLEUS, BUT NOT THE PEDUNCULOPONTINE NUCLEUS, AMELIORATE ASYMMETRY INDUCED BY STRIATAL DOPAMINE DEPLETION J.M. Phillips, V.J. Brown, S.P. Gupta, M.P. Latimer and P. Winn (SPON: Brain Research Association) School of Psychology, Univ. St. Andrews, St. Andrews KY16 9JU, Scotland.

The motor symptoms of Parkinson's disease result from striatal dopamine (DA) depletion, which in turns leads to overactivity of striatal outflow pathways via globus pallidus to thalamus and cortex and to pontine nuclei. Lesions in these pathways, at the level of the globus pallidus or subthalamic nucleus (STN), have been shown to ameliorate some of the Parkinsonian motor deficits. It is possible that the restorative motor effects of the STN lesion are due to the induction of a hyperactive state rather than a genuine restoration of impaired function by reestablishing normal levels of activity in the outflow pathways. Thus, it is important to examine the effects of STN lesions on deficits following striatal DA depletion on a range of tasks, rather than focussing solely on motor functions. Unilateral striatal DA depletion results in sensorimotor assymetry which is not due to an impairment in motor execution per se (Schallert et al. 1982, *Pharm. Bioch. Behav.* 16:455). We examined the effects of STN lesions and lesions of the striatopontine outflow pathway, at the level of the pedunculopontine nucleus (PPTg), on DA depletion-induced sensorimotor asymmetry. Whereas lesions of the STN restored symmetry following the striatal lesion, there was no effect (and, in particular, no evidence of hyperactivity) of the STN lesion on time to contact the patches. Lesions of the PPTg were without effect, failing to reduce the asymmetry induced by striatal lesion. These results suggest that specific deficits resulting from disordered basal ganglia function can be ameliorated by lesions of the STN and that the sensorimotor asymmetry following unilateral striatal DA depletion is due to "looped" thalamocortical striatal outflow, rather than striatopontine outflow.
(Supported by The Wellcome Trust, Project Grant # 040551/z/94)

165.16

BILATERAL 6-HYDROXYDOPAMINE LESIONS IN DOPAMINERGIC A8 CELL-GROUP PRODUCE LONG-LASTING DEFICITS IN MOTOR PROGRAMMING OF CATS. **M.P.M. Arts, M.A. Gingras* and A.R. Cools. Dept. of Psychoneuropharmacology, P.O. Box 9101, 6500HB, Nijmegen, The Netherlands.**

The A8 cell-group (A8) have been suggested to play a role in the progressive degeneration of dopaminergic cells in Parkinson's disease (1). However, the contribution of the degeneration of A8 cells to parkinsonian symptoms is unknown. Still, the feline A8 has been suggested to contribute to at least one parkinsonian symptom, namely the mask-like expression of the face (2). For these reasons, we studied the effects of 6-OHDA lesions (10 μg/0.5μL) in the A8 of cats (n=8) pretreated with 10 μg/0.5 μL desipramine. Behavioural deficits were studied with the help of a particular treadmill set-up that allows the assessment of subtle changes in motor programming (3). Effects were analyzed daily for 26 days (pre-lesion: 9 days; post-lesion: 17 days) and, thereafter, at increasing intervals for a minimum period of 7 weeks. The lesion being restricted to the A8 produced consistent and long-lasting deficits in all cats. The cats had a significantly reduced ability to switch arbitrarily motor patterns. The cats became fully bound to external stimuli provided by the apparatus and used these stimuli for switching their motor patterns. Moreover, the sequential patterning of their motor behaviour was fully changed. Finally, the kinetic melody of the movements was permanently changed. It is concluded that subtle lesions in the feline A8 produce long-lasting deficits in motor programming, implying that degeneration of dopaminergic cells in this brain region may also contribute to symptoms seen in Parkinson's disease. (1) Arts et al. in press; (2) Spooren et al. Synaps.15:104-123;1993 (3) Jaspers et al. Beh.Brain Res. 14:17-28;1984.

165.17

DEFICITS IN MOTOR MEMORY PRODUCED BY BILATERAL 6-OHDA CAUDATE LESIONS IN RAT.

X. Liu*, R. Strecker, C. Geronimo and J. Brener. Depts. of Psychology and Psychiatry, SUNY, Stony Brook, NY 11794-2500.

Patients with Parkinson's disease are reported to have greater difficulty in executing sequential moments when external cues are not provided than when external cues are provided. The current experiment was designed to model this phenomenon in rats. Subjects were rewarded with sucrose for pressing three force-sensitive beams in one of two sequences. Each was signalled by a stimulus light. In one of the sequences, the order was: Left, Right, Center, whereas in the other sequence, the order was: Right, Left Center. Upon completion of the signalled sequence, a reward was delivered to a central food tray. For one group of rats, each of three sequential beam presses was signaled by a corresponding light, whereas for another group, only the first press was signaled, while the two following presses were not. Time intervals between consecutive responses as well as sequence errors were recorded.

Bilateral 6-OHDA injections into the caudate putamen resulted in 50% DA depletion in this structure. In both groups the lesion caused lengthening of reaction times to the first signal light and to the time intervals between the first and second beam presses in the sequence. These changes were found to be due to the increases in the numbers of response sequence errors. Furthermore, for the noncued rats, there was an additional lengthening in the interval between the second and the third presses that was not shown by the cued rats. The results suggest that DA plays an important role in controlling sequential motor behaviors, and in keeping movement elements in motor working memory. (Supported by HL-42366 to JB.)

165.18

LESIONS OF THE VENTRAL-MEDIAL STRIATUM AND DORSAL HIPPOCAMPAL FORMATION INDUCE OVERLAPPING BUT DISTINCT BEHAVIORAL ABNORMALITIES. M. Burke, A. Cousins, A. Perez, M. Peterson, N. Dumont, J. McLear, S. Bheddah, S. Hye, S. King, M. Nistico, M. Allen-Racette, J. Losi, C. Engels, and J. P. Ryan*. Neurobehavioral Science Lab, Psychology Dept., State University of New York at Plattsburgh, Plattsburgh, New York 12901.

Disruption of the hippocampal system induces a variety of behavioral abnormalities that can be directly related to the hippocampus or to a disturbance in neural connections with other brain regions within the system such as the striatum. The present study attempted to delineate the aberrant behaviors of hippocampally lesioned animals in the T-maze, Activity Chamber, and Water Maze from the behaviors induced by lesions of the ventral-medial striatum. Using Long-Evans hooded rats, lesions of the dorsal hippocampus were induced with a total injection of 25 μg /μL of colchicine administered bilaterally. Lesions of the ventral-medial striatum were induced with a total injection of 30 μg/μL colchicine administered bilaterally. Preliminary results indicate that (1) marked deficits in Spontaneous Alternation Behavior are induced following both hippocampal and striatal lesions, (2) repetitive circumference swimming in the Water Maze is a unique characteristic of hippocampal lesions, and (3) increases in locomotion appear to be present in both lesioned groups with striatal lesioned animals demonstrating a unique pattern of activity in the T-maze.

165.19

LEARNED MOTOR PERFORMANCE IN RATS: PRODUCTION OF TARGET RESPONSE FORCES.

[1]J. Brener*, [1]X. Liu, [2]S. M. Mitchell and [1]S. Carnicom. [1]Dept. of Psychology, SUNY, Stony Brook, NY 11794-2500 and [2]Dept. of Psychology, University of New Hampshire, Durham, NH 03824-3567

Rats were rewarded with sucrose for pressing a force-sensitive beam with a criterion force. A concurrent feedback (CFB) group received auditory feedback as soon as their presses reached the criterion force whereas a terminal feedback group (TFB) only received auditory feedback on the termination of presses that met the force criterion. These contingencies permitted subjects in the CFB group, but not the TFB group to regulate force on the basis of external feedback. Fine-grain analyses of force-time trajectories under various conditions provided strong evidence that beam presses were composed of an initial ballistic phase followed by a controlled phase during which response force "homed in" on the force criterion under feedback control. The period of the feedback loop was found to be significantly longer in the CFB group (94.4 milliseconds between corrections) than in the TFB group (59.2 milliseconds between corrections). This difference is attributed to the use of exteroceptive feedback in force control by the CFB group in contrast to exclusive reliance on kinesthetic feedback by the TFB group.

Low doses of apomorphine and bilateral 6-OHDA SNpc lesions impaired force control under the TFB condition. The nature of the deficiencies suggest that under these conditions, the forebrain dopamine system was preferentially involved in the controlled phase of isometric beam presses: i.e. during the adjustment of response force on the basis of kinesthetic feedback. (Supported by HL-42366 to JB.)

OCULOMOTOR SYSTEM: CORTEX

166.1

MODULATORY ROLE OF D1-DOPAMINE RECEPTORS IN MNEMONIC CODING OF MONKEY PREFRONTAL CORTICAL NEURONS

T. Sawaguchi* and K. Kubota. Dept. of Behavioral and Brain Sciences, Primate Res. Inst., Kyoto Univ., Inuyama, Aichi 484, Japan.

To examine the role of dopamine receptors in visuospatial mnemonic coding of neurons of the prefrontal cortex (PFC), dopamine antagonists (SCH23390 for a D1-antagonist and sulpiride for a D2- antagonist) were applied iontophoretically to PFC neurons of two rhesus monkeys which performed an oculomotor delayed-response (ODR) task. The ODR task was initiated by having the monkeys fixate on a central spot in a CRT; this was followed by an eye fixation (1 s), cue (one of four peripheral cues, 0.5 s, 15° in eccentricity), delay (fixation cue only, 4 s), and go periods (memory-guided saccade to the cued-target location). A total of 62 neurons, sampled from periprincipal sulcal areas, showed a delay period activity with a preferred direction, i.e., directional delay period activity, and this activity fit well with a cosine function with a proportion (r^2) of mostly more than 0.8. Iontophoretic applications of SCH23390, but not sulpiride, with a 50 nA current decreased or increased the activities of most of the neurons with directional delay period activity (N=48/62), and the effect was mostly decreasing (N=43/48). For all of the neurons affected by SCH23390, their directional tuning, examined by the cosine curve fitting, was attenuated to become flatter during the application of SCH23390, whether the activity was increased or decreased by it. However, the preferred direction itself was not significantly altered during the application. These results suggest that the excitating and inhibiting activations of D1-dopamine receptors play modulatory roles in maintaining the directional or "mnemonic" coding of delay period activity of PFC neurons during ODR performance.

166.2

TRANSCRANIAL MAGNETIC STIMULATION OF THE PREFRONTAL CORTEX DELAYS CONTRALATERAL ENDOGENOUS SACCADES,

Tony Ro, Avishai Henik[†], Liana Machado and Robert D. Rafal*. Dept. of Neurology and Center for Neuroscience, UC Davis and [†]Dept. of Behavioral Sciences, Ben-Gurion Univ. of the Negev, Beer-Sheva, Israel

The contributions of the superior prefrontal cortex (SPFC) and the superior parietal lobule (SPL) in generating voluntary endogenous and reflexive visually guided saccades were investigated using transcranial magnetic stimulation (TMS). Subjects made horizontal choice saccades in response to a central arrowhead (endogenous signal) or a peripheral asterisk (exogenous signal) which were presented along with a single TMS pulse at varying intervals. When TMS was over the SPFC, saccade latencies in response to an endogenous go signal toward the hemifield contralateral to the TMS were delayed. No consistent effects were observed when the go signal was exogenous and TMS was over the SPFC or when TMS was over the SPL for either saccade type. The delayed contralateral endogenous saccades observed in this study are likely a consequence of disruption in the normal operations of the human frontal eye field.

Supported by US PHS grant MH41544 to R.D. Rafal and by US PHS training fellowship MH19930 to T. Ro.

166.3

TRANSCRANIAL MAGNETIC STIMULATION (TMS) OF THE LEFT POSTERIOR PARIETAL AND PREFRONTAL CORTEX DURING MEMORY-GUIDED SACCADES. IS THERE A HEMISPHERE ASYMMETRY IN SACCADE CONTROL ? R.M. Müri*[1], B. Gaymard, S. Rivaud, A.I. Vermersch, J.F. Cassarini, C.W. Hess[1], C. Pierrot-Deseilligny.
INSERM 289, Hôp. de la Salpêtrière, 47 Bld de l'Hôpital, F-75651 Paris, France, and [1]Dep. of Neurology, University of Bern, Inselspital, CH-3010 Bern, Switzerland.

In a previous study (1) using transcranial magnetic stimulation (TMS), we have shown that the right posterior parietal cortex (PPC) and the right dorsolateral prefrontal cortex (PFC) play different roles in the control of contralateral memory-guided saccades. This study investigated TMS over the left homologous regions. In 6 normal subjects, temporal mapping was performed by stimulating 160 ms, 260 ms, 360 ms after the flashed target, during the mid-memorization period (700 - 1500 ms), and 100 ms after the go-signal, i.e. the extinguishing of the central fixation point (2100 ms). Horizontal component of saccades were recorded by DC-electrooculography. When comparing with the percentage of error in amplitude (PEA) without stimulation (median: 9%, range:2-12%), a significant increase in the PEA was found for contralateral saccades with TMS applied over the PFC during the mid-memorization period (median: 16%, range:6-28%, Mann-Whitney test, $p < 0.01$). The PEA of ipsilateral saccades was not affected. Stimulation over the left PPC had no significant effect on PEA of contra- and ipsilateral saccades. The same was found in the control experiments with stimulation over the occipital cortex. These results show a difference between the previously reported effects of right TMS: for left stimulation, only prefrontal TMS (during the mid-memorization phase) had a significant effect on the right PEA. This suggests that 1) memory-guided saccades are mainly controlled by the contralateral PFC, and 2) an asymmetry between the right and left PPC exists concerning the control of these saccades.

Müri R.M. et al. Soc. Neurosci. Abstr. 21:469.13, 1995.

166.4

NEURAL CONTROL OF SACCADE INITIATION STUDIED WITH THE COUNTERMANDING PARADIGM: FRONTAL EYE FIELD. J.D. Schall* & D.P. Hanes, Vanderbilt University, Nashville TN 37240

A countermanding paradigm was employed to distinguish the functional role of neurons in macaque frontal eye field in generating saccades. On NO SIGNAL trials macaque monkeys shifted gaze to fixate a target that jumped to either side of the fixation spot which was removed. The target was positioned either in a neuron's movement field or in the opposite hemifield. On a fraction of trials the fixation spot reappeared at unpredictable intervals following the target jump. On these STOP SIGNAL trials monkeys were rewarded for countermanding the planned saccade and maintaining fixation. From a race model between independent GO and STOP processes, the time needed to inhibit the planned movement, STOP SIGNAL REACTION TIME (SSRT) was estimated (Hanes & Schall, 1995, Vis. Neurosci. 12:929). Based on the SSRT and the variation in saccade latency in NO SIGNAL trials the activity of individual neurons was compared between STOP SIGNAL trials when a saccade was inhibited and NO SIGNAL trials when a saccade was generated but would have been inhibited if the STOP SIGNAL had been given.

Two main populations of neurons were identified in frontal eye field. A population of cells with movement-related activity exhibited differential modulation before inhibited as compared to generated saccades. Because the difference in activity occurred before the SSRT had elapsed, these neurons can play a direct role in the voluntary control of movement. These neurons tended to be located deeper in the arcuate sulcus. A different population of visually responsive cells was recorded that did not exhibit differential activity before the SSRT had elapsed. These neurons tended to be located more superficially in the arcuate sulcus. These findings indicate the utility of the countermanding paradigm in distinguishing the functional role of neurons in movement production. (Supported by R01-MH55806 and F31-MH11178)

166.5

FRONTAL EYE FIELD VISUAL ACTIVITY PRECEDING SACCADES TO MOVING TARGETS. D. Shi*, H. R. Friedman, and C. J. Bruce. Section of Neurobiology, Yale Univ. Sch. Med., New Haven, CT 06520-8001.

Saccades to moving targets are usually accurate even though saccades are executed ballistically and take 100–200 ms to program, implying a predictive mechanism. Previously, we studied presaccadic movement activity in the macaque monkey's FEF in conjunction with such predictive saccades and found that this movement activity showed significant compensation for target velocity. Here we examined visual activity of both the purely visual and visuomovement types of FEF cells. For each cell, the optimal receptive field (RF) vector was first estimated and then parametric step-ramp experiments were conducted wherein targets moved either 1) towards the cell's RF center along paths orthogonal to the cell's optimal vector, or 2) in radial paths along the optimal vector, both centrifugal and centripetal. For Exp. 1 we analyzed the neuronal data by adding a parameter (K) to quantify any compensation of the cell's best direction for target velocity to our Gaussian formula for the polar tuning of FEF response fields: $R_i = B + R \cdot EXP(-0.5((\varnothing_t - (\varnothing_c - K \cdot d\varnothing_t)) / \partial)^2)$ wherein R_i is the spike rate for trial i [typically measured over the 50–200 ms interval after the step), $\varnothing_t$ is the target's initial polar coordinate on trial i, and $d\varnothing_t$ is its angular velocity. The remaining parameters are fit: $\varnothing_c$ estimates the cell's optimal polar direction, ∂ the breadth of its directional tuning, and K estimates (in ms) the velocity compensation. Almost all FEF visual responses showed highly significant compensation, with most K values in the 100–200 ms range. Moreover, the degree of velocity compensation increased with time following the step, indicating that FEF visual responses to moving targets are continually updated. Analogous compensation was seen for radial motion (Exp. 2). These data suggest a mechanism for the velocity compensation of FEF movement activity. They also help explain why projections from visual motion areas in posterior cortex (e.g., MT, MST) are not confined to FEF's smooth pursuit representation in the depths of the arcuate sulcus, but terminate extensively in the saccadic FEF as well, where they could afford predictive motion compensation to the visual responses there. Supported by PHS grants EY04740 & MH44866.

166.6

SELECTIVE DISRUPTION OF MEMORY-GUIDED SACCADES WITH INJECTION OF A CHOLINERGIC ANTAGONIST IN THE FRONTAL EYE FIELD OF MONKEY. E. C. Dias*, D. M. Compaan, M. M. Mesulam and M. A. Segraves, Institute for Neuroscience, Northwestern Univ., Chicago and Evanston, IL.

The frontal cortex receives a massive projection of cholinergic axons originating in the basal forebrain. There is a wealth of experimental results suggesting that the cholinergic muscarinic system plays a central role in learning and memory. For example, systemic injection of scopolamine, a cholinergic muscarinic antagonist, impairs memory in a variety of species, including primates. We tested the effect of local inactivation of the cholinergic system in the frontal eye field (FEF), a cortical region strongly involved in the generation of eye movements. Since many of the cells in the FEF have a memory component to their responses, we hypothesized that microinjections of scopolamine directly into the FEF might disrupt memory-guided saccades, but that visually-guided saccades would remain unchanged.

We made 6 microinjections (0.5-1.0 µl) of scopolamine hydrochloride (10mg/ml) within the FEF. Injections were made through a cannula with an electrode protruding from its tip, allowing constant monitoring of cell activity. The main effect of the scopolamine injections was a decrease in accuracy of memory-guided saccades, which increased as the memory delay period was increased. At some sites, this deficit was mainly for saccades whose amplitude and direction were similar to the optimal saccade vector represented by that site, but in other sites the accuracy of saccades in all directions was reduced. There was also an increase in saccade latencies and frequently a reduction in saccade velocities. There were no observable effects of the injection on the visually-guided saccades. In addition to these saccade-related deficits, the monkey had difficulty maintaining fixation after the injection, and was easily distracted from the task. These findings are in contrast to FEF injections of muscimol, a GABA agonist, which disrupt both visual and memory-guided saccades. In addition, muscimol silences cell activity at the injection site, whereas the cells remained active after scopolamine injection.

These preliminary data suggest that the cholinergic afferent system is important for the memory component of the responses of FEF cells, and may also be involved in the control of fixation.

Supported by NIH Grants EY08212 and EY06716

166.7

MACAQUE FRONTAL EYE FIELD INPUT TO SACCADE-RELATED CELLS IN THE SUPERIOR COLLICULUS. J. O. Helminski* and M. A. Segraves, Dept. of Neurobiol. and Physiol., Inst. for Neuroscience, Northwestern Univ., Evanston, IL 60208.

Frontal eye field (FEF) neurons with movement-related or foveal visual activity project to the intermediate layers of the superior colliculus (SC) providing information concerning the maintenance and release of fixation and parameters of the impending saccade. The purpose of this preliminary investigation was to identify and characterize SC neurons receiving this FEF input. Extracellular recordings were made simultaneously in the saccade-related regions of the FEF and SC. Antidromic excitation was used to identify pairs of interconnected FEF and SC sites. Then, the FEF site was stimulated and evoked potentials were recorded every 100 µm dorso-ventrally through the SC. Next, SC cells receiving short latency FEF input were identified with orthodromic stimulation and their activity characterized during visuomotor tasks.

Four pairs of interconnected FEF and SC sites in one monkey were identified with antidromic excitation. Field potentials were recorded and current source density was calculated, revealing FEF excitatory input concentrated at depths where cells with saccade-related activity were found. Nineteen superior collicular neurons receiving short latency, excitatory FEF input were identified and characterized in detail. An effective orthodromic stimulus was a pair of biphasic pulses (negative first, 0.02 ms total pulse width) with 3 ms separation between pulses applied 0.1-0.3 ms after the SC cell fired spontaneously. Current thresholds ranged from 100-800 µA (mean 392 µA); latencies from 2.0-5.8 ms (mean 3.9 ms). Every SC cell activated by FEF stimulation had movement-related activity. This activity was compared to a recent description of saccade-related activity in the SC (Munoz and Wurtz, 1995). Eighty-four percent of FEF-driven cells were classified as burst cells, discharging a burst of action potentials immediately before saccade onset. Sixteen percent were classified as buildup cells, discharging a long-lead buildup of activity between the signal for saccade initiation and saccade onset. There was no indication that the FEF contacts one of these cell types at earlier intervals than the other. Instead, it appears that the FEF makes short latency, presumably monosynaptic, contact with both buildup and burst cell types, allowing the FEF to directly influence all saccade-related activity in the SC.

Supported by NIH Grant EY08212 and the Illinois Physical Therapy Association Doctoral Fellowship.

166.8

INDIVIDUAL NEURONS IN THE VENTRAL ANTERIOR NUCLEUS OF THE THALAMUS OF *CEBUS* MONKEYS PROJECT TO BOTH THE SUPPLEMENTARY EYE FIELD AND THE FRONTAL EYE FIELD. J.C. Lynch*[1,2,3] J.-R. Tian[4,5], and A.W. Chiemprabha[1]. Departments of Anatomy[1], Ophthalmology[2], and Neurology[3], University of Mississippi Medical Center, Jackson, MS 39216; and the Brain Research Institute[4] and Department of Neurobiology[5], UCLA, Los Angeles, CA 90095.

Thalamocortical projections to the saccadic eye movement subregions of the supplementary eye field (SEFsac) and the frontal eye field (FEFsac) were studied in 3 *Cebus apella* monkeys. The SEFsac and FEFsac were localized using standard low-threshold electrical microstimulation. Retrogradely-transported fluorescent dyes were then injected into these areas. Neurons labeled by the SEFsac injections were found predominantly in area X of the VL complex and in the VA nucleus, with additional labeled neurons in the MD, VLc, VLo, and intralaminar nuclei. Neurons labeled by FEFsac injections were concentrated in the paralaminar MD nucleus, with additional labeled neurons in the VA nucleus, area X, VLc, VLo, and the intralaminar nuclei. In one monkey, the combination of tracers that was used permitted the detection of neurons labeled by *both* the SEFsac and FEFsac injections. Double-labeled neurons were observed *only* in the VA nucleus; 85.7% of these were in the magnocellular division (VAmc) and the rest were near the VAmc/VApc border. Within VAmc, 12.2% of the neurons that were labeled by the FEF injection also sent collaterals to the SEF; 5.7% of the neurons that were labeled by the SEF injection also projected to the FEF. The VAmc is known to receive input from the substantia nigra and to relay neural information from the substantia nigra to the FEF. The present results demonstrate that some VAmc neurons are able to provide identical neural information to both the SEFsac and the FEFsac.

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166.9

THE TRIPLE-STEP PARADIGM: AN ATTEMPT TO DISSOCIATE TARGET MAPPING FROM NEXT SACCADE SELECTION. J.-R. Tian*, J. Schlag, and M. Schlag-Rey. BRI and Dept. of Neurobiology, UCLA, Los Angeles, CA 90095-1763.

Quasi-visual cells (QV) are visual cells that discharge in the double-step paradigm when the subject (monkey) is ready to make a saccade toward a location where a target was flashed if - and only if - the initial saccade has brought this location in the cell receptive field. Such cells, first found in superior colliculus (Mays & Sparks, J.N. 1980, 43: 207) also exist in parietal cortex and frontal eye field (FEF). Their role can be interpreted in different ways: they can be elements of a dynamic map representing spatial relations between targets or they may signal the next, selected saccade.

To test these hypotheses, we designed a triple-step paradigm, consisting of one fixation point plus 3 targets, all flashed before the monkey had time to make the first saccade. The sites where targets were flashed were chosen so that only one of the last 2 target locations would be in the cell receptive field when the eyes had moved to the first target position. The "next saccade" hypothesis predicts QV cell activation at that position only if the next saccade is directed to the cell receptive field. In contrast, the "target mapping" hypothesis predicts cell activation even if the next saccade is directed away from the receptive field.

Unit recordings were made from visual FEF cells. Up to now, the majority of the QV cells tested were of the "target mapping" type. (Supported by USPHS grants EY02305 and 05879).

166.10

SUPPLEMENTARY EYE FIELD (SEF) NEURONAL ACTIVITY IN MONKEYS CAN PREDICT THE SUCCESSFUL PERFORMANCE OF ANTISACCADE TASKS. N.Amador, M. Schlag-Rey* & J. Schlag. BRI & Dept of Neurobiology, UCLA, Los Angeles, Ca. 90095-1763.

We studied neuronal activity in the SEF of 2 monkeys performing interleaved antisaccades (AS) and prosaccades (PS) in directions chosen to coincide with each cell preferred or null direction. The instruction to make AS or PS was conveyed on each trial by a small square or a dot appearing as the initial fixation point. The saccade target (always a dot) was flashed during or after fixation of the instruction cue. The go signal was provided by the offset of the cue.

On correct trials, out of 39 units (located in the SEF region responsive to low threshold electrical stimulation), 72% had a significantly higher firing rate for AS than for PS at one or several phases of the task (initial fixation, target onset, movement onset) while the opposite was true for only 5%. Activity differentiating AS and PS was measured for identical stimulus locations when related to initial fixation or target, and for similar trajectories when related to movement. The tendency for the cells to favor AS in competition with reflexive PS programmed in the other hemisphere was even higher for presaccadic units, which may reflect a final decision (90% vs. 0%). The greater activity seen on those AS trials can begin with fixation of the instruction cue. In that case, we propose that this differential firing rate sets the brain to block a compelling PS whatever its direction. Consistent with this result, when an AS instruction was given but a PS was made, the firing rate was generally lower than that observed on interleaved correct AS trials. Our results suggest a neuronal mechanism for the purposeful inhibition of visual grasping reflexes which is known to be impaired by frontal lobe lesions. *(Supported by USPHS grants EY02305 and EY05879).*

166.11

THE ROLE OF DORSOLATERAL PREFRONTAL CORTEX (DLPFC) IN THE EXECUTION OF SACCADES TO REMEMBERED LOCATIONS. E.P. O'Sullivan[12], S.C. Baker[2], C.D. Frith[3], L. Henderson[1], D.J. Brooks[2], C. Kennard[1]*. [1]Dept of Clinical Neuroscience, Charing Cross & Westminster Med Sch, [2]MRC Cyclotron Unit, Hammersmith Hosp, [3]Wellcome Dept of Cognitive Neurology, Inst of Neurology, London, United Kingdom.

Electrophysiological studies of non-human primates executing saccades to remembered locations suggest that DLPFC is primarily concerned with working memory for locations in body-centred space. However, the requirement to delay saccades until a command given after target offset makes severe demands on attentional control processes, notably suppression of the tendency for target onset to attract an immediate saccade and such executive control of the saccadic system has been shown to be impaired in humans with prefrontal lesions. We therefore decided to compare regional brain activation during the execution of delayed saccades (DS), with and without a memory requirement.

The bolus $H_2{}^{15}O$ PET technique was used to measure rCBF changes over a 30-40 second epoch. Eight normal volunteers were scanned while resting with eyes closed and during performance of a single trial in each of two DS tasks. In both a peripheral target was illuminated for 200 ms, and the saccade was summoned by a non-spatial cue after a delay of 45 seconds only, to exclude changes related to the motor component of the saccade. In the DS REM task target location had to be stored throughout the delay, whilst in the DS VIS task the target persisted until after the saccade. Four scans were obtained on each subject under each of these three conditions. Data were analysed with the statistical parametric mapping technique.

Comparison of either DS task to the baseline control revealed activation of DLPFC (right) but not the frontal eye field, whereas direct comparison of the DS tasks with and without the memory requirement failed to detect differential activations of DLPFC. This implies that activation relating to the motor component of the saccade was attenuated and that DLPFC was activated in relation to saccadic suppression, but not by the mnemonic component. *(supported by The Wellcome Trust).*

OCULOMOTOR SYSTEM: SMOOTH PURSUIT

167.1

RESPONSE PROPERTIES OF SMALL-FIELD PRETECTAL NUCLEUS OF THE OPTIC TRACT (NOT) NEURONS DURING SMOOTH PURSUIT EYE MOVEMENTS. M.J. Mustari*, A. Burrows, and C. Livingston. Marine Biomedical Institute, University of Texas Medical Branch, Galveston, TX. 77555.

The NOT comprises an essential part of the afferent limb for optokinetic response (OKR), elicited during coherent full-field visual motion. We reported previously[1] that the primate NOT contains units with large receptive fields (RFs) well suited to supporting the OKR. Here, we examine the potential role of parafoveal visual (PFv) neurons in the initiation and maintenance of smooth pursuit (SP). We recorded from 30 PFv NOT units in 2 monkeys (Macaca mulatta) during periodic and step-ramp SP tasks. Eye movements were measured by electromagnetic means, employing a scleral search coil. During SP, monkeys tended to have small tracking errors resulting in residual visual motion of ≤1 to 10°/s and modulation of PFv neuronal discharge. PFv units ceased their elevated discharge when the target spot was briefly extinguished during SP, despite maintained pursuit eye movements. PFv units respond preferentially for low velocity (<10^0/s) stimuli, moving ipsiversively. For PFv neurons to play a role in SP initiation their response latency to the onset of ipsiversive visual motion should lead smooth pursuit initiation. During step-ramp target tracking, we found that PFv unit discharge always lead SP initiation; by 20 to 80 ms. In summary, the sensitivity of PFv neurons to visual slip could be useful in initiating SP, perhaps through direct projections to the medial vestibular nucleus and nucleus prepositus hypoglossi[2]. Potentially, PFv units could play a role in adjusting the gain of SP through climbing fiber associated pathways[2]. [1]Mustari and Fuchs, J. Neurophysiol. 64: 77-90, 1990; [2]Mustari et al., J. Comp. Neurol. 349: 111-128, 1994; Supported by: N.E.I. grant EY06069.

167.2

ACTIVITY OF PURKINJE CELLS IN MONKEY CEREBELLUM DURING TRACKING OF TWO-DIMENSIONAL TRAJECTORIES H.-C. Leung* & R.E. Kettner Institute for Neuroscience & Department of Physiology, Northwestern University Medical School, Chicago, IL 60611

Purkinje cells in flocculus/paraflocculus of the cerebellum were recorded during tracking of a laser spot moving along circular (0.6 Hz, 5°), and sum-of-two-sines (0.6 Hz, 5°; 0.9 Hz, 3.3°) trajectories, as well as the component sinusoids used to create these trajectories. Saccadic and position sensitivity data were also obtained. Target position was computer controlled by horizontal and vertical mirror galvanometers. Eye position was monitored with a scleral search coil system. Juice reward was given for correct fixation and tracking. Pursuit directional sensitivity was determined from responses during tracking of a target moving sinusoidally in horizontal, vertical, and two diagonal (±45°) directions. Most cells were maximally modulated during either horizontal or vertical pursuit, although some cells had oblique preferred directions.

To assess the linearity of each cell's response, circular and sum-of-two-sines period response histograms were compared with histograms created by summing responses from the sinusoidal components used to create these trajectories. For example, combined horizontal and vertical sinusoidal motions 90° out of phase produce circular trajectories. For a linear system, responses during circular pursuit should equal the summed responses during pursuit of the horizontal and vertical components. Diagonal sinusoids that combined to generate circular trajectories were also used to study the linearity of the system. In a related set of studies, average responses during sum-of-two-sines pursuit were compared to summed component responses. Results showed approximate linearity for some cells, while other cells exhibited responses that were non-linear. (Supported by NIMH grants MH 48185 and NIH training grant T32 DC00015-13)

167.3

VECTOR AVERAGING FOR SMOOTH PURSUIT EYE MOVEMENTS INITIATED BY TWO MOVING TARGETS IN MONKEYS. Stephen G. Lisberger* and Vincent P. Ferrera, Department of Physiology and W.M. Keck Foundation Center for Integrative Neuroscience, UCSF, San Francisco, 94143.

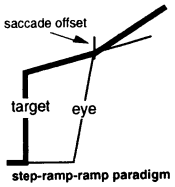
When targets move across the visual field, the distributed representation of motion in the visual cortex must be transformed into commands for the correct direction and speed of pursuit eye movements. Possible transformations fall along a continuum between "winner-take-all", which posits that pursuit is guided exclusively by the preferred direction and speed of the units with the largest responses, and "vector averaging", which posits the pursuit is guided by an equally-weighted combination of the firing of all active units. We have probed this transformation by recording the initiation of pursuit when two targets moved simultaneously in different directions at the same speed for 150 ms. Thereafter, one of the targets disappeared and the other became the tracking target. We used 8 directions of motion along the cardinal axes and the 45° oblique axes and an 8x8 design so that each combination of two initial target motions and one final motion were equally likely. All target motion was toward the position of fixation from 3° eccentric. Unlike our earlier studies, in which pursuit used a winner-take-all rule when cued which target to track and only horizontal target motion was eligible for tracking, this approach revealed a role for vector averaging when there was no cue and all directions of motion were "eligible" for tracking. Average eye acceleration in the first 100 ms of pursuit was consistent with vector averaging of the motion of the two targets when the angle between the two targets was 45°. The overall response amplitude was lower than predicted by vector averaging when angle between the two targets was 90° or more. Analysis of the eye acceleration in individual trials failed to reveal evidence for a winner-take-all rule. We conclude that the pursuit system uses a nearly-equally-weighted average of the two target motions when there is no cue about which target to track. *(Supported by NIH EY03878 and McDonnell-Pew JSMF 92-38)*

167.4

TOPOGRAPHIC ADAPTATION OF HUMAN SMOOTH-PURSUIT EYE MOVEMENTS REVEALED BY A STEP-RAMP-RAMP PARADIGM. T. OGAWA* and M. FUJITA, Neural Computation Sect., Communications Res. Lab., Koganei-shi, Tokyo 184, Japan

When a moving target appears in the peripheral visual field, saccade and pursuit eye movements are induced to acquire the target (Rashbass, 1961). Pursuit response is initiated immediately after the end of the saccade, and its velocity roughly matches to that of the target. Because pursuit has a latency of about 100 ms, this postsaccadic pursuit eye movement is probably generated based on information about the target moving in the peripheral visual field before the saccade (Newsome et al., 1985). The main purpose of the present study is to investigate the adaptive properties of these postsaccadic pursuit eye movements.

In adaptation trials, a target appeared in the peripheral visual field (2-10 deg) and moved away at a constant speed (10 deg/s). A subject was required to track it through eye movements, but the target speed was changed to a higher (20 deg/s) or lower (2 deg/s) constant speed at the termination of an initial saccade made to the target (step-ramp-ramp target motion). This adaptation paradigm induced adaptive modifications in postsaccadic pursuit responses and our results revealed the following properties in the pursuit adaptation system. *Topographic modification*: Modification of the initial pursuit velocities depends on the visual location of a moving target. *Pursuit gain change*: Pursuit velocity is modified not by the addition of a constant bias to the pre-adaptation pursuit velocity, but by a change in the pursuit gain (pursuit velocity/ target velocity). *Independence from saccade system*: Since pursuit adaptation did not change the amplitude and latency of saccades either to a moving target or to a stationary target, neuronal modifications for pursuit adaptation might occur at a level independent of the saccade system.



step-ramp-ramp paradigm

167.5

OFFSET RESPONSE OF THE HUMAN PURSUIT SYSTEM WHEN A TARGET JUMPS TO THE FOVEA: EFFECTS OF JUMP SIZE. J. Pola* and H. J. Wyatt, SUNY College of Optometry, New York, NY 10010.

Recently we showed (Soc. Neurosci. '94) that when a subject smoothly pursues a moving target and the target suddenly jumps to the fovea (and is stabilized there), smooth pursuit slows down with a time constant of about 0.5 sec. Here we explore effects that the target jump itself has on the dynamics of this pursuit offset. **Methods.** Subjects pursued a target moving horizontally at 15 deg/sec. When pursuit velocity became steady, the target jumped to the fovea with target velocity and feedback becoming 0 (target stabilized at the fovea). On 30% of the trials the target continued moving at 15 deg/sec with no jump. **Results.** The size of target jump varied from trial to trial due to variability of eye position relative to target position at the moment of the jump. (The jump was nearly always opposite to pursuit, since eye position trailed target position.) Pursuit offset following target jump had two components: an initial fast drop in velocity followed by a slow drop in velocity. As target jump increased, the fast component drop increased and the slow component drop decreased. However, as target jump varied from small to large, slow component deceleration remained roughly constant. **Conclusions.** Size of target jump had clear effects on pursuit offset. One possibility is that the target jump - a step change in target position - generated a "pulse" velocity signal opposed to the direction of ongoing pursuit, resulting in the fast component of pursuit offset preceding the slow component. However, the near constancy of the slow component deceleration suggests that the functional properties of the mechanism determining pursuit offset (e.g. parameters of an internal positive feedback loop) are not influenced by target jump. The fast component appears likely to be observed only in a laboratory environment; however, both components are suggestive of internal mechanism properties.

167.6

THE VALIDITY OF DIRECTIONAL CUEING INFLUENCES SMOOTH PURSUIT LATENCY. J.-L. Horng and M.J. Morrow*. Dept. of Neurology, Olive View-UCLA Medical Center, Sylmar, CA 91342

Maximally predictable step-ramp stimuli elicit smooth pursuit initiation with latencies of about 25 msec less than responses to unpredictable step-ramps. Recent work in our laboratory has shown that step-ramp stimuli in which ramp direction alone is constant evoke pursuit with similar latencies to targets in which step amplitude and ramp speed, direction and timing are all constant. In order to further explore this finding, we tested 4 normal subjects, presenting centripetal step-ramps of unpredictable direction and timing but constant speed and step amplitude. These stimuli were preceded by a directional cue consisting of a briefly-illuminated arrow. The arrow usually (80% of trials) pointed in the same direction as the subsequent ramp motion, but occasionally (10% of trials each) pointed in the opposite direction ("invalid cue") or in both directions ("no cue"). We found that pursuit latency was significantly determined by cue validity, having values averaging 20 msec lower for valid directional cues than for invalid cues, and intermediate values for the "no cue" task. We assessed the contribution of "motor habit" by segregating pursuit responses according to the relative directions of the previous two ramp movements; our analysis revealed no consistent effect of this factor upon pursuit latency. We conclude that the mechanism that expedites smooth pursuit initiation for predictable targets is driven chiefly by a cognitive synthesis of the likely direction of target motion. Motor habit based upon the repetition of a recently-executed movement plays no significant role in this process.

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167.7

ANTICIPATORY SMOOTH PURSUIT EYE MOVEMENTS IN RESPONSE TO REPETITIVE TARGET VELOCITY SEQUENCES S.G.WELLS, G.R.BARNES, D.M.WOLPERT* MRC HMBU, Inst of Neurology, Queen Sq, London WC1N 3BG

In response to an unexpected moving target there is a latency of ~100ms before smooth pursuit starts. However regular repetition of the same stimulus leads to the development of anticipatory smooth movements prior to target appearance. On average, anticipatory velocities increase with target velocity when only one velocity is used. This experiment tested the ability of 8 normal humans to control their anticipatory velocities when more than one target velocity was used in a repeated sequence of which they had full knowledge. Movements were recorded by infra-red oculography. Two velocities (Fast, 38°/s or Slow, 13°/s) of horizontal target motion were presented as *n* fast targets then *n* slow targets, repeated 12 times without a break. Three sequences (n=2,3 or 4) were used. The moving target was presented for 0.48s with 1.12s of darkness between presentations. In all 3 sequences, the response to the first slow target of each repetition was similar to the subsequent slow responses, but the first fast response was slower than subsequent fast responses for over 0.2s after target appearance. Anticipatory response magnitude was assessed by measuring smooth eye velocity (V_0) at target onset, prior to visual feedback influence. Mean V_0 decreased significantly ($p<0.05$;t-test) from 13.3+/-5.5°/s to 7.7+/-3.9°/s (mean±stdev) between the last fast and first slow responses, but did not increase significantly between the last slow (6.6+/-2.8°/s) and first fast responses (8.3+/-4.2°/s), although the appropriate velocity was achieved for the second fast response. Thus subjects graded anticipatory velocity appropriately for a decrease, but not when an increase was needed, probably due to the effect of the preceding slow targets. Although significant differences were shown by averaging many sequence repetitions, subjects had quite high variability in V_0, so the differences may not hold for all repetitions. Thus anticipatory movements may just crudely estimate target velocity whilst awaiting visual feedback. (Funded by MRC UK).

167.8

PREDICTIVE SMOOTH-PURSUIT EYE MOVEMENTS IN SCHIZOPHRENIA: REACTION TO SUDDEN PERTURBATIONS. W. Heide, P. Trillenberg, M. Blankenburg, K. Junghanns, V. Arolt, D. Kömpf. Depts. of Neurology and Psychiatry, Medical University, D-23538 Lübeck/Germany (SPON: European Brain and Behaviour Society).

A reduced gain of smooth-pursuit (SP) eye velocity has frequently been reported in schizophrenic patients. With respect to predictable stimuli, this could at least partly be due to a deficit in either generating an anticipatory component of SP or in modifying it according to unpredictable changes of the target trajectory. To test this we investigated the SP response to sudden perturbations of an otherwise sinusoidally moving visual target. Horizontal eye movements were recorded using infrared reflection oculography in 13 subacute schizophrenic patients and in 25 age-matched healthy adults, who were tracking a sinusoidally moving foveal target which after 5 cycles was suddenly stopped at one of the maxima of the position trace. Compared to the normal control group, the velocity gain of predictive SP was significantly ($p<0.02$) lower in schizophrenics, but the phase lag was normal. After the unpredictable stop of target motion, the eye accelerated away from the target for ca. 180 ms in both groups, but the resulting velocity and position error into the direction of the previous predictive SP was above normal limits in schizophrenics. We conclude that schizophrenic patients like normal subjects are able to internally generate an anticipatory component of predictive SP. The enlargement of this component in schizophrenics might help to compensate for their lower velocity gain, but impairs their ability to adapt predictive SP to sudden perturbations of the external stimulus.
(Supported by the DFG grant Ar 234/1-1)

168.1

MODULATION OF THE H-REFLEX DURING HUMAN WALKING IS NOT CORRELATED WITH JOINT MOVEMENT. B.A. Lavoie and C. Capaday. Centre de Recherche en Neurobiologie, Université Laval, Québec, (Qc) Canada, G1J 1Z4

It has been suggested that the soleus H-reflex modulation pattern during walking (Capaday and Stein, J. Neurosci. 6: 1308-1313) may be due to movement related afferent activity, especially from muscle afferents of the quadriceps muscle (Brooke et al. J. Neurophysiol 73:102-111; Misiaszek et al. J. Neurophysiol. 73:2499-2506). Additionally, these authors have suggested that the reduction of the H-reflex during walking compared to standing is also due to movement related afferent activity. One implication of this hypothesis is that the modulation pattern observed during walking should be closely correlated to the joint kinematics. We have thus re-examined in detail the modulation pattern of the H-reflex during human walking and simultaneously measured the angular displacements of the ankle, knee and hip. The very strong inhibition of the H-reflex at heel contact occurs with little or no knee flexion at that time. This period of inhibition is best correlated with a very low level of activity in the soleus and a large burst of activity in the tibialis anterior (TA). The H-reflex reaches its maximum value late in the stance phase and begins to decreases abruptly, as does the soleus EMG, at least 100 ms before the onset of knee flexion. It reaches its minimum value of essentially zero just before the burst of activity in the tibialis anterior, at a time when knee flexion is just beginning, and well before full knee flexion. We have also studied one leg stepping (i.e. swing) initiated voluntarily after an auditory 'go' signal. In this task, the H-reflex attained its minimal value of near zero before the onset of EMG activity, or movement of any joint. We conclude that during walking the pattern of H-reflex modulation is strongly correlated with the motor activity, but not joint movements; and that the task dependent differences in the input-output characteristics of this reflex are centrally determined. (Supported by the MRC of Canada)

168.2

MODULATION OF STRETCH REFLEX ACTIVITY AT THE HUMAN ANKLE DURING PASSIVE "WALKING" MOVEMENTS. R.E. Kearney*, R.B. Stein and M. Lortie Department of Biomedical Engineering, Faculty of Medicine, McGill University, Montreal, Quebec, Canada, H3A 2B4.

The objective of this study was to assess the role of peripheral mechanisms in modulating stretch reflex gain throughout the walking cycle.

Ankle position was recorded while human subjects walked on a treadmill at 3 km/hr and ensemble averaged over 60 strides. Average ankle position was "replayed" to supine subjects through a hydraulic actuator attached to the ankle via a rigid boot. Subjects maintained a constant level of muscle activation aided by visual feedback of low-pass filtered EMG. Small amplitude pulse displacements lasting less than 50ms were superimposed on approximately 20% of the cycles. Pulses were applied at eight different points in the walking cycle. Position, torque and EMG were ensemble averaged for both control and perturbed cycles. Reflexively evoked EMGs and torques were isolated by subtracting the ensemble average of the control trial from those of the perturbed trials. The residual EMG records were characterized by a phasic burst of activity occurring with a latency of about 40 ms. Residual torque records comprised two components: a short-latency component associated with intrinsic mechanisms and a longer latency transient component due to reflex activation

The amplitude of the reflex EMG and torque changed systematically throughout the walking cycle. Reflex EMG was near zero for several hundred milliseconds prior to heel strike. It then increased progressively for about 500 ms as the ankle was dorsiflexed, reaching a maximum near full dorsiflexion. Reflex EMG dropped rapidly to zero once the ankle began to plantarflex. Reflex torque was modulated similarly.

These results demonstrate that peripheral mechanisms, perhaps acting via pre-synaptic inhibition, are capable of producing the changes in reflex gain observed during walking.

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168.3

A COMPARISON OF THE SOLEUS STRETCH AND H-REFLEX DURING WALKING IN HUMANS. T. Sinkjaer* and J.B. Andersen. Center for Sensory-Motor Interaction, Aalborg University, Fredrik Bajersvej 7D, DK-9220 Aalborg, Denmark.

The short latency reflex is highly modulated during walking. Assuming that the size of the stretch reflex probe central as well as peripheral factors and the H-reflex probe central factors only, a comparison between the two reflexes might elucidate how peripheral factors contribute to the short latency reflex modulation during walking.

In nine healthy subjects both types of stimuli were applied to the ankle extensors during walking on a treadmill. A step was divided into 10-segments where both H-reflex stimulations and stretches were applied in the same experiment. The stretch reflex in the soleus muscle was elicited by imposing a well defined dorsiflexion of 8° with a velocity of approximately 300°/s by a portable stretch device (Andersen & Sinkjaer 1995, TRE VOL. 3, NO. 4, p 299-306). The H-reflex was elicited by an electrical stimulation of the tibial nerve of the investigated leg through an electrode in the popliteal fossa.

Both the stretch reflex and the H-reflex were modulated throughout the step with a maximal amplitude in the stance phase, a complete suppression of the amplitude in the transition from stance to swing. In late swing both reflexes again increased. At 60% of the stance phase the amplitude of the stretch reflex started to decrease. At the same time the H-reflex and the background EMG were still high.

When the force builds up in the ankle extensors during the stance phase, the soleus muscle fibres start to shorten at 62.2±4.3% of the stance phase (Voigt et al. 1996, this meeting). It is therefore conceivable that the decrease in the stretch reflex in late stance phase is caused by an unloading of the muscle spindles which not is detectable in the H-reflex. This imply that changes in muscle length are overriding the effect of alpha-linked gamma action on the stretch reflex during human walking.

Supported by the Danish National Research Foundation.

168.4

DIFFERENCE BETWEEN ORIGIN-TO-INSERTION AND MUSCLE-SPINDLE LENGTH-CHANGE IN SOLEUS DURING HUMAN WALKING M. Voigt, T. Sinkjaer and R.J.H. Wilmink* Center for Sensory-Motor Interaction, Aalborg University, Denmark.

In a few animal studies it has been shown that the patterns of length-change of the muscle-spindles and the whole muscle-tendon unit are different especially when the muscle-tendon unit is loaded. This is an effect of the predominant compliance of the tendinous structures. However, in the majority of studies on humans the tendon compliance and the possible consequences for reflex control has been overlooked. The objective of the present study was to map possible discrepancies between the soleus muscle-spindle and origin-to-insertion (OI) length-change during human walking at different speeds.

Five normal healthy subjects participated in the study (2 female, 3 male). They performed over-ground walking at three different speeds : 2.5, 3.5 and 4.5 km/hr. The movement of the left leg was recorded 120 frames/s and ground reaction forces were measured under the left foot. EMG was recorded with surface electrodes from soleus and anterior tibial muscles. The OI length-change was calculated by an ankle joint to OI length-change transfer function and the muscle-spindle length-change was calculated using a tendon model with the ankle joint moment as input.

At all three walking speeds the OI-lengthening preceded the muscle-spindle lengthening by 4.5±0.8 % (mean±SD) of the ground contact time (GCT). The muscle-spindle shortening began 61.0±3.5 % GCT after the heel contact which was 3.1±2.1 % GCT earlier than the beginning of the OI-shortening. Additionally, both the peak muscle-spindle lengthening and shortening velocities were attenuated on average 36.9±10.3 % and 52.0±15.4%, respectively, in relation to the OI velocities, and the attenuation increased with increasing walking speed.

Therefore, it is likely that the reflex control scheme for soleus during human walking is significantly influenced by tendon compliance.

168.5

Post-Exercise Facilitation of the Lateral Gastrocnemius H-reflex. **M.H. Trimble* & S.S. Harp. Dept. of Physical Therapy, University of Florida, Gainesville, FL 32610-0154.**

Following voluntary muscle activation, the amplitude of the soleus (S) H-reflex (HR) has been shown to be depressed for up to 10 s before returning to control values (Enoka, Hutton, & Eldred, 1980; Kukulka and Moore, 1991). We subjected 7 subjects to a vigorous bout of concentric-eccentric gastrocnemii exercise with the knee extended (8 bouts of 10 reps) using a modified Cybex II isokinetic dynamometer. Lateral gastrocnemius (LG) HRs were elicited before and after the exercise bout. Prior to the exercise, the test stimulus was set to produce a small M-wave and an HR with an amplitude of 10%-20% of the maximal M-wave. The small M-waves were used to monitor the stimulus amplitude pre and post exercise. All the subjects had an initial depression of the HR immediately post exercise. Some (5/7) but not all of the subjects demonstrated a large potentiation (30%-50%) of the HR over the control values following the initial period of inhibition. This potentiation often lasted 10 min. post exercise. The data suggest that at least 2 overlapping processes are occurring, an early inhibitory process of short duration, followed by or superimposed over a longer lasting facilitory process. The facilitory phase does not appear to be related to the background muscle activity since the LG or S RMS values did not change post exercise. A short term reduction of the tonic level of presynaptic inhibition of the Ia afferents may be responsible.

168.6

INTRINSIC AND REFLEX CONTRIBUTIONS TO SPASTIC ANKLE STIFFNESS: MODULATION WITH ANKLE POSITION. M.M. Mirbagheri[1]*, R.E. Kearney[1], H. Barbeau[2], and M. Ladouceur[2], Department of Biomedical Engineering[1], and School of Physical and Occupational Therapy[2], McGill University, Montreal, Canada H3A 2B4.

The objective of this study was to determine how the relative contributions of intrinsic and reflex mechanics to ankle mechanics varied with position in spinal cord injured (SCI) spastic subjects. Ankle stiffness dynamics were examined at different ankle positions in normal and SCI spastic subjects who maintained 10% MVC PF in the ankle extensors (gastrocnemius-soleus). Intrinsic and reflex contributions to the stiffness dynamics were separated using a new parallel-cascade identification method. Intrinsic stiffness dynamics were well modeled by a linear, second-order system relating intrinsic torque to joint position. Reflex dynamics were accurately described by a linear, third-order system between half-wave rectified velocity and reflex-torque.

In normal subjects, intrinsic and reflex stiffness gain increased as the ankle was progressively dorsiflexed. The relative contribution of reflex mechanics to ankle stiffness increased, nevertheless, the intrinsic torques were always dominant.

In SCI spastic patients, both intrinsic and reflex stiffness increased with ankle dorsiflexion. As for normal subjects, the relative contribution of intrinsic stiffness decreased and that of reflex stiffness increased as ankle was dorsiflexed. However, reflex stiffness gain was higher than in normal subjects, while intrinsic stiffness gain was similar. Consequently, the relative contribution of reflex mechanics to ankle stiffness was significantly greater in SCI spastic subjects than in normal subjects. Moreover, in contrast to normal subjects, the reflex torques were dominant in joint mechanics of the ankle as GS was stretched.

These results demonstrate that reflex mechanics are abnormal in spastic SCI patients and pathological muscle tone is due to enhanced reflex gain.

(Supported by a grant from the Medical Research Council of Canada)

168.7

RECIPROCAL INHIBITION BETWEEN QUADRICEPS AND HAMSTRING MUSCLES IN THE HUMAN

P. Evans and P.J.Harrison* Department of Physiology, University College London, Gower St., London WC1E 6BT, U.K.

In the cat reciprocal inhibition is much stronger between knee extensors and flexors than between ankle entensors and flexors. Curiously, while reciprocal inhibition has been demonstrated between ankle extensors and flexors in man there are no reports of reciprocal inhibition in man between knee extensors and knee flexors. We have therefore investigated this.

Surface electrodes were used to record EMG activity in the hamstring muscles in awake human subjects. The subjects maintained a low level of isometric activity while sitting with the legs supported and electrical stimuli were applied to the femoral nerve. An inhibition of EMG activity was revealed which was sufficiently large to be visible in individual sweeps. The inhibition occurred at short latency (5-6ms) and at low intensity (0.8x motor threshold) indicating a short reflex inhibitory pathway mediated by large afferent Ia fibres.

This finding is in contrast to the relatively weak reciprocal inhibition observed between soleus and the anterior tibial muscles in humans (e.g.Tanaka 1974, Exp Brain Res 21:529-540) and indicates a similar quantitive distribution to that seen in the cat.

Supported by UCL

168.8

THE JENDRASSIK MANEUVER DOES NOT AFFECT PRESYNAPTIC INHIBITION OF THE H-REFLEX.

E.P. Zehr* and R.B. Stein. Div. of Neuroscience, University of Alberta, Edmonton, Alberta, CANADA T6G 2S2.

Since its first description in 1883, the Jendrassik maneuver (JM) has been used in clinical neurological practice as an effective means of potentiating the tendon jerk stretch reflex in neurologically impaired patients. Subsequently, the JM potentiation of the electrical analogue of the stretch reflex, the Hoffman (H-) reflex has been studied. The purpose of the present study was to determine the mechanism of the reflex modulation, which has not been clearly established.

We studied soleus H-reflex modulation in neurologically intact subjects while at rest and during a mild plantarflexion contraction (EMG level equivalent to ~10 % MVC). The test H-reflex was elicited by stimulating the tibial nerve in the popliteal fossa with single pulses of 1 ms duration. Conditioning of the reflex was by either common peroneal (CP) nerve stimulation or JM, presented either alone or in combination. The JM was both by arm pulling and teeth clenching. As well, the conditioned reflex was elicited at 2 latencies after CP n. stimulation (10 and 100 ms; both of which are considered presynaptic effects) and JM (200 and 300 ms).

There was a significant ($p<0.05$) suppression of the H-reflex (50% H_{max}) at both CP latencies, while JM values were significantly ($p<0.05$) facilitated as compared to CP n. conditioning. There was no difference ($p>0.05$) between CP + JM and CP n. at any latency, thus revealing no interaction between the two types of conditioning. We conclude that the JM acts independently of the presynaptic mechanisms that can inhibit the H-reflex.

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168.9

PROPRIOCEPTIVE CONTROL OF VOLUNTARILY ACTIVATED WRIST EXTENSOR MOTOR UNITS IN HUMAN: DEPENDENCE ON MOTOR UNIT TYPE AND INFLUENCE OF SUBJECTS' HANDEDNESS.

J-M. Aimonetti, D. Morin, A. Schmied,J-P. Vedel*, S. Pagni, CNRS-NBM, 13402 Marseille cedex 20, France.

The proprioceptive control of human motoneurone activity was investigated in relation to motor unit type and subject's handedness. The activity of 411 single motor units was recorded in the Extensor carpi radialis muscles of the right and left arms of 5 right-handers and 5 left-handers during voluntary isometric contractions, while percussions were applied to their tendon with a constant post-spike delay (80 ms). The tendon taps induced an increase in the probability of discharge of the motor units with a delay compatible with a monosynaptic pathway. The amplitude and the latency of the responses were studied in relation to the motor unit functional characteristics (discharge frequency, recruitment threshold, twitch contraction time, force and macro-potential). In agreement with the size-principle, motor units with lower recruitment thresholds, longer contraction time, larger contraction force and smaller macro-potential were found to display most commonly larger reflex activation and to respond with longer latency independently on the discharge frequency. The strength of the motoneurone proprioceptive control was found to depend on the subject's motor lateralization. Whatever their functional characteristics, the motor unit responses and the tendinous reflex were found to be larger in the right arm of right-handers, when no differences could be evidenced in both arms of the left-handers. In left-handers as in right-handers no differences was found in the H-reflex tested in both arms. These results indicate the existence of a spinal asymmetry, apparently restricted to the right-handers which favours the right side in the control exerted by the muscle spindles on the discharge of the wrist extensor motoneurones during a voluntary contraction, in superimposition to the gradient related to the motor unit characteristics. The lack of H-reflex lateralization favours differences in the muscle spindle sensitivity between right and left arms in the right-handers.

168.10

EVIDENCE THAT LONG-LATENCY STRETCH REFLEXES IN THE HUMAN ANKLE DORSIFLEXORS MAY BE MEDIATED BY A TRANSCORTICAL PATHWAY.

N. Petersen*, [1] J. Nielsen, H. Morita, and[2] T. Sinkjær, Department of Medical Physiology, University of Copenhagen, DK-2200, Denmark. [1]Department of Physiology, Christian-Albrechts-University, Kiel, D-24098 Germany. [2]Center for Sensory-Motor Interaction, Ålborg University, DK-9100 Denmark.

Stretches applied to human ankle dorsiflexors muscles usually result in three distinct bursts of reflex activity (Toft et al., Exp Brain Res 74:213. 1989) labelled M1, M2 and M3. The latency of M1 and M2 is too short to be caused by a transcortical pathway, whereas M3 occurs at a latency of on average 97 ms, which would be just compatible with such a pathway. The present study was undertaken to investigate this possibility. With local ethics comittee approval human subjects (n = 15) were seated in an armchair with the left leg attached to a footplate which could be rotated by a strong motor. EMG activity was recorded by surface electrodes over the tibialis anterior (TA) muscle. During an isometric voluntary dorsiflexion a quick rotation of the footplate in plantar direction resulted in three distinct reflex bursts (M1, M2, M3) in the TA muscle. When a motor evoked potential (MEP), produced by transcranial magnetic stimulation (TMS) of the motor cortex, was superimposed on the late M3 stretch reflex a significant potentiation of the MEP was seen in all subjects. A similar potentiation was not seen when the MEP was superimposed on M1 or M2. In 18 single TA motor units from 7 subjects the monosynaptic peak in the post-stimulus time histogram (PSTH) following TMS was similarly increased when TMS was preceded by a stretch at the latency of M3. In none of 5 motor units was a similar increase of the monosynaptic peak observed when the magnetic stimulation was replaced by an eletrical stimulation. Electrical brain stimulation is assumed to activate the axons of the corticospinal tract cells and is therefore, unlike the magnetic stimulation which activates the soma, not influenced by the excitability of the corticospinal cells (Edgley et al., J Physiol (Lond) 425:301. 1990). The present data thus demonstrate that the long-latency stretch reflex (M3) in the TA muscle is likely mediated by a transcortical reflex pathway.

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168.11

VARIATIONS IN PERCEPTION THRESHOLD DURING DIFFERENT MOTOR TASKS.

C. Labrecque, J.P. Boucher, M. Bélanger* Dép. de Kinanthropologie, Univ. du Québec à Montréal, Montréal, Canada, H3C 3P8

The perception threshold (Th) is used as the reference value for adjusting the cutaneous stimulation intensity in most of the withdrawal-reflex studies. However, only one study has examined the variability of the Th during locomotion (Duysens et al., 1995). The present study measured Th variations during four different conditions; 1) standing, 2) cycling, 3) 8 static pedal positions with the Tibialis Anterior and Soleus EMG levels matching those recorded during cycling 4) 8 static pedal positions without EMG activity. A modified cycle ergometer (the subject is seated behind the pedals) was used for data acquisition. The Th was measured 10 times with ascending and descending stimulation intensities for standing and for each of the 8 pedal positions of the last 3 conditions. The skin-electrode impedance was also measured after each series of Th measurements. Each condition and each position was randomly evaluated with a 10 ms train of 5-1 ms pulses applied to the skin area overlying the sural nerve as it passes behind the external malleolus. The results show that there is a difference between the tasks where the Th is 13% higher during cycling (active movement). In contrast, there is no difference within the tasks (no position effect on the Th). These results suggest to use caution when examining cutaneous reflex modulation between tasks, particularly when comparing static and dynamic conditions, and if the stimulation intensity is based on perception threshold. (Supported by PAFACC, UQAM)

168.12

H-REFLEX EVOKED POTENTIAL (HEP) MONITORING DURING SURGERY: A SIMPLE SENSORY AND MOTOR EVALUATION OF PERIPHERAL NERVES AND SPINAL ROOTS

RD Rose.* Department of Neurological Surgery and Center for Clinical Neurophysiology, University of Pittsburgh Medical Center, PUH B-400, Pgh PA 15213

Neurologic complications from some surgical procedures placing peripheral nerves at risk are substantial (e.g. sciatic nerve in total hip arthroplasty: 1% for primary to 5% for revision; Sherry 1995 Sixth Int'l Symp on Spinal Cord Monitoring NY p78 and acetabular fracture repair: >5%; Fassler et al 1993 JBJS 75-A:1157). The development of a direct analysis of motor function during surgical procedures involving risk to motor components of peripheral nerves should enhance nerve injury detection and prevention and thus reduce neurologic complications. The H-reflex involves both sensory and motor components; i.e. the monosynaptic stretch (myotatic) reflex of muscle spindle Ia afferents to α-motoneurons to the homonomous muscle (continuing the sciatic example, HEPs from tibialis anterior provide the basis for a highly appropriate evaluation for the prevention of foot drop. Moreover, a concomitant SSEP can be recorded cervically or cortically (see Slimp et al 1996 Spine 21:99) in conjunction with the HEP as evoked activity continues rostrad). Because HEPs are observed as EMG responses, amplitudes are large. Additonally, novel HEPs are easily developed and adapted to particular situations as muscle innervation and nerve trajectories are well known.

168.13

REFLEX INSTABILITY IN SPASTICITY: ORIGINS OF CLONUS.

Joseph M. Hidler, W. Zev Rymer, and Julius P.A. Dewald* Sensory-Motor Performance Program and Dept. of Biomedical Engineering, Northwestern University, Chicago, IL 60611.

Clonus is defined as an involuntary rhythmic muscle contraction which usually accompanies spasticity of ankle muscles, and occurs in people who have sustained lesions involving descending motor pathways such as stroke and spinal cord injury. We hypothesize that clonus arises when three conditions occur simultaneously: the reflex pathway contains a slow muscle actuator (i.e. soleus), there are long pathway delay times, and the excitability of the motoneurons is enhanced. We tested this hypothesis, in particular how motoneuron excitability, loop delay, and muscle type each influence reflex behavior, by developing a computer model representing the ankle reflex pathway. Simulations showed that as the motoneuron current threshold was reduced (reflecting increased excitability of spinal motoneurons), normal reflex responses became unstable and oscillations developed similar to those observed in clonus patients. Furthermore, sustained oscillations occurred when the loop delay time was set to that found in the human ankle reflex (50 msec), while reductions in this time delay forced the oscillations to damp out. This explains why clonus is largely restricted to the ankle, where conduction distances are greatest. Finally, when slow twitch muscle characteristics were replaced with those of fast twitch muscles, the effect was similar to reducing loop delay as the oscillations rapidly decayed. This illustrates why clonus is mediated by the soleus muscle which contains nearly all slow twitch muscle fibers. The findings in this study support the hypotheses that when the motoneuron excitability increases in a reflex pathway containing long latencies and a slow muscle actuator, unstable behavior such as the oscillations observed in clonus will occur. Furthermore, it is demonstrated that sustained oscillations can occur in a reflex pathway through self re-excitation, which contradicts the theory that a "spinal generator" must be involved in clonus.

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168.14

SIMULATION STUDY OF INTERACTIONS BETWEEN SPINAL FEEDBACK LOOPS, M. G. Maltenfort *, J. He and T.M. Hamm. Div. of Neurobiology, Barrow Neurological Inst., Phoenix, AZ 85013

The spinal circuitry for control of muscle length and tension is not uniform across motor nuclei or functional tasks. beta axons innervate 70% of muscle spindles in distal muscles but 40-47% in proximal muscles (Scott, *et al.*: *Neurosci. Lett.*, 190: 1-4, 1995); in comparison, motor nuclei innervating distal muscles receive less recurrent inhibition, and those innervating digits receive virtually none (Hörner *et al.*: *Neurosci. Lett.*, 122: 183-183, 1991; McCurdy and Hamm: *J. Neurophysiol.*, 67: 1359-66, 1992). What is the functional significance of this obvious discrepancy in feedback regulation of distal and proximal muscles from recurrent inhibition and stretch reflex?

To explore possible interactions between feedback loops, we have constructed a simulation of a single muscle with force- and length-dependent mechanics; feedback from muscle spindles and tendon organs; beta innervation of muscle spindles; and recurrent inhibition. The muscle acts against a viscoelastic load. Feedback gains are adjusted to match published measurements and observations of motor behavior.

Two questions will be addressed: how does the simulated system respond to stochastic input or load perturbations? and how do the beta innervation and recurrent inhibition interact with each other in a motor control task?

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CONTROL OF POSTURE AND MOVEMENT: SENSORY CONTROL OF REACHING

169.1

THE EFFECTS OF ATTENTION ON MANUAL AIMING MOVEMENTS IN THE ELDERLY S.A. Morehouse and J.D. Fisk*, Dalhousie University, Halifax, Nova Scotia, Canada.

This study examined the effect of premovement target location information on the planning and execution of visually directed pointing movements by healthy elderly subjects. Central visual cues (60% valid, 20% invalid, 20% neutral) indicated which of four positions the subjects would be required to point to. Experiment 1 allowed for endogenous covert orienting of attention (shift of attention to the target position) plus premovement planning. This was followed by exogenous overt orienting of attention (i.e. pointing to the target). Experiment 2 required endogenous overt orienting and allowed for premovement planning only since the target position was indicated at the point of central fixation. Subjects' 3-dimensional hand position was recorded optoelectronically (WATSMART). Valid cues reduced movement onset time in Experiment 1 but did not affect peak or average velocity, or accuracy. Cue validity had no effect in Experiment 2. The results suggest that probable target location information did not provide a movement initiation advantage unless subjects also covertly shifted attention to the probable target position. Peak and average velocity were higher in Experiment 1 but accuracy did not differ. This demonstrates a general advantage in movement planning and/or execution for movements directed to targets that elicit exogenous overt orienting of visual attention.

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169.2

ORIENTING THE ATTENTION AFFECTS AIMING MOVEMENTS. [§]M. Badan, [§]N. Weideli and [§]D. Corbetta*. [§]Faculty of Psychology and Educational Sciences, University of Geneva, Carouge, Switzerland, [§]Department of HKLS, Purdue University, West Lafayette., In, US.

To investigate visuo-spatial attention in right-handed adults, we used a paradigm in which the programming and execution of visuo-manual aiming movements were interfered with by visual distractors. The target was a dot displayed on a computer screen at one out of 6 different positions along a transversal axis (left-right). After the disappearing of a central cue that specified the hemispace in which the next target would appear, the target was presented either alone, or with a left or a right distractor. Neutral, valid, and non-valid cues were given in the same proportion.

A pointer was mounted on a vertical handle placed in front of the screen in the midsagittal plane of the subject. When the target appeared, the subject had to orient the pointer to the target position as quickly and accurately as possible. The handle was connected to a potentiometer that recorded the spatio-temporal parameters of the manual responses (sampling rate: 200 Hz).

The results suggest that the type of cue, and the target position had an effect on movement programming time, whereas distractors did not affect this dependent variable. In contrast, the execution time of the movement was dependent on the presence of distractor but not on the type of cue. Execution time also varied as a function of target position. The results are interpreted in terms of the strategies developed by adults to allocate attention in the visual space prior and during aiming movements.

169.3

CONTRIBUTIONS OF THE CENTRAL AND PERIPHERAL VISUAL FIELDS TO VISUAL INTERCEPTION, P.J. Bryden, F. Allard, & M.P Bryden*. Departments of Kinesiology and Psychology, University of Waterloo, Waterloo, Ontario, N2L 3G1.

The relative contributions of both the central and peripheral visual fields to performance in a catching task was examined. Participants were asked to catch a ball attached to a pendulum, whose trajectory was close to the observer's head. The time of movement initiation, finger separation, and grasp were ascertained for each catch, and as well the total number of balls missed was determined. The first study examined the peripheral visual field and manipulated the observer's body and head alignment with respect to the oncoming ball. The peripheral visual field was found to be capable of mediating accurate catches, while no differences were found between the different body orientations investigated. The second study examined the central visual field. The acuity of the central field was manipulated by an optical defocus which reduced the acuity of the field to approximately that of the peripheral field. As well, a fixation control manipulation was introduced in order to restrain the participants from foveating the ball for its entire trajectory, and to equate the task demands between the two experiments. Catching performance was adversely affected by the defocusing of the central field when compared to performance under normal acuity. The manipulation of fixation also worsened catching performance. Equivalent conditions in the two experiments were compared statistically to evaluate the relative contributions of each field to catching performance. It was concluded that peripheral vision allows for more accurate interception of an oncoming ball than does central vision. It thus appears that the central field is dependent upon its superior acuity to perceive detailed information about the size and shape of the ball to mediate interception. Such detailed information appears to not be necessary for the peripheral field to mediate catching. The results seem to agree with recent research on the distinction between the two visual pathways (Goodale & Milner, 1995). *Supported by the Department of Kinesiology, University of Waterloo.*

169.4

2D-TRACKING HAND MOVEMENTS ARE INFLUENCED BY MOVING VISUAL BACKGROUND. W. Kruse*, S. Dannenberg and K.-P. Hoffmann, Allgemeine Zoologie & Neurobiologie, Ruhr-Universität Bochum, D - 44780 Bochum

The influence of linearly moving random dot patterns was studied during performance of visually guided circular tracking movements. Subjects used a 2-dimensional manipulandum to control a feedback cursor on a large video graphic display (43° * 52°). The vision of the limb was occluded during the task. Each tracking movement consisted of two consecutive cycles in clockwise direction, starting at the 12 o' clock position. The circular path of the visual target had a radius of 17.4°, corresponding to a tracking hand movement of 12.5 cm radius. After completion of the first target cycle (5 sec), the feedback cursor disappeared. Subjects were instructed to continue the tracking of the visual target for a second cycle („open loop" cycle). During both cycles, a full field random dot pattern moved horizontally with constant velocity (19.4 °/s), either to the left or to the right. Eight subjects performed the task using their preferred (right) hand. All subjects tended to draw an ellipse during the open loop cycle, which showed a prevalent pattern across all repetitions. In only 2 out of 8 subjects, the radial error at the end of the open loop cycle was significantly different between background movement from the left and from the right. At the end of the open loop cycle, in grand average, subjects were trailing the target by an angular error $\Delta\alpha = \alpha_{hand} - \alpha_{target}$ of −13.7°, measured from the origin of the circular target trajectory. The main effect of the moving background was a modulation of this angular error. All subjects showed a clear increase in the angular lag when the background moved in the same direction as the target at the end of the open loop cycle (near to 12 o' clock position), compared to the background movement in opposite direction. These results indicate that 2-dimensional tracking movements are influenced by the visual background. The effect on the angular error is in accordance with previous results derived from 1D- manual tracking movements (Masson et al., Vision Res. 35, (6): 837, 1994).

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169.5

THE ROLE OF VISION IN POINT-TO-POINT ARM MOVEMENTS. L.E. Sergio* & S.H. Scott CRSN, Dépt.Physiologie, Univ.de Montréal, Montréal, PQ, CANADA H3C 3J7

We wished to examine whether straight hand paths were an inherent property of goal-directed movements or an emergent property of visually-guided motor development. Comparisons were made between hand and joint paths produced during point-to-point arm movements by congenitally blind subjects and normally sighted subjects (with and without blindfolds). The planar horizontal movements covered many directions across the workspace.

Blindfolded sighted subjects produced hand paths with greater curvature compared to blind subjects in many movements. There was no systematic difference, however, between the paths produced by blind subjects and sighted subjects without blindfolds. These data suggest that visual input is not in itself essential for straight hand paths since blind subjects often produced straighter hand paths. Hand trajectories may be determined by constraints acting at a number of levels both perceptual and motoric in nature.

For some movements, sighted subjects made straighter paths in hand space with vision but straighter paths in joint space while blindfolded. The decrease in joint path curvature while blindfolded implies that the subjects may be linearizing the path in the perceptual space available to them. This may be a more general case of that seen by Flanagan & Rao (*J.Neurophys.***74**:2174-7,1995), whose subjects produced straight paths in the space (hand or joint) displayed to them on a screen.

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169.6

EFFECTS OF VISUAL FEEDBACK AND INITIAL START POSITION ON THE TEMPORAL COORDINATION OF BILATERAL WRIST MOVEMENTS. E.L. Flansburg, P.B. Blank, S.H. Brown*. Center for Human Motor Research, Division of Kinesiology, Univ. Michigan, Ann Arbor, MI. 48109-2214.

The degree of synchronization during bilateral arm movements depends upon such factors as movement direction, speed, and distance. In this study, we have examined the effects of initial start position and visual feedback on the temporal coupling of wrist movements. Six right-handed subjects (19-23 yrs) performed discrete flexion movements at the following initial start positions: self selected, matched extension of both wrists, and unmatched extension (extension/neutral wrist positions). For each position, movements were made without vision and with selective visual feedback of either the right or left hand. In the latter condition, subjects were required to point to a visual target with the displayed hand while simultaneously moving the non-displayed hand. Subjects were instructed to move both hands together at their own speed. Wrist displacement was recorded using electrogoniometry.

Differences in bilateral onset times were variable across subjects and were not significantly affected by selective visual feedback or start position. Mean onset difference was 13 +/- 30 ms across all conditions and subjects. In contrast, selective visual feedback had a marked effect on the matching of movement duration and amplitude in all subjects. Movement duration of the non-displayed hand was consistently greater than that of the displayed hand regardless of whether the dominant or non-dominant hand was displayed. Start position had no consistent effect on the degree of duration uncoupling. In some subjects, an increase in amplitude of the non-dominant hand was also observed which was independent of start position. These findings extend previous observations regarding the coordination of bilateral limb movements. It is also clear that visual information can exert a strong decoupling action, thus demonstrating the influence of sensory feedback in the control of bilateral motor tasks.

169.7

DELAY INDUCED PATTERNS OF SINUSOIDAL TRACKING MOVEMENTS

H. Hefter*, P. Tass, J. Salomon, K.R. Kessler, H.-J. Freund. Dept. of Neurology, H.-Heine-University, D-40225 Duesseldorf, F.R.G.

The overtrained matching between proprioceptive and visual feedback during sinusoidal forearm tracking movements was disrupted by introducing delays in the visual feedback loop.

During long term recordings (up to 15 mins) by making forearm flexion/extension movements, 10 normal subjects had to keep a thin response line in the middle of a sinusoidally moving target area on an oscilloscope screen. The movements of the forearm were displayed with different delays ranging from 0 to 100% of the target cycle duration. Target frequency was kept constant. Usually the most comfortable tracking rate was used. The Hilbert transformations of the target and response signal were determined to calculate the phase difference.

Analysis of the phase difference between the target and the response signal revealed four characteristic movement patterns: 1. A fixed point behavior for delays close to zero was found. 2. With increasing delays low-amplitude oscillations of the phase difference occurred. 3. A further increase of the delay led to cycle slipping, i.e. the subject preceeded the target or fell behind, kept a fairly constant phase relation for a few cycles but then slipped into the next cycle again. 4. With delays around 50 % a drift behavior was seen, the subject being unable to keep a stable phase relation. Delaying the visual feedback beyond 50% of the cycle duration led to an improvement of the tracking performance in all subjects with many of them returning to a fixed point behavior or low-amplitude oscillation at delays close to 100%.

A nonlinear model was developed describing qualitatively the different movement patterns. Fitting the model to the experimental data will allow to quantify the visuo-sensorimotor interaction. Thus, detailed analysis of tracking with visual delay can be used as a clinical method to test the mutual interaction of the visual and the sensorimotor system.

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169.8

TRANSFER OF GAIN ADAPTATION: EVIDENCE OF VECTORIAL CODING IN GOAL-DIRECTED MOVEMENTS. P. Vindras, P. Viviani and D. Pelisson*. Dept. of Psychobiology, FAPSE, Univ. of Geneva, 1227 Carouge, Switzerland.

It is still debated whether targets of goal-directed movements are coded by the coordinates with respect to the body, of vectorially, i.e. by the amplitude and direction of the vector from the starting to the final position. A key prediction of this second hypothesis is that any gain adaptation along one direction should transfer to any other arbitrary direction. Furthermore, between-arm transfer of adaptation is possible only according to the vector-coding scheme. In this study we tested both these predictions by altering movement gain through the use of non-veridical visual knowledge of the results (KR). In 3 separate sessions subjects pointed to 200 ms laser spot targets placed horizontally along 8 equally spaced (45°) directions at 6, 9 or 12 cm from a central starting position identified both visually and by a tactile landmark. Subjects, who could not see their arms, were instructed to point as precisely as possible. They received KR only in the case of right-hand movements to left or right 12 cm targets. KR was veridical during the first session. In the second and third session a biased KR was used to increase or decrease the gain by 15% along the left-right axis. Each session began and ended with 12 left-hand movements to 12 cm targets in the 4 cardinal directions. It also included 12 trials with KR, followed by 96 trials with one KR provided every 4 movements. For trials with KR, gain modulation was -13% and +8.4% with respect to the first session. About 2/3 of these modulations transferred to movements in other directions and amplitudes. The amplitude of left-hand movements at the end of the sessions also changed by -10.5% and +8.8% with respect to the initial left-hand movements. No systematic directional bias was observed. Our results suggest that hand-target distance is a relevant variable for reaching movements, and support the vectorial coding hypothesis. They are not compatible with the view that a transformation of target egocentric position into desired arm posture is the first stage of movement planning.

169.9

INTERACTION OF TREMOR AND MAGNIFICATION IN A MOTOR PERFORMANCE TASK WITH VISUAL FEEDBACK. K. Vasilakos, P. Cordo*, L. Glass, A. Beuter. Neurokinetics laboratory, UQAM, C.P. 8888, Station Centre-Ville, University of Quebec, Montreal, H3C 3P8, Canada.

This study investigates the interaction between increased gain in the visual feedback loop and motor control of the periphery. Subjects were asked to maintain a constant finger position while utilizing magnified visual feedback. The accuracy of each trial was quantified by taking the standard deviation (trial-error) of the finger position. Trials performed under magnification have lower trial-errors than trials without magnification. The change in trial-error between trials with and without magnification proves greater than the difference between trials at any two magnifications. In contrast, the differences between individual subjects is often greater than the difference between performances at individual magnifications. At higher magnifications performance seems to be limited by the tremor, the ratio of trial-error to tremor-intensity is constant. When applied to microsurgery these results are in accord with earlier research including results suggesting that the level of magnification used in microsurgery is not the most significant factor in achieving good results, and that tremor is the limiting factor in microsurgical tasks.

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169.10

PERCEPTION, APPLIED FORCE, AND MULTIPLE STRATEGIES IN A CONTINUOUS TRACING TASK. C. Barczys*, J. Boline, J. Ashe, and A.P. Georgopoulos, Brain Sciences Center, VAMC, Minneapolis, MN 55417.

To examine the relations between perception and applied force during movement, 4 subjects varying in motor-task experience traced a circle continuously at 1 Hz for 18 seconds, using a manipulandum that delivered combinations of spring (*S*), viscous (*V*), and inertial (*I*) torques. Subjects then rated the trial's difficulty from 1 to 100 (1 = easiest). Hand (*H*: left, right), movement direction (*D*: ccw, cw), and gravity (*G*: present, absent) were also varied in a randomized block design. There were 125 trials per session (*S*, *V*, and *I* varied), and 8 sessions per subject (*G*, *D*, and *H* varied). Using the last 6 seconds of each trial, a multiple linear regression model was applied to determine the effect of the following variables on each subject's perception of task difficulty: applied forces (*S*, *V*, *I*), task conditions (*H*, *D*, *G*), visual cues (RMS error of the trajectory from the template), motor performance (average velocity (Vavg) and standard deviation of Vavg), and output forces (subject force and net force). Regressions for all subjects were highly significant. The fit of the model and the relative importance of the different variables varied among subjects, with the more task-naive subjects showing lower R^2 values, lesser dependence on the applied forces and heavier dependence on the visual and motor cues. Of the applied forces, *V* had the strongest effect followed by *I* for 3 subjects; for the fourth subject *I* was strongest. Of the task conditions, *G* and *H* showed the strongest effects. Force effects were largely restricted to the 2 experienced subjects. These results suggest that perception of difficulty in a motor task is a composite of several cues whose relative importance varies among subjects depending on the degree of motor-task experience: for task-experienced subjects the relationship between perception and applied forces is strong, whereas for task-naive subjects that relationship is weaker and the dominant factors are visual and motor cues. (Supported by VA and NIH grants).

169.11

PSYCHOMETRIC FUNCTION FOR MOVEMENTS OF THE ELBOW JOINT L.A. Jones*[1], R.J. Irwin[2], and I.W. Hunter[1], Department of Mechanical Engineering, Massachusetts Institute of Technology, 77 Massachusetts Ave. Cambridge, MA 02139 USA[1] and Department of Psychology, University of Auckland, Auckland, New Zealand[2].

The ability to detect the movement of a limb depends on the velocity of the movement and the joint moved, with lower thresholds being associated with faster movements (10–80°/s) and more proximal joints. In such experiments unidirectional movements have been imposed on the joint and so the threshold estimates obtained may not represent the optimal sensitivity of the joint.

The objective of the present experiment was to determine the psychometric function for movements imposed on the forearm. Subjects were seated in an experimental rig and with each hand held a rod protruding from an electro–magnetic linear motor that was under computer control. On each trial two 5–s movement perturbations were delivered to the left and right arms. One stimulus was a 10 Hz signal embedded in a 5–15 Hz band of noise and the other a 5–15 Hz band of noise. Subjects indicated which perturbation included the signal by pulling against the appropriate motor at the end of the trial and were provided with feedback of the correct response. Eight different signal amplitudes were presented ranging from 1 to 4.5 times the rms amplitude of the background noise, and 100 trials were presented at each amplitude.

Over the amplitude range studied the psychometric function was steep for most subjects. The threshold (75%) for detecting the 10 Hz signal embedded in a 5–15 Hz band of noise ranged from 0.05 to 0.08 mm across subjects which corresponds to a movement of approximately 0.01°. This absolute threshold is larger than the differential threshold for elbow joint movements which is 5 μm (Jones et al.,1992).

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169.12

STOCHASTIC RESONANCE IN HUMAN MUSCLE SPINDLES. S.Verschueren, P.Cordo, J. T. Inglis, J.J. Collins, D. Merfeld* and F. Moss. R.S.Dow Neurological Sciences Institute, Portland, OR 97209

Noise—inherent to biological sensory systems—has traditionally been viewed as detrimental to signal detection and information transmission. Recently, however, it has been shown that noise can enhance the detection and transmission of weak signals in certain nonlinear systems via a mechanism known as "stochastic resonance." In the experiment reported here, we tested the hypothesis that the sensitivity of human muscle spindle receptors to a weak movement signal could be maximally enhanced by the presence of a particular, non-zero level of noise.

The firing activity of 8 single primary afferents from the finger and wrist extensors was recorded using microneurography in normal human subjects. The wrist was passively rotated with a sinusoidal waveform (0.5-0.8 Hz; ±1-3°), sufficient to excite the afferent weakly. A random noise signal (bandwidth 0-500 Hz; RMS amplitude 0-0.7 mm) was applied to the distal tendon of the parent muscle. The power spectrum of the spike train showed a peak at the wrist rotation frequency from which the output signal-to-noise ratio (SNR) was calculated as the area under the peak above the noise divided by the amplitude of the noise.

Six of 8 afferents showed stochastic resonance: the output SNR increased to a maximum and then decreased with increasing input noise intensity. Thus, low levels of noise improved the ability of muscle spindles to detect weak periodic movement signals. This positive effect of noise appears to be due to a direct effect on the spindle receptor rather than indirect (i.e., reflexive) fusimotor activation, as the same relation between input noise intensity and output SNR was found when the fusimotor system was inactivated by proximal block of the radial nerve.

169.13

THE CODING OF SPATIAL LOCATIONS BY JOINT ANGLES [§]G. Baud-Bovy,[§*]P. Viviani and [*]J. Requin* [§]Faculty of Psychology and Educational Sciences, University of Geneva, Carouge, Switzerland, [*]Department of Cognitive Science, Scientific Institute S. Raffaele, Milan, Italy, and * Center for Research in Cognitive Neuroscience, CNRS, Marseille, France

The pattern of errors in pointing to spatial targets (Flanders et al, 1992), and the recent demonstration that initial and final postures are not independent (Soechting et al, 1995) have rekindled the debate on the system of joint angles used by the CNS for coding posture and movement. We address this issue by separating the effects of the final posture from those of the target position. Targets located within the proximal part of the frontal space can be reached by either hand. However, outside the mid-sagittal plane, different sets of joint angles must be computed in the two cases. A robot arm guided the right (Condition 1), or the left hand (Condition 2) of blindfolded subjects to one of 27 targets arranged in a cubic array. After returning the arm to an invariable initial posture, the subject attempted to reach again the same position with the right hand. By measuring absolute and relative positions of all body segments involved in the task, we observed substantial systematic errors. Endpoints in the first condition were always shifted to the left in the first condition, and to the right in the second condition. Since pointings were performed by the same hand, starting from one posture, errors can be explained neither by biomechanical constraints, nor by factors related to movement execution *per se*. Instead, endpoint shifts must originate from the stage wherein the proprioceptive coding of the target is processed either to replicate the same set of joint angles (Condition 1), or to compute the new set of angles corresponding to the same final position as seen from the other arm (Condition 2). Mathematical analysis shows that the correspondence between joint angles that code for the same endpoint depends critically on the system of reference that the CNS is supposed to adopt. Our results suggest that this system may not be the Euler system that previous studies have favored.

169.14

POINTING TO PROPRIOCEPTIVE TARGETS: THE DISSOCIATION OF MOVEMENT AND POSITION ENCODING. J. Paillard* and G.E. Stelmach. CNRS-Neurobiologie du Mouvement, Marseille (*) and Motor Control Laboratory, Arizona Sate University.

Position sense under active and passive conditions was reexamined using the protocol first introduced by Paillard and Brouchon (1968), dissociating movement and position encoding. In a dynamic mode, subjects moved the target index finger to a location along a vertical track and immediately pointed at it with the left hand. In a stabilized position, subjects pointed at the stabilized finger, 15 seconds after reaching final position. In both modes, tactile or/and visual cues were added to the basic proprioceptive condition in separate blocs of trials. In the dynamic mode, the active was better than the passive condition. In the active trials, providing additional tactile or/and visual cues yielded no difference in pointing accuracy. However, under passive condition, tactile and visual cues both reduced pointing errors, with tactile cues showing the largest reduction.The combination of cues reduced the errors to the level of the tactile condition alone. In the stabilized mode, where the dynamic receptors were adapted, there was no more difference between active and passive pointing accuracy.Moreover, providing tactile cues had no impact on error reduction, whereas providing visual cues significantly reduced pointing errors. Providing both cues maintained errors at the level of the vision condition alone.The data suggest that proprioceptive cues lead to different location encodings according to the dynamic or stabilized modes. Proprioceptive encoding, following active positioning, clearly dominates tactile and visual cues, whereas visual encoding of target location dominates in a stabilized mode. Moreover tactile cues, which greatly improve pointing accuracy at passively positioned targets, do not influence the location encoding of passively or actively maintained limb.

169.15

PROPRIOCEPTIVE INFLUENCES ON A STIMULUS-RESPONSE COMPATIBILITY REACTION TIME TASK. G.K. Kerr* and C. J. Worringham. School of Human Movement Studies, Queensland University of Technology, Brisbane, Q4059, Australia.; Department of Movement Science, The University of Michigan, Ann Arbor, MI 48109-2214.

This study examined how alterations in proprioceptive information induced by tendon vibration affected performance in a visual reaction time (RT) task. In the compatible instruction set group subjects responded with the finger that was on the same side as the visual stimulus. Whereas, in the incompatible instruction set group, subjects responded with the finger on the opposite side. For the present experiment subjects arms were passively moved to different positions by the experimenter such that subjects fingers were either crossed or uncrossed. These positions were chosen such that there were different amounts of separation between the fingers (2.4 to 12 cm). There was no contact between the limbs. On half of the trials subjects completed the task while the tendon of their right triceps brachii was vibrated (60 Hz).

RTs for the compatible group were significantly shorter than those for the incompatible group. For both groups there was an increase in RT as the amount of crossover of the finger tips increased. This systematic alteration in RT was correlated with subjects' perception as to whether their fingers were crossed or uncrossed at these positions. When vibration was applied RTs were longer than in the non-vibrated condition. RTs also demonstrated a systematic alteration as a function of the amount of finger crossover. This trend was consistent with subjects responding as if their fingers were in the "perceived" rather than their actual positions. That is, subjects behaved as if their limbs were crossed when they were not. These results demonstrate a strong proprioceptive influence on neural processes subserving stimulus response compatibility tasks.

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169.16

SOMATOSENSORY INPUT ENHANCES NEUROMUSCULAR ACTIVATION DURING MOVEMENTS PERFORMED WHILE FREE-FLOATING IN MICROGRAVITY. C.S. Layne*, A.P. Mulavara, P.V. McDonald, C.J. Pruett, J.J. Bloomberg. Neuroscience Movement and Coordination Laboratory, Space Biomedical Research Institute, NASA-Johnson Space Center, Houston, TX 77058.

Substantial evidence suggests that somatosensory input can modify neuromuscular activation characteristics (Burke, et al., 1991; McCloskey, 1995). What is not well understood is the integrated effects of somatosensory and other sensory input (e.g. visual, vestibular) on neuromuscular activation. This investigation utilized the microgravity environment of space flight to assess how foot pressure modifies neuromuscular activation in the absence of vision, a gravitational reference and a support surface. Four crewmembers performed two conditions of rapid, unilateral arm raises while free-floating during the course of two long duration missions (66 and 110 flight days) aboard the MIR space station. One condition consisted of arm movements without foot pressure. A second condition was performed with pressure being applied to the feet with the use of an inflatable insole encased in a specially constructed boot. All movements were performed with the eyes closed. Arm accelerations and surface electromyography from shoulder, trunk and lower limb muscles were obtained. Patterns of muscle activation were determined for each condition and then compared with ground-based patterns collected prior to flight. Results indicate that the temporal sequencing of muscle activation patterns associated with rapid arm raises performed while free-floating were very similar to those observed during ground-based performance. However, the addition of foot pressure enhanced the magnitude of activation relative to that observed during arm raises performed without foot pressure. These findings indicate that in the absence of vision, a gravitational reference, and the proprioceptive input resulting from the interaction of the performer and the support surface, the perceptual-motor system is able to generate neuromuscular activation patterns which support rapid arm movements. Importantly, the addition of somatosensory input from the feet can be used to enhance the magnitude of neuromuscular activation.

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169.17

GENERALIZABILITY OF EVOKED POTENTIAL GAIN DURING PASSIVE MOVEMENT. J.D. Brooke*, W.R. Staines, P.A. Angerilli and W.E. McIlroy[1]. Department of Human Biology and Nutritional Sciences, University of Guelph, Ontario, N1G 2W1, and [1]Sunnybrook Health Science Centre, Toronto, Ontario, M4N 3M5, Canada.

Somatosensory evoked potential (SEP) gain attenuates during passive movement. In the upper limb, this gating is restricted mainly to nerve stimulation supplying the moved limb segment. We hypothesized that movement-related gain attenuation in the lower limb would be generalizable from ipsi- to contralateral limb movement, but not to other sensory modalities. Evoked potentials were recorded in eight subjects from Cz, Cz' (2 cm posterior to Cz), O1, and O2 in response to stimulation of the tibial nerve at the popliteal fossa (SEPs), pattern reversal visual stimuli (VEPs), and binaural auditory stimuli (AEPs) during unilateral passive movement at 40 rpm of the leg ipsi- and contralateral to the stimulated limb. SEPs, referenced to Fpz, and soleus H reflexes were attenuated in both movement conditions compared to non-movement controls ($p<0.05$). VEPs (N75-P100), referenced to Fz, and short latency AEPs, referenced to the right earlobe, were not significantly altered by passive movement. Middle and long latency AEPs were inconsistent across subjects. We conclude that in contrast to sensory gating in the upper limb, gain attenuation in the lower limb is generalizable to passive movement contralateral to the stimulated limb. This gain attenuation is not generalized to other sensory modalities. Supported by NSERC (Canada).

169.18

REACHING TRAJECTORY AND ENDPOINT ERRORS INDUCED BY CORIOLIS FORCE PERTURBATIONS IN LABYRINTHINE-DEFECTIVE SUBJECTS. P.DiZio* and J.R.Lackner. Ashton Graybiel Spatial Orientation Laboratory and Volen Center for Complex Systems, Brandeis University, Waltham , MA 02254.

We measured the effects of inertial Coriolis force perturbations on visually open-loop reaching movements in a fully enclosed rotating room. Five profoundly labyrinthine-defective (LD) individuals and five age-matched controls participated. The LD subjects do not experience self-rotation even during exposure to high levels of angular acceleration. Ss were tested at 10 rpm, constant velocity and all felt stationary during testing.

The normal Ss performed like Ss in our earlier experiments (Lackner & DiZio, 1994. J Neurophys, 72:299-313), showing initial deviations of reaching trajectory and endpoint in the direction of transient Coriolis force perturbations, followed by complete adaptation to baseline within 40 reaches, and mirror-image aftereffects post-rotation. The LD Ss showed the same pattern of initial errors but fragmentary adaptation. They achieved 100% adaptation of trajectory but only 25% of endpoint after 40 per-rotation reaches, trajectory but not endpoint aftereffects were present post-rotation. Thus, 1) Coriolis forces and not vestibular stimulation are responsible for reaching errors in our paradigm during constant velocity rotation, 2) brachial proprioceptive feedback involved in adaptation depends on vestibular regulation, and 3) adaptation of posture (endpoint) and movement (trajectory) are separately controlled.

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169.19

REACHING MOVEMENTS DURING ILLUSORY SELF-ROTATION SHOW COMPENSATION FOR EXPECTED CORIOLIS FORCES. J.V.Cohn*, P. DiZio and J.R.Lackner. Ashton Graybiel Spatial Orientation Laboratory and Volen Center for Complex Systems, Brandeis University, Waltham , MA 02254.

Subjects exposed to constant velocity, body rotation (60°/s) at the center of a fully enclosed rotating room feel stationary. When reaching to targets they show large trajectory deviations and endpoint errors in the direction of the transient Coriolis forces generated by their movements. By contrast, subjects who turn voluntarily (365°/s, peak) reach accurately indicating that they compensate for their large self-generated Coriolis forces. We predicted that stationary subjects experiencing illusory self-rotation would make reaching errors because of "automatic" motor compensations for "expected" Coriolis forces.

We studied reaching movements of stationary Ss (N=10) experiencing illusory self- rotation and displacement induced by full-field visual scene rotation (60°/s) in a LEEP Cyberface2 (140° FOV) head mounted display. Ss experiencing illusory CCW self-rotation exhibited leftward trajectory deviations and endpoint errors in pointing to targets. These errors are mirror image to those generated during passive CCW body rotation in a rotating room. This means that Ss can compensate for anticipated Coriolis forces. These observations emphasize the importance of forward models in understanding human movement control and the necessity to take into account information about whole body orientation.

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169.20

TRANSLATING INSTRUCTIONS TO SPATIALLY-DIRECTED LIMB MOVEMENT: ELDERLY vs YOUNG. R.K.Parasher*, A.M. Gentile, Z.M.Pine & P.Kringas Teachers College, and College of Physicians & Surgeons, and Presbytarian Hosp., Columbia Univ. NYC, NY. 10027

Previously, spatially-directed limb movement was found to be be initiated and executed faster following instructions using pictures (visuospatial - VS) than words (visuowritten - VW) (Parasher & Gentile, 1995). We proposed that VS instructions rapidly yield a visuospatial representation of the goal (associated with right hemisphere processing) and is used to guide movement organization. In contrast, VW (left hemisphere processing) necessitates interhemispheric transfer and recoding of input to derive the goal-representation. In the present study, an interaction of Age x Instruction was predicted: differences between VS and VW were hypothesized to be greater for elderly than young subjects as interhemispheric transfer of information may be disrupted. The task required reaching forward to grasp a dowel and placing its light or black end into one of 4 containers located left/right, near/far of the subject. Instructions specifying the goal were presented visually: VS - a line drawing depicted final dowel-placement; VW - 3 words described placement (e.g. BLACK FAR RIGHT). Right-handed females, 12 elderly (70-79 yrs) & 12 young (20-40 yrs), were tested under 8 conditions derived from right/left; near/far; black/light combinations of dowel-placements. Reaction time (RT) and movement times (MTs) were measured. Prior findings were replicated: RT & MTs were faster for VS than VW which appears due to simultaneous vs. serial processing. As predicted, differences between VS and VW were greater for elderly than young. The significant Age x Instruction interaction suggests a delay in interhemispheric transfer of information, which may be related to prior findings of structural changes in the corpus callosum with aging (cf, Doraiswamy, et al, 1991).

CONTROL OF POSTURE AND MOVEMENT: HAND MOVEMENT

170.1

HUMAN INDIVIDUATED FINGER MOVEMENTS: HOW INDEPENDENT ARE THE DIGITS? R. Hayes and M. H. Schieber*. Department of Neurology, University of Rochester, Rochester, NY, 14642.

Whereas nonhuman primates are capable of only relatively independent finger movements, the human fingers and thumb often are assumed capable of completely independent motion, except for some couplings produced by interconnections between tendons. In both unskilled tasks and skilled performances, however, humans typically make simultaneous movements of multiple digits. To quantify the degree to which human subjects move one finger without moving others, we used either a DataGlove or a video movement analysis system to record the movements of the thumb and fingers as subjects flexed and extended different digits in a verbally instructed pseudorandom rotation. Most human subjects made measurable movements of non-instructed digits, which were smaller than those previously reported for the individuated finger movements of rhesus monkeys, but showed generally similar characteristic patterns (Schieber, J Neurophys 65:1381-91, 1991). Consistent with common experience, thumb and index finger movements were highly independent, while movements of the middle, ring and little fingers were less so. Explicitly requesting that human subjects keep non-instructed digits still while moving the instructed digit failed to improve performance. We used the previously developed Individuation Index to quantify the degree to which non-instructed digits moved during each instructed movement, and the Stationarity Index to quantify the degree to which a digit remained stationary when it was a non-instructed digit. These indices confirm that human finger movements, though more highly individuated than those of Rhesus monkeys, are not completely independent. The causes may include tendons, multitendoned muscles, and descending control. Support: R01-NS-27686, P41-RR-0283.

170.2

COORDINATING THE MANY JOINTS OF THE HAND

J. F. Soechting and M. Flanders*. Dept. Physiology, University of Minnesota, Minneapolis, MN 55455

The hand and fingers comprise an enormous number of kinematic degrees of freedom and the question arises: to what extent can each of them be controlled independently by the nervous system? We investigated this question by studying a well-practiced motor task involving the hand and fingers, namely typing, and by obtaining a fairly complete kinematic description of these movements. This description included 3 degrees of freedom at each of the fingers: flexion/extension at the metacarpophalangeal and proximal interphalangeal joints and abduction/adduction. It also included a 4 degree of freedom description of thumb motion and 2 more degrees of freedom at the wrist (pitch and yaw). We found principal component analysis and cluster analysis to be useful in providing a concise description of the experimental data. In particular, very few (<4) principal components were needed to describe motion at all of the degrees of freedom for all of the keystrokes executed with the right hand. The data were found to be clustered, both for the patterns of motion for one degree of freedom as well as for the relations between degrees of freedom, but we did not find any obligatory coupling of motions of any of the fingers.

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170.3

AUTOMATIC GRASP FORCE ADJUSTMENT IN A FES HAND NEUROPROSTHESIS FOR TETRAPLEGIC PATIENTS, A. Lickel, M. Haugland*, Center for Sensory-Motor Interaction, Aalborg University, Fredrik Bajersvej 7D 9220 Aalborg, Denmark

When normal humans are grasping and holding an object between two fingers, the cutaneous mechanoreceptors are used for estimation of the precise force necessary to hold the object in a secure grasp without using excessive force. We have investigated the possibility of implementing such a mechanism in a system for re-establishing lateral handgrasp in a tetraplegic patient by functional electrical stimulation, by means of signals from a cuff electrode placed on a cutaneous nerve in the hand. A 26 yr male C5 tetraplegic volunteer subject was instrumented with 8 percutaneous stimulation electrodes implanted into four finger muscles (FPL, AdP, EPL and FDS) and a tripolar cuff electrode implanted around the palmar digital nerve to the radial side of the index finger. A scheme for detecting slips across the index finger was developed and used as a trigger for increasing the stimulation intensity, so that slips were stopped before the object being held was dropped. In a controlled setup, where the arm of the subject was stabilized in a vacuum cast, the object held in a key grip between the thumb and index finger and being pulled out of the grip horizontally, the performance of the tetraplegic subject using the FES system was compared to the performance of normal subjects in the same situation. The necessary stimulation intensity to hold a given load could be probed by slowly decreasing the intensity until a slip occurred. Using this scheme it was possible to automatically adjust the stimulation intensity and hold a constant load with the same or lower average grasp force than normal subjects would usually do. It was also shown that for sudden increases in load, the average slip length for the FES system was the same as for the normal subjects.

This project was supported by the Danish National Research Foundation.

170.4

CONTROL OF 2-DIMENSIONAL FINGERTIP FORCE VECTORS DURING PRESSING: FORCE AND MUSCLE ACTIVATION PATTERNS. K.J. Cole* and W.G. Darling. Dept. Exercise Science, Univ. of Iowa, Iowa City, IA 52242.

Manipulating objects with the fingertips requires that we control the resultant fingertip force vector's direction and magnitude. It is not clear how this control occurs, given the complex geometry of the finger. For example, when pressing the distal, palmar surface of the index finger against a flat, rigid surface the long flexor muscles will produce the desired force normal to the surface and a tangential force directed towards the wrist. In the present experiment subjects pressed against a force plate with their index finger. They generated normal forces from 5% to 30% of their maximum pressing force (MPF). Only target force and normal force were displayed oscillographically to subjects in real time.

Tangential forces were minimal in all subjects; the resultant force vector was nearly normal to the force plate. In most subjects the vector was aimed no more than 3 degrees off the perpendicular, while in a few subjects the vector deviated up to 15 degrees. Thus, frictional forces were not exploited for stability (measured friction would permit 45 degree deviation from perpendicular). Intrinsic and extrinsic muscles coactivated during this pressing task, including extensor muscles. All scaled their activity with the size of the resultant fingertip force vector. The long flexors and lumbrical modulated most steeply, while the long extensors and dorsal interosseous modulated much less. Lumbrical was active at 20% of its maximal activity at 5% MPF, but was at 100% by 30% MPF. In contrast, the long flexors were between 25 and 50% of maximal activity at 30% maximal pressing force. Because of lumbrical's origin from the flexor digitorum profundus (FDP) tendon and insertion into the extensor expansion, it produces extensor torques at the distal interphalangeal joint, while removing flexor torque at this joint from FDP. This muscle may play a crucial role in directing the resultant fingertip force vector during dexterous manipulation.

170.5

INTERDIGITAL CONTROL OF ISOMETRIC FINGER FORCES H. Henningsen*, S. Knecht, M. Deppe, M. Fischer, B. Ende-Henningsen, A. M. Gordon. Dept. of Neurology, Univ. of Münster, 48129 Münster, Germany; Dept. of Movement Sciences, Teachers College, Columbia Univ., New York, NY 10027, USA.

Previously we have demonstrated asymmetries in the ability to match the finger forces exerted with the right and left hands which depend on the handedness of the subject. It can be hypothesized that functional asymmetries may exist in the ability to match the finger forces exerted within each hand as well. In the present study we investigated the ability to match the forces between adjacent and non-adjacent fingers of the same hand. Subjects were trained to produce an isometric flexion force of 200 $\pm$ 50 g separately with each of two fingers of the right hand at a time. During the experiment, they were instructed to match the forces of these two fingers simultaneously within the trained force range. The training and testing were repeated for all possible two-finger-combinations of the index, middle, ring and little finger of the right hand in a randomized order. The results showed that the ability to match the force of two fingers depended on the specific fingers involved. Subjects were fairly accurate at matching forces of adjacent fingers, but performance progressively decreased as the number of digits between the two tested fingers increased. We suggest that the recently shown overlap of representation of the individual fingers within the primary motor cortex may facilitate the comparison of forces exerted by adjacent fingers.

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170.6

MUSCLE COORDINATION DURING MAXIMUM INDEX FINGER ADABDUCTION FORCES F.J. Valero-Cuevas*§∞, C.G. Burgar°∞, F.E. Zajac°§∞, V.R. Hentz°†, K.C. McGill∞, K.N. An¥
∞Rehab. R&D Ctr. and †Hand Ctr., VA PAHCS, Palo Alto, CA 94306; §Dept. Mech. Eng. Stanford U. and °Dept. Funct. Rest. Stanford U. Med. Ctr. Stanford, CA 94305; ¥Orthop. Biom. Lab., Mayo Clinic, Rochester, MN 55905

The muscle excitation pattern (MEP) that produces index finger force in key pinch is relevant to the restoration of hand function as it may reveal surgical factors and principles that contribute to successful grasp outcomes. Due to the biomechanical complexity of the fingers, index finger force in key pinch may be produced by different MEPs. Our four degree-of-freedom (DOF) model of the index finger (flexion-extension at MCP, PIP and DIP joints, ad-abduction at MCP joint) included all seven finger muscles and predicted the unique MEP which produces maximum static force originating at the midpoint of the distal phalanx, in neutral MCP ad-abduction, in extended (10° flexion at all joints) and flexed (45° MCP and PIP, 10° DIP joint flexion) finger postures. For abduction force (analogous to force during key pinch), MEP predictions for the first dorsal (FDI) and palmar (FPI) interossei agonist-antagonist pair consisted of maximum excitation of FDI and zero excitation of FPI, and viceversa for adduction force, invariant with posture.

We recorded intramuscular EMG from all seven muscles of the index finger during maximum voluntary static ad-abduction forces in eight adults in the extended and flexed postures. As predicted, we found no co-activation of the agonist-antagonist pair in the extended posture, but found unpredicted co-activation in the flexed posture ($p<0.05$).

We propose this co-activation of FDI and FPI may be evidence of active stabilization of proximal phalanx MCP torsion, a DOF generally omitted in finger models, which may be needed to produce key pinch force and control the MCP joint.

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170.7

STRETCH REFLEXES ARE ATTENUATED IN HAND MUSCLES DURING HUMAN PRECISION GRIP. D.F.Collins* and A.Prochazka. Div. of Neuroscience, University of Alberta, Edmonton, Alberta, CANADA, T6G 2S2.

The aim of our study was to analyze the role of reflexes in hand muscles during grasping and lifting of weights. Stretch reflexes were reduced just prior to contact with the weights when compared to isometric contractions. Tasks involved the right hand only. Vision of the arm distal to the mid-forearm was prevented. Grip aperture and the full-wave rectified EMG activity of 4 muscles involved in the grip were monitored. The thumb and index finger were splinted to restrict interphalangeal joint movement. Step stretches (12 mm, 8 ms rise time) were applied using a servo-motor connected to a ring around the distal inter-phalangeal joint of the index finger during 2 grip tasks and one static hold task. Prior to each grip trial thumb and index finger positions were standardized. Task 1: grasp, lift and replace the 1.5x1.5x3" high, 750g weight using a precision grip between the index finger and thumb. Task 2: weight absent, move the index finger to contact the thumb. Task 3: constant isometric force between thumb and index finger. During each task reflexes were evoked at the same grip aperture, corresponding to just before finger contact with the weight in task 1 and 20 trials of perturbed and unperturbed trials were collected. During the static trials short latency (25-30 ms) and long latency (60-70 ms) excitatory responses were recorded. The amplitude of the short latency component was substantially reduced (as much as 90%) during tasks 1 and 2. The longer latency responses were depressed in some cases. The attenuation of these reflexes likely arises from central inhibition and possibly spindle unloading in the shortening muscles and suggests that this phase of human grip relies more on central than proprioceptive reflex control.

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170.8

ANTICIPATORY CONTROL OF PRECISION GRIP FORCE IN TOURETTE'S SYNDROME. L.S. Jakobson*, J.R. Flanagan and K.G. Munhall. Queen's Univ., Kingston, K7L 3N6, Canada.

When lifting or transporting an object held in a precision grasp, grip force normal to the sides is finely modulated in anticipation of changes in load force tangential to the surface such that the grip force tends to be just slightly greater than the minimum required to prevent slip. Such predictive control may be contrasted with reflex-mediated increases in grip force that follow an unexpected load force perturbation to the object. In this paper, we examine the control of grip forces in an individual with Tourette's Syndrome. A key issue is whether increases in load force associated with tics are anticipated by appropriate grip force adjustments or act as unexpected load perturbations requiring feedback based grip force modulation. Our results suggested that the subject employed a grip force safety margin that was greater than that used by control subjects. In addition, we observed a number of cases in which load forces due to tics were anticipated by an increase in grip force. These results suggest that "involuntary" tics are anticipated by the motor system in a manner similar to voluntary movements. The extent to which grip force adjustments were sensitive to the magnitude and/or direction of the load will be reported.

170.9

MATCHING VISUALLY PERCEIVED OBJECT SIZES BY CONTROLLING FINGER SPAN. M. Santello and J.F. Soechting*. Department of Physiology, University of Minnesota, Minneapolis, MN 55455.

During the transport phase of grasping, finger span is found to be scaled to the object size. In order to study the accuracy of this scaling, we asked 5 subjects to match the size of cubes (ranging from 0.5 cm to 12.4 cm) by adjusting the finger span. The experiments were performed in a 'static' condition (*i.e.*, in absence of the transport phase) and without visual feedback of the hand. The experiments were designed to study the dependence of accuracy on (a) object size, (b) the finger coupled to the thumb, (c) shape of the object, (d) distance of the viewed object and (e) object orientation.

In all experimental conditions the error (underestimation of cube's size) tended to increase for larger sizes ($p<0.01$). A significant effect of finger was also found, the little finger being less accurate (larger underestimation of cube's size) than the rest of the fingers. A significant difference in the error was found when comparing the accuracy in estimating the same sizes of cubes and cylinders, but not when comparing cubes and rectangular prisms. No effect of viewing distance and object orientation was found.

The fact that the accuracy in reproducing a cube's size was not affected by changing the cube's retinal projection (experiments *e* and *d*) indicates that the subjects were able to infer the actual cube's size. Object's shape, on the other hand, altered the perception of size. The accuracy in the transformation from visual information to motor commands was found to be dependent on the finger used.

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170.10

DIGITAL INTERACTIONS IN THE CONTROL OF REACTIVE MOTOR RESPONSES IN BIMANUAL AND UNIMANUAL TASKS. Y. Ohki* and R. S. Johansson. Dept. of Physiology, Univ. of Umeå, S-901 87 Umeå, Sweden

When we manipulate objects the finger tip forces are controlled by digit-specific mechanisms for grasp stability, but higher level controllers account for aspects of inter-digital coordination. We addressed the relative importance of those control policies when subjects used two fingers to restrain a manipulandum with two horizontal grip plates. In a bimanual task they used the right and left index fingers, and in a unimanual task the index and middle fingers of the right hand. Trials of distally directed loads were unpredictably delivered to either one of the two grip plates or simultaneously to both. The probabilities of these three loading conditions were set differently among test series. Subjects prevented frictional slips by always responding to the load increase by rapidly increasing the normal force of a loaded finger. Although the very initial part of the dynamic response could be stronger in certain test series, simultaneous loading of the cooperating finger tended to attenuate the response in both grasp configurations, but most markedly in the unimanual task. Automatic responses also occurred in a finger that was not loaded. These were considerably smaller than those evoked while the same finger was loaded, but their sizes increased with the probability of loading of that particular finger in the test series. Such responses were essentially confined to a brief force rate pulse coinciding in time with the initial part of the dynamic response of the loaded finger. However, in the bimanual task the responses in a non-loaded finger lagged those of the loaded finger by some 20 ms. In conclusion, in both bi- and uni-manual grasp configurations digit-specific afferent inputs are necessary for eliciting a response strong enough to restrain an object. But the response is to some extent influenced by the loading of the cooperating finger, and its size depends on subjects' expectations of the behavior of the manipulandum. Finally, the features of the responses in loaded and non-loaded fingers indicate that these are in part mediated by fundamentally different neural processes both in bimanual and unimanual tasks. This work was supported by the Swedish MRC and the ONR, Arlington, VA.

170.11

SIGNIFICANT LEFT-RIGHT SYNCHRONIZATION OF PULSATILE MOTOR OUTPUT IN A HUMAN BIMANUAL FINGER MOVEMENT TASK.
J. Wessberg*. Dept. of Physiology, Göteborg University, Medicinaregatan 11, S-413 90 Göteborg, Sweden.

Slow finger movements in humans are often characterized by velocity peaks or discontinuities recurring at 8-10 Hz, produced by a pulsatile modulation of the activity of agonist and often antagonist muscles as well (Vallbo & Wessberg, J Physiol 1993, 469, 673-691). It has been shown that the pulsatile modulation of muscle activity is not dependent on peripheral mechanisms, but generated within the central nervous system (Wessberg & Vallbo J Physiol 1995, 485, 271-282; J Physiol *In press*). The aim of this study was to analyze if the 8-10 Hz modulations of motor output to the left and right hand exhibit bilateral synchronization during a bimanual precision task. Movements were recorded in 5 healthy volunteers. The subjects were sitting with the hands resting on a table, and were asked to flex the two index fingers slowly and simultaneously towards a target object located mid way between the fingers. Movements were recorded with a video array tracking the location of a set of reflective patches.

Intermittent synchronization of the 8-10 Hz velocity peaks was observed during the movements. Statistical signal analysis in the time- and frequency-domains confirmed a significant synchronization in all subjects. Overall coherence of the joint angle acceleration for all movements in individual subjects peaked in the 9-10.3 Hz range, with values ranging from 0.023 to 0.071. Analysis of the coherence in relation to the instant of contact with the target object revealed that significant coherence was not limited to the onset of the movements.

The findings support the conjecture that the 8-10 Hz modulations of motor output are due to a modulation at similar frequency of the descending central command during slow finger movements.

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LIMBIC SYSTEM AND HYPOTHALAMUS I

171.1

FUNCTIONAL SEGREGATION OF SYNAPTIC INPUTS TO CA3 USING MULTIELECTRODE PROBES. C.Y. Perron*, M.F. Yeckel and T.W. Berger. *Dept. of Biomedical Engineering and Program in Neuroscience. University of Southern California, Los Angeles, CA 90089-1451.*

The electrophysiological isolation of mossy fiber (mf) inputs to CA3 pyramidal cells by *in vivo* extracellular field potential recordings remains a challenge due to factors such as the spatial restriction of the mf pathway in *s. lucidum* and the proximity of other current sinks produced by CA3 collateral inputs and somatic spike generating processes. In this study, vertical multielectrode probes were lowered through CA3c to perform current-source density (CSD) analyses on halothane-anesthesized rabbits for different stimulation sites, which include the hilus of the dentate gyrus, the perforant path (pp) in the angular bundle and the Schaffer collaterals (Sch) in CA1 *s. radiatum*. Hilar stimulation was expected to produce a short latency, monosynaptic mf excitatory sink within 100 μm of the CA3 population spike sink, as well as a longer latency, disynaptic sink higher in the dendrites due to activation of CA3 collaterals. The pp stimulation was expected to produce a monosynaptic sink in the apical dendrites of CA3 and a disynaptic mf sink if the stimulation intensity was high enough to activate granule cells. The purpose of Sch stimulation was to observe recurrent collateral sinks in the absence of mf inputs.

Recordings with the multielectrode probes revealed four main findings. (1) Hilar stimulation produced a short latency population spike, indicating that CA3 cells were monosynaptically activated by mf. (2) The expected short latency mf sink was weak. This may have been due to either the proximity of both the recurrent collateral sink and its associated source or to insufficient spatial resolution of the present probes. (3) The collateral sink in response to hilar stimulation was distinct both temporally and spatially from the population spike sink and occurred at the same location as the sink produced by Sch stimulation, although shifted in time. (4) Single pp stimulation did not produce a detectable disynaptic mf sink, but paired pulse or low frequency (10Hz) stimulation did. In summary, the present probes clearly identified some response components such as the recurrent collateral sink. Probes with higher spatial resolution may be required to accurately dissociate the mf sink from the recurrent collateral sink.

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171.2

EONS: A MULTI-LEVEL MODELING SYSTEM. J.-S Liaw[1]*, Y. Shu[2], T.W. Berger.[1] [1]Dept. of Biomedical Engineering & Program in Neuroscience, [2]Dept. of Computer Science, Univ. of Southern California, Los Angeles, CA 90089

The capability of simulating neural systems at different levels of organization is essential for an understanding of brain function. We present EONS (Essential Objects for Neural Systems), a simulation system for developing neural models with different levels of analysis. Unlike traditional approaches of tightly coupling the user interfaces and the simulation systems, EONS tries to separate these two components to facilitate the reuse and portability of existing models so that scalability and extensibility of the simulation systems can be realized. In the approach presented here, object-oriented design methodology is used to form a hierarchy of neural models. Each model, e.g., for a synapses or a neuron is self-contained. This provides the flexibility to compose complex system models from models of basic elements. Furthermore, by including a modularity which is independent of any user interface, EONS will facilitate collaboration among geographically distributed groups (e.g., over the Internet).

As an example of this utility, we have used EONS to develop a neural network model for speech recognition that incorporates detailed synaptic mechanisms. In particular, short-term presynaptic plasticity such as facilitation, and feedback modulation are included. Due to presynaptic mechanisms, the synaptic strength changes dynamically, which we call the dynamic synapse. The probability of neurotransmitter release is determined by the temporal pattern of the spike train. That is, the dynamic synapses transform the temporal pattern of a spike train into a spatio-temporal pattern of discrete events of neurotransmitter release. As a result, the dynamic synapses provide an exponential increase in the computing capability of a neuron. We demonstrate the power of synaptic computation by performing speech recognition from unprocessed, noisy raw waveforms. Supported by ONR, NCRR, Human Frontiers Org., and NIMH.

171.3

NONLINEAR SYSTEMS CHARACTERIZATION OF DENTATE GRANULE CELL EPSPs BY FULL WAVEFORM KERNEL MODELS. S.S. Dalal* and T.W. Berger. *Dept. of Biomedical Engineering and Program in Neuroscience, University of Southern California, Los Angeles, CA 90089-1451.*

The perforant pathway provides glutamatergic input to the granule cells of the dentate gyrus. Electrical stimulation of this pathway yields a granule cell EPSP comprised of both AMPA and NMDA receptor-mediated components, each of which is facilitated or depressed depending upon the pattern of prior stimulation. Hence, both components of the system are nonlinear with respect to interstimulus interval. We present here an experimentally based nonlinear characterization of this system through use of a Volterra functional power series.

Intracellular sharp microelectrode recordings of granule cell EPSPs were performed in the rabbit hippocampal slice preparation. The perforant pathway was stimulated by a random impulse train which consisted of 4064 pulses and had a mean frequency of 2 Hz. Recordings were conducted in both physiological and low magnesium concentrations (1.2 mM and 0.1 mM, respectively). Picrotoxin (20 μM) was included in the perfusate to block gabaergic inhibition, and the AMPA and NMDA components were pharmacologically isolated by adding CNQX (10 μM) and APV (50 μM), respectively, to the medium.

First, second, and third order kernels for the full waveform of the EPSP response were computed. Results show that the measured, composite EPSP was not merely a linear superposition of the NMDA and AMPA components. Because of the different kinetic properties of the two receptor-channels, such as voltage-dependent blockade of the NMDA receptor, the nonlinearities exhibited by each channel type were different. Analysis of the full waveform was critical in that the nonlinearities expressed by each receptor subtype were observed to occur during both the initial and late phases of the composite EPSP. Supported by ONR, NCRR, and NIMH.

171.4

INTRACELLULAR EVIDENCE FOR GREATER PERFORANT PATH INPUT IN THE VENTRAL VS. DORSAL *in vitro* DENTATE GYRUS. Choi Choi* and T.W. Berger, *Dept. of Biomedical Engineering and Program in Neuroscience, Univ. of Southern California, Los Angeles, CA 90089*

Previously, we reported evidence from field EPSP recordings for greater perforant path excitatory input to dentate granule cells in slices prepared from the ventral vs. dorsal third of the hippocampus (with respect to the longitudinal axis). Input-output (I/O) curves for amplitudes of EPSPs elicited by twin pulse stimulation of the perforant path showed a steeper increase with increasing stimulus intensity and a larger maximum for ventral slices. In the present study, we examined intrinsic granule cell electrophysiology and intracellular EPSP I/O curves for 600 μm thick transverse slices obtained from dorsal and ventral thirds of the hippocampus.

Sharp microelectrodes (impedance 100-180 MΩ) filled with a 2 M $KMeSO_4$ salt solution, alone or combined with 50 mM QX-314, were used to impale and record (in current-clamp mode) from neurons in *s. granulosum*. Granule cells were identified by their location in the *s. granulosum* and by electrophysiological criteria (e.g. burst spike adaptation, action potential and/or EPSP to molecular layer stimulation). Orthodromic stimulation consisted of twin pulses (30 ms interval) delivered to the perforant path in the middle third of *s. moleculare*.

No dorsal-ventral differences were observed in granule cell resting membrane potential (RMP), input resistance (R_N), threshold for action potential, or membrane time constant (τ). In the subthreshold portion of the intracellular EPSP I/O curve, a steeper increase in EPSP amplitude with increasing stimulus intensity was observed for ventral slices. The addition of QX-314 to the microelectrode solution depolarized the RMP 15 to 20 mV in all cells and eliminated the action potential in most cells for both groups of slices. In the cells where an action potential was not observed, the maximal EPSP was larger in ventral slices for both first and second responses. In addition, at maximal stimulus intensities, a greater proportion of cells from ventral slices continued to generate action potentials.

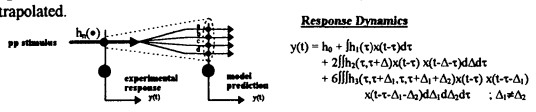
These results are consistent with previous extracellular results, and provide strong evidence for greater excitatory input to the dentate gyrus in slices from the ventral third of the hippocampus. Supported by ONR, NIH BMSR, NIMH.

171.5

COMPUTATIONAL MODEL OF A POPULATION OF DENTATE GRANULE CELLS BASED ON A NONLINEAR SYSTEMS ANALYTIC APPROACH. G.L. Wehrer* and T.W. Berger. *Department of Biomedical Engineering and Program in Neuroscience*, University of Southern California, Los Angeles, CA 90089

We have shown previously that the response dynamics of dentate granule cells can be accurately modeled as the kernels of a functional power series. The kernel functions are determined on the basis of random impulse stimulation of the perforant path and represent higher order nonlinearities with respect to the inter-pulse interval of the stimulus. These experimentally determined kernel functions ($\mathbf{h_n(\bullet)}$ in diagram below) were used as the basis of a computational model of a population of dentate granule cells. In addition, since the actual contribution of each synapse of a particular granule cell cannot be determined experimentally, it is necessary to estimate the distribution of the cell's dynamics over the estimated number of synapses in terms of a statistical description (**a-d** in diagram below).

The model utilizes the stochastic methods used in artificial neural networks to assign and modify individual weights to each of its processing units which correspond to each of the estimated pp-gc synapses. Each processing unit weight is modified in order to minimize the error between the network output and the experimental response. The optimum weight matrix represents the dynamics of the network which correspond to the dynamics of the cell. It is from this optimum weight matrix that the relative statistical distribution of pp-gc connectivity can be extrapolated.



Response Dynamics

$$y(t) = h_0 + \int h_1(\tau)x(t-\tau)d\tau + 2\iint h_2(\tau,\tau+\Delta)x(t-\tau)\,x(t-\Delta-\tau)d\Delta d\tau + 6\iiint h_3(\tau,\tau+\Delta_1,\tau,\tau+\Delta_1+\Delta_2)x(t-\tau)\,x(t-\tau-\Delta_1)\,x(t-\tau-\Delta_1-\Delta_2)d\Delta_1 d\Delta_2 d\tau \quad ;\Delta_1 \neq \Delta_2$$

Supported by ONR, NCRR , and NIMH

171.6

MIXED-SIGNAL VLSI IMPLEMENTATION OF A HIPPOCAMPAL MODEL. T.W. Berger*, B.J. Sheu, and R.H. Tsai. *Depts. of Biomedical Engineering, Electrical Engineering, and Program in Neuroscience, Univ. of Southern California, Los Angeles, CA 90089.*

We have demonstrated previously that a nonlinear systems analytic approach can be used to develop an experimentally-based model in the form of kernels of a functional power series of the dynamics of single neurons in the hippocampus. The kernels include the contribution of all possible sources of nonlinearities and represent the cell's response to the present input in the context of a "sliding window" of the input history. We used the kernel models of dentate granule cells and inhibitory interneurons to implement a hardware model of the dentate gyrus. In a previous design, a table-look-up approach was used that involved storage of discretized kernel values. Here we report a new design which utilizes a model-based approach in which extracted parameters are used to present the first and second order kernels in analog current-mode to save silicon area and maintain high accuracy. The model-based approach includes the following: i) the input is a series of impulses that can be conveniently specified, with effects from the history of input events accumulated in a set of capacitors; ii) the first order kernel function, $h_1(\tau)$, represented in analog, using capacitors charged to different voltage levels depending on the kernel value; the second order kernel, $h_2(\tau,\Delta)$, approximated by a characteristic curve of an analog circuit with parameter values that are externally programmable; iii) with each input event, output currents are generated from $h_1(\tau)$ and $h_2(\tau,\Delta)$; iv) summation of the currents from $h_1(\tau)$, $h_2(\tau,\Delta)$ determines the output of each silicon neuron. The above design has been incorporated into a 4x4-neuron prototype fabricated through MOSIS using 2-μm technology. Supported by ONR, NCRR, NIMH.

171.7

IDENTIFICATION OF HIGHER ORDER NONLINEARITIES OF HIPPOCAMPAL GRANULE CELLS BY FEEDFORWARD ARTIFICIAL NEURAL NETS M.A. Saglam*, V.Z. Marmarelis, and T.W. Berger *Dept. of Biomedical Engineering and Program in Neuroscience, Univ. of Southern California., Los Angeles, CA, 90089*

A traditional approach to characterizing biological systems is the use of Volterra-Wiener (V-W) functional expansions and kernels. Nonlinear input/output properties of hippocampal dentate granule cells are characterized experimentally by stimulating perforant path input fibers with a Poisson process pulse-train and recording the evoked population spike amplitudes. Kernels express the response of the system to every pulse in the train as a function of the temporal pattern of the previously occurring pulses. However, the kernel-based model is practically limited in terms of the order of nonlinearity it can capture, and also requires long data sequences for its estimation. On the other hand, the nonlinear relation between the intervals and amplitudes can be easily modeled by feedforward Artificial Neural Networks (ANN). For example, the output of a second order system in response to an input pulse would be dependent only on the interval between the current pulse and the previous pulse and a three-layer perceptron with only one input unit would be able to model this second order relation. Higher order nonlinearities can be captured simply by including additional input units.

We compare the V-W and ANN models in terms of their prediction ability on test data. Results show two major advantages of the ANN approach: (1) a significant reduction in the required data length (by a factor of 10-20) and (2) an ability to model high-order nonlinearities. Since a net with 8-input units gave the minimum prediction error, we can conclude that nonlinearities up to 9th order may be present in the dentate granule cells, an order of nonlinearity that cannot be modeled with V-W kernels. This work was supported by ONR, NCRR, and NIMH.

171.8

CHARACTERIZING UNOBSERVABLE NEURAL ELEMENTS IN THE HIPPOCAMPUS WITH NONLINEAR SYSTEMS ANALYSIS. M.T. Chian*, V.Z. Marmarelis, and T.W. Berger. Dept. of Biomed. Eng. & Prog. in Neurosci., Univ. of Southern California, Los Angeles, CA 90089

The hippocampal formation includes multiple feedback loops which are difficult to characterize through direct electrophysiological recording. However, the input/output properties (kernels of a functional power series) of these feedback connections may be quantified indirectly through nonlinear systems analysis. The input/output properties of the feedback system may be mathematically derived from those measured for the feedthrough and overall systems. Performing this calculation requires nonlinear algebraic system decomposition in the frequency domain. This method has been applied to characterization of the inhibitory effect of GABAergic basket cells on granule cells of the dentate gyrus. The granule cells of the dentate gyrus may be considered the feedthrough subsystem while the basket cells may represent an inhibitory feedback subsystem within an overall system. Since the feedthrough input/output properties may be isolated pharmacologically by blocking the feedback component, it is possible to mathematically deduce the feedback subsystem properties when the properties of the overall system and the feedthrough system are known.

Application of this method revealed that successful identification of unobservable subsystems depends primarily on accuracy of the initial estimates of observable system input/output properties and on the relative frequency bandwidths of the respective subsystems. Comparison of two input/output estimation methods showed that a new estimation method utilizing artificial neural networks allowed accurate recovery of the linear and nonlinear input/output properties of a given system. It also was discovered that in order to recover the nonlinear properties of the feedback element, the feedthrough element must have a larger frequency bandwidth than the feedback element. The influences of noise and different degrees of nonlinearities within each system were also studied. Supported by ONR, NCRR, and NIMH.

171.9

ARE FEEDFORWARD AND FEEDBACK INHIBITION MEDIATED BY THE SAME OR DIFFERENT SETS OF INHIBITORY NEURONS? J.S. Fitzpatrick*[2], H.E. Scharfman[3], and T.W. Berger[1,2]. [1]*Dept. of Biomedical Eng. and* [2]*Prog. in Neurosci., Univ. of Southern Calif., Los Angeles, CA 90089 and* [3]*NRC, Helen Hayes Hospital, and Columbia Univ. NY, NY.*

Granule cell activity in the hippocampal dentate gyrus is subject to both feedforward and feedback inhibition. The present experiments were carried out to determine whether these two types of inhibition are mediated by two distinct sets of inhibitory neurons or by one common set of inhibitory neurons. Field recordings and sharp electrode intracellular recordings were made simultaneously from transverse rabbit hippocampal slices. Stimulating electrodes were placed in two locations: (1) the molecular layer (ML) to stimulate the perforant path, thereby activating (a) solely feedforward mechanisms if no granule cells generate action potentials or (b) both feedforward and feedback mechanisms if granule cells do generate action potentials; and (2) *s. lucidum* of area CA3c to stimulate mossy fibers (thereby activating feedback inhibition) and CA3 pyramidal cells (which could also activate feedback inhibition). We assume that stimulation at this second site did not activate feedforward inhibition. The extent to which feedforward and feedback inhibition originate from common or distinct sets of inhibitory neurons was assessed by comparing the IPSP resulting from simultaneous stimulation at both locations ("simultaneous IPSP") to the calculated sum of the two IPSPs produced by stimulation at the two locations individually ("calculated IPSP"). At maximal stimulation intensity, the results were mixed. Sometimes the "simultaneous IPSP" was identical to the "calculated IPSP," suggesting that the two stimulation locations activate distinct sets of inhibitory neurons. Other times the "simultaneous IPSP" was no larger than the IPSP elicited by ML stimulation, suggesting that feedforward and feedback inhibition are mediated by a common set of inhibitory neurons. At low or moderate stimulation intensities, the "simultaneous IPSP" was usually similar to the "calculated IPSP," indicating that sub-maximal stimulation at these locations activates distinct sets of inhibitory neurons. Supported by an NDSEG Fellowship to JSF; grants from ONR, NCRR, and NIMH to TWB; and NINDS to HES.

171.10

CHARACTERIZATION OF Ca^{2+} RESPONSES INDUCED BY ACTIVATION OF mGluRs IN HIPPOCAMPAL INTERNEURONS USING CONFOCAL MICROSCOPY AND WHOLE CELL RECORDING. G.Woodhall*, R. Robitaille and J.-C. Lacaille. Centre for Research in Neurological Sciences, Department of Physiology, Univ. of Montreal, Montréal, Canada, H3C 3J7

Hippocampal CA1 interneurons show diverse Ca^{2+} responses to excitatory amino acid receptor activation, including Ca^{2+} oscillations, fast transients and biphasic responses. Prolonged oscillations are associated with specific interneurons in the stratum oriens and alveus area (OA) and involve ionotropic and metabotropic glutamate receptors (mGluR). The aim of this project was to examine the response of interneurons in OA to mGluR activation using whole cell recording and confocal Ca^{2+} imaging in rat hippocampal slices. Interneurons in OA were visually identified and whole cell current clamp recordings obtained using patch electrodes (5-10MΩ) filled with (in mM) 120 K-gluconate, 20 KCl, 5 NaCl, 2 $MgCl_2$, 10 HEPES, 2 ATP-tris, 0.2 GTP-tris. Biocytin (0.1%) was also included for morphological characterization of interneurons. The fluorescent Ca^{2+} indicator calcium green-1 (95μM) was included in the recording solution and responses monitored with a BioRad MRC-600 confocal microscope. The mGluR agonist 1S,3R-ACPD (250μM) was applied onto discrete regions of the cell (soma, dendrites) using pressure ejection (10-40 ms) from a patch pipette. OA cells responded to ACPD in two ways. Some cells showed an oscillatory Ca^{2+} response lasting 200-500ms and consisting of multiple rises in Ca^{2+} level ($\geq$ 3 peaks). Other OA cells responded with a gradual rise in Ca^{2+} level. The electrophysiological responses evoked by ACPD consisted of membrane depolarizations associated with either phasic discharges and occasional plateau potentials, or a more tonic firing pattern. These results suggest that activation of mGluRs produces two types of Ca^{2+} rise and electrophysiological response in OA cells. Ca^{2+}-mediated mGluR actions may thus be heterogeneous and selectively expressed in specific interneurons.

(Supported by the MRC, FRSQ and FCAR)

171.11

INTRACELLULAR Ca^{2+} CHANGES INDUCED BY TETANIC STIMULATION OF AFFERENTS IN RAT HIPPOCAMPAL INTERNEURONS. M. Ouardouz*, R. Robitaille and J.-C. Lacaille. Centre de Recherche en Sciences Neurologiques et Département de Physiologie, Université de Montréal, Montréal, Canada, H3C 3J7.

Hippocampal CA1 interneurons located in different layers show different Ca^{2+} responses to local application of glutamate. The aim of the present study was to characterize Ca^{2+} responses induced by synaptic activation of glutamate receptors in two types of interneurons in stratum oriens (OR) and lacunosum-moleculare (LM) in rat hippocampal slices. Interneurons were identified visually and whole cell recordings obtained with patch electrodes containing (in mM) 120 K-gluconate, 20 KCl, 5 NaCl, 2 $MgCl_2$, 10 HEPES, 2 ATP-tris, 0.2 GTP-tris, as well as 0.1 calcium green-1 to measure intracellular Ca^{2+} changes. Changes in fluorescence were measured using a BioRad MRC-600 confocal microscope and expressed as percentage change from resting level. Synaptic responses were evoked using a monopolar electrode placed in the vicinity of the recorded cell. Tetanic stimulation (100 Hz, 1s) induced a larger Ca^{2+} increase in dendrites than soma (36.8±6.6% and 9.9±2.4%, respectively, n=11, p<0.01 t-test). Dendritic Ca^{2+} responses were similar in LM and OR cells, and were of two types. First, transient responses in LM cells (n=18) had an amplitude of 43.3±7.0%, a time to peak of 1.4±0.2s and a recovery time of 7.0±2.4s, *versus* an amplitude of 34.2±8.6%, a time to peak of 1.0±0.2s and a recovery time of 6.6±1.1s for OR cells (n=12). Second, biphasic responses consisted of an initial Ca^{2+} peak (66.0±2.8%) followed by a secondary peak (n=3 LM and 3 OR cells). Preliminary results indicate that Ca^{2+} responses were reduced by bath application of antagonists of ionotropic (20μM CNQX and 50μM AP-5) or metabotropic (250μM MCPG) glutamate receptors. These results show that synaptically released glutamate can induce an elevation of intracellular Ca^{2+} in dendrites of both OR and LM interneurons. These Ca^{2+} responses may involve non NMDA, NMDA and metabotropic glutamate receptors.

(Supported by MRC, FRSQ, FCAR and the Savoy Foundation)

171.12

INTRACELLULAR CORRELATES OF HIPPOCAMPAL DENTATE EEG SPIKES IN GRANULE CELLS, INTERNEURONS AND PYRAMIDAL CELLS IN VIVO.

M Penttonen*, A Sik, and G Buzsaki. CMBN, Rutgers University, Newark, NJ 07102

Dentate spikes (DS) are large amplitude (1-4 mV), short duration (10-40 ms) field potentials that occur during behavioral immobility and slow wave sleep. DS are associated with burst discharge of putative hilar interneurons and suppressed firing of CA1-3 pyramidal cells. Here we recorded intracellularly from granule cells, CA3 and CA1 pyramidal cells and hilar interneurons during DS in urethane anesthetized rats. The neurons were anatomically identified. DS were associated with depolarization of granule cells and occasional action potentials. CA3 and CA1 pyramidal cells were hyperpolarized or not affected. A HICAP interneuron as well as a trilaminar interneuron with axon collaterals in CA3, CA1 and subiculum fired bursts during DS. Hilar interneurons with extradentate projections may be responsible for the DS-induced suppression of the CA3-CA1-subiculum circuitry.

171.13

DISCORDANCE WITHIN ENSEMBLES OF CA1 AND CA3 CELLS IN CODING FOR SPATIAL RELATIONSHIPS. H.Tanila (1)*, M.S. Shapiro (2), H.B. Eichenbaum (3). [1]Dept. Neurology, Univ. Kuopio, Finland, [2]Dept. Psychology, McGill University, Montreal, Canada, [3]Cntr Behav. Neurosci, SUNY at Stony Brook, NY, USA.

Place fields of hippocampal pyramidal cells are controlled by salient local as well as distal environmental cues (Young et al., J Neurosci 14:6553, 1994). It is not known, however, whether all cells in an ensemble respond to a change in an environment in a concordant way. We recorded the activity of single hippocampal CA1 and CA3 cells in rats performing a win-shift task in a radial maze. Prominent distal visual stimuli were attached to the dark curtain surrounding the maze, and inserts with distinctive visual, tactile, and olfactory stimuli provided local cues on each maze arm. To test the influence of the different stimulus sets, the distal and local cues were rotated 90 degrees in opposite directions. In response to this manipulation, place fields could: maintain a fixed position to room coordinates, rotate with either the local or the distal cues, disappear, or new fields could appear. With one tetrode per animal we simultaneously recorded 2 to 10 complex spike cells with distinct place fields, providing a sample of 336 cells in 58 cell ensembles. On average 74% of cells within an ensemble responded in the same way (82% in 24-26 mo old rats who were impaired in spatial learning, 76% in aged learning-intact rats, and 68% in young rats.) Overall 31% of the ensembles were concordant (40% in aged learning-impaired, 33% in aged learning-intact, and 23% in young rats). Half of the discordant ensembles had place fields that rotated with one set of cues whereas the other fields disappeared or new fields appeared. In 19% of the discordant ensembles some cells had rotated fields whereas others maintained their fields in the original location. In 10% of the discordant ensembles there was one (but only one) cell with fields rotating in the direction opposite to that of the majority of cells. These findings suggest that hippocampal ensembles can simultanously represent both the original and the altered environment and, in rare cases, conflicting orientations of cues within the same environment.

171.14

THE DORSO-VENTRAL DIFFERNCE IN THE DISTRIBUTION OF NON-PRINCIPAL CELLS IN THE RAT HIPPOCAMPUS.

TAKUO NOMURA[1], TOSHIO KOSAKA[1] MASAHIKO SAKAGUCHI[2]* [1]Dept. of Anat. and Neurobiol., Faculty of Med., Kyushu Univ. Fukuoka 812-82, Japan. [2]Dept. of Biol, Faculty of Educ., Shinshu Univ. Nishinagano, Nagano 380, Japan.

In the present study we examined major subpopulations of GABAergic nonprincipal neurons in the hippocampus, focusing on the dorsoventral differences in their distributions. The subpopulations analyzed are those immunoreactive for parvalbumin (PV), calretinin (CR), nitric oxide synthase (NOS), somatostatin (SOM), calbindin D28k (CB), vasoactive intestinal polypeptide (VIP) and cholecystokinin (CCK). Using a confocal laser scanning light microscope, we could confirm that the penetration of each immunostaining was complete throughout 50μm thick sections under our immunostaining conditions (except that of CB). So we counted the number of positive cells according to the disector principle and calculated the numerical densities (ND). Generally speaking, PV, CB, CCK and VIP neurons showed no significant dorsoventral differences in the NDs in any subdivisions of the hippocampus, whereas the NDs of CR, NOS and SOM neurons were significantly larger in the ventral than in the dorsal hippocampus. The ND of SOM neurons was significantly larger in the dentate gyrus. That of NOS neurons was significantly larger in the CA3 region as well as in the DG. The ND of CR neurons was larger in all hippocampal subdivisions. We concluded that the dorsal and ventral hippocampus differ from each other in the composition of GABAergic non-principal cells. Support: Grants-in-Aid for Scientific Research from Japanese Ministry of Education, Science & Culture, Uehara Memorial Foundation, Mitsubishi Foundation

171.15

RAPHE INFLUENCES ON GRANULE CELL EXCITABILITY: EFFECTS OF LOCAL ACTIVATION AND 5-HT ANTAGONISTS. K.Kao* and E.J. Green, Dept. of Psychology, Univ. of Miami, Coral Gables, FL 33124

Prestimulation of the median raphe (MR) can markedly augment the flow of information through the hippocampus. Recent neurophysiological studies indicate that electrical activation of the MR increases granule cell excitability through inhibition of inhibitory interneurons (Kao, Sanders and Green, 1995), a finding consistent with anatomical evidence for MR-interneuron connections (Freund et al.,1990). The present experiments were designed to 1) assess whether local activation of MR cell bodies can support granule cell facilitation, and 2) determine whether facilitative MR prestimulation effects are mediated by 5-HT.

Anesthetized rats were prepared for stimulation of the perforant path (PP) and median raphe (MR), and recording of field potentials in the dentate gyrus. Prestimulation of the MR consistently produced substantial (> 50%) facilitation of the perforant path-evoked population spike without affecting the evoked field EPSP. Microinjections of 0.1 M D,L-homocysteic acid (50-200 nL) into the MR over a period of 5-10 sec produced a dose-dependent facilitation of the population spike without altering EPSP slope. Mean ± S.E. % spike facilitation for 50, 100, and 200 nL's were 39.0±19.8, 71.7± 25.6, and 86.2± 9.9, respectively, (all $p<.05$). Facilitation started within 30 sec of injection, peaked in approximately two min and dissipated within four to five min. Preliminary results from separate experiments showed that electrically-induced MR facilitation was not attenuated by a 5-HT1A antagonist *p*-MPPI (10 mg/kg, i.p.).

The present study demonstrates that increases in granule cell excitability observed following electrical prestimulation of MR do not require activation of fibers of passage. While the systemic *p*-MPPI results suggest that 5-HT1A receptor activation is not necessary for the electrically-induced facilitation, it remains possible that activation of facilitative non - 5-HT hippocampal afferents masked the effect. Alternatively, other 5-HT receptor subtypes might mediate the hippocampal disinhibition. Supported by BNS-9021632

171.16

CHANGES IN THE LEVEL OF GLUTAMATE (GLU) IN THE MEDIAN RAPHE NUCLEUS (MRN) ASSOCIATED WITH HIPPOCAMPAL THETA ACTIVITY IN THE ANESTHETIZED RAT. V.Varga, B.Kocsis, A.Kekesi, G.Juhasz (SPON: European Brain and Behaviour Society) National Institute of Neurosurgery and Department of Comparative Physiology, Eotvos University, Budapest, Hungary

Serotonergic input from the median raphe nucleus (MRN) to the septum exerts a pronounced desynchronizing influence on hippocampal (Hipp) EEG. Long-lasting, uninterrupted theta elicited by antagonizing excitatory amino acid (EAA) transmission in the MRN (Kinney et al., Brain Res., 1994) suggested that, in turn, the activity of MRN serotonergic cells is under control of a tonically active EAAergic drive.

This study was designed to investigate the relationship between spontaneous changes of Hipp activity and the release of EAAs in the MRN, using the in vivo microdialysis technique. Dialysates were collected from the MRN during 2 to 6 sampling periods of equal length (20 min). The percentage of theta rhythmic pattern in Hipp recordings was calculated for each sampling period and the corresponding average GLU level was determined. The relationship between Hipp activity and GLU release in the MRN was characterized by comparison of GLU levels measured during consecutive sampling periods with different ratios of theta vs. non-theta segments in the Hipp EEG. We found high level of GLU in the MRN during periods dominated by desynchronized Hipp activity as compared with those mostly containing long or frequently occurring theta segments indicating that the desynchronizing serotonergic influence originating from the brainstem is maintained by strong tonic excitatory input to the MRN. During theta activity, due to suppression of this EAAergic activation, serotonergic neurons in the MRN become disfacilitated and the resulting decrease of their effect on the septum/Hipp serves to assist natural switching of the activity in the septum and Hipp from desynchronized to synchronized theta rhythmic pattern. (Supported by OTKA Grants T17778 and T16552)

171.17

DIVERGENT PROJECTIONS FROM THE PEDUNCULOPONTINE TEGMENTAL AREA CO-OPERATE IN GATING THETA RHYTHM N. R. Swain and N. McNaughton*. Dept. Psychology and Centre for Neuroscience, University of Otago, POB 56, Dunedin, New Zealand

Hippocampal theta rhythm (θ) can be elicited from several 'synchronizing' pathways in the pons (Vertes, 1981, *J. Neurophysiol.* **46**, 1140). One ascends from n. pontis oralis, relays in the supramammillary nucleus (SUM), and contributes to the frequency of θ. A second ascends from the pedunculopontine tegmental nucleus (PPT), apparently diffusely through the reticular formation (Vertes, 1981). We used injections of procaine (20% w/v, 0.2 or 0.5 µL) in urethanized (1.5g/Kg) rats to map the course of the fibres originating in PPT. Using 100 Hz, 150 µA, 0.1ms stimulation, two optimal synchronizing sites were found - in PPT and 1 mm dorsal to PPT. The same results were obtained with both sites. Injections of procaine into the ascending system eliminated θ with latencies under six minutes and for durations of 30-60 minutes. In total contrast to procaine in the region of SUM, frequency of θ was never changed and amplitude only showed small reductions. These data are consistent with a θ-gating function for PPT and are similar to the effects of anti-cholinergic drugs. Effects were obtained throughout the ascending system as mapped by Vertes (1981) and also in two additional, distinct sites: in the region of the superior colliculus and the substantia nigra, respectively. All these areas are targets of the ascending cholinergic fibres originating in PPT. These results suggest 1) that output from PPT (possibly cholinergic) gates theta; 2) that this output relays in at least three distinct areas, two of which are in or near the superior colliculus and substantia nigra; and 3) that θ-gating in the hippocampus can result from co-operation between subthreshold inputs from all three relays concurrently. *Supported by the Health Research Council of New Zealand*

171.18

HIPPOCAMPAL UNITS CHANGE PHASE RELATION TO LOCAL SLOW WAVE DURING RABBIT CLASSICAL CONDITIONING. R.L. Borgnis*, M.A. Seager, & S.D. Berry. Center for Neuroscience, Miami Univ., Oxford, OH 45056.

Many years of previous research has established that hippocampal cells fire with a consistent phase relation to the local slow wave. However, recent evidence from Recce & O'Keefe (1991) and Brankack, Stewart, and Fox (1993), suggests that cells may change their preferred phase in special situations such as when a rat traverses a place field.

Analysis of *multiple* unit/slow wave cross-correlations in rabbit classical jaw movement conditioning (CJM) has shown that the pattern and timing of the correlation function differs from free-running activity to tone (CS) period, indicating a changing relationship between units and slow waves (Borgnis & Berry, 1996). To expand these findings, we undertook a *single* unit analysis of unit/slow wave relations during CJM.

Following recovery from surgery (to cement a capped metal cylinder over the left hemisphere), rabbits were trained to criterion in CJM. The next day, a microdrive unit was used to lower a single unit electrode (5-20 um tip) into dorsal hippocampus. Once a unit was isolated, a few minutes of spontaneous activity were recorded. The rabbit was then given up to three 9-trial blocks during which time-locked unit and slow wave responses were recorded.

EEG and unit activity were analyzed before and during the trial to note the preferred phase relation of the unit to the slow wave. Consistent with many reports, every unit had a consistent relation to the slow wave if theta was present. During the trial, however, this relation changed. To date, all conditioned responses include firing on the phase opposite to pre-trial preferred phase. These results suggest that, in rabbit CJM, as in rat place fields, as hippocampal neurons become engaged in task-specific processing, the change in synaptic inputs causes a shift in unit/slow wave phase relations.

171.19

CORTICOSTERONE MODULATES HIPPOCAMPAL CHOLINERGIC THETA ACTIVITY VIA MINERALOCORTICOID RECEPTORS. D. Murphy*, B. Costall and J.W. Smythe, Dept. of Pharmacology, Univ. of Bradford, Bradford, U.K., BD7 1DP

Arousing stimuli elicit cholinergic theta activity in alert, immobile rats. Recently, we reported that intrahippocampal cholinergic blockade increased corticosterone (CORT) secretion induced by restraint stress. These data suggest that CORT modifies hippocampal cholinergic function and we examined the effects of blocking mineralocorticoid receptors (MR) on theta activity in urethane-anesthetized rats which exhibit only cholinergic theta. Adult male, Lister hooded rats (n=10) were anesthetized with urethane and a theta recording electrode was positioned in the hippocampus. A bipolar stimulating electrode was placed in the dorso-medial posterior hypothalamus (DMPH) to activate theta. Baseline recordings of spontaneous and DMPH-stimulated activity (0.1-0.7 mA) were obtained. Rats were then administered the MR antagonist spironolactone (50 mg/kg IP) and basal and DMPH-stimulated activities were monitored for 60 min. Changes in theta frequency (Hz) were analyzed using ANOVA followed by a Bonferroni t-test, and by linear regression. ANOVA revealed a main effect of DMPH stimulation intensity on theta frequency ($P<.001$). Theta frequencies increased in response to tail pinch ($P<.01$) and with increasing stimulation intensities prior to drug administration. ANOVA also demonstrated a significant effect of time post-spironolactone ($P<.03$). Spontaneous theta was absent by 5 min following spironolactone. Overall, maximal theta frequencies elicited by DMPH stimulation declined with the passage of time post-injection. A regression analysis on the 0.1 mA stimulation level looking at the time x frequency relationship revealed a significant negative correlation $r= -0.64$ ($P<.02$). Hippocampal cholinergic theta activity is modulated by CORT acting through MR. These data raise the possibility that hippocampal cholinergic systems and theta activity are involved in CORT-mediated negative-feedback control of the hypothalamic-pituitary-adrenal axis. *(Supported by U. of Bradford)*

171.20

THE EFFECTS OF SCOPOLAMINE, DELIVERED VIA INTRAHIPPOCAMPAL MICRODIALYSIS, ON THE FIRING OF LOCAL PLACE CELLS. S.E. Fox*, N.Ludvig, J.L. Kubie, R.U. Muller, M. Stead and A. Fenton Dept. of Physiology, SUNY Health Sci. Ctr., Brooklyn, NY 11203.

The hippocampus receives a dense cholinergic innervation from the septum that contributes to the EEG theta rhythm. The firing of hippocampal place cells within the place field is enhanced during theta rhythm, whereas the out-of-field firing is suppressed. This suggests that acetylcholine may modulate the firing of place cells. In previous studies using intracerebroventricular injections of scopolamine the in-field firing of place cells was dramatically reduced. With such injections it is not clear whether the effects are local to the hippocampus.

To test local drug applications, simultaneous microdialysis and single cell recording were performed in the CA1 region of freely moving rats. The electrode tips were in the stratum pyramidale, while the 1 mm microdialysis probe tip extended from stratum oriens to stratum lacunosum-moleculare, at a 400 µm horizontal distance from the electrodes. The firing of place cells was recorded while the rat chased food pellets in a 70 cm dia. cylindrical chamber. Data collection periods of 10 to 60 min were used, separated by intervals when the rat was in a smaller "resting" box for at least one hour. Either artificial cerebrospinal fluid (ACSF) or a selected concentration of scopolamine (10-50 mM, dissolved in ACSF, applied for 15-20 min) was perfused through the microdialysis probe.

When ACSF was perfused, place cells displayed their normal location-specific firing. Adding scopolamine into the microdialysis fluid caused a concentration-dependent reversible suppression of the firing of recorded place cells. An initial, transient firing facilitation was also observed. The effects were not attributable to changes in the shapes or amplitudes of spike waveforms. These data support the notion that acetylcholine increases the sensitivity of CA1 place cells to the firing of afferents coding spatial information. *Supported by NIH grant NS-17095.*

172.1

AN ARTIFICIAL NEURAL NETWORK SIMULATING PERFORMANCE OF NORMAL SUBJECTS AND SCHIZOPHRENICS ON THE WISCONSIN CARD SORTING TEST. S. Berdia, J. T. Metz*. University of Chicago, Chicago, IL 60637.

A major research challenge in psychiatry today is to relate the classic signs and symptoms of schizophrenia to underlying neurobiological processes. In addition, artificial neural networks are now offering clues about how biological events can accomplish cognitive tasks. By perturbing these neural models, one may better understand the biological basis for disordered cognition. Recently, a few researchers have developed neural network models that provide a framework for understanding some of the pathological processes in schizophrenia. However, the abilities to abstract and learn, which are some of the most appealing features of artificial neural networks, have not been incorporated into the models.

The Wisconsin Card Sorting Test (WCST) is a neuropsychological test considered sensitive to frontal lobe functions including abstracting and learning. The test requires subjects to match trial cards to target cards depending upon a sorting criterion. After subjects make ten consecutive correct matches, the sorting principle shifts without warning. We studied hospitalized psychiatric patients that met the DSM-IIIR criteria for schizophrenia (N=28), and normal subjects with no psychiatric history (N=19). Performance on the WCST by schizophrenic patients was poorer than performance by normal subjects as estimated by various scoring measurements including perseverative errors.

We have modeled an artificial neural network, motivated in part by biological considerations, that provides a framework for understanding performance on the WCST. The network can be perturbed by inhibiting feedback and causing axonal instability so as to simulate different types of failure that occur in normals and schizophrenics. We expect this model to provide insight into the neurobiological pathology in schizophrenia.

Supported by Calvin Fentress Research Fellowship Award to Dr. Berdia

172.2

SINGLE TRIAL ANALYSIS OF EVENT RELATED POTENTIALS IN SCHIZOPHRENICS AND DEPRESSIVES.

J. Röschke*, P. Wagner, K. Mann, Dept. of Psychiatry, University of Mainz, Germany

The aim of the present paper was to perform a single-trial analysis of event-related potentials in order to elucidate the mechanisms behind a reduced P300 amplitude occurring in unmedicated depressives (n = 11) and schizophrenics (n = 18). For this purpose, tools from linear system theory were applied to single trials in an oddball paradigm. This analysis provides estimates of the magnitude of the positive deflection occurring around the latency of P300 following target and nontarget stimuli. According to the density functions of these amplitude distributions, we operationally defined „false-negative" (P300 amplitude lower than an individual threshold under target conditions) as well as „false-positive" responses (P300 amplitude higher than the threshold following nontarget stimuli). Ford and coworkers recently published an article that schizophrenics have fewer and smaller single trial P300 amplitudes. Due to the different psychopathology and clinical signs of schizophrenia and depression we hypothesized that the mechanisms of reduced averaged P300 measurement might be of different nature in these two diagnostic groups. Therefore, we hypothesized that due to different psychopathology (a) schizophrenics manifest fewer single trial P300 and (b) depressives manifest attenuated single trial P300. Our investigations revealed a reduction of the single trial P300 amplitude in depression and a combination of amplitude reduction along with fewer elicited single trial P300 waves in schizophrenics.

172.3

ERP AND QUANTITAIVE EEG STUDY OF SCHIZOPHRENIC ABNORMALITY OF INFORMATION PROCESSING -FROM ASPECTS OF AUTOMATIC PROCESS AND CONTROLLED PROCESS- E. Kirino, M. Shinomiya, R. Inoue, Y. Minami, Y. Okada and H. Imai* Department of Psychiatry and Neurology Juntendo Univ. Sch. of Med., Tokyo 113

The present study examined the relationship between the automatic and controlled processes in schizophrenic disorder and changes of the relationship in various clinical course of the illness using ERPs. Thirty schizophrenic patients were classsified into two groups according to ERP variations. Group-A consited of schizophnics who showed higher mismatch negativity (MMN) amplitudes and lower P300 amplitudes than group-B patients. On quantitative EEG, delta, theta and slow alpha activity of Group-B was higher than Group-A. After medication, Group-A showed decreased MMN amplitudes and increased P300 amplitudes and Group-B showed increased MMN amplitudes, but no significant changes in P300 amplitudes. In Group-A, theta activity decreased after medication. A negative correlation were obtained between MMN amplitudes and the left Sylvian fissure and the bilatreral lateral ventricles on CT scans. It suggests that MMN might be a helpful marker of trait-dependent cerebral dysfunction in schizophrenic disorder. In information processing of schizophrenics, processing capacity is limited and automatic process and controlled process complement each other. Before treatment, that compensation are inbalanced but defensive to severe manifestation. In Group-A, higher MMN amplitudes suggested plasticity of processing, which respsnced well to psychopharmacological therapy. In Group-B, a lower MMN amplitudes and higher slow activity suggested more severe vulnerability, which responsed to medication less favorably.

172.4

PROCEDURAL AND STRATEGIC MEMORY IN SCHIZOPHRENIA. J. Singh*, K. Gopal, D. Yohanna, A. De Wolfe, J. T. Metz, M. Mesulam. Department of Psychiatry and Behavioral Sciences, Northwestern University.

In the present study, we examined procedural and strategic memory in 10 patients with schizophrenia (SC, DSM-IV)) and 10 age- and education-matched normal controls (NC). Procedural memory was examined with two widely used tests: pursuit rotor learning and mirror tracing (RP, MT). Subjects performed 3 blocks of the RP test, with each block consisting of 8 trials. In the MT test, the subjects traced a star by looking into a mirror for 5 consecutive trials in 2 blocks; the blocks were separated by a short time interval. Temporal Ordering and a modified version of Tower of Hanoi tasks were used to assess strategic memory. In the Temporal Ordering task, subjects are asked to reconstruct an original sequence of 10 words which are presented visually in a fixed sequential order during the study phase. A degree of correlation between the subject and test order was used as an index of the level of performance. In the Tower of Hanoi task, subjects were asked to move discs from one peg to another. Numbers of moves within each level (levels 1 through 7) were averaged and compared between the two groups.

The results of this study show that SC were unimpaired on both MT and RP tasks (NC vs SC, $p > .05$). However, they were markedly impaired on strategic memory tasks relative to their controls (Tower of Hanoi, $< .001$; Temporal Ordering, $p < .001$). These results suggest that SC have no difficulty in acquiring simple sensori-motor skills. However, they are unable successfully to complete tasks that make strategic memory demands and require complex cognitive processing. The strategic memory tasks used in the present study are known to be impaired in patients with frontal-lobe lesions. Impaired strategic memory in SC may indicate frontal lobe dysfunction in schizophrenia.

Supported by NARSAD

172.5

HUMAN BRAIN RHYTHMS AND ELECTROCONVULSIVE THERAPY. R. Salmelin*, J.P. Mäkelä, P. Heikman, K. Kuoppasalmi, and R. Hari. Brain Research Unit, Low Temp. Lab., Helsinki Univ. of Technology, 02150 Espoo, and Dept. of Psychiatry, Univ. of Helsinki, 00180 Helsinki, Finland.

Electroconvulsive therapy (ECT), efficient in treatment of severe depression, is known to increase slow EEG rhythms. Where these rhythms originate and whether they have a beneficial or even an essential role in the patient's recovery have remained open questions.

We employed whole-head magnetoencephalography (MEG) to track the temporal and spatial modulation of spontaneous oscillations in five depressive patients (all females, 24-46 yrs). The patients were measured prior to the beginning of ECT, after the 4th and the last (8-13 in total) treatments, and one month after the end of ECT (4 patients). The normal human brain shows spontaneous magnetic rhythms with maximum signals typically around 10 and 20 Hz. Interestingly, ECT often resulted in a prominent increase of frequencies below 8 Hz. These anomalous slow oscillations largely disappeared within one month after the last treatment.

In 3 patients, relief of depression was accompanied by an increase of the 4-8 Hz oscillations by at least 50% after the first 4 treatments and up to 500% after 8 treatments, particularly over the temporal and rolandic areas. All frequency components were affected, most notably in the central and precentral areas, in the posterior temporal lobes, and around the temporo-parieto-occipital junctions. The signals also suggest a contribution from subcortical structures to the modulation of brain rhythms in response to ECT.

Supported by the Academy of Finland.

172.6

ANALYSIS OF PARTICULAR DOTTING PATTERN OF SDAT PATIENTS FROM THE VIEW POINT OF MEMORY. Hayashi Mikio *, Sakamoto Atuko, Kashiwamura Hiroko. Dep. of neuropsychiatry, Osaka Koseinenkin Hosp. Osaka, JAPAN

METHOD and OBJECT: 30 SDATs ,19 asymptomatic CVDs and healthy persons were tested 3 psychological tests(minimal dementia test, memory test(long and short memory), Benton's test) and CT/MRI and computorized test(hard and soft)which were developed by us, which is dotting 30 in the 12x20 grid at random, and programed variables were 19.

RESULTS:1:multipule regression analysis was significant with 3 psychological tests in the inner samples. 2: transional probability and number of transition was small in SDATs if, compared with another objects. 3:dotting patern of SDATs were clasified 4.4:particular dotting pattern is only dotting same point from the view of transition. This was thought as procedual memory. 5:this phenomena was relatively paraleled with detoriation. 6: SDAs lossed firstly themantic memory and next lossed procedual memory. 7:dotting patten is expression, and expression was quantitatively analysed.

CONCLUSION:Dotting pattern of SDAT was analysed by our developed computarised system. and phenomena of particular dotting pattern of SDAT was explained from the view of memory.

172.7

MODULATION OF A STEADY-STATE VISUAL EVOKED POTENTIAL BY SHIFTS OF VISUAL SPATIAL ATTENTION IN NORMAL SUBJECTS AND IN PEOPLE WITH AUTISM. M.K. Belmonte* <mkb4@Cornell.edu>
http://www.cnl.salk.edu/~matthew/SSVEP/

Previous behavioural studies of autism have demonstrated a deficit in the rapid shifting of attention between sensory modalities, between object features, and between spatial locations. This study applied electroencephalographic measures to shifts in visual spatial attention in an effort to elucidate the time courses of such shifts both in normal subjects and in people with autism. A general-purpose software package for analysis of steady-state evoked potentials was constructed, and applied to quantify phase-locked and non-phase-locked EEG power levels during a behavioural task that required rapid, repetitive shifts in visual spatial attention. Background stimuli and occasional targets were flashed at 9Hz in both hemifields in order to drive a steady-state evoked potential. A target in the attended hemifield cued not only an overt behavioural response but also a covert shift of attention to the opposite hemifield. In normal subjects, attended targets produced a transient increase in phase-locked amplitude, in the interval 0 to 300ms post-stimulus, contralateral to the hemifield in which the target appeared. The latency of this increase varied with the length of time since the previous shift, attaining a minimum at shift intervals between 800ms and 1500ms. In addition, in the range 300ms to 600ms post-stimulus, phase-locked amplitude increased in the hemisphere contralateral to the newly attended visual hemifield and decreased in the ipsilateral hemisphere. Conversely, non-phase-locked amplitude decreased contralaterally to the newly attended visual hemifield and increased ipsilaterally. These modulations may reflect the sequential process of orienting to a target stimulus, reorienting to the opposite, cued location, and redistributing attention in anticipation of a subsequent shift back to the previous location. At the time of submission of this abstract, a comparison of this normal data to data from autistic subjects is underway. The previously documented behavioural impairment of people with autism in the rapid shifting of attention suggests that the reorienting component, which in normals manifests between 300ms and 600ms post-stimulus, may be delayed or diminished in autistic brains. Part of this work was funded by NIMH project 2-R01-MH36840-11A1.

172.8

BILATERAL OCCIPITAL-TEMPORAL DYSFUNCTION AND VISUAL AGNOSIA IN A CHILD: ROLE IN THE PATHOPHYSIOLOGY OF AUTISM. L. Carmant* and L. Mottron. *Division of Neurology, Hôpital Ste-Justine and Department of Psychiatry, Hôpital Rivière des prairies, Université de Montréal. Montréal, Qc.

The purpose of studying this child is to understand the role of occipital-temporal structures in the pathophysiology of autism. We report neuropsychological, electrophysiological, neuroimaging, and clinical findings of a 5 year old boy with bilateral occipital and right temporal lobe lesions secondary to neonatal meningitis. Early investigations did not reveal significant abnormalities. At age three years, during an evaluation for developmental delay, autism was diagnosed with the Autism Diagnostic Interview. Repeated measures of his IQ showed subnormal results only on tests involving visual material. Neurology and ophthalmology evaluations confirmed the presence of severe prosopagnosia and visual agnosia. MRI showed extensive occipital-temporal encephalomalacia. Autism is a developmental disorder of neuronal organization. Abnormalities involve mainly the limbic system and cerebellum with growing evidence supporting disturbances of the associative cortex. This child's history suggests that a deficit in the construction of perceptive representation during development could play a role in the etiology of autism. We discuss these findings in view of the description by Jambaqué et al of autistic features in children with infantile spasms, a catastrophic epileptic syndrome often associated with posterior brain dysfunction.

172.9

LOCALIZED ABNORMALITIES OF rCBF AT REST IN CHILDHOOD AUTISM. M. Zilbovicius*, B. Garreau, Ph. Remy, P. Belin, C. Barthélemy, A. Syrota, G. Lelord and Y. Samson. SHFJ, CEA, Orsay and INSERM U 316, CHU Bretonneau, Tours, France.

We previously failed to detect regional cerebral blood flow (rCBF) abnormalities at rest in autistic children older than five years. However, this negative result was obtained with a low spatial resolution SPECT camera, suggesting that well-localized rCBF abnormalities may have been overlooked. To test this hypothesis, we measured rCBF at rest with $H_2{}^{15}O$ and a high resolution PET camera (31 slices, 5 mm resolution) in 11 children with primary autism (mean age ± sd : 8 ± 3.1 years) and six non-autistic control children (7 ± 1.6 years). All rCBF studies were performed under the same premedication, and the data were analyzed by comparing the regional pattern of rCBF in both groups using the SPM software. A significant ($p < 0.01$) decrease in rCBF was found in the autistic group in four brain regions : superior cerebellar vermis (x: -14, y: -40, z: -12 mm in Talairach's coordinates), left insula/superior temporal gyrus (-42, -8, 0 mm), left insula/inferior frontal gyrus (-32, 12, 12 mm) and right anterior cingulate (6, 28, 20 mm). In contrast, we did not find any significant rCBF increase in the autistic group. These results indicate that rCBF abnormalities may be detected at rest in restricted but well defined brain regions using high resolution imaging techniques in autistic children. The cerebellar vermis hypoperfusion may be related to previous reports of MRI vermal hypoplasia. The left temporo-insular and left fronto-insular hypoperfusion may be related to an alteration of an auditory-verbal brain network. Finally, the anterior cingulate hypoperfusion is consistent with a limbic dysfunction in autism. *Supported by INSERM network # 489001 and PHRC 95.*

172.10

DIFFERENTIAL PATTERN OF AMPLITUDE REDUCTION FOR ENDOGENOUS COMPONENTS WITH VARIOUS PROCESSING LOAD IN AUTISM, OBSESSIVE COMPULSIVE DISORDER AND NORMAL CONTROLS. K. T. Ciesielski , R. Elmasian, and A. Reeve*Clinical Neuroscience Laboratory, Departments of Psychology and Psychiatry, University of New Mexico, Albuquerque, NM 87131.

We presented four stimuli, a green and red flash, a 903 Hz and 1923 Hz tone; each 50 msec duration and equally probable at random intervals that ranged between .5-3.5 secs. While stimuli were kept constant the processing load was varied only by changing task instructions. A designated target was the repetition of a stimulus in all conditions, except for the passive observation session. We hypothesized that subject would invest the most processing resources to target stimuli, less to non-targets in the same stimulus modality, less to passively observed stimuli and least to non-targets in the opposite modality. The results in 16 normal controls were in agreement with predictions. A parietal P3 at 330-390 msec and frontal brain negativity SNW at 470-530 msec systematically decreased with reduced processing resources to target and non-target stimuli, suggesting that background engenders similar processing although to a similar degree. The preliminary results in high functioning subjects with autism did not reveal significant modulation of the above components, although P3 and SNW were generally reduced in amplitude. A similar deficit in modulation of amplitudes was observed in the obsessive-compulsive group with significant amplitude enhancement. The role of frontal-parietal subsystem for selective inhibition mechanism is discussed in norm and disorders with frontal brain abnormalities.

172.11

A MULTIDIMENSIONAL SCALING APPROACH TO CONTEXT-DEPENDENT FUNCTIONAL REPRESENTATIONS. K. C. Whang*, D. A. Crowe, and A. P. Georgopoulos. Brain Sciences Center, VAMC, Minneapolis, MN 55417.

Functional deficits in constructional apraxics suggest that the internal representations used for a given set of objects or actions could differ considerably depending on specific task contexts. We present a psychophysical approach to characterizing such representations using standard parametric methods as well as Shepard-Kruskal multidimensional scaling (MDS).

Pairs of items from a set of 25 geometric fragments of a square were presented to healthy human subjects making two different judgments using two response methods for each judgment. The first judgment concerned similarity: in separate blocks, subjects either rated the similarity of items on a numerical scale or made a choice reaction time (RT) response depending on whether the two were the same or different. The second judgment concerned object completion: subjects made a numerical or choice RT response based on how well the two presented fragments fit together to form a complete square. Such a judgment might be expected to be an important component in object construction.

Analysis of choice RTs showed that the completion judgment took 100 msec longer on average than the similarity judgment. This is consistent with a first-order model of the completion task in terms of two additive component processes: one concerned with raw object identification and another specific to completion or construction. MDS configuration spaces derived from rating data suggest that the underlying representations used in the two judgments may be organized along qualitatively different dimensions. (Supported by USPHS grant NS17413)

172.12

HUMAN OSCILLATORY BRAIN ACTIVITY NEAR 40Hz: CORRELATION WITH COGNITIVE TEMPORAL BINDING AND ALTERATION DURING LONG TERM UNCONSCIOUSNESS.
Schiff, N.++, Ribary, U.+, Plum, F.++*, and Llinas, R+. +CNM, Dept. Physiology and Neuroscience, New York University Medical Center, ++Dept. Neurology and Neuroscience, Cornell Medical Center, New York, 10021.

A dual 37-channel MEG system (BTi) localized and monitored dynamic brain function from healthy controls and from a 49 year old woman continuously vegetative for 19 years who has shown no evidence of attention, recognition, or interaction. Atypically, she occasionally uttered single, understandable words with no accompanying expression or relevance to the environmental stimuli. MRI of the brain revealed no remaining right thalamus and only the anterolateral portion of left thalamus. Varying areas of cerebral cortex remained. Spontaneous and evoked magnetic activity in response to auditory clicks and tones, and somatosensory stimulation were used to evaluate global brain activity. Spontaneous oscillatory electrical activity at near 40 Hz in the human brain, and its reset to following sensory stimulation in normal subjects has been reported elsewhere, indicating that oscillatory activity near 40-Hz represents a neurophysiological correlate to the temporal cognitive binding of auditory stimuli.

In contrast to these normal findings, preliminary data in the unconscious patient indicate a loss of high frequency (gamma) activity especially in the right hemisphere, and a reduced correlation or even an anticorrelation of brain oscillatory activity between the two hemispheres. The data also suggest the existence of coherent and synchronized spontaneous oscillatory activity at around 40 Hz in the left hemisphere, including partial reset with incomplete synchronization in response to sensory stimulation. These findings suggest that the human cerebrum can express some gamma band activity, words, and an incomplete and disorganized coding of sensory stimuli in the absence of any behavioral evidence of the conscious state.

172.13

TWO MODES OF FUNCTIONAL REORGANIZATION FOLLOWING PARTIAL CALLOSOTOMY IN HUMANS. E. Zaidel[1], N. Weekes[1], M. Iacoboni[1,2], I. Fried[3] and J. Bogen*[1].-[1]Department of Psychology, UCLA ; [2]Reed Neurological Institute, UCLA; [3]Division of Neurosurgery, UCLA

We measured callosal transfer through motor and auditory channels before and after anterior callosal section for intractable epilepsy in a 30 year old male. The surgery extended from the rostrum to the anterior splenium. Motor transfer was indexed by the Poffenberger paradigm, using unimanual simple reaction time to lateralized light flashes. Auditory transfer was indexed by the left ear score in dichotic listening to consonant-vowel syllables. This task is exclusively specialized in the left hemisphere (LH) and exhibits complete ipsilateral suppression, so that the left ear (LE) signal must relay from the RH to the LH through the corpus callosum.

Pre-surgically, the subject exhibited normal motor transfer, insensitive to visual parameters, in the Poffenberger paradigm. It changed to a visual channel, sensitive to eccentricity, within two days following surgery. This reflects immediate takeover by a parallel pre-existing but sub-dominant channel of the corpus callosum.

Pre-surgically, the patient exhibited a normal right ear advantage in divided attention in dichotic listening. It changed to a massive right ear advantage post-surgically. Over the next two years the patient gradually started reporting the LE in the divided, but not focused, attention condition. This reflects a slow shift of an attention-dependent auditory channel from the isthmus to the anterior splenium.

The results show radically different modes of functional reorganization of callosum channels for motor and auditory relay.

172.14

CHANGES IN BRAIN METABOLISM INDUCED BY EEG-BIOFEEDBACK: AN FMRI-STUDY.
N. Birbaumer *, F. Pulvermüller, H. Preißl, C. Tempelmann, H. Scheich, H.-J. Heinze, B. Mohr & W. Lutzenberger
Universities of Tübingen and Magdeburg, Germany

Metabolic changes in brain activity caused by EEG biofeedback were investigated using a 3 Tesla functional magnetic resonance imaging (fMRI) scanner. EEG biofeedback makes it possible to control the vertex potential, that is, to voluntarily shift it in the negative or positive direction. After 24 sessions of successful feedback training, fMRI experiments were carried out determining brain regions activated and deactivated by feedback. In the condition where negativity of vertex potentials was to be produced, pronounced and consistent feedback-induced enhancement of activity was seen in thalamus, putamen, and in medial prefrontal and temporo-occipital cortex. When vertex positivities were induced, relative reduction of activity was seen at these sites, but activity enhancement in lateral prefrontal and inferior occipital areas. Clinical implications of these results on biofeedback therapy of epilepsy are discussed.

Supported by the DFG (SFB 307/B1, Pu 97/5).

172.15

REVISED CHILDREN'S VERSION OF THE ALTERNATIVE IMPAIRMENT INDEX: A NEUROPSYCHOLOGICAL PILOT STUDY, A. M. Horton, Jr.,* Psych Associates, Towson, Maryland, 21204.

The Revised Children's Version of the Alternative Impairment Index is a new measure of neuropsychological impairment in older children derived from the Halstead-Reitan Neuropsychological Test Battery for Older Children. A sample of 16 normal and heterogeneously brain damaged children between the ages of 9 and 14, including the following conditions, normal controls (4), brain tumors (3), brain abscess (1), hemispherectomy (1), encephalitis (1), traumatic brain injury (4), learning disabled (3), behavior problems (1), was assessed. The hit rate obtained for agreement on level of severity for the Children's Version of the Alternative Impairment Index and the Older Children's Neuropsychological Deficit Score (i.e., 50% or 8/16 correct agreement) does not support further development of the Revised Children's Version of the Alternative Impairment Index.

172.16

THE EFFECTS OF HYPOBARIC HYPOXIA ON COGNITIVE FUNCTIONING AND EEG. C.R. Swain*[1], C. Kourtidou[2], G.F. Wilson[3]
[1]Logicon Technical Services, Inc., Dayton, OH 45437-7258
[2]Department of Aerospace Medicine, WSU, Dayton, OH 45401-0927
[3]Armstrong Laboratory, USAF, Wright-Patterson AFB, OH 45433-7022

Cognitive impairment at high altitudes is of vital interest to those working in aviation and aerospace medicine. Despite the need, there is a paucity of research examining the relationship between complex task performance and brain electrical activity at altitudes greater than 20000 feet. In this study, 10 USAF personnel served as subjects in a repeated measures within subject investigation of cognitive performance at hypobaric simulated high altitude. EEGs were recorded from 19 sites (positioned in accordance with the 10-20 system) as subjects performed a three minute computer generated multitask flight simulation (MATB) during three conditions (hypobaric normoxia, hypobaric hypoxia, and recovery) at five altitudes (5000, 10000, 15000, 20000 and 25000 ft). Respiration, heart rate and $PsCO_2$ were monitored throughout. FFTs were calculated based on 2 minute samples of EEG at each condition and altitude and the absolute and relative power of five frequency bands (delta, theta, alpha, beta1 and beta2) were assessed. While all bands yielded significant hypoxia related changes at specific scalp sites, most notable were the widespread increases in absolute delta and alpha power at 25000 ft and increases in absolute theta, alpha, beta1 and beta2 at 20000 ft that were significantly correlated with decreases in continuous tracking task performance. At lower altitudes, decreases in monitoring task performance were significantly associated with increases in delta, theta and beta2 powers.

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LEARNING AND MEMORY: PHARMACOLOGY IV

173.1

ENHANCED DELAYED RESPONSE ACCURACY IN RATS ADMINISTERED THE CHOLINERGIC CHANNEL ACTIVATOR, ABT- 418 A.V. Terry, Jr.[1,2]*, J.J. Buccafusco[2] and M.W. Decker[3] [1]The University of Georgia Clinical Pharmacy Program and [2]Department of Pharmacology and Toxicology, Medical College of Georgia, Augusta, GA 30912, [3]Neuroscience Research, Abbott Laboratories, Abbott Park, IL 60064-3500

The purpose of this study was to evaluate the effects of ABT- 418, a recently developed isoxasole bioisostere of nicotine, in rats trained to perform a delayed response task, the Delayed Stimulus Discrimination Task (DSDT). ABT- 418 has recently been described as a selective nicotinic-acetylcholine receptor (nAChR) agonist and cholinergic channel activator (ChCA), which possesses memory enhancing properties (in animal models) while exhibiting a reduced side effect profile when compared to nicotine.

In a previous study, improved DSDT accuracy was observed in rats after nicotine and lobeline administration, but was diminished after mecamylamine administration, indicating a sensitivity of the task to nicotinic-cholinergic manipulation. The goal of these experiments was to assess the potential mnemonic effects of ABT-418 with this task and to compare the results to the previously characterized alkaloids, nicotine and lobeline. ABT- 418 improved DSDT accuracy, exhibiting a significant dose-effect relationship in the μg/kg range of doses, and produced significant treatment and treatment x delay effects after optimal dose readministration. Post-hoc analyses revealed that the predominant improvements in DSDT performance were rendered at the shorter delay intervals. Surprisingly, mecamylamine (1.0 mg/kg) when combined with ABT- 418 failed to prevent improvements in accuracy of the task (as it had with nicotine), although the delay-dependent pattern of improvement was altered. These data confirm the findings of previous rodent and non-human primate studies, which indicate that the nicotinic ligand ABT- 418 has the potential to improve memory. In addition the lack of sensitivity to mecamylamine blockade by ABT- 418 and lobeline (in this task) may reflect a difference in nicotinic receptor subtype mediated effects produced by these compounds when compared to nicotine.

Supported by Abbott laboratories, 100 Abbott Park Road, Abbott Park, IL 60064-3500

173.2

LOBELINE AND ANALOGS EXHIBIT DIFFERENTIAL AGONIST ACTIVITY AND SENSITIVITY TO ANTAGONIST BLOCKADE WHEN COMPARED TO NICOTINE. G.F. Carl[1]*, R. Williamson[2], J.W. Beach[3], C.R. McCurdy[3] J.R. Pauly[4], J.A. Sparks[4], and A.V. Terry Jr.[1,2,3] [1]Departments of Neurology and [2]Pharmacology and Toxicology, Medical College of Georgia, Augusta, GA 30912, [3]College of Pharmacy, University of Georgia, Athens GA, 30602 [4]College of Pharmacy, University of Kentucky, Lexington KY, 40536

Significant preclinical evidence supports the potential role of nicotine (nic) in the therapeutics of a number of CNS disorders. Nic associated dependence and cardiovascular side effects provide the impetus to evaluate other existing agonists (and develop novel compounds) which possess the desirable properties of nic without the adverse effects. Lobeline (lob) is one such compound which is currently being evaluated pharmacologically for this purpose. Lob shares some properties with nic in animals including a high affinity for nicotinic receptors, the ability to improve memory and decrease anxiety. However, lob is less potent in stimulating dopamine release, does not upregulate nicotinic receptors or substitute for nic in drug discrimination experiments and at least some of its effects are not reversed by nicotinic antagonists.

In the present study, lob and two structurally simplified analogs, CRM 1-13-1 and CRM 1-32-1 were compared to nic in ligand binding studies and rubidium (Rb^+) efflux experiments to elucidate the agonist pharmacophore and compare agonist activity. Nic and lob displaced [^{3}H]-cytisine from rat cortical syaptosomes with K_i values in the nM range, while each lob analog exhibited K_i values in the μM range. Nic over a range of 1.0 to 20 μM stimulated efflux of Rb^+ from rat striatal synaptosomes above baseline levels in a dose dependent manner and 10 μM mecamylamine (mec) reversed these effects. Lob in contrast, appeared to produce a completely different concentration-effect relationship with 1.0 to 10 μM slightly increasing Rb^+ efflux over baseline levels, while 50 and 100 μM reduced efflux significantly below baseline levels. In addition none of the effects of lobeline were reversed with mec (10 μM). Although less potent, the two lob analogs exhibited a similar pattern of activity. These data may suggest that lob and structurally similar compounds bind with different subtype selectivity than nic or to a mec insensitive receptor. (Supported by UGA Research Foundation).

173.3

PROTRACTED IMPAIRMENT OF WORKING MEMORY IN RATS, BUT NOT REFERENCE MEMORY IN MONKEYS EXPOSED TO LOW-LEVELS OF DFP. B.D. Goldstein*, M.A. Prendergast, A.V. Terry, Jr., and J.J. Buccafusco. Medical College of Georgia, Alzheimer's Research Center, Dept. Pharm. Tox., and Dept. Veterans Affairs Medical Center, Augusta, GA 30912 , Univ. Georgia Clin. Pharm., Augusta, GA.

Chronic, low-level exposure to cholinesterase inhibitor organophosphate (OP) insecticides or chemical warfare agents produces marked abnormalities in CNS acetylcholine (ACh) function and may impair cognitive abilities. However, the duration of cognitive impairment following this exposure is unclear. In the present study, adult rats were administered the OP diisopropyl fluorophosphate (DFP; 50, 250, or 500 µg/kg) daily for 14 days. Spatial learning ability was assessed 3 or 16 days after completion of drug injections. Spontaneous locomotor activity and olfactory reactivity were suppressed beginning on Day 4 by the two higher doses. With the 250 µg/kg dose, behavioral tolerance was observed in that the frequency of these behaviors returned to baseline levels by Day 7. Spatial learning was impaired by the 250 µg/kg dose (to 65-70% of control levels) on Days 3-6 following the end of drug administration. On Days 16-19 after the end of DFP administration, learning was still impaired (to 60-80% of control levels). Acetylcholinesterase (AChE) levels in frontal cortex, cerebellum, medulla, and hippocampus were suppressed to 41%, 65%, 18% and 50%, respectively, of control levels at the end of the 14 day DFP treatment regimen. By the end of the first time-point of cognitive testing (Day 6) cortical AChE levels had recovered to 90% of control levels. In 3 rhesus monkeys well trained in the performance of a delayed response task, chronic daily exposure to 10-20 µg/kg of DFP produced severe toxicity and task impairment after 8 weeks of treatment. However, task performance was at near control levels by 6-10 days after DFP withdrawal. Withdrawal from chronic exposure to low levels of DFP may compromise working memory or learning of a new task, but fails to inhibit the performance of a well learned task if no toxicity is present. Supported by DAMD17-95-1-5036.

173.4

REACTIVATED MEMORIES ARE IMMUNE TO THE AMNESTIC EFFECT OF CHOLINERGIC ANTAGONISM IN DEVELOPING RAT PUPS. N. J. Sandstrom* and H. M. Arnold. Department of Psychology: Experimental, Duke University, Durham, NC 27708.

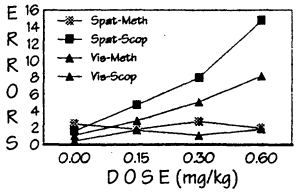
The cholinergic system of the rat has been shown to undergo rapid physiological and behavioral maturation during the third and fourth postnatal weeks. In order to learn more about the role of the developing cholinergic system in memory, we examined the effects of scopolamine on performance of an odor preference task following odor aversion conditioning in rat pups. Twelve-day-old rat pups received mild footshock (1.0 mV) explicitly paired or unpaired with the presentation of a novel odor. Pups were then tested in an odor preference chamber either 2 or 48 hours after conditioning. When tested after a 2 hour retention interval, pups injected with saline or scopolamine hydrobromide (0.2 or 0.5 mg/kg) both showed significant aversion to the odor previously paired with shock relative to unpaired controls. After a 48 hour retention interval, pups injected with scopolamine 30 minutes prior to testing showed no evidence of an aversion while pups injected with saline continued to show an aversion to the conditioned odor. This amnestic effect of scopolamine was prevented by a reactivation treatment involving the administration of the US used during training (footshock) in the absence of any odor 90 minutes prior to injection. These data indicate that the memory for the odor aversion remains active and immune to the amnestic effect of scopolamine for at least 2 hours after conditioning. But after a long retention interval, the cholinergic system does appear to be necessary for expression of the memory. However, even at the long retention interval, expression of a recently *reactivated* memory is not affected by cholinergic antagonism. (Supported by HD17458 & AG09525)

173.5

WITHIN-SUBJECT, WITHIN-SESSION ANALYSIS OF SCOPOLAMINE'S EFFECT ON SPATIAL ALTERNATION AND VISUAL DISCRIMINATION IN THE RAT. W. J. Wilson*, G. S. Lyons, M. L. Griffith, T. Vanhorn, & J. D. Dillman. Psychological Sciences, Indiana-Purdue University, Fort Wayne, IN 46805 USA

Eight female adult Sprague-Dawley rats learned a combined spatial alternation-visual discrimination task in a phi-maze, then were tested 15 min after i.p. injection of scopolamine or methylscopolamine. Performance of both tasks was impaired by centrally-acting scopolamine, but the spatial alternation task was more disrupted (see Figure), suggesting a greater effect of scopolamine on working memory than on reference memory. The same subjects were retested with delays of 5 or 20 s imposed between trials. Scopolamine (.1 & .2 mg/kg) impaired delayed alternation more than it did the other tasks. In a second experiment, scopolamine (.3 mg/kg) impaired choice performance in a visual discrimination task.

These results suggest that scopolamine's effects are not limited to working memory, but that perhaps working memory is more sensitive to muscarinic blockade than is reference memory. Research supported by IPFW Neuroscience Underground & Psychological Sciences.



173.6

ENHANCING AND IMPAIRING LEARNING AND SPATIAL MEMORY THROUGH MANIPULATING THE CHOLINERGIC SYSTEM. R.L. Galli*[a,c], B.C. Thorpe*, R.E. Fine[d,e], & H.R. Lieberman[b]. [a]Military Performance & Neuroscience and [b]Military Nutrition Divisions, United States Army Research Institute of Environmental Medicine, Natick, MA 01760, [c]Brain, Behavior & Cognition Program, Psychology Department, Boston University, [d]Departments of Biochemistry & Neurology, Boston University School of Medicine and [e]Geriatric Research, Education & Clinical Center, Bedford V.A. Hospital.

Decrements in cognitive abilities associated with normal aging and with age-related disorders such as Alzheimer's disease have been associated with decreases in cholinergic neurotransmission. A number of treatments to reverse these impairments have been evaluated in attempts to increase brain acetylcholine levels. In general, these strategies have met with only limited success. The experiments described here employed a pharmacological and a molecular manipulation to affect cognitive performance in rats by modulating cholinergic neurotransmission.

Behavior was tested using a two day version of the Morris water maze. On day 1, both the start position and the hidden platform location remained constant. On day 2, trials 1-7, the start position was varied in a semi-random manner and for trials 9-12 the platform location was moved 180°. Day 1, trial 10 and day 2, trial 8 were probe trials during which the platform was removed from the pool. It was determined that 0.2 mg/kg scopolamine significantly impaired performance on several measures of learning and spatial memory. The molecular treatment, previously tested in cell culture, significantly reversed two of the three scopolamine-induced decrements.

Supported by Department of Defense intramural funding.

173.7

LOW DOSES OF ATROPINE SULFATE ATTENUATE SPATIAL LEARNING IMPAIRMENTS PRODUCED BY NPC 17742 IN RATS IN THE WATER MAZE TASK. G. Tirado-Santiago*, I.C. Katsarkas and M.L. Shapiro, Department of Psychology, McGill University, Montreal, Quebec H3A 1B1 Canada.

The effects of the combined administration of an NMDA and a muscarinic antagonist on spatial and cued learning in rats (male Long Evans, 3 months old) were assessed in the Morris water maze. Although the administration of either type of drug alone impairs spatial learning in rats in the water maze, the possibility of a synergistic effect between the cholinergic and glutamatergic systems on spatial learning has not been adequately explored. To test this possibility, a competitive NMDA antagonist, NPC 17742 (3 mg/kg, given i.p. 60 min before training), and a sub-threshold dose of a muscarinic antagonist, atropine sulfate (3 mg/kg, given i.p. 20 min before training), were administered separately or in combination during water maze training. Rats were given four 30 sec trials / day with a 10 sec rest period between trials. Cue training to find a visible platform was given for 4 days, which was followed by place training to find a hidden platform for 8 days. Escape latency measured performance. Rats given atropine sulfate alone (n=8) learned both the cued and spatial tasks as well as rats given either physiological saline (n=4) or distilled water (n=4). NPC 17742 (n=8) impaired learning in the place task (ANOVA, $F(1,14) = 11.43$, $p < 0.005$), but not the cue task. Rats given both NPC 17742 and atropine (n=8) learned both tasks normally. Thus, atropine sulfate given at low doses attenuates the effects of NMDA antagonists on spatial learning, perhaps by increasing cholinergic activity through muscarinic autoreceptors.

(Supported by NSERC of Canada)

173.8

ATTENUATION OF SCOPOLAMINE INDUCED MEMORY DISRUPTION IN A WATER MAZE TASK BY AN ANGIOTENSIN IV ANALOG. E.S. Pederson*, K.E. Foley, R.E. Nielson, J.W. Harding and J.W. Wright . Prog. In Neuroscience, Wa. State Univ., Pullman, WA 99164

Recently, the Angiotensin II(3-8) (AngIV) hexapeptide receptor (AT_4) has been localized in the hippocampus, suggesting its possible role in learning and memory. An analog of AngIV, norleucine^1-AngIV (Nle^1-AngIV). was synthesized by our laboratory and was shown to posses added resistance to degradation and binds to AT_4 receptors with high affinity. The present study examined the effects of scopolamine (SCOP), a cholinergic antagonist, previously demonstrated to disrupt spatial learning, and Nle^1-AngIV on learning hidden platform locations from various entry points in a circular water maze task. Twenty-four female Sprague-Dawley rats were intracerebroventricularly (ICV) pretreated with either SCOP or artificial cerebrospinal fluid (aCSF) 30 minutes prior to behavioral testing. Five minutes before testing, either Nle^1-AngIV or aCSF was delivered ICV. The rats were then tested for 8 consecutive days (5 trials per day) and latency to locate the platform (in seconds) and path distance (in meters) were measured. A sixth trial on day eight was run with the platform removed. Latencies in the target quadrant and number of anulus crossings were compared. The results indicate that animals treated with SCOP/Nle^1-AngIV had shorter latencies and distances as compared with SCOP/aCSF treated animals. Furthermore, SCOP/Nle^1-AngIV treated rats attained the low latencies and distances achieved by controls Thus, these results suggest that Nle^1-AngIV compensates for the disruption of spatial learning induced by SCOP.

173.9

EFFECTS OF NICOTINE ON ACTIVE AVOIDANCE LEARNING: GENDER DIFFERENCES. O.Yılmaz, L. Kanıt, B. Erdem, S. Demirgoren* S. Pogun Center for Brain Res. and Dept. of Physiology. Ege Univ. Sch.of Medicine Bornova 35100 İzmir Turkey

Nicotine, an addictive pharmacological agent, is reported to have an improving effect on cognitive functions and this cognition enhancing property may play an important role in nicotine's addictive potential. Recent work from our laboratory has revealed gender differences in central nicotinic receptor binding and response to chronic nicotine treatment in rats. The aim of the present study is to see the effect of nicotine on active avoidance learning with emphasis on gender differences.

Three-months-old Sprague Dawley rats received varying doses (0.2, 0.4, 0.6, 0.8 mg/kg) of nicotine or saline s.c. either before or after active avoidance learning trials which were done daily and consisted of 15 trials each for a period of 5 days. Behavioral testing was continued after the termination of nicotine treatment once a week for 4 or 12 weeks. Effects of nicotine on motor performance was tested using an inclined plane. Possible effects of stress arising from injections were also assessed. Female rats were significantly better than males in active avoidance learning as expected, and nicotine improved cognitive function during the acquisition phase of active avoidance learning trials in a dose dependent manner. Male rats benefited from nicotine at all doses tested whereas in females, at a dose of 0.6 mg/kg, learning performance deteriorated. In conclusion, nicotine pre-treatment affects active avoidance learning in a sexually dimorphic and dose dependent pattern and this outcome is not secondary to effects of stress or motor performance.

173.10

STRAIN-DEPENDENT DIFFERENCES FOR THE ROLE PLAYED BY DOPAMINE-ACETYLCHOLINE INTERACTION IN MEMORY CONSOLIDATION IN THE MOUSE. A. Zocchi*, S. Cabib, S. Puglisi-Allegra Dept. Psychology, University "La Sapienza", and Inst. Psychobiology and Psychopharmacology C.N.R., Italy

A number of studies indicates that an interaction between dopamine and acetylcholine systems is involved in the modulation of memory processes. Such a regulation appears to be genotype-dependent, as direct and indirect dopamine agonists improve memory consolidation in the C57BL/6 strain of mouse and impair it in the mice of DBA/2 strain. The opposite effect is obtained with DA antagonists. The observed difference might be correlated with a different susceptibility of the cholinergic system in the hippocampus to dopaminergic manipulation. In fact, microdialysis experiments have shown a strain-dependent response of the hippocampal extracellular acetylcholine levels after administration of DA agonists.
These data suggest that dopaminergic projections from VTA interact in a genotype-dependent manner with the hippocampal cholinergic pathway. This interaction might result in a different modulation of hippocampal acetylcholine release with a different effect on memory consolidation.
C.N.R. and M.U.R.S.T funded research.

173.11

LEARNING IMPAIRMENT BY CHOLINERGIC DEAFFERENTATION AFTER CORTICAL NGF DEPRIVATION H. Gutiérrez, M.I. Miranda, A. Uribe and F. Bermúdez-Rattoni* *Depto. de Neurociencias, Instituto de Fisiología Celular. UNAM, México, D.F. 04510. México.*

Cholinergic basal forebrain neurons (CBF) have been shown to respond *in vivo* to exogenous administration of nerve growth factor. Although neurotrophins and their receptor are widely expressed in the central nervous system, little data exists for the physiological significance of endogenous neurotrophin signaling in CNS neurons. In order to test directly whether cortically derived NGF is functionally required for the cholinergic functions mediated by the cerebral cortex, repeated injections of anti-NGF monoclonal antibodies were locally applied into the insular cortex (IC). Our results showed a dramatic decrease in the local extracellular levels of acetylcholine as determined by the *in vivo* microdialysis technique. Furthermore by using small injections of fluorogold, we have found a corresponding disruption in the connectivity between the IC and CBF. Behavioral experiments showed that the NGF antibodies into the IC produced a significant impairment in the acquisition of conditioned taste aversion and inhibitory avoidance learning. However, the same animals were able to recall the taste aversion when the conditioning trial was established before the blockade procedure. Given these results it seems that cholinergic activity from CBF is no longer necessary for recalling an aversive stimulus, but is necessary for the acquisition of aversively motivated conditionings. Supported by DGAPA-UNAM IN201893.

173.12

CORTICAL ACETYLCHOLINE EFFLUX AND OPERANT PERFORMANCE: ROLE OF ATTENTIONAL AND NON-ATTENTIONAL COMPONENTS. A.M. Himmelheber*, N.M. Katovic, M. Sarter and J.P. Bruno. Department of Psychology and Neuroscience Program, Ohio State University, Columbus, OH 43210.

Converging evidence in animals strongly supports the existence of a critical relationship between cortical acetylcholine (ACh) efflux and performance in a vigilance task (Sarter et al., Cog. Brain Res., in press). This relationship was assessed directly using microdialysis techniques to measure cortical ACh efflux while animals performed in the vigilance task. The presence of a distractor (flashing houselight, 0.5 Hz) impaired vigilance performance and concurrently increased medial prefrontal cortical ACh efflux (Sarter et al.). To aid in interpreting these results more thoroughly, a series of control experiments was designed to assess the effects on cortical ACh efflux of non-attentional aspects of operant performance such as sensory stimulation, motor activity, and reward loss. Animals were trained either to perform in a simple visual discrimination task or to respond on a variable interval schedule of reinforcement. In general, performance in these tasks did not correlate with medial prefrontal ACh efflux. Additionally, the presence of the flashing houselight did not affect either discrimination accuracy or cortical ACh efflux. The failure of these non-attentional variables to systematically affect cortical ACh efflux provides a framework within which the effects of environmental and pharmacological manipulations on cortical ACh efflux and operant vigilance performance can be assessed and interpreted.
Funding: NIA 10173 to M.S. and J.P.B.

173.13

INTERACTIONS BETWEEN THE EFFECTS OF AGE AND CHOLINERGIC LESIONS ON CORTICAL ACETYLCHOLINE RELEASE. J. Fadel*, M. Sarter, and J.P. Bruno. Department of Psychology and Neuroscience Program, Ohio State University, Columbus, OH 43210.

Investigations of the effects of normal aging on the cortical acetylcholine (ACh) system in aged rodents have led to inconsistent and heterogeneous results. The use of age as an interacting, rather than independent variable in assessing susceptibility of animals to cholinergic lesions and manipulations may represent a more productive approach to understanding age-related cholinergic deficits. Using in vivo microdialysis, we have previously demonstrated that young adult rats which received cortical infusions of the cholinergic immunotoxin 192 IgG-saporin showed significant decreases in cortical ACh efflux concomitant with a decrease in cholinergic (acetylcholinesterase-positive) fiber density; However, the ability of a benzodiazepine receptor (BZR) partial inverse agonist alone or weak inverse agonist in combination with an activating conditioned stimulus (darkness plus palatable food) to increase cortical ACh efflux was unaffected in these animals. Here, we extend these findings to aged (24-26 mos.) rats. Basal ACh efflux values did not differ between aged and young rats. Cortical infusions of 192 IgG-saporin (5 ng/0.5uL/ infusion site; 3 infusion sites/hemisphere) resulted in significant (40-50%) decreases in basal cortical ACh efflux, relative to age-matched controls, a lesion magnitude similar to that seen in young rats following administration of the same dose of toxin. These animals also demonstrated the capacity for increased cortical ACh efflux in response to the aforementioned complex behavioral stimulus. The ability of the BZR weak inverse agonist ZK 93426 to potentiate this effect and of the BZR partial inverse agonist FG 7142 to increase basal efflux will also be discussed.
(Funding: NIA 10173 to M.S. and J.P.B.)

173.14

CHANGES IN CENTRAL ACETYLCHOLINE RELEASE RELATED TO HABITUATION LEARNING R.K.W. Schwarting*, C. M. Thiel & J.P. Huston Inst Physiol Psychol, and Biologisch-Medizinisches Forschungszentrum, Univ of Düsseldorf, 40225 Düsseldorf, Germany

The septo-hippocampal input and the striatal interneurons (nucleus accumbens, neostriatum) constitute major cholinergic systems in the brain. Functionally, these have been related to mechanisms of learning and memory, behavioral activity, arousal and, more recently, to motivational aspects of behavior. With respect to the study of learning and memory, one of the most elementary behavioral paradigms is that of habituation learning in the open field, where the decrease of exploration during repeated exposure to the same environment is taken as an index of memory. We monitored extracellular acetylcholine (ACh) in the hippocampus and nucleus accumbens during performance in this paradigm. Male Wistar rats were placed singly into a home cage next to the testing environment (open field). On the next day, the animal was taken from the home cage and exposed to the open field under dim light for 10min and locomotion and rearing was measured. On the following day, the animal was re-exposed to the open field in the same way. Two control groups were used: "Handling" controls were treated like experimental animals, however, they were placed into their home cage instead of the testing environment. "Lighting" controls were exposed only to dim light for 10min in their home cage. Our results show that extracellular ACh levels in hippocampus and nucleus accumbens usually increased when behavioral activity increased. Furthermore, ACh levels increased during and after exposure to the open field or when animals were handled. Most importantly however, the cholinergic increase was even more prominent during re-exposure to the testing environment, to which the animals had been habituated. Since re-exposure was accompanied by a decrease in exploration (indicating habituation) and presumably in arousal, the ACh increase may be a concomitant of an active process of recognition of the environment which is the basis or prerequisite for the habituation response.

173.15

GENDER DIFFERENCES IN THE EFFECTS OF CARBARYL ON THE REPEATED ACQUISITION AND PERFORMANCE OF RESPONSE SEQUENCES IN RATS. [1]J Cohn* and[2] R MacPhail. [1]Curriculum in Toxicology, UNC, Chapel Hill, NC and [2]Neurotoxicology Division, NHEERL, U.S. EPA, RTP, NC.

The cholinesterase-inhibiting pesticide carbaryl decreases in a dose-dependent manner the accuracy of response sequences emitted by male rats trained under a repeated acquisition (RA) and performance (P) task (Cohn and MacPhail, Soc. Neurosci. Abstr. 20(2):1563, 1994). Some evidence suggests female rats may be more sensitive than males to the effects of cholinesterase inhibitors. In the present experiment we examined whether carbaryl would differentially affect males and females repeatedly learning response sequences. 24 adult male Long-Evans rats, maintained at 300 g, and 20 adult females maintained at 250 g, performed under a multiple schedule of RA and P. The RA component required rats to learn a new 3-member sequence of responses during each session (e.g., Center Right Left, RLC, etc.); the correct sequence of responses for the P component remained constant (CLR). Components alternated twice during a session. Rats were administered 0, 12.5, 25.0, 37.5 or 50.0 mg/kg carbaryl in corn oil by oral gavage. No significant gender differences were obtained under baseline conditions. Carbaryl produced dose-related decreases in both RA and P response accuracy and rate, regardless of gender. Females were, however, significantly more affected by carbaryl than were male rats. These data indicate an important gender difference in the behavioral effects of a carbamate pesticide.

173.16

EFFECTS OF CHOLINERGIC AND ADRENERGIC DRUGS ON VISUAL SIGNAL DETECTION ("VIGILANCE") IN RATS. P.J. Bushnell*, W.M. Oshiro and B.K. Padnos. Neurotoxicology Division, NHEERL, U.S. EPA, Research Triangle Park, NC 27711.

Both the adrenergic and cholinergic neurotransmitter systems have been implicated in the control of attention. Drug challenges were used to evaluate the effects of cholinergic and adrenergic agonists and antagonists on sustained attention ("vigilance") in rats, using a visual signal detection task. Daily 60-min test sessions consisted of 3 100-trial blocks. Signal detection analysis provided estimates of sensitivity (SI) and bias (RI) in each block; response time (RT) for each correct signal detection was also recorded. The role of muscarinic cholinergic receptors was tested with pilocarpine (0, 1.0, 1.8, and 3.0 mg/kg sc, 10 min prior to testing) and scopolamine (0, 0.03, 0.01, and 0.30 mg/kg ip, 15 min prior to testing); that of nicotinic cholinergic receptors with nicotine (0, 0.083, 0.25, and 0.75 mg/kg sc, 15 min prior to testing) and mecamylamine (0, 1.8, 3.0, and 5.6 mg/kg ip, 15 min prior to testing); that of α_2-adrenergic receptors with clonidine (0, 0.003, 0.010, 0.030 mg/kg sc, 15 min prior to testing) and idazoxan (0, 1.0 , 3.0, and 10.0 mg/kg sc, 30 min prior to testing). SI was decreased, and RT was increased by pilocarpine in Block 1; both measures of performance returned to normal in Blocks 2 and 3. RI was not affected. Scopolamine reduced SI and increased RT during the entire test session, without affecting RI. Nicotine at 0.75 mg/kg decreased SI in Block 1; both 0.75 and 0.25 mg/kg improved SI in Block 3. Nicotine did not affect RT. Mecamylamine reduced SI and increased RT across the whole session without affecting RI. Clonidine reduced SI and RI and increased RT in all blocks. Idazoxan reduced SI and increased RT across the whole session; RI was reduced in Block 1 only. Thus all drugs except nicotine reduced SI, and most slowed responding. Nicotine facilitated target detection in the last block of trials.

LEARNING AND MEMORY: PHARMACOLOGY V

174.1

SPATIAL MEMORY DEFICITS FOLLOWING QUISQUALATE ACID-INDUCED LESIONS OF THE NUCLEUS BASALIS MAGNOCELLULARIS: EFFECT OF NICOTINE. N.R. Katz*, J. Rochford, S.A Welner. Douglas Hospital Research Center, Dept. Neurology & Neurosurgery, McGill University, Montreal, Quebec, CANADA.

Previous studies have shown that rats with ibotenic acid lesions of the nucleus basalis magnocellularis (NBM) display deficits in spatial memory (Hodges et al., 1991). This finding has been taken as indirect support for the hypothesis that basal cholinergic forebrain systems mediate spatial memory. Ibotenic acid, however, is not particularly selective for cholinergic neurons. Quisqualic acid has been demonstrated to cause less non-selective damage to non-cholinergic systems, and typically leads to a greater reduction in cortical choline acetyltransferase (Chat) activity. Therefore, we examined the effects of NBM quisqualic acid lesions on spatial memory. To provide further evidence that this deficit may be the result of cholinergic neuronal destruction, we assessed the effect of the nicotinic receptor agonist nicotine. Two groups of rats were lesioned with quisqualic acid, and two groups served as controls. Following recovery, one group within each set received an injection of nicotine fifteen minutes prior to each testing session. Spatial memory was assessed using the eight arm radial arm maze. Food was placed at the end of each arm and errors were recorded for each re-visited arm. NBM-lesioned animals displayed a marked deficit in the acquisition of radial arm maze performance in comparison to non-lesioned animals. Performance of lesioned animals administered nicotine did not differ from controls, indicating that nicotine was able to reverse the deficit provoked by NBM-lesions. Nicotine did not influence performance in non-lesioned animals. These results add further to the evidence implicating basal forebrain cholinergic neurons in the mediation of spatial memory (Supported by the Medical Research Council of Canada).

174.2

DIFFERENTIAL EFFECTS OF IgG-192 SAPORIN ON PERFORMANCE OF TWO MEMORY TASKS: REVERSAL OF DEFICITS BY CDD-0097. A.A. El-Assadi*, M.A. Shepherd and W.S. Messer, Jr. Department of Medicinal & Biological Chemistry, Center for Drug Design & Development, College of Pharmacy, The University of Toledo, Toledo, OH 43606

Selective muscarinic agonists might be useful in the treatment of Alzheimer's disease. Previous studies identified CDD-0097 as a selective and efficacious m1 agonist with a low side-effect profile. The cognition-enhancing properties of CDD-0097 were examined using two paradigms to assess memory function and IgG-192 saporin to deplete acetylcholine levels.

In rats, injections of IgG-192 saporin (0.20 µg total) into the diagonal band produced impairments of performance on a paired-run, delayed alternation paradigm in a T-maze (78 ± 2.8 %). Vehicle-injected controls were unimpaired (100 ± 0.0 %). Comparable IgG-192 saporin injections did not affect performance (94.8 ± 4.0 %) on a spatial discrimination task in a water maze relative to controls (97.9 ± 2.4 %).

CDD-097 (1.0 mg/kg) completely reversed the memory deficits induced by IgG-192 saporin in the T-maze task, and did not impair performance of control animals in either task. Taken together, the data implicate the basal forebrain cholinergic system in some, but not all forms of spatial memory function. Furthermore, the beneficial effects of CDD-0097 on memory function warrant further examination of the compound as a selective M_1 agonist for the treatment of Alzheimer's disease.

This work was supported by NS 01493 and NS 31173.

174.3

SELECTIVE LESIONING OF DISCRETE REGIONS OF THE BASAL FOREBRAIN USING INTRAPARENCHYMAL INJECTIONS OF 192IgG-SAPORIN. G.A. Ramirez+, J. Winkler+, D.P. Pizzo+ and L.J. Thal.*+• +Dept. of Neurosciences, UCSD, and •VAMC, San Diego, CA 92161.

Intracerebroventricular (ICV) infusions of the immunotoxin 192IgG-saporin result in a decrease in choline acetyltransferase (ChAT) activity in the projection areas of the basal forebrain system and in loss of cerebellar Purkinje cells. We used intraparenchymal injections to lesion the nucleus basalis (NBM) and/or medial septum (MS) using a broad range of concentrations of 192IgG-saporin (18.75 ng to 300 ng) to develop selective lesions, sparing Purkinje cells. Immunotoxin was infused into a single site at a rate of 0.05 µl/min in a volume of 0.5 µl. Dose response curves for ChAT activity were established to maximize depletion in the projection fields of the lesioned structure. The optimal dose for lesions of the NBM was 75 ng/site which resulted in an extensive depletion of ChAT activity in the frontal (>75%), cingulate (>60%), parietal (>55%), and occipital (>35%) cortex, and olfactory bulb (>75%) whereas hippocampal activity was reduced less than 20%. Optimal lesions of the MS were obtained with 37.5 ng/site and resulted in a depletion of ChAT activity in hippocampus (>90%), cingulate cortex (>80%), occipital cortex and olfactory bulb (>60%), whereas ChAT activity of the frontal and parietal cortex were least affected (<30%). This study defined the parameters for selective and topographically restricted lesions of components of the basal forebrain. Supported by VA Medical Research Service.

174.4

BEHAVIORAL EFFECTS AFTER INTRAPARENCHYMAL INJECTIONS OF 192IgG-SAPORIN INTO DISCRETE REGIONS OF THE BASAL FOREBRAIN. D.P. Pizzo*+, J. Winkler+, and L.J. Thal+. +Dept. of Neurosciences, UCSD, and •VAMC San Diego, CA 92161.

Cholinergic lesions using intracerebroventricular (ICV) administration of the immunotoxin 192IgG-saporin result in a broad range of behavioral impairments including deficits in learning and retention. However, Purkinje cells are destroyed at doses required to obtain behavioral impairments. At present it is unclear to what extent the destruction of cerebellar structures contributes to the behavioral impairment. We therefore selectively lesioned cholinergic neurons in the medial septum (MS) and/or the nucleus basalis (NBM) using intraparenchymal injections of 192IgG-saporin at a dose of 75ng/MS or 75ng/NBM in 0.5µl phosphate-buffered saline (PBS). These intraparenchymally-lesioned animals were behaviorally compared with ICV-lesioned, intraparenchymally-injected PBS controls, and non-lesioned animals. ICV-lesioned animals showed significant impairments in acquisition and spatial probe in the water maze, but retention was unaffected. In comparison, combined intraparenchymal-induced lesions of the NBM/MS resulted in a deficit in acquisition, retention and spatial probe. Single lesions of the MS or the NBM did not result in any behavioral impairment. These data indicate that single lesions of MS or NBM are not sufficient to induce behavioral impairments in the water maze. Supported by VA Medical Research Service.

174.5

ATTENTIONAL AND MEMORY DEFICIT FOLLOWING INJECTION OF 192-IgG-SAPPORIN INTO THE RAT NUCLEUS BASALIS MAGNOCELLULARIS (NBM). P. Curzon* A.W. Bannon and M.W. Decker. Neuroscience Research, D-47W, AP-9A, Pharmaceutical Products Div., Abbott Labs, 100 Abbott Park Rd., Abbott Park, IL 60064-3500

192-IgG saporin (SAP) selectively destroys cholinergic neurons when injected into the NBM, reducing cortical acetylcholine (ACh) levels. Disruptive effects of NBM SAP lesions on attention have been reported but effects on performance of spatial and other memory tasks have been inconsistent. We used a rapidly acquired, operant, Go/No-go task (Winocur, Behav. Brain Res., 1985, 16:135) that involves both attention and short-term memory. The ratio of latency to press the lever during alternating rewarded and non-rewarded trials separated by delays of 5, 20, and 40 s is used as an index of performance. Injections of either 21 or 10.5 μg of 192-IgG saporin in 0.5 μl or vehicle were made into the NBM (-0.6 mm, ±2.8 mm lat. from Bregma and -7.5 mm from skull surface). Male Long-Evans rats (group sizes of 10-11) were lesioned either before or after training in the Go/No-go task to assess effects on acquisition and performance. For acquisition, a dose dependent SAP lesion effect was observed on the 3rd week of training ($p=0.01$). The high dose SAP lesioned rats were significantly impaired ($p=.015$) at the 20 s delay and there was a marginal effect ($p=.066$) at the 5 s delay. In the group lesioned after training, there was an overall lesion effect ($p=.002$). The high dose of SAP impaired performance at the 5 s delay during the 1st but not during the 2nd week. Similarly, there was a marginal impairment during the 1st ($p=.06$) but not the 2nd week at a 20 s delay. In contrast, high dose SAP significantly impaired performance at a 40 s delay during both weeks post lesion. This somewhat more persistent impairment at the longest delay suggests that impaired memory plays a role in the disuptive effects of reduced cortical ACh on Go/No-go performance. However, impairments at the shortest delay suggest that impaired memory may not completely account for the lesion-induced deficit and that impaired attention may also be important. [Supported by Abbott Laboratories]

174.6

IgG SAPORIN POSTNATAL DAY (PND) 7 LESIONS DO, BUT PND 1 LESIONS DO NOT, PRODUCE SIGNIFICANT BEHAVIORAL IMPAIRMENTS AND REDUCED CHOLINERGIC MARKERS IN YOUNG MALE RATS. L. Ricceri[1], G. Calamandrei[1], and J. Berger-Sweeney[2]*. [1]Comparative Psychology Secion, Lab. Fisiopatologia, Istituto Superiore di Sanita, I-00161 Rome (ITALY) and [2]Dept. Biological Sciences, Wellesley College, Wellesley, MA 02181.

We have shown previously that 192 IgG-saporin, a p75 (NGF receptor)-specific antibody linked to a ribosomal inactivator produces cholinergic losses and behavioral impairments in adult rats (*J. Neurosci.* 14:4507-4519). The purpose of the current experiment was to examine the developmental role of the cholinergic system in behavior. Rats were injected with the neurotoxin into the 3rd ventricle on either PND1 or PND7; vehicle injected pups served as controls. Passive avoidance acquisition was assessed on PND15 and open field behaviors (locomotor activity, wall rearing and grooming) were assessed on PND 19. All rats were sacrificed on PND20 and choline acetyltransferase (ChAT) activity in hippocampus and cortex was determined. Passive avoidance acquisition was impaired in the PND7 lesioned group, but not in the PND1 lesioned group. Open field locomotor activity was not altered in either neurotoxin treated group. The PND7 group, however, exhibited significantly reduced wall rearing in an open field, while the PND1 group exhibited increased grooming in an open field. The PND1 group showed 14% and 17% reductions in hippocampal and cortical ChAT activity, respectively, as compared to controls. The PND7 group showed 78% and 65% reductions in hippocampal and cortical ChAT activity, respectively. It is clear that the more significant reduction in ChAT activity in the PND7 group was associated with impaired acquisition performance. The PND1 group results could indicate either that the toxin was not effective at that age, or that there was significant recovery of the cholinergic system by the time of testing such that a behavioral deficit was not detected. (Supported by a Fogarty Fellowship F06TW002115-01).

174.7

THE ACTIONS OF GALANIN AND M40 ON DELAYED NON-MATCHING TO POSITION IN ^{192}IgG-SAPORIN-LESIONED RATS. M. P. McDonald*[1], G. L. Wenk[2], & J. N. Crawley[1]. [1]Section on Behavioral Neuropharmacology, ETB, NIMH, Bethesda, MD 20892, and [2]Division of Neural Systems, Memory and Aging, University of Arizona, Tuscon, AZ 85724.

Galanin is a 29-amino acid neuropeptide that coexists with acetylcholine (ACh) in the medial septum/diagonal band in the rat and inhibits acetylcholine release in the septohippocampal pathway. Galanin is overexpressed in the basal forebrain in Alzheimer's disease (AD). Galanin impairs performance on several rodent learning and memory tasks, including delayed non-matching to position (DNMTP). M40 (galanin[1-12]-Pro_3-$(Ala\text{-}Leu)_2$-Ala-NH_2), a peptidergic galanin receptor ligand, has previously been shown to antagonize galanin-induced impairment on DNMTP. The current experiments used a lesion model of AD to evaluate the actions of galanin and M40 on DNMTP when cholinergic transmission was reduced. Rats were injected with ^{192}IgG-saporin, an immunotoxin that selectively lesions cholinergic cells in the basal forebrain and produced a 54% reduction in hippocampal choline acetyltransferase in the present study. After recovery, rats were injected with galanin in the lateral ventricle and with M40 in the lateral ventricle or the ventral hippocampus. Galanin treatment significantly reduced choice accuracy in both the lesioned and sham groups. M40 alone did not affect choice accuracy. These results suggest that blocking endogenous galanin is not sufficient alone to improve performance in lesioned rats, indicating the potential need for a combined cholinergic-galaninergic treatment strategy.

174.8

MODERATE LOSS OF CORTICAL CHOLINERGIC INPUTS AS A MODEL OF AGE-RELATED IMPAIRMENTS IN ATTENTION AND EFFECTS OF PUTATIVE THERAPEUTIC DRUGS. J. McGaughy* and M. Sarter. Department of Psychology and Neuroscience Program, The Ohio State University, Columbus, Ohio 43210.

Previously, we demonstrated that cortical cholinergic deafferentation by intra-basalis infusions of 192 IgG-saporin produced severe, prolonged deficits on behavioral vigilance. The lesion-induced impairments were not attenuated by the administration of either physostigmine, a cholinesterase inhibitor, or FG 7142, a benzodiazepine receptor partial inverse agonist (Behav Neurosci 110:247-265). These results did suggest, however, that FG 7142 was more efficacious in attenuating the behavioral deficits in animals with less severe depletions. In the current study, the effects of moderate cholinergic depletions were produced by diffuse intra-cortical infusions1 of the immunotoxin 192 IgG-saporin. Such depletions represent a more valid model of the extensive, but incomplete loss of cortical cholinergic inputs observed in aged and demented humans. As predicted, intracortical infusions of the toxin produced decreases in cortical AChE-positive fibers and attentional impairments that were less severe than those previously found following intrabasalis infusions of the immunotoxin. The efficacy of physostigmine and FG 7142 in ameliorating these more moderate deficits, and the implications of these results for the development of novel treatment strategies will be discussed.

Supported by PHS Grants AG10173 and MH01072.

174.9

NEUROCHEMICAL PROFILE OF MHP-133, A NOVEL MIXED CHOLINERGIC AGONIST DEVELOPED FOR THE TREATEMENT OF ALZHEIMER'S DISEASE AND RELATED DISORDERS. J.J. Buccafusco*, J.C. Powers, K.M. Starkes, and M. Gattu. Alzheimer's Research Center.,and Dept.Pharmacol. & Tox., Medical College of Georgia, and the VA Medical Center, Augusta, GA 30912, and the School of Chem. & Biochem., Georgia Institute of Technology, Atlanta, GA.

MHP-133 (3-(N,N-Dimethylcarbamoyl)hydroxy-1-methyl-2-[[N-phenylaminocarbonyl) hydrazono]methyl] pyridinium chloride) was one of a series of oxime, semicarbazone, and acyl hydrazone derivatives of 2-formyl-1-methylpyridinium chloride and 2-formyl-3 or 5-hydroxy-1-methylpyridinium chloride originally designed as potential pretreatment antidotes against poisoning by irreversible organophosphorus acetylcholinesterase (AChE) inhibitors. MHP-133 was demonstrated to be an effective protectant, and a moderate post-poisoning antidote in mice intoxicated with organophosphorus AChE inhibitors. Unlike many of the other compounds of the series, the effectiveness of MHP-133 as a protectant against organophosphorus poisoning could not be correlated with reversible inhibition of AChE. In fact, MHP-133 was shown to have weak affinity for the enzyme ($K_i < 100$ μM) and was extremely slow in its ability to carbamylate the enzyme's active site ($k_{obs}/[I] \sim 2.5$ M^{-1} sec^{-1}). Nevertheless, the drug was chosen for further study, because it was shown to be effective by the oral route of administration. Also, MHP-133 combined high effectiveness as a protective agent with the highest margin of safety (LD_{50}/Maximal effective dose ~ 300 for both i.m. and oral administration). The quaternary nature of this compound also may impart some ability to directly bind to neuronal nicotinic receptors. In this regard it is about 600 fold less potent than nicotine and about 30 fold less potent than carbachol. Despite its charged nature, however,the drug has actions on the central nervous system. Since both inhibition of brain AChE and stimulation of nicotinic receptors have been demonstrated to improve cognitive performance in animal models and in humans, MHP-133 was examined for potential memory enhancing ability in two animal models (see accompanying poster). Supported by a GIT/MCG grant and the Dept. of Veterans Affairs. Medical Center.

174.10

MHP-133-INDUCED ENHANCED PERFORMANCE OF MEMORY-RELATED TASKS IN RATS AND MONKEYS M.A. Prendergast*, J.J. Buccafusco and J.C. Powers. Alzheimer's Research Center, and Dept.Pharmacol. & Tox., Medical College of Georgia, and the VA Med. Ctr., Augusta, GA 30912, and School of Chem. & Biochem., Georgia Institute of Technology, Atlanta, GA.

In the Morris Water Maze task involving a stationary slightly submerged platform, i.p. pretreatment with MHP-133 to adult Wistar rats significantly improved the learning curve over 4 consecutive trials. Of 3 doses tested, 50, 100 and 200 μg/kg, the two lower doses were associated with significant improvement in task performance. A group of 12 macaques were well-trained in the performance of an automated delayed matching-to-sample (DMTS) task. The monkeys were represented by individuals from 3 species, both genders and two age groups, mature and aged. All, but 1 animal exhibited marked improvement in DMTS performance at 1 or more doses. Virtually all of the improvement was obtained for trials involving the longest delay intervals imposed between stimulus presentation and choice presentation. At the most effective dose (30 - 100 μg/kg, i.m.), performance at long delay trials increased on average by 32.2% over baseline performance for the entire group. Thus, MHP-133 improves short-term memory ability in animals, including aged monkeys. MHP-133 was also examined for its ability to displace muscarinic receptor and neuronal nicotinic receptor ligands from rat cortical membranes using standard ligand binding techniques. The drug completely displaced the [^{3}H]labeled receptor ligands, methylscopolamine and cytisine, with IC_{50} values in the μM range. Finally, MHP-133 was compared with physostigmine for the ability to increase blood pressure in conscious rats,and to produce other signs of acetylcholinesterase (AChE) inhibitor toxicity in rats. Over i.v. doses ranging from 25 - 750 nmol/kg physostigmine produced a dose-dependent increase in blood pressure up to about 70 mmHg. Over the same range, MHP-133 evoked no significant change in resting blood pressure. MHP-133 may improve the performance of memory-related tasks through a combined mechanism including weak AChE inhibtion and nicotinic receptor stimulation. Supported by a GIT/MCG grant, & Dept. VA Med. Ctr.

174.11

COMPARATIVE STUDIES OF HUPERZINE A AND TACRINE ON SCOPOLAMINE-INDUCED AMNESIA OF SHUTTLE-BOX ACTIVE AVOIDANCE IN ADULT AND AGED RATS. Z.J. Dong, Y.L. Lo, K.Y. Chan and Y.F. Han. (SPON: The Hong Kong Society of Neuroscience). Department of Biochemistry, The Hong Kong University of science and Technology, Hong Kong

Huperzine A (Hup A), isolated from a Chinese medicinal herb *Huperzia serrata*, has been well established as a potent and reversible cholinesterase inhibitor. In this study Hup A was compared with tacrine in both improving the learning process of normal rats and in reversing scopolamine-induced amnesia. Sprague-Dawley rats were trained to have active avoidance response to foot shock in the shuttle-box. Hup A effectively increased the learning process of adult and aged rats at the dose range of 0.2-0.4 mg/kg and 0.1-0.3 mg/kg (IP, immediately after training), respectively (all $p<0.05$). A similar effect of tacrine was observed at the dose range of 6-12 mg/kg. (IP). In addition, Hup A (0.3 and 0.4 mg/kg, IP) significantly ameliorated the consolidation impairment in active avoidance responses induced by scopolamine (1 mg/kg, SC, $p<0.05$). These results suggest that Hup A, which is much more potent than tacrine, can potentially improve the learning process through a central cholinergic mechanism in both adult and aged animals. [Supported by Hong Kong RGC Direct Allocation Grant (94/95.SC11)]

174.12

REVERSAL EFFECTS OF HUPERZINE A AND TACRINE ON MEMORY DEFICITS IN BILATERAL NBM LESIONED RATS. O.Y. Yeung and Y.F. Han. (Spon: The Hong Kong Society of Neuroscience). Department of Biochemistry, The Hong Kong University of Science and Technology, Hong Kong.

Huperzine A (Hup A), a novel selective acetylcholinesterase inhibitor, was first isolated from a Chinese medicinal herb *Huperzia serrata*. The specific aim of this study was to evaluate the efficacy between Hup A and tacrine on nucleus basalis of magnocellularis (NBM) lesion-induced spatial memory deficits. Male Sprague-Dawley rats were trained to achieve the criterion level of performance in the radial maze in which eight arms were baited with reinforcement. Bilateral NBM lesion with ibotenic acid (6 μg/μl, per side) caused significant impairment in rats' ability to perform this working memory task as demonstrated by fewer correct choices before the first error, more total errors to complete the task and lower the percent efficiency. The behavior impairment was correlated to about 40% decrease of choline acetyltransferase activity in neocortex. Hup A (0.3-0.6 mg/kg, IP, 30 minutes before task) significantly reversed this memory deficits induced by NBM lesion. Tacrine (3-6 mg/kg) also ameliorated the memory impairment. The results suggest that the integrity of NBM lesion is critical for spatial memory processing, and the working memory impairment induced by NBM lesion can be more effectively reduced by Hup A than tacrine. Acknowledgment: To Prof. X.C. Tang and Mr. Z.Q. Xiong for their helps during the course of the experiment. [Supported by HK RGC DAG (94/95.SC11)].

174.13

NEFIRACETAM REVERSES MUSCARINIC AND NICOTINIC ANTAGONISTS IN EYEBLINK CONDITIONING IN RABBITS. D.S. Woodruff-Pak* & R. M. Hinchliffe. Psychology Dept., Temple Univ., Philadelphia, PA 19122.

The septo-hippocampal cholinergic system plays a modulatory role in eyeblink classical conditioning (EBCC), and this provides a point of contact with Alzheimer's disease (AD). The focus of the role of the cholinergic system in EBCC has been on the muscarinic cholinergic receptors. Our research was the first to demonstrate a role for nicotinic cholinergic receptors in EBCC. However, we are not aware of research on the effect of nefiracetam on nicotinic cholinergic receptors. Given that nicotinic cholinergic receptors as well as muscarinic cholinergic receptors are involved in the modulation of learning of EBCC, experiments were carried out to examine reversal of cholinergic antagonists to nicotinic (mecamylamine) and muscarinic (scopolamine) receptors in young rabbits with nefiracetam. Two experiments were completed by a total of 88 young SPF female New Zealand white rabbits tested for 15 days in the 750 ms delay paradigm: Both paired and explicitly unpaired conditions were tested with drugs and vehicle. Nefiracetam at a dose of 10 or 15 mg/kg reversed the effects of both mecamylamine and scopolamine. The vehicle alone and nefiracetam alone groups performed similarly to the groups treated with mecamylamine and nefiracetam or scopolamine and nefiracetam. An interesting outcome in this experiment was that nefiracetam alone did not facilitate acquisition of EBCC in young rabbits over the rate of vehicle-treated young rabbits. Young rabbits were apparently performing at close to ceiling levels. In summary, nefiracetam acted to reverse the effects of both a nicotinic and a muscarinic cholinergic antagonist in young rabbits trained in the 750 ms delay EBCC paradigm. Our research has indicated that it is the disruption of the hippocampal cholinergic system that impairs EBCC in AD. Thus, the reversal of nicotinic and muscarinic cholinergic antagonists by nefiracetam suggests that nefiracetam has the potential to ameliorate impaired cognition in AD. ***Supported by Daiichi Pharmaceutical Co., Ltd., Tokyo, Japan.***

174.14

INFLUENCE OF NEFIRACETAM ON NGF-INDUCED NEURITOGENESIS AND NEURAL CELL ADHESION MOLECULE POLYSIALIC ACID EXPRESSION: IN VIVO AND IN VITRO COMPARISONS. T. Shiotani, S. Watabe*, O. Odumeru, K.J. Murphy, A.W. O'Connell and C. M. Regan. Dept. Pharmacology, University College Dublin, Ireland and Daiichi Pharmaceutical Co. Ltd, Tokyo, Japan.

Co-administration of nefiracetam (DM-9384) during acquisition of an avoidance response prevents attenuation of learning-associated polysialylation of dentate neurons in scopolamine-induced amnesia at a 12h post-training period. We now provide *in vitro* evidence for these enduring effects. Exposure of primed PC-12 cells to nefiracetam for an 8-12h period and subsequently to NGF alone for 36h augmented neurite growth and polysialylation in a dose-dependent manner and increased significantly the percentage of cells with neurites in excess of 30um. In all cases the maximal effect was observed at the lowest dose evaluated (0.1uM). A 48h co-exposure of NGF and nefiracetam to naive PC-12 cells resulted in a 2-fold increase in PSA expression which could not be attributed to increased NCAM polypeptide prevalence. Chronic *in vivo* exposure of nefiracetam (3mg/kg; 40days), a dose known to reverse scopolamine-induced amnesia, failed to significantly prevent the basal expression of dentate polysialylated neurons suggesting the action of nefiracetam to be stimulus-dependent as provided by NGF *in vitro* and by the learning event *in vivo*. In contrast, higher doses of nefiracetam (≥9mg/kg) prevented age-dependent decline of dentate polysialylated neurons following chronic administration. This suggests that doses higher than those required to ameliorate scopolamine-induced amnesia may increase neuroplastic potential and account for the neuroprotective actions attributed to nefiracetam. *Supported by Daiichi, EU Biotechnology Programme and Health Research Board of Ireland.*

NEURAL PLASTICITY I

175.1

INCREASED DENDRITIC ARBORIZATION OF CEREBELLAR STELLATE NEURONS FOLLOWING MOTOR SKILL LEARNING IN THE RAT. W.T. Greenough*, J.A. Kleim, R.A. Swain, C. M. Czerlanis, J. Kelly & M. A. Pipitone. Depts. Psych., Cell & Struct. Biol., Neurosci. Prog. and Beckman Inst., Univ. Illinois, Urbana, IL. 61801.

Following complex motor skill acquisition, increases in the number of parallel fiber to Purkinje cell synapses have been observed in the cerebellar cortex. In this study we examined whether motor skill learning affects the morphology of the stellate interneurons within the cerebellar cortex. Female rats were randomly assigned to either an Acrobatic condition (AC) or a Motor control condition (MC). The AC animals were trained to traverse a complex obstacle course which required a significant amount of motor coordination to complete. Each AC animal was pair matched with an MC animal which was forced to travel down a flat, obstacle free runway equal in length to the acrobatic course. Following ten days of training, the animals were sacrificed and neurons within the cerebellum were visualized using Golgi impregnation. Approximately ten stellate cells from both the upper and lower half of the molecular layer of the paramedian lobule were traced using camera lucida. Concentric ring analysis revealed the AC animals to have significantly more dendritic material than their MC littermates. In addition, cells in the lower half of the molecular layer had significantly more dendritic material than those in the upper half irrespective of condition. These results indicate that the inhibitory interneurons within the cerebellar cortex undergo dendritic remodelling in association with motor skill learning. Supported by AG 10154, NSF BNS 88 21219 and NSERC Scholarships.

175.2

AN EXAMINATION OF LATERAL CEREBELLAR NUCLEUS MORPHOLOGY FOLLOWING COMPLEX MOTOR LEARNING IN THE RAT. J.A. Kleim*, M. A. Pipitone, J. Kelly, J. Drew, C. M. Czerlanis, & W.T. Greenough . Depts. Psych., Cell & Struct. Biol., Neurosci. Prog. and Beckman Inst., Univ. Illinois, Urbana, IL. 61801.

The cerebellar cortex has been observed to undergo profound structural remodelling in association with motor skill learning but not with motor activity. In this experiment we examine how motor skill learning affects the morphology of the lateral cerebellar nucleus (LCN), one of the primary targets of cortical output. Female rats were randomly assigned to either an Acrobatic condition (AC), Motor control condition (MC) or an Inactive condition (IC). AC animals were trained to traverse a complex obstacle course which required a substantial amount of motor skill to complete. Each AC animal was pair matched with an MC animal which was forced to traverse a flat, obstacle free runway equal in length to the acrobatic course. IC animals received no motor training or activity but were handled daily. Following 14 days of training, the animals were sacrificed and serial 100μm sections were taken in the horizontal plane through one hemisphere of the cerebellum. The LCN was dissected from two of these sections and prepared for electron microscopy. Unbiased stereological techniques were then used to obtain estimates of LCN volume and neuron density from the remaining sections. Preliminary results indicate no significant effect of CONDITION on either LCN volume or neuron density. Ultrastructural analysis of synapse number is currently in progress. Supported by AG 10154, NSF BNS 88 21219 and NSERC Scholarships.

175.3

CHANGES IN EXPRESSION OF bZIP PROTEINS AND DNA BINDING ACTIVITY IN MOUSE BRAINSTEM DURING TASTE AVERSION LEARNING. D.S. Kotchmar[1], K.R. Pennypacker[2] and M.W. Swank[1]*. *Dept. Psychology, Furman U., Greenville, SC 29613[1], Dept. Pharmacology, U. South Florida, Tampa, FL 33612[2]*

Previous immunohistochemical studies from this lab[1] have demonstrated CS- and US-specific patterns of bZIP expression in rodent brainstem during taste aversion learning. Using a micro-punch technique, we demonstrate the feasibility of performing Western blots and gel-shift assays with tissue collected from the intermediate NTS. Preliminary Western blot analysis using polyclonal antibodies for detection (Santa Cruz Biotechnology) reveals that a LiCl US produces an up-regulation of a number of bZIP proteins, including c-Fos, FosB, JunD, and a Fos-related antigen. Gel-shift analysis reveals increases in both AP-1 and CRE binding activity following US LiCl or CS saccharin. Treatment with both CS saccharin and US LiCl produces a pattern of AP-1 and CRE binding activity which resembles neither that of the CS nor US alone, suggesting an associativity of these two stimuli which is not merely additive. In light of the known different affinities and binding specificities of various bZIP heterodimeric combinations, present studies are directed at identifying the specific heterodimers formed as a result of the different behavioral treatments involved in taste aversion learning.

(Supported by NIH/NICHHD #HD33138-01 to MWS)

175.4

MEASUREMENT OF PRIMED BURST POTENTIATION DURATION VARIES ACROSS THE POPULATION SPIKE INPUT/OUTPUT FUNCTION IN THE DENTATE GYRUS OF FREELY BEHAVING RATS. E.L.Hargreaves* & M.L. Shapiro, Psychology, McGill University, Montreal, CANADA

Primed Burst Potentiation (PBP) is a form of neural plasticity that is similar to long-term potentiation (LTP), but of brief duration. Because PBP is repeatable, it may be a more useful tool for studying memory mechanisms in behaving animals than LTP. Here, we measured the duration of PBP across the population-spike (PS) input/output (I/O) curve.

I/O functions were recorded from the perforant-path/dentate-gyrus circuit of behaving Long-evans male rats (350-400g). Diphasic test pulses (0.1 ms/phase) were used to generate I/O functions and PBP. I/O functions consisted of 9 levels ranging from 50μA above PS threshold to maximal responses tailored for each animal. All I/O test pulses were delivered at a frequency of 0.1Hz. I/O functions were recorded continuously during PBP sessions. PBP was induced using 1000 uA stimuli: 1 priming pulse followed 170 msec later by a 200Hz pulse burst.

As in LTP, greater PS potentiation was measured at lower I/O test pulse intensities than at more maximal I/O test pulse intensities. Although greater potentiation was recorded at the lower test pulse intensities, the measured duration of this potentiation was shorter than that of the smaller potentiation recorded at the more maximal test pulse intensities. Potentiation at the more maximal test pulse intensities could be detected up to 2.7 hours after PBP was induced, whereas potentiation measured at the lower test pulse intensities could return to baseline levels within 30 min.

Thus, measurement of the degree and duration of PS enhancements, following PBP, are not uniform across the I/O curve. Lower test pulse intensities exhibit greater potentiation, but return to baseline faster than test pulse intensities evoked near or at maximal responses.

Supported by NSERC and MRC of Canada.

175.5

ELECTROPHYSIOLOGICAL CORRELATES OF THE ESCAPE BEHAVIOR, IN THE ABDOMINAL MUSCLES, OF JUVENILE AND ADULT AMERICAN LOBSTERS, OVER THE MOLT CYCLE. S.I. Cromarty*[1] and G. Kass-Simon[2]. [1]Department of Biology, Georgia State University, Atlanta, GA 30302. [2]Biological Sciences Department, University of Rhode Island, Kingston, RI 02881.

Neuromuscular plasticity over the molt cycle has been well documented in the claw-opener musculature of the American lobster (Schwanke et al., 1990). Here we present evidence that similar molt-related differences occur in the flexor muscles effecting escape swimming.

We compared excitatory junction potentials (EJPs) in juvenile and adult lobsters in each of four molt stages (A, B, C, D) and analyzed the results with respect to the following parameters: EJP amplitude, EJP area, EJP rise and decay time, and facilitation and/or failure rates.

Our results show that increasing the frequencies from 1 to 6 Hz differentially affected the production of EJPs in the different molt stages among juvenile lobsters: EJPs of hard-shelled (C and D) juvenile lobsters failed at 4 and 5 Hz, while soft-shelled (A and B) juveniles continued to produce EJPs beyond 6 Hz. Among adult lobsters however, EJPs of soft-shelled lobsters, failed at 4 and 5 Hz, while hard-shelled lobsters continued to produce EJPs to 5 and 6 Hz. Soft-shelled adult (Stage A) lobsters produced the largest EJPs, indicating that there is a tendency for A lobsters to respond to a stimulus with a maximal transmitter release which is then not replenished at the higher frequencies resulting in EJP failure.

Our results therefore indicate that changes in the neuromuscular synapse occur not only as a function of relative weight and/or developmental period (small juveniles, large adults), but also as a function of molt-related physical condition. The differences in escape swimming (Cromarty, et al., 1991) among juvenile and adult lobsters in different molt stages can be in part accounted for by correlated differences in the electrical activity at the abdominal neuromuscular junction.

Supported by grants (Sigma Xi and Lerner Gray to SIC; Whitehall to GKS)

175.6

MEG AND FMRI INVESTIGATIONS OF CROSS-MODAL PLASTICITY IN A CONGENITALLY DEAF SUBJECT Gregory Hickok†*, David Poeppel‡, Kevin Clark†, Richard Buxton#, Howard A. Rowley‡, and Tim P.L. Roberts‡. †Salk Institute-LCN, 10010 N. Torrey Pines, La Jolla, CA 92037, #UCSD, and ‡UCSF, 513 Parnassus Ave, San Francisco, CA 94143.

It has been proposed that auditory cortex of deaf subjects may provide an example of cross-modal compensatory plasticity. This study investigated whether visual or somatosensory stimulation could elicit responses from primary auditory areas of a congenitally deaf subject. Neuromagnetic fields were recorded using a 37-channel biomagnetometer (Magnes, Biomagnetic Technologies, Inc., San Diego, CA) under conditions of (i) visual stimulation, (ii) somatosensory stimulation, and (iii) a simple motor task. Visual items were a reversing checkerboard and single spots of light, presented in various portions of the visual field and at different rates; somatosensory stimuli were pneumatic taps delivered to one digit-segment at a time; the motor task was self-paced finger tapping. In addition, functional magnetic resonance imaging was performed with a full-field checkerboard stimulus. Results: no obvious responses to passively presented visual or somatosensory stimuli were observed in auditory cortex In contrast, somatosensory, motor, and visual cortices reveal evoked responses comparable to hearing control subjects, indicating canonical physiological organization in these areas. These data are consistent previous ERP and fMRI data, as well as with analogous MEG studies of congenitally blind subjects, and suggest that primary projection areas do not reveal obvious plastic effects. Rather, compensatory plasticity effects emerge only in non-primary areas of cortex and only under attentionally demanding conditions. NIH R01 DC00201

175.7

SIGNS OF MAP-2 DEGRADATION IN THE HIPPOCAMPUS WITH CONTEXTUAL MEMORY

N.J. Woolf*, M.D. Zinnerman and G.V.W. Johnson, Dept. of Psychology, UCLA, Los Angeles, CA 90095-1563 and Depts. of Psychiatry and Behavioral Neurobiology, University of Alabama, Birmingham. AL 35294-0017

We have previously found signs of MAP-2 degradation with Pavlovian conditioning to tone. In our previous studies, MAP-2 degradation in the auditory cortex correlated with conditional responses to tone. These changes were detected the day after the last training session, and, at that time, there was no sign that MAP-2 was degraded in hippocampus.

With Pavlovian conditioning to tone, rats also develop a memory of the context in which they are conditioned. Consolidation of a configuration, like the training context, is more complex and takes longer than the learning of elemental stimulus like a tone. For these reasons, we decided to look at longer intervals after training for signs of degradative changes in the hippocampus.

Sprague-Dawley rats were trained with a 2 KHz tone and a 1 mAmp grid shock in a novel chamber. Two weeks later, the rats were sacrificed for immunohistochemical studies or immunoblot analyses. Immunohistochemistry for MAP-2 was increased in CA1 of the hippocampus of conditioned animals as compared to controls ($p<0.01$). This suggested the possibility of increased breakdown products. Immunoblots confirmed that more trained animals exhibited a 90 kD breakdown product in the hippocampus than did the controls.

Previous studies have shown a 90 kD breakdown product when purified MAP-2 is exposed to calpain. Thus, calpain-mediated breakdown of MAP-2 in the hippocampus may underlie contextual memory.

175.8

SYNAPTIC CORRELATES OF SPATIAL LEARNING IN *DENTATE GYRUS* AND AREA CA1 OF THE RAT HIPPOCAMPUS. M.G.Stewart , D.A.Rusakov, H.A.Davies, E.Harrison, G.Diana and T.V.P.Bliss. (SPON: Brain Research Association). Dept. of Biology, The Open University, Milton Keynes MK7 6AA, UK; Istituto Superiore di Sanita', 00161 Roma, Italy; National Institute for Medical Research, London NW7 1AA, UK.

Structural correlates of a hippocampal-dependent learning task, water maze escape in the rat, were investigated at the level of synapses from *dentate gyrus* (granule cells) and CA1 (apical dendrites of pyramidal cells) of hippocampus. Nine rats were trained in 13 sessions to locate an invisible escape platform. Eight control rats were similarly trained but using a visible platform. Five days after the last session, rats were perfused and hippocampi dissected and processed for electron microscopy. Synaptic densities were estimated using disector routines. Sizes of synaptic active zones, and planar co-ordinates of synapses in sampling windows were recorded.

Each data set 'session vs. escape latency' showed an exponential regression, and we considered its time constant as an individual 'performance index'. Preliminary morphometric data show no significant changes in the spatial density, or active zone sizes, of synapses located either on dendritic spines, or the shaft, in either of the hippocampal areas, after training. However, a negative correlation was indicated between the 'performance index' and the density of shaft synapses in *dentate gyrus* (both groups) and CA1 (control group). In both areas, inter-synapse distances showed a significant trend towards lower values (in the range from 0 to 1.5 μm) after the task.

The results indicate that memory processing for the water maze task may involve subtle re-arrangement of synapses, including a switch between shaft and spine contacts, rather than dramatic changes in synaptic numbers.

Supported by *BBSRC Grant S02085*

175.9

PERFORMANCE IN A FREE SWIM TEST WITH VISUAL CUES IN ADULT MICE WITH BRAIN LESIONS INDUCED BY PRENATAL X-IRRADIATION R.W.F. Vitral (1,2), G. Campos (1), G. Paiva (1) and S.L. Schmidt* (1), (1)Dept. Ciências Fisiológicas, Inst. Biol., UERJ, (2)Inst. Biof. Carlos Chagas Filho, UFRJ, Rio de Janeiro, 20551030, BRAZIL.

Exposure of pregnant mice to ionizing radiation on the sixteenth gestacional day (E16) with doses greater than 2 Gy produces extensive depletion of neocortical superficial layers (II-IV) and callosal agenesis in the progeny. In this investigation, mice were exposed to X-rays at E16 receiving a total dose of 3 Gy. At adulthood, 49 irradiated (28 female and 21 male) and 61 non-irradiated (32 female and 29 male) animals were tested in a white free-swimming box. In one side there was a 11 cm high escape platform painted black. The platform was always visible to the animals because the water level was kept at a height of 10 cm. Each mouse was placed in the opposite side to the escape platform, facing the wall. They were submitted to two testing blocks that consisted of five consecutive trials each. The time interval between the two blocks was 24 hours. The trials were recorded with a video camera. From the recorded images, a microcomputer calculated the mean speed of each animal. In the first trial of the first block there was no significant difference between the velocity of the irradiated (v = 20,6 cm/s) and non-irradiated (v = 24,5 cm/s) animals. However, in the fifth trial of the first block there was a significant difference between irradiated (v = 21,9 cm/s) and non-irradiated (v = 30,1 cm/s) animals. This pattern was maintained in all trials of the second block. We concluded that, for both sexes, the irradiated mice do not improve their performance with repeated testing. This might be the result of a visuomotor integration deficiency of the irradiated mice.

Supported by CNPq and UERJ.

175.10

NEURAL CHANGES IN FORELIMB CORTEX AND THE BEHAVIOURAL DEVELOPMENT OF REACHING AND BIMANUAL COORDINATION IN THE RAT. B. L. K. Coles* and I. Q. Whishaw. Dept. of Psychology, University of Lethbridge, Lethbridge, AB, Canada, T1K 3M4.

Neural changes in the forelimb cortex were studied at Postnatal (P) 10, 15, 20, 25, 30, and 100 days. Three biological markers of brain development, glial fibrillary acidic protein (GFAP), c-Fos, and Acetylcholinesterase (ACh), as well as the morphology of motor cortex cells obtained from Golgi-Cox prepared tissue, were correlated with the behavioural development of reaching and bimanual coordination during feeding behaviors. Behaviors were filmed from P10 until P30 and then also in adults. For both behaviors there was a gradual development of the skilled patterns of paw and digit use seen in adults. The development of the adult patterns of movement were correlated with the morphological changes in the cortex. The results suggest that the maturation of skilled movement depends upon morphological maturation of the neocortex as well as upon experience.

This research was funded by NSERC.

175.11

ALTERATIONS OF SPONTANEOUS UNIT DISCHARGES IN THE DENTATE GYRUS FOLLOWING LTP INDUCTION. Akihisa Kimura*[1] and Constantine Pavlides[2]. [1]Wakayama Medical College, Wakayama, Japan; [2]The Rockefeller University, New York, NY.

Long-term potentiation (LTP) is a use-dependent modification of synaptic plasticity following high-frequency stimulation (HFS) to a set of afferents. Typically, field potentials or intracellular recordings are made to assess changes in synaptic plasticity. In the present experiment we were interested in determining changes in spontaneous neuronal activity in relation to induction of LTP, in the dentate gyrus (DG) following HFS of the perforant pathway. Experiments were performed in rats under chloral hydrate anesthesia. Field potentials and unit recordings were obtained alternatively from the same recording electrode.

Following baseline recordings of both field potentials and unit firing, HFS (400Hz, 20-50 pulses, 5 times, 10 sec apart) was applied and recordings of field potentials and spontaneous single or multi-unit discharges were continued. Experiments, in which LTP of field potentials was induced, were analyzed. In a quarter of cases, the enhancements of the field potentials were accompanied with an increase of unit discharges. A subgroup of cells, however, decreased their firing following enhancements of field potentials. In a subset of experiments, 30-40 min following induction of LTP, low frequency stimulation (1Hz, 10 min) was applied to the perforant pathway. This depotentiated the field potentials. Similar to the findings with LTP, depotentiation was accompanied with either enhancements or decrements in unit discharges. These results suggest that there exists a dynamic modification of unit activity in the DG, following LTP induction.

Supported by the grants from Wakayama Medical College and the Whitehall Foundation.

175.12

DIFFERENTIAL SYNAPTIC LOCALIZATION OF AMPA AND NMDAR1 RECEPTOR SUBUNITS IN THE RAT HIPPOCAMPAL DENTATE GYRUS. N. L Desmond[1]* and R. J. Weinberg[2]. [1]Dept. of Neurosurgery, Univ. of Virginia, Charlottesville, VA 22908 and [2]Dept. of Cell Biology & Anatomy, Univ. of North Carolina, Chapel Hill, NC 27599.

We are interested in the localization of glutamate receptor subunits at synapses in the adult hippocampal dentate gyrus (DG) and changes therein with synaptic modification. Toward this end, we used postembedding immunogold labeling for three glutamate receptor subunits (GluR1, GluR2/3, and NMDAR1) to characterize axospinous synapses in the middle third of the normal, adult DG molecular layer. These synapses are predominantly entorhinal-DG contacts. Synapses with gold particles within ±25 nm of the postsynaptic density (PSD) trace length were evaluated. Axospinous synapses were substantially more likely to be NMDAR immunopositive than GluR1 or GluR2/3 immunopositive. If we compare immunopositive axospinous synapses as a function of their PSD form, another picture emerges. Axospinous synapses with either perforated or nonperforated PSDs are about equally likely to be NMDAR immunopositive. On the other hand, the probability of axospinous synapses with perforated PSDs being AMPAR (GluR1 or GluR2/3) immunopositive is about twice that of axospinous synapses with nonperforated PSDs. Given that perforated PSDs are hypothesized to be potentiated synapses, these data suggest that LTP leads to the insertion of AMPA receptors at the PSD.

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175.13

HIPPOCAMPAL LTP DECAYS MORE RAPIDLY IN RATS EXPOSED DEVELOPMENTALLY TO LEAD. M.E. Gilbert*[1,2], C.M. Mack[2], and S.M. Lasley[1], National Research Council, US EPA, RTP, NC 27711, and U.Ill. Coll. Medicine, Peoria, IL 61656.

Childhood lead (Pb) exposure has been associated with impaired cognitive function. We have previously shown an increase in the threshold for LTP induction in the urethanized rat developmentally exposed to low levels of Pb. The present study examined the time course of decay of LTP in the dentate gyrus of the conscious rat chronically exposed to Pb. Pregnant female rats received 0.2% Pb-acetate in the drinking water and male offspring were maintained on this solution. As adults, animals were prepared with chronic stimulating/recording electrodes in the hippocampus. Following recovery from surgery and the establishment of stable field potentials evoked by single pulse stimulation (0.1 ms) of the perforant path, a pretrain input/output (I/O) function was collected. Saturable levels of LTP were induced by delivering 20 train pairs (4 pulses/pair at 400 Hz), 200 ms apart, at an intensity of 1000μA. Post-train I/O functions were collected 1hr and 1,2,4,7,14,21,28 days after LTP induction to monitor the time course of its decay. Preliminary analysis comparing maximal levels of LTP measured 1 hr after tetanization with later time points indicated faster declines in Pb-exposed animals within the first week. These data indicate that in addition to threshold shifts, mechanisms supporting the maintenance phase of LTP are also impacted by developmental Pb exposure. (Supported by [1]NIH ES06253, [2]NRC, and [2]US EPA).

175.14

CORRELATES OF LONG-TERM SENSITIZATION IN MOTOR NEURONS AND INTERNEURONS OF *APLYSIA*. W.L. Lee, L.J. Cleary* and J.H. Byrne, Dept. of Neurobiology and Anatomy, Univ. Texas Houston Medical School, Houston, TX 77225.

Long-term sensitization is an important form of learning exhibited by the tail-siphon withdrawal reflex. Previous work demonstrated that long-term sensitization was lateralized, and was correlated with changes in the membrane properties of tail sensory neurons (Scholz and Byrne, 1987; Lee et al. 1995). In this study, we extended these observations to include tail motor neurons and interneurons.

Animals were trained as described previously (Scholz and Byrne, 1987). Enhancement of siphon withdrawal elicited by weak tail shock was greater on the sensitized side of the animal than on the contralateral control side. The median was increased from 102% (41 Interquartile Range (IR)) to 186% (95 IR) [$P < 0.0001$]. In these same animals, two properties of motor neurons were affected. Resting membrane potential was increased from a median of -59 mV (9 IR) to -63 mV (6 IR) [$P < 0.05$]. In addition, the threshold membrane potential for generation of an action potential was decreased from a median of -44 mV (9 IR) to -46 mV (7 IR). [$P < 0.05$]. Two other biophysical properties of tail motor neurons were unaffected by long-term sensitization: input resistance at -80 mV and excitability. LPl17 is an excitatory interneuron that provides long-lasting synaptic input to motor neurons in pedal and abdominal ganglia (Cleary and Byrne, 1993). LPl17 was not affected by training, nor was the strength of the synaptic connection between LPl17 and tail motor neurons.

To our knowledge, this is the first report that long-term sensitization in *Aplysia* affects biophysical properties of motor neurons. Hyperpolarization may serve to bias the motor neuron to respond to strong stimuli. The reduced spike threshold would compensate in part for the increased membrane potential. Although LPl17 was unaffected by training, other elements of the circuit mediating tail-induced withdrawal reflexes have not yet been examined, and they may be important sites of plasticity.

Supported by NIH grant NS 19895.

175.15

REGULATION OF THE GLYCOLYTIC ENZYME, PHOSPHOGLYCERATE KINASE, BY TREATMENTS PRODUCING LONG-TERM FACILITATION IN *APLYSIA* NEURONS. H. West*, R. Homayouni, J. H. Byrne*, and A. Eskin. Dept. of Biochem. and Biophysical Sci., Univ. of Houston, Houston, TX 77204. *Dept. of Neurobiology and Anatomy, Univ. of Texas Med. School, Houston, TX 77225.

Serotonin-induced long-term facilitation (LTF) of pleural sensory neurons requires protein and mRNA synthesis. Previously, using *in vitro* translation of mRNA and two-dimensional polyacrylamide gel electrophoresis (2D-PAGE), we found 4 proteins whose mRNA was altered by treatments with 5-HT (Zwartjes et al., 1992). Microsequencing revealed one protein to be 88% identical to human 3-phosphoglycerate kinase II (PGK II). To obtain *Aplysia* PGK (*ap*PGK), we used human PGK II as a probe to screen a Lambda Zap *Aplysia* head ganglia cDNA library. A 2.9kb cDNA clone containing the 45 amino acid peptide obtained by microsequencing was isolated and partially sequenced. Complete sequencing of this cDNA is underway.

To confirm the changes in the mRNA of *ap*PGK observed previously, ribonuclease protection assays were employed. The level of *ap*PGK mRNA in pleural sensory neurons was increased 33±10% (n=9) after treatment of ganglia with 5-HT (5uM) for 1.5hr. However, similar treatments produced no change in the level of mRNA for another glycolytic enzyme, aldolase. These data confirm our previous findings and suggest that 5-HT might affect glycolysis through a specific effect on PGK. Perhaps treatments that induce LTF alter glycolysis to fulfill an increased energy demand during LTF. In other studies, we have shown that 5-HT treatments increase the rate of glycolysis in sensory neuron clusters (Homayouni, et al, 1994).
Supported by NS28426.

175.16

AGE-RELATED IMPAIRMENT OF LTP RETENTION IS ASSOCIATED WITH A DEFICIENCY IN SYNAPSE RESTRUCTURING TYPICAL OF THE MAINTENANCE PHASE OF LTP. Y. Geinisman*, L. deToledo-Morrell, F. Morrell, I. S. Persina and E. A. Van der Zee. Dept. of CM Biol., Northwestern Univ. Med. Sch. and Dept. of Neurol. Sci., Rush Med. Coll., Chicago, IL 60611.

Old animals are known to be impaired in LTP retention since they loose potentiated synaptic responses more rapidly than young adults (Barnes, *J. Comp. Physiol. Psychol.*, 1979, *93*:74; deToledo-Morrell et al., *Neurobiol. Aging*, 1988, *9*:581). The aim of the present study was to determine whether the rapid decay of LTP in old animals has its structural synaptic substrate. LTP was induced in aged (28 months old) rats by high frequency stimulation of the medial perforant path carried out on four consecutive days. The animals were examined morphologically 13 days after the fourth stimulation when synaptic responses decayed and were no longer significantly enhanced relative to baseline. Stimulated but not potentiated rats served as controls. Synapses were quantified in the middle molecular layer of the dentate gyrus. Estimates of the number of synapses per postsynaptic granule cell were differentially obtained for various synaptic types with the aid of the double disector method. The results show that aged rats, which were potentiated and examined 13 days after LTP induction, do not significantly differ from control ones with respect to the number of synapses. In marked contrast, young potentiated animals were found to exhibit a selective increase in the number of axodendritic asymmetrical synapses during LTP maintenance over a period of 13 days (Geinisman et al., *Soc. Neurosci. Abstr.*, 1995, *21*:444). Thus, the data presented here indicate that a deficiency in synapse restructuring characteristic of the maintenance phase of LTP is associated with and may be responsible for a rapid decay of LTP in aged animals.

Supported by NIH (AG 08794) and NSF (BNS-891237) grants.

175.17

REDUCTIONS IN SYNAPTIC TRANSMISSION (LONG-TERM DEPRESSION AND PAIRED-PULSE DEPRESSION) UPON REPEATED STIMULATION IN THE RAT PERIRHINAL CORTICAL SLICE. M.W. Brown, Z. Ziakopoulos and Z.I. Bashir (SPON: Brain Research Association). Anatomy Department, Bristol University, Bristol BS8 1TD, UK.

The results of recording, c-fos, and ablation experiments indicate the importance of perirhinal cortex to the judgement of the prior occurrence of individual visual stimuli in both rats and monkeys, such judgement being central to recognition memory. Neuronal responses in perirhinal cortex typically show long-lasting reductions with stimulus repetition. Two processes may be involved in these reductions as the relative familiarity and recency of occurrence of individual stimuli are separably encoded by different sets of neurons. In preliminary investigations using perirhinal cortical slices we are seeking changes in synaptic transmission that might provide potential neural substrates for these memory processes.

Standard techniques were used to record field excitatory postsynaptic potentials (epsps) from the superficial layers of slices of adult rat perirhinal cortex maintained *in vitro*. Test stimuli were delivered alternately (at 0.033 Hz) to each of two stimulating electrodes placed in the same layer to either side of the recording electrode. When two consecutive stimuli were delivered to the same pathway, significant paired-pulse depression (PPD) was observed ($n = 5$ slices; $P < 0.005$). PPD was found at intervals of 10, 100, 200, 500 and 1000 ms, the depression being greatest at 200 ms. Low frequency stimulation (1 Hz, 900 stimuli) delivered to one pathway resulted in significant homosynaptic long-term depression (LTD) lasting for $\geq$ 30 min ($n = 10$; $P < 0.005$). Preliminary findings indicate that the magnitude and duration of the LTD is reduced in the presence of the NMDA receptor anatgonist D-AP5. Thus both PPD and LTD can be induced in the adult rat perirhinal cortex. The mechanisms responsible for these synaptic changes *in vitro* could be those that underlie memory for the prior occurrence of stimuli *in vivo*.

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175.18

DEVELOPMENT OF ENVIRONMENTAL MODIFICATION OF HIPPOCAMPAL LONG-TERM POTENTIATION (LTP). Nicholas S. Waters* and Thomas C. Foster, Department of Psychology, University of Virginia, Charlottesville, VA 22903.

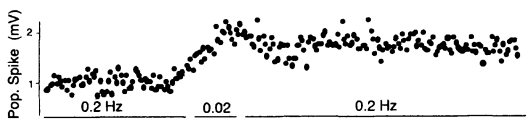
Juvenile rats are unable to perform a number of learning and memory tasks, which, in adults, appear to require an intact hippocampus. This inability appears despite competent sensory-motor systems, and the ability to perform other learning tasks. In adults, hippocampal long-term potentiation is reduced following a number of environmental manipulations, including the presentation of novel or stressful stimuli. To assess the maturity of hippocampal function, the induction of LTP was studied following behavioral testing at the ages during which hippocampally dependent learning emerges. At post-natal day 16 (P16; birth=P0), P23, and P30, subjects were tested for 5 min in a water motivated alternation (Y-maze) task, placed into a novel environment for 55 min, then sacrificed for *in vitro* electrophysiology in the hippocampal slice. Controls were sacrificed immediately after removal from the home cage. Paired pulses were delivered with a bipolar stimulating electrode in the Schaffer collaterals, and extracellular field potentials were recorded from *s. radiatum* in area CA1. LTP was induced with tetanic stimulation (two 1 sec bursts at 100Hz, 10 sec between bursts), and changes in the EPSP slope were measured as percent of baseline. The behavioral manipulation resulted in a decreased expression of LTP (compared to Controls) only in P30 subjects, the same age at which alternation behavior emerged. These results suggest that the emergence of the mechanisms which underlie hippocampal responsiveness to the environment coincides with the emergence of hippocampally dependent behavior.

This work was supported, in part, by NIH Grants NS31830 to TCF, and HD07323.

175.19

LONG-TERM SYNAPTIC PLASTICITY IN THE BEE BRAIN. S. Oleskevich*, J. D. Clements and M. Srinivasan. Visual Sciences, Research School of Biological Sciences, Australian National University, P.O. Box 475, Canberra, A.C.T., Australia 2601.

Synaptic plasticity such as long-term potentiation (LTP) has been hypothesized as the basis for learning and memory. The honeybee, with a highly developed learning capacity and relatively simple central nervous system, provides a good model to study the basic mechanisms of memory formation. The mushroom body in the bee brain contains 4×10^5 neurons and, like the vertebrate hippocampus, is an important site for memory consolidation. Focal stimulation of a major afferent input to the mushroom body evoked an extracellular field response which consisted of a presynaptic afferent fibre volley followed by a postsynaptic potential and a superimposed population spike. Continuous low frequency stimulation (0.02 - 0.1 Hz for 10 min) induced a long lasting potentiation of the population spike (100-400%; > 3.5 hrs). The potentiation was input specific, reached saturation, and was maintained in the absence of stimulation. The potentiation was also stimulus frequency dependent (170% increase at 0.02 Hz stimulation and insignificant at 1 Hz). This is the first demonstration of long-term synaptic plasticity in the insect brain. (S.O. supported by Ramaciotti Foundations).



175.20

AUDITORY-SOMATOSENSORY CONDITIONING PRODUCES LONG-LASTING CHANGES IN THE RESPONSE PROPERTIES OF SINGLE NEURONS IN THE VIBRISSAE REGION OF THE RAT SOMATOSENSORY CORTEX. **A.E. Butt*, A.A. Myasnikov, & R.W. Dykes. Département de Physiologie, Université de Montréal, Québec, Canada H3C 3J7.**

We hypothesized that repeatedly pairing an auditory stimulus with the vibratory stimulation of an individual vibrissa would produce a conditioned response to the auditory stimulus in single neurons in the somatosensory cortex of awake rats. Rats were surgically implanted with a light-weight frame attached to the skull and, upon recovery, were habituated to partial restraint in a chamber with their heads immobilized. Five days later, rats were anesthetized again prior to receiving bilateral craniotomies over the vibrissae representation of the somatosensory cortex. To prevent spontaneous whisker movement during electrophysiological recording, rats also received bilateral transections of the facial nerves innervating the vibrissal pad. On the following two days, awake rats were restrained, a small opening was made in the dura, and a tungsten-in-glass electrode was lowered into the vibrissae region of the somatosensory cortex. Upon isolation of a single neuron that responded to whisker stimulation, the whisker driving that neuron was attached to an electromechanical stimulator. Rats then received 20 consecutive tone (750 ms, 2000 Hz) presentations, followed by 20 consecutive whisker stimulations (750 ms, 33 Hz), each at an average inter-stimulus interval (ISI) of 8 s. Next, rats received 100 tone-whisker stimulation pairings (300 ms ISI, avg 8 s inter-trial interval). Finally, rats received another 20 presentations of each stimulus alone. Controls underwent identical procedures except that the 100 tone and whisker stimulations were explicitly unpaired. Results showed that 2 of 5 cells developed a conditioned response to the tone following the tone-whisker stimulation pairings, compared to 0 of 3 cells in the control condition. These data show that cross-modal associative conditioning can produce long-lasting changes in the response properties of single neurons in the rat somatosensory cortex. (Supported by the Programme Québécois de Bourse D'Excellence and by the MRC).

176.1

RECOGNITION OF EMOTIONAL FACIAL EXPRESSIONS IN PARKINSON'S DISEASE. R. Schul, R. Adolphs, H. Damasio* and A.R. Damasio, Dept. Neurology, Division of Cognitive Neuroscience, University of Iowa, Iowa City, IA 52242.

The role of the basal ganglia in processing emotion in humans is unclear. Damage to parts of the basal ganglia as a consequence of Parkinson's Disease (PD) results in impaired ability to express facial emotion, and it has been suggested that the recognition of emotions might also be impaired (Jacobs et al., 1995). In order to clarify this issue, we examined PD patients (n=18) and normal controls (n=13) on a task of recognition of multiple emotions within a single facial expression. No patient was demented, and none had impairment in basic visuoperceptual function. The control subjects were matched to the PD group in age, education and verbal IQ. Both groups were asked to rate the facial expressions of six basic emotions (from Ekman and Friesen), happiness, sadness, disgust, surprise, fear and anger, by using appropriate verbal labels. The ratings were correlated with the mean ratings given by normal controls, as described previously (Adolphs, et al., 1994). A Group x Emotion (2x6) ANOVA revealed no significant difference between the PD patients and the normal controls on this task ($F=0.16$, $P>0.05$, no interaction). Our results suggest that the basal ganglia components affected in PD do not play a critical role in the recognition of emotional facial expressions.

(R.S. is a recipient of the Fulbright Postdoctoral Scholarship. R.A. is a Burroughs Wellcome Fund Fellow of the Life Sciences Research Foundation. The study was supported in part by a grant from NINDS).

176.2

AN fMRI STUDY OF EMOTION PROCESSING: VALENCE-DEPENDENT HEMISPHERIC LATERALIZATION. T. Canli[1]*, J. Desmond[1,2], G. Glover[2], J. Gross[1], J. D.E. Gabrieli[1]. Departments of Psychology[1] and Radiology[2], Stanford University, Stanford, CA 94305.

There are at least two views on hemispheric specialization of emotion processing: one argues for a general dominance of the right hemisphere for all emotions, while the other holds that positive and negative emotions are processed by the left and right hemisphere, respectively. These two views were tested in the present study using functional magnetic resonance imaging (fMRI) in seven right-handed healthy female volunteers (age 18-32).

Subjects viewed alternating sets of emotionally positive or negative pictures that were selected from Lang's standardized stimuli set [Lang et al., IAPS Reports 1A-1C, Univ. of Florida, Gainesville]. Coronal slices of 6 mm or horizontal slices of 9 mm thickness were acquired, using a T2* sensitive gradient echo spiral sequence (TR = 720 ms, TE = 40 ms, flip angle = 65^0) in a 1.5 T GE Signa MR imager, and analyzed using the cross-correlation methods described by Friston et al. [Human Brain Mapping, 1994].

Viewing negative, compared to positive, pictures activated predominantly the right hemisphere, with significant ($p<.025$) activation most frequently seen in the inferior and middle frontal gyrus and middle temporal gyrus. Significant activation of the right amygdala was also observed in some subjects. Viewing positive pictures, compared to negative ones, was predominantly associated with significant ($p<.025$) left hemisphere activation, most frequently the superior, middle, and inferior frontal gyrus, and middle and superior temporal gyrus. The left amygdala was significantly activated in some subjects, as well. These data add to earlier evidence from EEG and other studies in favor of valence-dependent hemispheric lateralization of emotion processing. Supported NIH MH53673.

176.3

AMYGDALA ACTIVATION WITH EMOTIONALLY VALENCED AND NEUTRAL FACES: AN FMRI STUDY. N. Etcoff, H. Breiter *, P. Whalen, W. Kennedy, S. Rauch, S. Hyman, B. Rosen MGH NMR Center & Dept. of Psychiatry, Charlestown, MA 02129

Humans with bilateral amygdala lesions can have selectively severe impairments for visual recognition of fear. We sought to extend these observations using functional MRI (fMRI) to characterize amygdala involvement with visual recognition of fearful, angry, happy, and neutral faces in normal subjects. Whole brain imaging was performed on nine healthy right-handed males with an asymmetric spin-echo sequence. The experiment employed an A-B-C-B-C-B design with blocks of 72 to 120 tachistoscopic presentations of faces from Ekman and Friesen's Standardized Pictures of Facial Affect. In A, subjects saw 36 presentations of a fixation point (0.3 seconds) followed by a blank screen (0.2 seconds). In B, subjects saw neutral expressions (0.2 seconds) followed by a fixation point (0.3 seconds). In C, subjects saw faces with one emotion presented with the same timing parameters as in B. Subjects were scanned twice per emotion (6 total runs in fixed order), with 4 minutes rest between scans. Time-course data were motion-corrected, Talairach transformed, averaged, and mapped using Kolmogorov-Smirnov (KS) statistics. At the Bonferroni level (KS $p<10exp-7$), bilateral signal increases were noted in the amygdala for fearful vs neutral faces. Right-sided amygdala activation ($p<10exp-5$) was noted for angry vs neutral faces. No amygdala signal change was noted for happy vs neutral runs. Surprisingly, the first neutral face block had higher signal than subsequent neutral face blocks, suggesting a novelty effect or assessment of threat effect. Post-scan, all subjects recognized the target emotions; some reported low-level experience of emotion congruent with the valence of the emotional faces which declined with time. Heart rate changes were not noted for anyone between experimental blocks. In summary, the amygdala was differentially active for faces valenced with fear and anger. The amygdala was not similarly active during visual recognition of happiness. These findings suggest a potential focus for the amygdala on the registration of threat-related stimuli. Supported by NIDA DA00265-01 and NARSAD.

176.4

D2 and D4 DOPAMINE RECEPTOR POLYMORPHISMS AND TEMPERAMENT. E.P. Noble, T. Ozkaragoz, T. Ritchie*, X. Zhang. Department of Psychiatry and Biobehavioral Sciences and the Brain Research Institute, University of California, Los Angeles, CA 90024.

Personality characteristics, using the Tridimensional Personality Questionnaire (TPQ), and polymorphisms of the D2 dopamine receptor (DRD2) and D4 dopamine receptor (DRD4) genes were determined in 119 healthy Caucasian (non-Hispanic) boys (12.1 ± 1.2 yrs). Novelty Seeking (NS) score was significantly higher in subjects who carried the A1 allele ($P=.032$) or the B1 allele (.039) but not the 1 allele of intron 6 ($P=.085$) of the DRD2 gene compared to those who did not carry these alleles, respectively. Reward Dependence (RD) score was higher, but not significantly different, in subjects who carried the A1 allele ($P=.248$) or the B1 allele ($P=.051$) but were significantly higher ($P=.037$) in the 1 allele subjects when compared to those who did not carry these alleles, respectively. No significant differences were observed when these comparisons were made for the Harm Avoidance (HA) score. When the repeat alleles of exon 3 of the DRD4 gene were studied, NS score was significantly higher ($P=.049$) in subjects who carried the 7 repeat (7R) allele than those without this allele. However, no significant differences were found among the DRD4 alleles in RD ($P=.089$) or HA scores ($P=.539$).

The relationship of TPQ scores and haplotypes of the DRD2 polymorphisms in individual combinations with DRD4 polymorphisms showed a stronger effect. Subjects with A1 and/or 7R alleles (A^1), B1 and/or 7R alleles (B^1) and 1 and/or 7R alleles (C^1) had significantly higher NS scores ($P=.009$) than the respective groups without these alleles (A^2, B^2, C^2). RD scores were also significantly higher in A^1 compared to A^2 ($P=.014$), B^1 compared to B^2 ($P=.004$) and C^1 compared to C^2 ($P=.004$). However, in none of these comparative groups were there significant differences in the HA scores.

In conclusion, polymorphisms of both the DRD2 and DRD4 gene are associated with certain characteristics of temperament suggesting a genetic basis for some aspects of personality. (Supported by Smithers Foundation)

176.5

NEUROANATOMICAL CORRELATES OF POSITIVE AND NEGATIVE EMOTION, R.D. Lane*, E.M.Reiman, M.M. Bradley, P.J. Lang, G.L. Ahern, R.J. Davidson and G.E. Schwartz. U. of Arizona, Tucson, AZ; U. of Florida, Gainesville, FL; U. of Wisconsin, Madison, WI and The Samaritan PET Center, Phoenix, AZ.

Positron emission tomography (PET) and the International Affective Picture System (IAPS) were used to investigate the brain regions that participate in picture-generated positive and negative emotions. The ECAT 951/31 system, 40 mCi intravenous bolus injections of ^{15}O-water, 60-sec scans, and a 10-15 min between-scan interval were used to acquire three images of regional cerebral blood flow (CBF) in each of four conditions. Twelve healthy female subjects viewed counterbalanced sets of 20 pictures previously demonstrated to elicit: 1) positive, pleasant emotions; 2) negative, aversive emotions; and 3) neutral emotion. Subjects also viewed 4) a central cross-hair.

Positive and negative emotion conditions were each distinguished from neutral emotion conditions by significantly increased CBF in the vicinity of medial prefrontal cortex (BA 9), thalamus, hypothalamus and midbrain ($p<.005$) and by significantly decreased CBF in the vicinity of middle and superior temporal (BA 21,22,42), inferior parietal (BA 40), and lateral prefrontal (BA 10) and frontal (BA 44) areas ($p<.005$). Negative emotion was distinguished from neutral and positive emotion conditions by significantly increased CBF in the vicinity of occipitotemporal cortex (BA 18,19,37), cerebellum, amygdala, and left parahippocampal gyrus (BA 28) ($p<.005$). Negative emotion conditions were also distinguished from positive emotion conditions by significantly greater CBF decreases in the vicinity of anterior cingulate cortex (BA 24,32), superior and middle temporal gyri (BA 22,42) and inferior parietal cortex (BA 40). This study provides new information about the brain regions that participate in the perception of and reaction to emotional pictures and their relationship to emotional valence.

Supported in part by MH00972-02 and P50 MH52384 from N.I.M.H.

176.6

Depression and cardiovascular regulation in Multiple Sclerosis, **B.J.Diamond, J.DeLuca, H.Kim ,S.Kelley, R.Engel, K. Fontaine, D.Cordero, Kessler Institute ,Pleasant Valley Way, West Orange, NJ 07052.**

Individuals with Multiple Sclerosis (MS) can exhibit dysregulation in Heart Rate Variability (HRV) and depression (i.e. Beck Depression Scale(BDI)). Becauses linkages between HRV and depression are not well understood the goal of this study was to examine this relationship. Subjects consisted of 18 clinically definite MS and 20 matched controls. The major findings showed that the MS group was significantly more depressed (total score:z=2.85, p=.004), exhibiting higher mean somatic subscores on the BDI (z=-3.7, p=.001); and a trend towards higher scores on the vegetative (z=-1.7, p=.07) and self-reproach (z=-1.6, p=.10) subscores. Higher self-reproach and somatic subscores were correlated with slower heart rates (rho=.67, p=.006 and rho=.55, p=.02), respectively. Relatedly, higher self-reproach scores correlated with increased HRV (rho=.73, p=.008). In contrast, high versus low somatic score groups exhibited lower HRV during paced breathing (H=7.2, p=.02). Overall, during unpaced breathing, higher somatic and self-reproach scores were associated with a diminished sympathetic response. In contrast, during paced breathing, the level of entrainment of respiration and heart rate in the high somatic score group was diminished and manifested as lower HRV and reduced parasympathetic control. Mechanisms mediating patterns of physiological reactivity may, therefore, be differentially associated with certain types of depression.

176.7

HOSTILITY and IRRITABILITY PREDICT PARIETAL P300 EVOKED POTENTIAL AMPLITUDE. Mallery D. Gilbert* and Jim H. Patton. Neuroscience Program, Baylor University, Waco, TX 76798-7334.

Twelve college females were selected from a larger group based on their total scores on the 75-item Buss-Durkee Hostility Inventory (BDHI). Six subjects had scores in the lowest quartile (below 27) and six had scores in the highest quartile (above 45) of the larger sample. Evoked potentials were recorded from a 24-lead Neurodata system running on a Macintosh Power Mac 7100 while subjects performed an auditory P300 oddball task. Subjects with the highest hostility scores had the lowest P300 peak amplitudes. While there was a trend toward significance over the entire parietal region (PZ, P3, P4) the effects were statistically reliable in the central (PZ: $t = 2.715$; $p<0.035$) and left (P3: $t = 2.649$; $p< 0.027$) parietal areas. When female subjects were classified by BDHI Irritability subscale scores the P300 amplitude was again reduced over parietal areas, but the statistically reliable effect was limited to the left parietal lobe (P3: $t = 2.587$; $p < 0.0181$). Interestingly, female subjects who scored at the extremes on the BDHI, either total or Irritability, also scored reliably different on the Barratt Impulsivity Scale (BIS-11) total score and all three subscale scores. These results support the thesis that there may be a relationship between predictors of violence and aggression such as hostility, irritability and impulsivity and left parietal lobe function.

176.8

TICKLISH RELATIONSHIPS: AN ENIGMA RESOLVED. R. R. Provine*, B. Fischer and K. Taylor. Dept. of Psychology, UMBC, Baltimore, MD 21228.

Tickle is an ancient and elusive behavior associated with laughter that has not yielded its secrets to anatomists and physiologists. The present questionnaire study (N = 215) of who tickles who and what they think about it reveals the essential social nature of tickle, a critical variable missing in most laboratory studies of cutaneous sensation. Tickle is the product of a social interaction between a "tickler," the person administering the stimulus, and the "ticklee," the person being stimulated. There is no sensation of tickle without the complicity of the tickler. Solo tickle is even emptier than solo sex. This insight is consistent with one of the few points of agreement about tickle, that you can't tickle yourself. (False alarms from self-stimulation are prevented by an efferent process that also enhances the detection of non-self animate objects on the skin surface.) The social nature of tickle is emphasized further by patterns of who tickles who. People both tickle and are tickled, and desire to tickle and be tickled, by members of the opposite sex, especially those with whom they have a close or romantic relationship, a pattern consistent with the report of tickle as an expression of affection. Tickle often brings pleasure and laughter to both ticklee and tickler. Tickle is a central component of the intimate and emotionally charged medium of touch (e.g., caress, stroke, hug, fondle, grope) that is important in establishing and maintaining social bonds between mother and infant, relatives, friends, and especially lovers. Tickle is present in chimpanzees and is a principal stimulus for what may be the most ancient form of laughter (Am. Sci., 84 (1996) p. 38). Tickle is a reminder that we must consider the social and emotional contexts of neurobehavioral evolution and function.

176.9

CONDITIONED PATTERN PREFERENCE WITHOUT AWARENESS IN HUMANS. I.S. Johnsrude*, V. W. Zhao, A.M. Owen, and N.M. White. Cog. Neuroscience Unit, Montreal Neurol. Inst., McGill Univ., Montreal, QC, Canada H3A 2B4.

Conditioned preferences for places or visual cues have been demonstrated previously in rats and in other non-human species. In this study, a conditioned pattern preference was induced in neurologically normal human participants without their explicit knowledge using a novel computerized touchscreen procedure. Three abstract monochrome patterns, presented incidentally over 180 trials in the context of a cognitive estimation task, were randomly assigned to one of three reinforcement contingencies. One pattern was paired with positive visual and auditory feedback together with food reward on 90% of the trials in which it was presented, and with negative visual and auditory feedback together with no food reward on the other 10% of trials. The other patterns were similarly reinforced, but at ratios of 50%:50% and 10%:90% with positive and negative feedback respectively. Subsequently, participants preferred the 'positive' pattern (the pattern paired most often with positive reinforcement), to the 'negative' pattern (the pattern paired least often with positive reinforcement). Importantly however, the participants were not able to relate their preference explicitly to the conditioning procedure. In a subsequent study, we tested a group of patients who had undergone unilateral temporal-lobe resection, a procedure that includes removal of the amygdaloid region. Preliminary results suggest attenuation of the conditioned preference in these patients. Thus, conditioned preferences similar to those demonstrated previously in non-human species may be induced in humans in the absence of conscious awareness, and, as in non-human species, may depend on circuits involving the amygdaloid region.

This work was supported in part by the Medical Research Council of Canada.

176.10

CONTRIBUTION OF CORTICAL AND SUBCORTICAL BRAIN STRUCTURES TO AFFECTIVE PROCESSING. C. Breitenstein, I. Daum, H. Ackermann and W. Larbig*. Institute of Medical Psychology, University of Tuebingen, 72074 Tuebingen, Germany.

Deficits in the processing of facial and prosodic expressions were studied in four groups of patients with cortical lesions (n=32), in two groups of patients with subcortical dysfunctions (Parkinson's disease=PD; n=14) as well as in healthy control subjects (n=12). A standardized test battery for measuring emotional perception and production was administered to all subjects. The results show that both patients with damage to the frontal right hemisphere and patients in advanced stages of Parkinson's disease were significantly impaired in affect recognition in both modalities (facial expression, prosody). Affective prosodic expression, as measured by three acoustic parameters, was reduced in the PD group only. The findings imply the involvement of the fronto-striatal circuitry in emotional processing.

Supported by the Deutsche Forschungsgemeinschaft (SFB 307).

MOTIVATION AND EMOTION: LESIONS

177.1

EXCITOTOXIC LESIONS OF THE PEDUNCULOPONTINE AND LATERODORSAL TEGMENTAL NUCLEI IN RATS: II. EFFECTS ON CONDITIONED PLACE PREFERENCE. G.L. Keating* and P. Winn. School of Psychology, Univ. St Andrews, Fife, KY16 9JU. Scotland.

The pedunculopontine and laterodorsal tegmental nuclei (PPTg, LDTg) both contain heterogeneous neuronal populations and are organized in parallel. In a series of lesion studies, we and others have shown that PPTg loss does not impair basic activities such as feeding, drinking or locomotion but does impair various reward-related behaviors, including acquisition of conditioned place preferences. We have argued that this disruption is a consequence of interference with striatal outflow (*see Inglis WL and Winn P (1995) Prog. Neurobiol. 47 1*). However, PPTg and LDTg also affect striatal input: PPTg innervates A9 dopamine neurons while LDTg neurons project to A10. Since A10 rather than A9 dopamine neurons have been associated with reward, the LDTg, by virtue of its direct excitatory connections to these, might be expected to influence reward-related behavior. Having shown that LDTg lesions do not alter daily food or water intake, or locomotion, we investigated the acquisition of place preferences. We made bilateral ibotenate lesions in the LDTg and, in a separate group of rats, the PPTg. Control rats received phosphate buffer injections. Following surgery we habituated rats to a standard place preference apparatus after which systematic side pairings were run: one side of the apparatus had food present, the other side had no food. Different groups of rats were tested in food-deprived and non-deprived states. Unlike sham lesioned control rats, neither PPTg nor LDTg lesioned rats acquired normal place preferences. This confirms that the PPTg is important in regulating reward-related behavior, and shows for the first time that, as might be expected from their anatomical connections with mesolimbic dopamine neurons, LDTg lesions also interfere with reward-related behavior.

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177.2

LESIONS OF THE CAUDAL PERIAQUEDUCTAL GREY DO NOT ATTENUATE RATS' DEFENSIVE AVOIDANCE OF A PREDATOR. B. M. De Oca* and M. S. Fanselow. Dept. of Psychology, University of California, Los Angeles, CA 90095-1563.

The midbrain periaqueductal grey (PAG) is a structure implicated in fear and defense-related behaviors. Lesion studies suggest that the caudal region ventral to the aqueduct (vPAG) is critical for the defensive behavior of freezing while the region dorsal and lateral to the aqueduct (dlPAG) is implicated in circa-strike defensive behavior. Studies using chemical stimulation of the PAG suggest that avoidance behaviors are also mediated by the PAG, particularly the dlPAG. However, these studies did not examine responses to a natural predator. In order to determine whether the PAG is necessary for both freezing and avoidance behaviors, rats with lesions of either the dlPAG, vPAG or sham lesions were presented with a cat in a long runway apparatus. Significant levels of freezing, and avoidance were observed in response to the cat but not a stuffed animal used as a control stimulus. Lesions of the vPAG or dlPAG did not alter the pattern of avoidance demonstrated by rats in response to a cat. However, lesions of the vPAG did attenuate freezing. Thus, while the vPAG is necessary for freezing, neither the vPAG nor the dlPAG are necessary for defensive avoidance. In contrast to the specificity shown by lesions of the PAG, lesions of the amygdala have been reported to attenuate both the autonomic and behavioral components of defensive responses in a variety of measures. As the PAG is an efferent of the amygdala, the possibility that different defensive behaviors are mediated by different efferents of the amygdala was examined. Supported by MH 39786 to MSF.

177.3

EXCITOTOXIC LESIONS OF THE PEDUNCULOPONTINE AND LATERODORSAL TEGMENTAL NUCLEI IN RATS: I. EFFECTS ON INTAKE AND DAILY PATTERN OF LOCOMOTION. P. Winn,* M.P. Latimer, A.J. Brewin and C. O'Nions. School of Psychology, Univ. St Andrews, Fife, KY16 9JU, Scotland.

The pedunculopontine and laterodorsal tegmental nuclei (PPTg, LDTg) both contain heterogeneous neuronal populations and are organized in parallel. In order to understand better PPTg functions we have undertaken a series of lesion experiments (*Inglis WL and Winn P (1995) Prog. Neurobiol. 47, 1-29*) and shown in acute tests that neither drug-induced nor spontaneous locomotion is affected by PPTg lesion. Feeding and drinking are also unaffected by PPTg lesions, though reward-related responding is altered. The LDTg has not been closely examined to determine its psychological functions. Before doing so it is necessary to consider what effect LDTg lesions have on basic processes such as feeding, drinking, and locomotion. In these experiments we measured, for the first time in LDTg lesioned rats, intake and locomotion over 24h; daily locomotion has not previously been measured in PPTg lesioned rats. We made bilateral ibotenate lesions in LDTg and, in a separate group, PPTg. Control rats received phosphate buffer injections. Following surgery we measured feeding and drinking in the home cage, followed by measurement of locomotor activity over 24h in cages equipped with photocell beams to which the rats had been habituated for 4 days. Neither lesion affected food or water intake, total locomotion over 24h or the amount of locomotion in the different phases of the light-dark cycle. PPTg lesioned rats showed fewer, longer bouts of locomotion than either LDTg or sham lesioned rats. These data show that neither PPTg nor LDTg lesions affect basic activities such as feeding, drinking or amount of locomotion, but PPTg, not LDTg, lesions did affect the pattern of locomotion.

Supported by the St Andrews University School of Psychology Research Budget.

177.4

ANXIETY-RELATED BEHAVIOR OF THE MAS KNOCK-OUT MOUSE J.-P.Voigt*, M. Bader[1], Th. Walther[1] and H.Fink Institute of Pharmacology and Toxicology, Medical Faculty (Charité) of the Humboldt University at Berlin, D-10098 Berlin, [1]MDC, D-13125 Berlin-Buch, Germany.

The *mas* proto-oncogene is expressed in brain regions (hippocampus, cerebral cortex) and is developmentally regulated. Since the product of the *mas* gene may be involved in the development of the brain, behavioral consequences are likely to occur following a knock-out of the gene. The present study was performed to detect effects of the knock-out on anxiety related behavior. For this purpose, $mas^{+/0}$ mice, $mas^{0/0}$ and their wild-type controls were subjected to the murine Plus-maze. Each animal underwent a test of 10 min duration. The behavior was recorded and videotaped. Two indices of anxiety were obtained in the present experiment: the number of entries into open arms expressed as percentage of the total number of arm entries, and the amount of time spent on the open arms as percentage of total time. In addition, the total number of entries into both open and closed arms was counted as a measure of locomotor activity. Homozygous knock-out mice entered less often the open arms of the maze and spent less time on this section. Heterozygous mice showed an intermediate behavior, but they were not significantly different from either the homozygous or the wild-type control. However, no significant overall group differences were found in total entries. The lack of overall statistical significance on this parameter suggests specificity of the anxiety measure. Upon a second exposure to the maze the behavioral profile remained the same, except that locomotor activity was reduced in all groups. The latter suggests memorization of the first exposure in all experimental groups. In conclusion, the behavioral data obtained from the Plus-maze experiment suggest a significantly higher level of anxiety in the $mas^{0/0}$ mice compared to wild-type control mice. (Supported by DFG INK 21/A1-1, Germany)

177.5

LESIONS OF THE NUCLEUS BASALIS MAGNOCELLULARIS ENHANCE CONDITIONED APPROACH RESPONSES TO STIMULI PREDICTIVE OF FOOD. M.C. Olmstead*, T.W. Robbins and B.J. Everitt. Dept. of Exper.Psychology, Univ. of Cambridge, Downing St., Cambridge, CB2 3EB, UK.

The nucleus basalis magnocellularis (NBM) receives efferents from the ventral striatum (VS) and projects to neural structures implicated in reward including the basolateral amygdala and prefrontal cortex. NBM neurons are in turn influenced by dopamine signals from the VS and respond to reward and stimuli associated with reward. These findings suggest that the NBM may be part of the neural systems that mediates incentive motivation. The present study examined this hypothesis by investigating the effects of NBM lesions on locomotor responses to appetitive stimuli and the acquisition of responding with conditioned reinforcement (CR). Male Lister-Hooded rats received bilateral lesions of the NBM induced by infusions of 1*u*l (0.5 *u*l per side) of a 0.01M solution of the excitotoxic amino acid AMPA, which relatively selectively destroys magnocellular cholinergic neurons but not surrounding pallidal areas. Brain sections were processed immunohistochemically to reveal neurons immunoreactive for nerve growth factor and cholinergic cell loss was quantified by image analysis. NBM lesions enhanced the locomotor response to a novel environment and increased the magnitude of hyperactivity conditioned to the presentation of food, but had no effect on the unconditioned locomotor stimulating effects of d-amphetamine (0.5, 1.5 and 5.0 mg/kg i.p). During CR training animals underwent Pavlovian conditioning in which the presentation of a CS (5 s illumination of a tray light) preceded a US (5 s elevation of a sucrose filled dipper). The ratio of responding during the CS compared to the pre-CS period increased across trials in both groups of animals, but to a lesser extent in rats with NBM lesions. In contrast, NBM lesions did not disrupt the acquisition of responding with CR and appeared to enhance its potentiation by systemic amphetamine (0.2, 0.4, 0.8 mg/kg i.p.). The effects of quinolinic acid lesions of the basal forebrain, which preferentially destroy non-cholinergic pallidal neurons but relatively spare cholinergic NBM neurons, on the two behavioural tasks are currently being investigated.

MC Olmstead is supported by a Human Frontier Science Program Organization Fellowship.

177.6

DYNAMIC CHANGES IN CYTOCHROME OXIDASE ACTIVITY IN AMYGDALOID AREAS AFTER LESION OF REWARDING LATERAL HYPOTHALAMIC SITES. R. Shiao, and C. Bielajew . School of Psychology, University of Ottawa, ON K1N-6N5, Canada.

There are limited amygdaloid areas, such as the central and cortical nuclei, that receive direct projections from the lateral hypothalamic sites. Here we report the changes in cytochrome oxidase activity (COA) induced by electrolytic lesion of lateral hypothalamus that support self-stimulation, using histochemical and photometric semi-quantitation techniques. Sixteen adult rats with unilaterally implanted electrodes aimed at the lateral hypothalamus that give rise to vigorous bar-press behaviour were perfused to examine the changes in COA following different periods after lesion. Overall we observed a discrete reduction of oxidative metabolism in the cortical nucleus of the amygdala compared with the contralateral side. These effects were seen two to four weeks after lesion, and there was some recovery in COA 8 weeks after lesion. A lesser decrease in COA in the neighbouring piriform cortex was also observed. No similar changes were found in the basolateral, central, and medial nuclei of the amygdala. One characteristic of the COA activity is that it occurred in specific areas of cortical nucleus, namely, the frontal-middle region. The functional significance of these alterations in cytochrome oxidase activity in discrete amygdaloid areas to rewarding lateral hypothalamus is under investigation. Project supported by an NSERC grant to CB.

177.7

FIMBRIA-FORNIX LESIONS AND MODULATION OF PROGRESSIVE RATIO RESPONDING FOR FOOD IN THE RAT. A. M. Bratt*, J. P. Knowles and G. Mittleman. Dept. of Psychology, University of Memphis, Memphis, TN 38152.

Our laboratory has reported on the role of the hippocampus in rewarded responding (Schmelzeis & Mittleman, 1996). In a progressive-ratio (PR) operant task, bilateral neurotoxic lesions of the total hippocampus resulted in enduring increases in breakpoint (BP); an indicator of the amount of effort an animal will exert to obtain a reinforcer. The current experiments aimed to investigate PR responding in rats with electrolytic lesions of the fimbria-fornix (FIFO).

Twenty young adult male rats (350-380g), food deprived to 85% free-feeding weight, were trained to lever press for a food reward (Noyes, 45mg grain-based pellet) in operant chambers, initially on a fixed ratio (FR) schedule (10 trials), progressing on to a PR10 schedule. Efficient PR responding required the rat to exert progressively more effort to obtain successive reinforcement, until breakpoint was reached. Upon attaining a stable baseline response, 12 of the subjects received electrolytic FIFO lesions (1mA, 40s, anodal current, coordinates: AP: -1.3, -1.5mm; L: ±1.5, ±0.5mm; DV: -3.6, -3.3mm) and PR responding was monitored immediately thereafter for 50 post-operative trials. Significant increases in breakpoint were observed in FIFO rats, as compared to controls, over the initial 25 trials, however, this effect was lost in subsequent trials, with control rats increasing responding with training. Prefeeding both groups of rats with 50, 100, 150 & 200 grain pellets prior to PR testing decreased breakpoint for both groups, however FIFO rats showed no performance effects indicative of differential satiety levels. These data suggest that partial deafferentation of the hippocampal formation following destruction of the FIFO pathway does not mimic the effects on rewarded responding obtained with total hippocampal loss. This may indicate the role of more specific intrinsic hippocampal circuitry in addition to input/output FIFO pathways in rewarded responding.*A. Bratt holds a Welcome Trust post-doctoral travel grant.

177.8

ELECTROLYTIC LESIONS OF THE VENTRAL TEGMENTAL AREA ATTENUATE HYPERACTIVITY ELICITED BY INFUSING LOW DOSES OF KAINIC ACID INTO THE LATERAL HYPOTHALAMUS IN THE RAT. M.R. Pitzer* and D. Wirtshafter. Dept. of Psychology, University of Illinois at Chicago, Chicago, IL. 60612.

Previously we have reported that low doses of the L-glutamate analog kainic acid (KA) elicit dose-dependent increases in locomotor activity when injected into three separate rostro-caudal levels of the lateral hypothalamus (LH). These results suggest that the activation of cells throughout the LH region, without the recruitment of fibers of passage, is capable of eliciting hyperactivity. One theory to account for these results is that the relevant LH fibers either pass through or terminate in the ventral tegmental area (VTA). In order to investigate this possibility, intra-LH KA-elicited hyperactivity was measured following unilateral electrolytic VTA lesions. In rats implanted with bilateral cannulae directed toward the LH, intra-LH KA (10ng/0.25ul) elicited hyperactivity was significantly attenuated when injections were made into the ipsilateral LH. In contrast, lesions had less of an effect when injections were made into the contralateral LH. These results suggest that the relevant LH fibers may pass through the region of the VTA or that, perhaps, VTA cells are involved in LH-elicited hyperactivity. To explore these possibilities, experiments are currently being conducted to assess intra-LH KA elicited hyperactivity following excitotoxic lesions of VTA neurons. Research supported by NS33992.

177.9

REACTION TIME PERFORMANCE IN RATS FOLLOWING OCCLUSION OF THE ANTERIOR CEREBRAL ARTERY

N.M. Ward*, J. Sharkey and V.J. Brown Sch. of Psychology, Univ. St. Andrews, St. Andrews KY16 9JU, and Fujisawa Institute of Neuroscience, Dept. of Pharmacology, Univ. Edinburgh, Edinburgh EH8 9JZ, Scotland.

Occlusion of the anterior cerebral artery (effected by stereotaxic application of the vasoconstrictor, endothelin-1, at the artery anterior to the Circle of Willis) results in widespread ischemic damage to cingulate and infralimbic cortex and medial septum. The cortical areas compromised by the lesion have been thought to be involved in limbic functions, whereby motivational information can influence motor output. Therefore, to assess the behavioral consequences of this lesion, rats were tested on a reaction time task in which motivation is manipulated from trial to trial by the provision of visual cues indicating the proximity of reward.

Rats were trained to maintain a sustained nose-poke into a response hole until a temporally unpredictable tone sounded, whereupon they withdrew the snout from the hole and turned to press a panel covering a food hopper. Visual cues (a progressive change in brightness of peripheral lights) indicated the availability of reward, which was either on the current trial or after two or three correct responses (a cued multiple-ratio schedule of reinforcement).

As fewer trials intervened before reward, reaction times were faster and there were fewer errors. Following the lesion, there was no behavioral impairment: the rats were still able to interpret the meaning of the cues which continued to be reflected in both speed and accuracy of performance. Furthermore, when the meaning of the cue lights was reversed, the lesioned rats were able to learn the new relationship as rapidly as unlesioned control rats.

(Supported by Fujisawa Institute of Neuroscience. NMW is supported by an MRC (UK) collaborative studentship with Fujisawa Institute of Neuroscience).

177.10

LESIONS OF THE RAT VENTRAL STRIATUM CHANGE PERFORMANCE IN A PROGRESSIVE FIXED-RATIO SCHEDULE OF REINFORCEMENT WITHOUT AFFECTING REACTION TIMES WHEN VISUAL CUES INDICATE REWARD COST

E.M. Bowman* and V.J. Brown School of Psychology, Univ. St. Andrews, St. Andrews KY16 9JU, Scotland.

This study examined the possibility that lesions of the nucleus accumbens in rats impair the perception of the "cost of reward", as defined by the number of operant responses to obtain it. In the first experiment, visual cues indicated the cost of reward, which was either available on the current trial or after two or three correct responses (a multiple-ratio schedule of reinforcement). Reaction time was faster and performance more accurate as fewer trials intervened before reward. Following lesions centred on the core of the nucleus accumbens, this pattern was preserved, demonstrating that the integrity of the nucleus accumbens is not required for the interpretation of visual cues carrying motivational information or for the translation of this motivational signal to response vigor. In a second experiment, the cost of reward in terms of the number of lever presses required for each reward incremented with each trial (i.e., a progressive ratio schedule of reinforcement). In this task, there were no external cues to signal the increasing cost: only the internalized knowledge of the position in the schedule gave indication of the current cost of reward. Following lesions of the nucleus accumbens, rats showed a pattern of behaviour indicating an impairment in the ability to monitor position within the schedule of reinforcement. Specifically, "breaking points" were higher and latency to resume responding with each increment in the schedule was lower. The results are considered in terms of the traditional idea of the nucleus accumbens as a "limbic-motor interface". We suggest that the nucleus accumbens serves functions which might be regarded as those of an interface between the limbic and motor systems in only limited contexts. (Supported by the MRC (UK): G9319670N.)

177.11

SOCIOEMOTIONAL BEHAVIOR IN ADULT RHESUS MONKEYS AFTER EARLY VERSUS LATE LESIONS OF THE HIPPOCAMPAL FORMATION.

J. H. Chaudhuri, L. Malkova, J. Bachevalier[2], S. J. Suomi[1], and M. Mishkin*, Lab. of Neuropsychology, NIMH, 1 Lab. of Comparative Ethology, NICHD, NIH, Bethesda, MD 20892, 2 University of Texas, Houston, TX 77030. USA

Our earlier studies in rhesus monkeys demonstrated that early damage to the hippocampal formation yielded only subtle changes in socioemotional behavior at 2 months of age and these changes became even less evident by 6 months of age. When the same monkeys were observed as adults in dyads with their normal peers, the amount of time they spent in social contact was markedly less than that in normal dyads, and they exhibited more locomotor stereotypies than normal controls (Beauregard et al., NeuroReport 6:2521-2526, 1995). To determine whether this degree of behavioral abnormality would follow the same damage to the mature brain, we prepared adult monkeys with bilateral removals of the hippocampal formation and the underlying cortex (H) and compared their behavior with that of i) normal adult monkeys and ii) adult monkeys with the same lesions sustained early in infancy from our previous study. Dyads consisting of either one operated monkey and its normal control (2 pairs, each including a monkey operated in adulthood, H+N; 5 pairs, each including an adult monkey operated in infancy, neoH+N), or two normal monkeys (7 pairs, N+N) were examined in a large enclosure and their behavior videorecorded for two 5-minute intervals (10 AM and 4 PM) on each of five consecutive days. The average amount of social interaction per observation in the H+N dyads (196 sec) was less than that in N+N dyads (281 sec) but more than in the neoH+N dyads (66 sec). In addition, the monkeys operated in adulthood did not exhibit any locomotor stereotypies. These results indicate that early damage to the hippocampal formation yields more profound socioemotional effects than the same damage in adulthood, a pattern that is similar to that we reported for combined lesions of the amygdala and hippocampus (Malkova et al., Soc. Neurosci. Abstr. 20:368,1994). Interestingly, the hippocampal lesions alone appear to produce a decrease in social contact comparable to that of combined amygdalohippocampal removals. Supported by NIMH-IRP, NICHD-IRP, and NIMH 49278.

177.12

EARLY CENTRAL SEROTONIN DAMAGE INCREASES "ANXIOUS" BEHAVIORS IN JUVENILE RATS.

B. Knutson*, J. Panksepp, T. K. Narayanana, and J. Rossi III. Department of Psychology, Bowling Green Univ., Bowling Green, OH 43402.

Although the "serotonin-anxiety" hypothesis postulates that enhanced serotonergic tone can increase anxiety, recent clinical trials suggest that serotonin-enhancing compounds (e.g., SSRIs) can effectively remediate some anxiety disorders in humans (e.g., social phobia). Comparative studies on the effects of serotonergic manipulations on anxious behavior have yielded mixed results (Johnston & File (1986), Pharm., Biochem., & Behav., 1467-70). As a strong test of the yet ambiguous role of serotonin in anxiety behaviors, we lesioned the central serotonergic systems of 35 young rats with 5,7 dihydroxytryptamine and observed the effects on behavioral tests of anxiety, relative to 35 sham-lesioned controls. In Experiment 1, subjects were placed in an open field for 5 minutes, and line crosses, rears, locomotion away from the wall, and fecal bolli were recorded. In Experiment 2, subjects were placed in an elevated plus maze and the number and duration of entries into closed and open arms was recorded, as well as "risk assessments." Finally, in Experiment 3, subjects were placed in a habituated shuttlebox and their locomotor activity was recorded. In both Experiments 1 and 2, 5HT-lesioned subjects showed increased "anxious" behavior compared to sham-lesioned subjects, but also decreased locomotion. However, in Experiment 3, there was no difference in the locomotor activity of 5HT-lesioned versus sham-lesioned subjects. HPLC analyses were conducted on brain amines and their metabolites in these subjects and correlated with indices of anxious behavior. The behavioral results suggest that the serotonin system is not necessary for the expression of anxious behavior. Further, as a whole, brain serotonin may play more of an inhibitory than a facilitative role in behavior linked to this negative emotional state.
Supported by Naval Medical Research and Development Command.

NEUROETHOLOGY: OTHER SYSTEMS

178.1

STRIKE BEHAVIOR IN THE MUSKELLUNGE, *ESOX MASQUINONGY*: RELATIVE CONTRIBUTIONS OF LATERAL LINE AND VISUAL SENSORY SYSTEMS.

L. Alborg, S. Coombs and J.C. New*, Dept. of Biology and Parmly Hearing Institute, Loyola University Chicago, Chicago, IL 60626

The muskellunge, *Esox masquinongy*, possesses both well-developed visual and lateral line systems. They are voracious predators, displaying robust feeding behavior in captivity. The purpose of this study is to analyze the strike feeding behavior of the muskellunge and to determine the role played by the visual and lateral line senses in organizing the strike.

Juvenile muskies in a test arena were presented with live fathead minnows (*Pimephales promelas*) and their strikes and feeding behavior was videotaped and analyzed. Muskies were tested under three conditions: (1) control animals, (2) animals with the lateral line suppressed via immersion in 0.1 mmol $CoCl_2$, and (3) animals blinded via bilateral optic nerve transection. Quantitative measures (distances, angles and times) were obtained for two positions during discrete phases of the attack, and compared across the three groups.

Muskies initiate their approach to prey with a slow, directed stalk using fin sculling but little body trunk movement to decrease angle and distance to the prey. This phase is followed by an explosive C- or S-start strike directed at the target. Additionally, muskies employ different strategies in striking at near or far prey. Our data indicate that both lateral line and vision play important roles in directing the strike behavior: muskies with suppressed lateral line systems align themselves with prey but hesitate or have difficulty in intiating the rapid strike. Blinded muskies will strike at minnows, but the apparent detection range of prey is greatly decreased.

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178.2

SEXUALLY DIMORPHIC SWIMBLADDER MUSCLES IN AN *ELECTRIC FISH*.

X Huang, J Kozloski, & JD Crawford.* Dept of Psychology, University of Pennsylvania, Philadelphia, PA 19104.

In the African fish *Pollimyrus* (Mormyridae), males produce acoustic signals when courting females. While it is not know how these sounds are produced, these fish have a swim bladder, and other teleost fishes are known to generate sounds by coupling rapid muscle contractions to a bladder. We have recently discovered that *Pollimyrus* has a pair of extrinsic muscles enveloping the posterior end of the swimbladder. These muscles are clearly distinguished from the adjacent trunk muscles by their orientation orthogonal to the trunk muscles (ie the swim bladder muscles are aligned in the dorso-ventral plane). Just like the acoustic behavior of breeding adults, there is a conspicuous sexual difference in these muscles: the muscles are 5-fold larger in males than in females ($P<0.02$, 5 df, t-test). In behavioral tests males became temporarily mute when this muscle was injected with local anesthetic, but not when anesthetic was injected into the trunk muscles, nor when saline was injected into the swimbladder muscles. We suspect that these muscles are involved in male sound production. The mechanisms of sound production in these fish are of interest since their sonic repertoire is unusually complex and because analysis of the sounds indicates that the sonic muscles may normally contract at high rates (340 Hz at 28°C), exceeding rates known for other sonic muscles. NIH R01 DC01252; PA Lions HRF GA1205; U of Penn Res Foundation.

178.3

TIME CODING IN THE AUDITORY MEDULLA OF A SONIC FISH. James Kozloski*, John D. Crawford. Institute of Neurological Sciences & Dept. of Psychology, Univ. of Pennsylvania, Philadelphia, PA 19104.

We are examining temporal coding in the auditory medulla of the mormyrid fish *Pollimyrus*. The auditory periphery of this vertebrate is simple and shows little specialization for mechanical frequency analysis. *Pollimyrus* uses a repertoire of temporally structured sonic communication signals in its courtship behavior, and cells in the midbrain show selectivity for temporal features of sounds. We have identified two types of neurons in the medulla which appear to represent critical steps in the processing of temporal information. The first type shows broad tone-frequency selectivity in the 100 to 900 Hz band, has shallow, non-saturating rate level functions, and fires small bursts of spikes synchronized to each stimulus cycle. Intracellular dye fills indicate that these are primary afferents recorded as they course through the medulla. The second type shows similar broad tuning, but has steep, saturating rate-level functions, and generates a single spike per stimulus cycle over a wide range of frequencies; these cells show exceptionally strong phase-locking. Intracellular fills indicate that these cells are second-order auditory neurons in the descending nucleus of the medulla. These cells thus transform intensity-dependent afferent input into a relatively intensity-invariant output of a single spike synchronized to each stimulus cycle. We conclude that this part of the ascending auditory pathway functions to isolate temporal information from intensity information for further analysis in the midbrain. [NIH RO1 DC01252-04; PALHRFGA1205; U of P Res F]

178.4

CHARACTERISTICS OF DIRECTIONAL AUDITORY AFFERENTS IN THE TOADFISH (*OPSANUS TAU*). P.L. Edds-Walton, S.M. Highstein, and R.R. Fay*. Marine Biological Laboratory, Woods Hole, MA 02543

The sensory hair cells on the saccule of fishes are arranged in different orientation patterns that may be important for encoding the direction of acoustic particle motion for directional hearing analyses in the CNS. We have been working with the toadfish saccule because directional responses to sound have been reported for this species, and the saccule is an auditory organ in toadfish. A particle motion stimulus was produced by sinusoidal oscillations (nm displacements) of a rigid, water-filled dish in which the fish was firmly held by the head. The fish was stimulated along six axes (0, 30, 60, 90, 120, 150°) in both horizontal and midsagittal planes. Initially, we recorded extracellularly from over 200 afferents to characterize directional response from the rostral, middle, and caudal regions of the saccule. Most afferents were highly directional with wide-ranging "best directions." We are currently recording intracellularly from saccular afferents. Once characterized, the cell is filled with neurobiotin (Vector) to compare the dendritic arbors and central projections of cells with different response properties. Axon diameters (1.7-8.8 μm), minimum number of terminal endings (7-114), and arbor lengths, widths, and areas have been determined for directional fibers from different regions of the saccule. Dendritic arbor drawings will be presented along with the response properties of each (threshold, best direction, spontaneous activity). (Funded by Program Project Grant 1P01DC018737 from NIH, NIDCD).

178.5

VOCAL PATHWAYS IN CATFISH: COMPARISON WITH OTHER VOCAL TELEOSTS. F. Ladich* and A.H. Bass. Section of Neurobiology and Behavior, Cornell Univ., Ithaca, NY 14853; Institute of Zoology, Univ. Vienna, Althanstraße 14, 1090, Vienna, Austria.

The diversity of sonic/vocal organs, and central pathways delineated by retrograde transport of biotin compounds, was analyzed in four families of catfish (order Siluriformes) and compared to representative families of two distantly related orders, the Scorpaeniformes (families Cottidae and Triglidae) and the Batrachoidiformes (family Batrachoididae). In all groups, sonic motoneurons (SMNs) are located in the medulla oblongata and rostral spinal cord and exit the CNS via occipital or occipital and spinal nerves. Some catfish have two sonic mechanisms - pectoral spine stridulation and swimbladder (SB) vibration established by "drumming muscles". The latter differ between catfish families in origin and insertion and in motoneuron supply. Paired motor nuclei are located on the midline between the fourth ventricle or central canal and the medial longitudinal fasciculus (MLF) (Doradidae, Mochokidae), lateral to the MLF (Ariidae) or at both locations (Pimelodidae). Pectoral spine-associated motoneurons, however, are always located within the ventrolateral motor column (VMC), lateral to the ventral fasciculus. Contrary to catfishes, the diversity of sonic mechanisms in scorpaeniforms is not correlated with different SMN positions. Cottids lack a SB although sonic muscles originate at the occipital cranium and insert at the pectoral girdle (PG); SMNs are in the VMC. Triglids also have VMC-positioned SMNs but "intrinsic" muscles directly attached to the SB only; ontogenetic data however report a transitory attachment of SB muscles to the PG (Rauther, 1945; Zool. Jb. Anat. 69:159). Batrachoidids possess intrinsic SB muscles that are never attached to the PG and always supplied by midline SMNs positioned as in doradid and mochokid catfish. We propose that a VMC vs. non-VMC position for SMNs is coupled to respectively PG vs. non-PG associated sonic muscles. (Austrian Science Foundation FWF Grant 10295 to FL and NSF Grant IBN-9421319 to AHB.)

178.6

AUDITORY PATHWAYS IN A VOCAL FISH: INPUTS TO A MIDBRAIN NUCLEUS ENCODING ACOUSTIC BEATS. A.H. Bass*, D. A. Bodnar and M. Marchaterre. Neurobiology & Behavior, Cornell Univ., Ithaca, NY 14853.

Male midshipman fish, *Porichthys notatus*, generate long duration (minutes to > 1 h) vocalizations known as "hums" that attract females to nest sites. Males often vocalize simultaneously which results in beat-like waveforms with envelope modulations at the difference frequencies (dF) between hums. Recordings with indium and 5% neurobiotin-filled electrodes revealed neurons in the midbrain's nucleus centralis (NC) that synchronize their spike outputs to the dF of synthetic beats. (NC is a division of the torus semicircularis, homologue of the mammalian inferior colliculus.) The locations of dF-encoding neurons were identified in a far medial region of NC following iontophoretic injections of neurobiotin. Retrograde transport distinguished multiple sources of input to NC (survival times, 1-6h). The majority of labelled neurons were clustered throughout various divisions of a secondary octaval nucleus (SO) comparable in location to that of other teleosts (McCormick & Hernandez, Brain Behav. Evol. 47:113, 1996). However, a SO appears more expansive in midshipman, extending ventrolaterally to far rostral levels of the lateral division of the trigeminal motor nucleus (see Bass et al., J. Neurosci. 14:4025, 1994). The SO identified here includes the positions of neurons previously shown to receive input from VIIIth nerve-recipient hindbrain nuclei (Bass et al. 1994). Though less abundant, neurobiotin-filled cells afferent to NC were also located in the descending octaval nucleus (an VIIIth nerve recipient nucleus), hindbrain paraventricular groups, a pretoral dorsal tegmental nucleus, and just dorsolateral to nucleus glomerulosus. To conclude, the majority of inputs to midbrain, dF-encoding neurons arise from secondary rather than primary octaval-targets in the rostromedial medulla. This study now delineates the circuitry underlying central computation of dF in the auditory system of midshipman fish. (NIH, NSF & Cornell Univ.).

178.7

TEMPORAL COMPUTATION OF THE DIFFERENCE FREQUENCY OF CONCURRENT ACOUSTIC SIGNALS IN THE CENTRAL AUDITORY SYSTEM OF A VOCAL FISH. D. A. Bodnar*, J.R. McKibben and A.H. Bass. Neurobiology & Behavior, Cornell Univ., Ithaca, NY 14853.

Male midshipman, *Porichthys notatus*, generate long duration, sinusoidal-like advertisement calls ("hums") with a fundamental frequency (F0) of 90-100 Hz. Males often cluster and hum simultaneously. The summation of two hums generates a beat-like waveform characterized by amplitude and phase modulations at the difference frequencies (dF). To identify and localize individual vocalizations, the auditory system must segregate the concurrent signals of the beat waveform. Responses to beat stimuli with varying dFs (F0A =90 Hz; F0B =90 ±10 Hz in 2 Hz increments) were recorded from auditory VIIIth nerve afferents and midbrain neurons. Synchronization to the individual components is significantly higher for afferents than midbrain units. In contrast, the maximum vector strength (VS) of synchronization to dF is significantly higher for midbrain units. Afferent VS vs. dF profiles are generally V-shaped; synchronization is lowest for dF= ±2 Hz and highest for dFs ≥ ±10 Hz . Auditory midbrain VS vs. dF profiles reflect both untuned and tuned response types for beat stimuli. Untuned units exhibit moderate synchronization to all dFs between ±10 Hz, but no significant changes in their VSdF. DF tuned units exhibit significant changes in their VSdF (ANOVA, effect of dF, $p<0.05$) with distinct peaks at a particular dF. Among the dF tuned units, a subset of units exhibit different responses to positive and negative dFs (asymmetric units). Thus, there is a transformation from an afferent periodicity code of the individual components of low frequency beats to a midbrain periodicity code of their dF. This indicates that in midshipman, a temporal computation of the dF of concurrent signals occurs centrally, suggesting that dF information is utilized in the segregation of concurrent vocal signals. Support from NIH, NSF & Cornell Univ.

178.8

PERIPHERAL ENCODING OF BEHAVIORALLY RELEVANT ACOUSTIC SIGNALS IN A VOCAL FISH. J.R. McKibben* and A.H. Bass. Section of Neurobiology & Behavior, Cornell Univ. Ithaca NY 14853.

The midshipman fish, *Porichthys notatus*, produces simple, constant frequency (F0 90-100 Hz) harmonic sounds by vibrating intrinsic swimbladder muscles. Different vocalizations are used in agonistic and courtship behaviors (Brantley & Bass, Ethology 96:213) and playbacks of signals resembling the long duration "hum" of nesting males elicit phonotaxis by gravid females. We recorded from individual VIIIth nerve afferents innervating the sacculus, the major inner ear end organ, while presenting computer synthesized sounds via an underwater loudspeaker (UW-30 beneath the fish in a 35 cm diameter tank). Auditory afferents (n>200) responded best from 60 to 200 Hz, encompassing the frequency range of midshipman vocalizations. Iso-intensity plots ranged from "low-pass" (highest spike rate at 60-70Hz), to "band pass" (highest spike rates at 140-180Hz) to more complex, multi-peaked curves. Synchronization to pure tones was very high, resulting in more sensitive and less ambiguous encoding of frequency than given by changes in average spike rate. Spike rate increases encoded stimulus level over a 10 to 50 dB range. The ability to maintain firing rates to long duration (5-10 s) stimuli varied with frequency and among units. However, synchronization remained strong at all stimulus durations. Two-tone harmonic stimuli were presented in order to examine the effects of higher harmonics on the encoding of an individual hum; units synchronized to both F0 and F2. Two tones at slightly different frequencies were added to generate stimuli with phase and amplitude variations, or beats, at the difference frequency (dF). This stimulus corresponds to the natural listening task, where the receiver's ear must process the overlapping calls of nearby fish. At most dFs, cells synchronized best to one of the two tones, usually the lower frequency. Generally, synchronization to the dF was much lower than to either of the component tones. In conclusion, the peripheral auditory system of midshipman precisely encodes the temporal waveforms of species-typical signals. (NSF, NIH, NIMH & Cornell U.)

178.9

THE ROLE OF DORSAL TORUS LONGITUDINALIS IN PROCESSING LUMINANCE INFORMATION IN THE GOLDFISH VISUAL SYSTEM. M.A. Gibbs*[1] and D.P.M. Northmore[2]. Depts. of Biology[1] and Psychology[2], University of Delaware, Newark, DE 19716.

The torus longitudinalis (TL) is a unique actinopterygian structure situated along the medial borders of optic tectum, from which dorsal TL receives a topographic visual input. Based on electrophysiological recordings in goldfish TL, which revealed a sustained neural discharge inversely related to luminance (Northmore, 1984), we hypothesize that TL is a specialized luminance processor acting in parallel with the optic tectum that is more involved in color vision. Behavioral and electrophysiological experiments were conducted to determine the role of TL in processing luminance information and whether it is part of a parallel processing visual system. Bilateral ablation of TL resulted in a 66% reduction in the dorsal light reflex, which involves comparison of luminance levels by the two eyes, indicating a significant role for TL as a luminance processor. Additionally, an examination of the dorsal light reflex in TL-ablated fish revealed a loss of gain in the luminance-response function. Multiunit recordings from TL revealed relatively flat, broad-band action spectra that matched behaviorally derived luminance spectra. However, recordings in tectum yielded a variety of spectra, many with pronounced peaks and troughs indicative of chromatic opponent processing. The tectal action spectra averaged about 1 log unit less sensitive than TL spectra. It appears, therefore, that tectum processes chromatic information, but also elaborates a broad-band luminance signal that is relayed to TL without the chromatic information. Together, the results provide evidence for parallel processing of chromatic and luminance processing in the goldfish.

178.10

HOW DO FROGLETS RESPOND WHEN THEY SEE A BUG FOR THE FIRST TIME? N. Licata and A. Weerasuriya*. Mercer University School of Medicine, Division of Basic Sciences, Macon, GA 31207.

Herbivorous tadpoles metamorphose into carnivorous frogs. This entails drastic changes in their gut as well as in their behavior. The objective of this study was to characterize the emergence of prey catching behavior in newly metamorphosed froglets (*Rana pipiens*). Two to three week old tadpoles were maintained in pond water at room temperature. Development was monitored and staged according to Gosner (1960). Emergence of air breathing was noted, and upon reaching stage 46 their prey catching behavior was examined. Prey was either mutant wingless fruit flies (*Drosophila* sp.), small (4-8 mm) wood lice (*Armadillidium* sp.), or newly emerged (<8 mm) mealworm larvae (*Tenebrio* sp.). For each trial, a froglet was placed in a testing arena and presented with one or more of the above prey. The behavior of the froglets was recorded with a high resolution video camera at 30 frames per second. In 10 froglets, prey catching behavior was observed for upto 4 weeks after reaching stage 46. The froglets became air breathers 3 to 9 days before reaching stage 46. The first prey-elicited snap was observed 4 to 12 days (6 ± 1 (S.E.)) after reaching stage 46. All responses were directed at prey and a fully protracted tongue contacted the prey. Eight of the ten snaps were successful in retrieving the prey into the mouth. From the third day after the first snap, seven froglets were presented with 30 mm mealworm larvae; these prey were too big for the froglets to ingest. Nevertheless, all 7 froglets responded to these larvae with targeted prey catching behavior. Therefore, in *Rana pipiens*, within a few days of completing metamorphosis, an unlearned, fully functional and efficient prey catching motor program emerges. On the other hand, visual discrimination of prey objects is less than optimal in froglets. (Supported by NSF IBN-9420525).

178.11

PREY CAPTURE EFFICIENCY IN NORMAL AND MONOCULAR FROGS P. Patton* and A. Weerasuriya. Mercer University School of Medicine, Division of Basic Sciences, Macon, GA 31207.

Anurans capture small prey by means of a ballistic snap, involving a lunge and tongue flip. Capture efficiency was studied with 6 frogs (*Rana pipiens*) between 7 and 9 cm in snout/vent length presented with mealworm targets at distances between 2 and 11 cm. During each presentation, animals were videotaped at 30 frames per second using a camera held vertically above the frog. The images were analysed to determine the location of the tip of the frog's tongue, at greatest extension, with respect to the prey. A total of 193 snaps were observed, of which 91% resulted in successful capture, with success rates ranging from 81-97% for individual frogs. 67% of all misses were due to errors of distance, usually undershooting, with the remainder due to errors in elevation (22%) or azimuth (11%). 85% of snaps directed at targets placed frontally within 30° of the midsaggital plane were successful, whereas a 95% success rate was observed for prey placed more laterally. This tendency to respond inappropriately to frontally placed targets was especially pronounced in frogs rendered monocular by occlusion of one eye by a plastic eyepatch. Only 64% of the 55 frontal snaps observed for such animals resulted in successful capture. This deficit was quite pronounced for two of the tested animals, but was less evident, or absent, in 3 others. Frogs and toads rendered monocular by enucleation or optic nerve transection have not been reported to exhibit such a deficit (Ingle Psychon. Sci. 29(1): 37-38; Collett et al. Exp. Brain Res. 66:35-40; Grobstein et al. J. Comp. Physiol. A 156:775-785). These results suggest that some readjustment of visual distance discrimination occurs following destruction of the optic nerve, which does not occur so readily when an eye is simply occluded (Supported by NSF IBN-9420525).

178.12

THE EVOLUTION OF BIASES IN MATE CHOICE: MODELING CALL RECOGNITION IN THE TUNGARA FROG WITH NEURAL NETWORKS. S.M. Phelps, M.J. Ryan and W. Wilczynski*. Departments of Zoology and Psychology, Univ. of Texas, Austin, TX 78712.

The origins of female preferences for male traits are a pivotal concern in current sexual selection theory. Recent data from neural network models suggest that significant biases in mate choice may arise as by-products of selection for mate recognition. To date these models have used simple abstractions of visual stimuli to represent traits, such as tail length and symmetry, that are preferred by females of some species. We asked whether artificial neural networks could evolve to recognize an actual acoustic signal used in mate choice, the call of the tungara frog, *Physalaemus pustulosus*. We chose a simple recurrent network architecture and evolved weights using Goldberg's simple genetic algorithm and "roulette-wheel selection.". Networks were selected for their ability to discriminate call spectrograms from noise presented in the same amplitude envelope. We found that these networks can learn to recognize calls, and that they generalize to calls they have not been selected to recognize. Recent field studies exploring the ability of *P. pustulosus* females to generalize to the calls of other species demonstrate that phylogenetic relatedness is a better predictor of call recognition than call similarity--suggesting biases may be the result of the evolutionary history of the female sensory apparatus. We are currently investigating whether the artificial neural networks have biases that correspond to those of real females, and whether the evolutionary history of the networks shapes these biases.
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178.13

FORAGING STRATEGY AND SPATIAL MEMORY IN LIZARDS. L. B. Day*, W. Wilczynski and D. Crews. Institute for Neuroscience, Univ. of Texas, Austin, TX 78712.

Mental capacities for spatial memory and ecological demands for these abilities appear to be integrally related in several species of birds and mammals. We explored whether a similar relationship exists between spatial ability and foraging strategy in lizards. *Acanthodactylus boskianus* (n=6), an active forager that collects clumped, immobile prey, was expected to perform better than *A. scutellatus* (n=9), a sit-and-wait predator that collects distributed, mobile prey, on a spatial memory task. No species difference was expected for a similar visually cued task that did not require use of spatial memory. In the spatial memory task, lizards were placed in the center of a circular arena (1.5m D) in a cold (23°C) room where they could escape to a insulated hot rock (40°C) that was identical to 7 other unheated rocks placed along the perimeter of the arena. There were no local cues and we controlled for olfactory detection. The lizard had to use distal cues to locate the hot rock. For visually cued trials a small red light was moved randomly each day to a different heated brick. We ran 3 trials a day for 12 days for both spatially and visually cued trials. Latencies to the goal declined across trials for both species, but contrary to expectations, there were no species differences on latency to the goal in the spatial cued task ($p>.05$). There may, however, be species differences in distance traveled to reach the goal. These data are currently being analyzed. Unexpectedly, when the goal was visually cued we found a species difference. The sit-and-wait predator was significantly faster than the active forager at locating the goal ($F = 5.13$, $p<.04$). Sit-and-wait predators are more likely to detect prey using vision and thus species differences in ability to attend to or form associations with visual cues might explain this difference. Supported by grant MH 41770 to D. C. and NIMH grant MH 45696 to W. W.

178.14

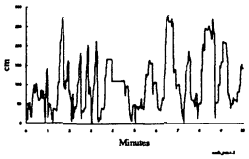
DISSOCIATION BETWEEN MK-801- AND CAPTIVITY-INDUCED STEREOTYPIES IN BANK VOLES (*Clethrionomys glareolus*). I. Vandebroek, R. Meeusen* and F.O. Ödberg. Dep. of Animal Nutrition, Genetics, Production and Ethology, Univ. of Ghent, Heidestraat 19, B - 9820 Merelbeke, Belgium.

Pharmacologically-induced stereotypies (PHIS) following amphetamine and, more recently, the non-competitive NMDA-antagonist MK-801, are used to study human psychopathology and for screening potential neuroleptic drugs. On the other hand, it is also possible to elicit stereotypies by captivity, for example in barren environments. In this study, the relationship between PHIS and captivity-induced stereotypies (CIS) was investigated by repeated administration of MK-801 (RBI, Natick, MA, 0.3 mg/kg) to bank voles that had respectively developed a typical jumping stereotypy in captivity (ST) or not (NST). ST and NST received saline for 9 days followed by 9 daily sc injections with MK-801, with a 48 h break between both treatments. Their behaviour was subsequently recorded during 2 min every 5 min for a total of 112 min with a pocket computer loaded with the Observer 3.0 software (Noldus Information Technology, Wageningen, The Netherlands) on days 1, 5, 9, 11, 15 and 19. According to our results, jumping was not evoked in NST voles, nor increased in ST. Instead, other stereotypies such as intensive sniffing, followed by locomotor stereotypy characterized as running in unidirectional circles, were elicited by the drug. Furthermore, differences between both groups were encountered with repeated injections. ST showed a progressive increase in levels of locomotor stereotypy (one-way ANOVA for repeated measures: $F = 5.71$; $p = 0.015$) and no change in levels of sniffing, while in NST intensive sniffing was progressively enhanced ($F = 5.64$; $p = 0.016$) and levels of locomotor stereotypy did not vary significantly. These results seem to point, at least in part, to a different regulation of the neurobiochemical background of CIS and PHIS. This research was supported by a specialization grant of the Belgian IWT.

178.15

GERBILS REGULARLY RETURN TO THEIR ENTRY POINT WHEN EXPLORING A NOVEL ENVIRONMENT. D.S. Touretzky,* S. J. C. Gaulin,† A.D. Redish. Computer Science Dept. and Center for the Neural Basis of Cognition, Carnegie Mellon Univ., Pittsburgh PA 15213. †Dept. of Anthropology, University of Pittsburgh, Pittsburgh PA 15261

Recent theories of rodent navigation have suggested that rodents associate visual cues with path integrator coordinates as they explore a novel environment. Because path integration is subject to cumulative error, these theories predict that rodents must periodically return to places where the association has already been made, in order to correct for drift. One likely reference point is the location where the animal first entered the environment. Informal observations of rodents exploring a novel environment suggest that they return to their entry points at regular intervals. We quantify this in two ways: by measuring the distance from the entry point as the animal explores, and by counting the number of times the animal returns to a small circle around this point. Eight gerbils (four male and four female) were individually released into an open, 3-meter diameter arena and allowed to explore for 10 minutes while their position was tracked at 18.2 frames per second. The gerbils explored a large portion of the arena during this period. The graph at left shows distance from entry point (in cm) vs. time for one animal. Each time the value drops to zero, the animal has returned to its entry point (14 times for this trial). This plot differs significantly from that produced by a random walk.



Funded by the Center for the Neural Basis of Cognition.

178.16

GABA$_A$ RECEPTORS OF AMYGDALA ARE INVOLVED IN THE MODULATION OF DORSAL IMMOBILITY. W. I. C. Albuquerque-Araújo*, M. R. Brentegani and A. Hoffmann. Lab. of Neurophysiology, University of São Paulo, Ribeirão Preto, SP 14049-900 Brazil.

Dorsal immobility (DI) is an inhibitory behavioral response that occur in the prey-predator relationship and in fight between conspecifics. It is elicited by grasping the animal by the dorsal skin at the nape of the neck and lifting it off its feet. In the rat, the animal immediately exhibits a stereotypical immobility posture that persists for a period of time until the animal suddenly emits escape-like behaviors. Extensive evidence indicates that the amygdala plays a crucial role in fear and anxiety. Many of the amygdaloid projection areas are critically involved in specific signs (changes in autonomic activity, simple reflexes) that are used to measure fear and anxiety. Electrical stimulation of the amygdala elicits a pattern of behaviors that mimic natural or conditioned states of fear. Local infusion of drugs (opiate agonists, benzodiazepines) have anxiolytic effects in several behavioral tests. In view of the above considerations the purpose of the present study was to investigate if the amygdala and the GABAergic system, acting through GABA$_A$ receptors, are involved in the modulation of dorsal immobility. The changes in the duration of dorsal immobility in seconds, were determined before (control 26.8 ± 2.55) and after microinjections of saline (25.2 ± 2.87; 0.2µl), muscimol (22.47 ± 4.26; 0.1µg/0.2µl), muscimol (25.6 ± 3.85; 0.3µg/0.2µl), muscimol (20.03 ± 3.21; 0.6µg/0.2µl), bicuculline (11.94 ± 0.98; 0.1µg/0.2µl), bicuculline + muscimol (22.8 ± 2.95; 0.6µg/0.2µl) and midazolam (48.6 ± 5.81; 20nmol/0.2µl) in the amygdala of adult Wistar rats (± 250g) chronically implanted with stainless steel guide cannulae. The results show: 1) muscimol alone don't interfere in the duration of DI, but blocks the inhibitory effect of bicuculline; 2) the anxiolytic action of benzodiazepines (injected in the amygdala) potentiate the duration of DI.

Research supported by CNPq and FAPESP.

178.17

VESTIBULAR INFORMATION DEFINES A DIRECTIONAL REFERENCE NECESSARY TO ELABORATE A SPATIAL REPRESENTATION. P. Lavenex (1), V.M. Bajo-Lorenzana (2)*, L.F. Jacobs (1). 1-Dept of Psychology, University of California, Berkeley, CA 94720. 2-Inst of Physiology, University of Lausanne, CH-1005 Lausanne

The aim of this study was to investigate the importance of vestibular information in the definition of a directional reference necessary to elaborate a spatial representation of the environment. The caching and retrieval behavior of kangaroo rats was analysed in a visually controlled environment. The experiment took place in a square arena surrounded by curtains, which prevented the access to distant visuospatial information. This apparatus constituted a symmetrical world, where internal spatial coordinates could be disambiguated only with the help of a directional reference. K-rats were transported in their home-cage from the housing room to a fixed position in the experimental room. They were then transferred in an opaque plastic transport box from their home-cage into the arena and allowed to cache seeds. During the caching trials, k-rats were always introduced into the arena from the same entrance (SW). Three different types of retrieval trials were conducted. (1) The k-rat was given access to the arena from the same entrance (SW) as in the caching session. (2) The k-rat was not transported directly from its home-cage to the experimental arena. Instead, the animal, in the plastic transportation box, was placed on a rotating platform for one minute with a rotation speed of 16 turns/min. It was then introduced to the arena from the opposite entrance (NE). This attempted to control for the integration of vestibular information and prevent the animal from maintaining an internal direction of reference. (3) The k-rat was introduced to the arena from the opposite entrance (NE), but was transported in the plastic box directly from its home-cage into the experimental arena. Animals should be able to integrate the vestibular information and maintain a correct directional reference. In (1), k-rats found the seeds that they had previously cached in the arena. In (2), they searched for their caches in positions exactly symmetrically opposite to their original caches. In (3), k-rats searched at the correct locations and at symmetrical positions in the arena. These results show that the integration of vestibular information enables the maintenance of an internal directional reference necessary to elaborate a spatial representation of the environment. If this integration is disrupted, the animals reset the integration of the vestibular information at the entrance of the arena. They consider this point to be the new point of reference (i.e. the origin), from which to calculate their position using vestibular and kinesthetic information. Supported by the Swiss National Science Foundation and the University of California, Berkeley.

178.18

THE DEVELOPMENT OF ROTATORY MOVEMENTS AROUND THE LONGITUDINAL AXIS DURING CONTACT-RIGHTING: SEX DIFFERENCES IN THE ORGANIZATION OF ROTATORY MOVEMENTS D. J. Martens, E. F. Field, V. C. Pellis* & S. M. Pellis. Dept. of Psychology, University of Lethbridge, Lethbridge AB Canada T1K 3M4

Sex differences in the development of righting on the ground, from supine to prone, were studied. Righting, from the prone position, is initiated by a rotation of the head and shoulders to supine. Rotation then progresses cephalocaudally and is complete when the pelvis has also rotated to a supine position. Males take less time to rotate their pelvis, following rotation of their head and shoulders to prone, than do females. Males and females also use different combinations of axial rotation and limb movements to rotate from supine to prone. These differences appear to be due to differences in the way males and females integrate their fore- and hindquarter movements. These results suggest that males and females have different developmental patterns of movement organization in the rotatory plane. This research was supported by NSERC.

NEUROETHOLOGY: ELECTRORECEPTION

179.1

PULSE TRAIN CHARACTERISTICS OF THE ELECTRIC ORGAN DISCHARGE (EOD) DURING THE COMMUNICATIVE BEHAVIOUR OF TWO SKATE SPECIES (*RAJA OCELLATA* AND *R. ERINACEA*). L.D. Hayes, B. Bratton[1]. Dept. of Biology, Bates College, Lewiston ME 04240 & [1]Oberlin College, Oberlin OH 44074.

The electric organ of marine skates (Chondrichthyes: Rajidae) produces weak electric organ discharges (EODs) that may occur within the detectable range of the ampullae of Lorenzini. Although it is believed that the electric organ is employed during social communication, the precise function of the EOD during behaviour has yet to be determined. This study examined the characteristics of the EOD pulse trains of grouped Winter skates (*Raja ocellata*) and Little skates (*R. erinacea*) observed in 4-11 day periods. EOD activity produced during intra-specific interactions were quantified into five defined behaviour types. Qualitative observations of EOD activity were also examined. Skates produced an average number of EODs per train that differed between the five behaviour types. Similar differences in the average EOD pulse train duration and frequency were not found. The EOD pulse train of *R. ocellata* was lower in frequency than that produced by *R. erinacea*. Two *R. ocellata* individuals developed a constant EOD pulse train of 0.4-0.6 pulses per second (pps) that occurred during behavioural interactions with other skates and in isolation. *R. erinacea* did not produce a constant EOD pulse train. Although distinct EOD pulse train patterns were not observed during specific behaviours, the occurrence of high frequency responses (>1.0 pps) to certain behavioural encounters suggests that the electric organ is used as a communicative signal. Differences between the EOD pulse trains produced by *R. ocellata* and *R. erinacea* provide evidence that the electric organ is used as an intra-specific signaler, possibly during reproductive and antagonistic behaviour.

(Supported by HHMI Grant to Bates College)

179.2

LOCATING THE SITE OF ADAPTIVE REAFFERENCE SUPPRESSION: EVIDENCE FOR A ROLE OF PARALLEL FIBERS. D. Bodznick*, M.R. Carey and B.W. Larner. Dept. Biol., Wesleyan Univ., Middletown, CT 06459.

Second order electrosensory neurons (AENs) in the skate medulla selectively suppress sensory inputs associated with the fish's ventilatory movements. This reafference suppression is based in part on an additive adaptive filter mechanism in which internal reference signals (central motor commands and sensory feedback) provide a cancellation signal input to each AEN that is the negative of the expected reafference in that cell. The cancellation signal is adaptive. Coupling electric field stimuli to the fish's ventilatory movements leads, within minutes, to a suppression of the AEN response to the stimulus. Immediately after the coupling, the modified cancellation signal is apparent as a negative image of the expected reafference in the neuron's firing pattern during ventilation. We report here the results of two experiments to determine the site of the plasticity and the source of the cancellation signal. First, we maintained stable intracellular recordings of AENs in freely-ventilating animals for periods up to 1 hr. and coupled intracellular current pulses with the fish's ventilatory movements. This pairing resulted in changes in the cancellation signal to the AEN (evidenced by the negative image after the coupling) similar to those seen following the coupling of sensory stimuli to ventilation. This indicates that the plasticity is due to changes in the strength of synapses onto the AEN itself. Second, we tested the hypothesis that the parallel fiber projection, which supplies central motor command and sensory feedback information to the AENs, is the source of the cancellation signal. Large extirpation lesions to DGR, the granule source of the parallel fiber projection, resulted in a significant decrease in the proportion of AENs exhibiting cancellation signals in response to coupling. This suggests that parallel fiber input to the AEN plays an important role in the plasticity mechanism. Plasticity in some AENs after lesioning may be due to incomplete lesions or may indicate an additional source of cancellation signal inputs.

Funded by NSF grant IBN-9409829 to DB.

179.3

BEHAVIORAL SIGNIFICANCE OF MULTIPLE SENSORY MAPS IN THE ELECTROSENSORY LATERAL LINE LOBE (ELL) OF THE ELECTRIC FISH, *EIGENMANNIA*. W. Metzner * (1), J. Juranek (2) (1) Dept. of Biology, UCR, Riverside, CA 92521-0427, (2) Program in Neuroscience, Dept. Psychology, UCR, Riverside, CA 92521-0426.

Multiple representations of the environment are a common component of vertebrate sensory systems. For instance, four electrosensory maps are found in the hindbrain of weakly electric gymnotiform fish. Each of the four segments of the ELL contains a topographic map of electroreceptive input from the body surface. The medial segment receives information from low-frequency (ampullary) receptors and the three remaining maps (centromedial, centrolateral, and lateral) receive information from high-frequency (tuberous) receptors. Differences in the neuronal activity between the three tuberous maps indicate highest temporal resolution of neurons in the lateral and highest spatial resolution of neurons in the centromedial map. The behavioral significance of these differences, however, is purely speculative and suggests that the lateral map may be involved mainly in communication behavior and the centromedial map in the detection of jamming signals caused by neighboring conspecifics (jamming avoidance response; JAR) and possibly during electrolocation. We use pharmacological lesions (kainic and ibotenic acid) of various ELL maps in combination with a, in this context, new stimulation technique to determine the effects on the JAR: The fish is positioned in a chamber with multiple, electrically isolated compartments. This allows us to apply external electric fields only those body surface areas projecting to that particular portion of the ELL map where the multiple barrel electrode used for simultaneous recording and lesioning is located. Thus, compensatory effects of highly convergent input from different receptor afferents is minimized. Results indicate a strong separation of the behavioral significance of the maps, and even suggest that the ampullary map may be involved in the JAR.

Support from UCR, Acad.Sen.Res.Grant

179.4

ROLES OF ADAPTATION, PASSIVE ELECTRICAL FILTERING AND VOLTAGE-DEPENDENT CONDUCTANCES IN THE TEMPORAL SELECTIVITIES OF TORAL NEURONS, G.J. Rose* and E.S. Fortune. Dept. Biology, Univ. Utah, Salt Lake City, UT 84112.

In its jamming avoidance response (JAR), the weakly electric fish Eigenmannia changes its frequency of electric organ discharges (EODs) to increase the frequency difference between its EODs and those of a neighbor. Frequency differences (beat rates) of approximately 3-8 Hz elicit largest JARs. A neural correlate of this behavior is that spiny neurons in the torus semicircularis respond best to beat rates in this range and the magnitude of EPSPs declines for higher rates. Processes that might contribute to this temporal selectivity include 1) passive electrical properties of the neuron 2) voltage-dependent conductances 3) adaptation. The contribution of each process to the temporal selectivity of toral neurons was determined by recording intracellularly (whole-cell patch method) at several levels of negative current clamp while 1) varying the frequency of sinusoidal current injected 2) varying the electrosensory stimulus frequency or beat rate and 3) presenting AM or sinewave bursts as electrosensory stimuli. Results indicate that all three processes can contribute to the temporal filtering properties of toral neurons. The importance of each process varies across neurons. Supported by NSF grant IBN-9421039.

179.5

CELLULAR EFFECTS OF DIFFERENT PREMOTOR CIRCUITRY ON THE PACEMAKER NUCLEUS IN TWO SPECIES OF ELECTRIC FISH. J. Juranek *(1) and W. Metzner (2). (1) Program in Neuroscience, Dept. Psychology, UCR, Riverside, CA 92521; (2) Dept. Biology, UCR, Riverside, CA 92521.

Eigenmannia raises and lowers its electric organ discharge (EOD) frequency in response to jamming signals from neighboring conspecifics. The premotor control of this jamming avoidance response (JAR) is mediated by two anatomically distinct, antagonistically acting inputs to two cell types, pacemaker cells and relay cells of the medullary pacemaker nucleus (Pn). Input from a diencephalic prepacemaker (PPnG/CP) terminates mainly on pacemaker cells and is normally inactive but can be activated to raise the EOD frequency above the resting level. An input from a sublemniscal prepacemaker (SPPn) terminates mainly on relay cells and is normally active but can be reduced to lower the EOD frequency below resting level (Metzner, 1993). In contrast, the closely related *Apteronotus* is unable to lower its EOD frequency below the resting level during a JAR. Although both inputs to the Pn and its cellular components exist, only the SPPn mediates EOD frequency changes during a JAR (Heiligenberg et al., 1996). The SPPn is normally inhibited and only when this inhibition is released, the EOD frequency raises above the resting level during a JAR. The aim of this study is to characterize the effects of this different premotor circuitry on pacemaker and relay cells in the two species. Thus, intracellular recordings from these cells were performed during iontophoretic stimulation of PPnG/CP (with L-Glutamate) and of SPPn (with GABA in *Eig.* and with L-Glutamate in *Apt.*) as well as during a JAR elicited by externally applied jamming signals. In both species, pacemaker cells are only affected by PPnG/CP input whereas relay cells respond only to SPPn input. Baseline voltage, spike amplitude, and rising and falling phases of action potentials revealed drastic differences between pacemaker and relay cells in the two species.

Support from UCR, Acad. Sen. Res. Grant

179.6

COMPLEX ELECTRIC SIGNAL STRUCTURE IN REPRODUCING GYMNOTIFORM ELECTRIC FISH. P.K.STODDARD*, M.D.KILBURN, K.H.PATTERSON. Dept. Biological Sciences, Florida International Univ., Miami FL 33199.

Infrared video & DC-coupled electric field recordings of 7 pairs of the gymnotiform electric fish *Brachyhypopomus pinnicaudatus* revealed 3 behavioral stages to reproduction: female approach, male courtship of female, and spawning. Each stage was associated with unique electric signaling patterns. (1) The male discharged at a steady rate until the female approached. (2) The male courted the female by giving rapid EOD accelerations as he prodded her with his snout. This rapid EOD acceleration interacts with the monophasic region of the male's local electric field to generate a large, head-positive pulse in the frequency range of the female's ampullary electroreceptor system. (3) Spawning occurred when the male and female were parallel. The male alternated his steady discharge rate with repeated periods of high frequency, low amplitude, EOD oscillation, each of which produced a low frequency pulse. Our data suggest the sexually dimorphic, biphasic, EOD waveform of the male acts as the mate attraction signal, and that courtship and spawning signals consist of more complex interactions of local electric field structure, EOD rate, and EOD amplitude modulation. Sexual advertisement appears to involve only the high-frequency tuberous electroreceptive pathway, whereas courtship and spawning signals stimulate both this pathway and the evolutionarily older low-frequency ampullary electroreceptive pathway. Funding was provided by the FIU Foundation and NIGMS/NIH grant GM08205 to P.Stoddard.

179.7

NATURAL AND EVOKED SEXUAL DIMORPHISM IN THE WEAKLY ELECTRIC FISH *HYPOPOMUS SP.* A. Silva, A. Caputi, M. Galeano, P. Errandonea, G. Eiris and R. Budelli*. Labs. de Neurofisiologia y Neuroanatomia Comparada, IIBCE-Fac. de Ciencias, 11600 Montevideo, Uruguay.

The Río de la Plata is the southern boundary of the geodistribution of Gymnotiforms in South America. Hundreds of freshwater sources within Uruguay were explored in order to determine the existence of native species and characterize their habitats. *Gymnotus carapo* is the more ubiquitous one, whereas *Hypopomus sp.* is restricted to the northeast region, and *Eigenmannia virescens* to the northern region of the country.

Hypopomus sp. showed natural morphological and electrophysiological sexual dimorphism during the breeding season in late spring and summer (November to February). On the other hand, 20 non differentiated individuals under captivity were intramuscularly injected with 20-50 μgr/gr of testosterone ciclopentil propionate (Dispert).

Since the electric organ discharge (EOD) depends on complex spatio-temporal patterns, we used the multiple air-gap technique (Caputi et al., J. Comp. Physiol. A. 1993, 173:227-232) to record independently and simultaneously the contribution of different portions of the electric organ (EO) in the generation of the EOD.

We found a strong correlation between natural and evoked sexual EOD changes. The discharge generated by the intermediate portion was more affected. We observed an increase in the amplitude and duration (mainly of the negative component) of the electromotive force generated by this portion in the natural differentiated male and after hormonal treatment.

Data suggest that: a) testosterone is responsible for male changes as reported elsewhere, b) testosterone evokes an increase in the efficiency of action potential invasion of electrocyte rostral faces, c) this effect is not homogeneous along the EO. Partially supported by CSIC, Proj. N. 052 and CEC, Proj. N. CI1*-CT92-0085

179.8

ACCURACY OF PHASE-LOCKED NEURONS, ELECTRIC ORGAN DISCHARGES AND THE JAMMING AVOIDANCE RESPONSE OF THE AFRICAN WAVE-TYPE ELECTRIC FISH, *Gymnarchus niloticus*. Y.X. Guo and M. Kawasaki*. Department of Biology, University of Virginia, Charlottesville, VA 22903.

A behavioral study on the jamming avoidance response (JAR) of the African wave-type electric fish, *Gymnarchus niloticus* (Kawasaki 1993 J. Comp. Physiol.173:9-22), indicated that the JAR requires timing comparison in the order of microseconds between electrosensory signals from different body surfaces. In this study, accuracy of phase-locking neurons in the electrosensory lateral line lobe (ELL) and behavioral threshold for phase differences were measured.

The S-type primary afferent fibers, which fire an action potential in response to each cycle of electric organ discharges (EODs), project to the somata of giant neurons and to the inner cell layer (ICL) of the medial zone of the ELL. The giant neurons also fire in the phase-locked fashion and terminates also on the ICL. Thus the phase information is represented by action potentials of these phase-locking neurons at the differential-phase-sensitive neurons in the ICL (Kawasaki and Guo 1996 J. Neurosci.16:380-391). Mean of standard deviations of jitter of these phase-locking neurons over several seconds were 5.6 μsec. Of 134 neurons tested, the most and least accurate neurons showed standard deviations of 1.3 μsec and 18.4 μsec, respectively.

The JAR could be induced when available phase differences were only a few hundred nanoseconds.

Although jitter of EODs was less than one microsecond on average, artificially induced jitter of an EOD replacement signal (up to 60 μsec) did not have any significant effects on the JAR even when phase differences of submicroseconds were available to curarized fish.

These results indicate that the differential-phase-sensitive neurons in the ELL receive phase information with accuracy of single-digit microseconds and further phase processing yields an approximately ten times higher behavioral acuity.

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179.9

GLUTAMATERGIC INPUT TO PACEMAKER NUCLEUS FROM MAUTHNER CELL NETWORKS IN *GYMNOTUS CARAPO* A. Falconi, M. Borde, S. Curti and F.R. Morales*. Departamento de Fisiología. Facultad de Medicina- Facultad de Ciencias Montevideo 11800-Uruguay.

Activation of Mauthner cells (M-cells) in *G. carapo*, results in an abrupt increase in the rate of discharge of its electric organ (Mauthner cell initiated Abrupt Increase in Rate, M-AIR). This response is mediated by an excitatory input to the medullary pacemaker-cells (PM-cells). In the present work we explored, utilizing pharmacological tools, the nature of the neurotransmitter released by synapses responsible for this excitatory input. In addition we investigated the subtypes of postsynaptic receptors activated by this neurotransmitter. The experiments were conducted *in vivo* as described previously (Falconi et al. 1993, J. Comp. Physiol.). Glutamate and its agonists, as well as blockers for different receptor subtypes, were applied to the PMn.by pressure injection.

Glutamate (1 to 10 mM), NMDA (500 µM), Kainate (1 to 5 mM) and AMPA (500 µM) injections induced abrupt and relatively short lasting acceleration of the electric organ discharge (EOD). *Trans*-ACPD (5 mM) induced a long lasting (about 60 s) biphasic increase in EOD rate. None of this agonist-induced responses were accompanied by any variation in EOD waveform. M-AIR was reversibly blocked (up to 80%) by AP5 (500 µM), MCPG (5 to 10 mM) reduced both its amplitude and duration. CNQX (1 to 10 mM) either had no effects or slightly affected the onset of some M-AIR responses. All these data taken together indicate that the excitatory input to the PM-cells responsible for M-AIR responses appears to involve mainly NMDA and metabotropic subtype glutamate receptors To our knowledge this is the first demonstration of the involvement of metabotropic glutamatergic actions in a specific behavior.

Supported by CSIC, Universidad de la República and CONICYT-Uruguay.

179.10

PACEMAKER REGULARITY: INTER-SPECIES COMPARISON OF ELECTRIC ORGAN DISCHARGE FREQUENCY. K.T. Moortgat[1]*, T.H. Bullock[2], and T.J. Sejnowski[3]. [1]Physics and [2]Neuroscience Depts., University of California, San Diego, 92093; [3]Computational Neurobiology Lab, Salk Institute, La Jolla, CA 92037.

Certain species of weakly electric fish produce pulses, commanded by a central pacemaker, orders of magnitude more regular than other known biological oscillations. Here we investigate the inter-species differences in electric organ discharge (EOD) timing regularity among some South American wave-type fish, *Sternopygus macrurus*, *Eigenmannia virescens*, and three species of *Apteronotus*, as well as *Microsternarchus* sp. Measurements were made during day and in physical isolation. The natural EOD of these species ranges from 20 to 1300 Hz.

We show that EODs of all three *Apteronotus* species (*leptorhynchus*, 650 Hz; *albifrons*, 980 Hz; sp., 1300 Hz) are regular to sub-microsecond resolution (standard deviation (sd) as low as 0.2 µsec; coefficient of variation (sd /mean), cv ≈ 0.00014) over thousands of EOD cycles. In contrast, the low frequency (20 Hz) *Microsternarchus* is much less regular (ca. 0.2 msec ; cv ≈ 0.00403). A 500 Hz *Eigenmannia* EOD was very stable (0.4 µsec; cv ≈ 0.00019) while *Sternopygus* (100 Hz) had less timing regularity (2.1 µsec; cv ≈ 0.0002). The regularity appears to be species-specific but independent of mean frequency.

The cv will remain constant over thousands of cycles but can vary by a factor of ten on longer time scales (hours). One hypothesis under study is that regularity is adjusted by the individual, depending on its behavioral state. The ability of a species to maintain regular EODs largely depends on the medullary pacemaker nucleus, which varies in cell number, degree and type of coupling, and ratio of cell types among species. We are studying which of these features is most important in attaining the observed EOD regularity. Support: NIMH fellowship to KTM; NINDS, NIH to W. Heiligenberg (deceased), and HHMI to TJS.

179.11

SENSORY EVOKED RESPONSES IN THE PALLIUM OF THE WEAKLY ELECTRIC FISH, *GNATHONEMUS PETERSII*. T.H.Bullock*, J.C.Prechtl, G.von der Emde[§], C.J.H. Wong, G.N.Akoev[‡], G.N.Andrianov[‡]. Neurobiology Unit, Scripps Inst Oceanography, UCSD, La Jolla, CA 92093; [§]Zool Inst, Univ Bonn, 53115 Bonn, Germany; [‡]Pavlov Inst Physiol, Russian Acad Sciences, 199034 St. Petersburg, Russia.

Little is known about the physiology and functional organization of the teleost forebrain. The everted pallium, well developed in this mormyrid species, was examined for structure and distribution of evoked responses (local field potentials, LFP and multiunit activity, MUA) in the dorsal medial nucleus and immediately ventral area in locally anesthetized, curarized fish. In each of 21 l tracks, responses to 4 stimulus modalities were sampled from ca. 30 depths, in ca. 50 µm steps, with 1-4 semi-microelectrodes, at 22-23°C. Stimuli were water displacement (adequate for lateral line receptors), light flash, electric field and acoustic tone pip, with intervals of >7 s between them.

Results are described from single sweeps. 1) Sharp, usually negative LFPs with MUA are designated DEP, VEP, EEP & AEP (according to the 4 modalities), and are usually very local. Loci as little as 100 µm from one with a good EP may show no hint of an EP to the same stimulus or quite a different wave form or latency (e.g. 25 vs 40 ms). Some high frequency LFPs with MUA have only a small amplitude slow component. 2) EPs often include induced rhythms between 40 & 80 Hz. 3) AEPs are usually obtained without concomitant EEPs but most EEP loci also yield some AEP activity. Some EEP loci are purely EEP. Loci with the large sharp DEPs show no EEP or AEP overlap activity. 4) AEPs do not follow at rates greater than 1 Hz. 5) AEPs and EEPs are almost always in the upper 600 µm, begin ca. 300 µm below the surface and span ca. 200 µm. DEPs are deeper, ca. 600-800 µm and caudal to the best AEP loci. 6) AEP onset latencies average 35ms, principal peak N20-100 ms (mean 64 ms). EEPs were similar, principal peak is N45-80 ms. DEPs have longer latencies (N105-130 ms). 7) VEPs are seen very locally >1,000 µm deep. Supported by NINDS.

179.12

TISSUE PRINTED CELLS FROM THE ELECTROSENSORY LATERAL LINE LOBE (ELL) OF A WEAKLY ELECTRIC FISH. Suhas A. Kotecha, Douglas W. Eley, and Ray W. Turner* Dept. Anatomy, University of Calgary, Calgary, Alberta, Canada T2N 4N1.

Several techniques have been used to isolate vertebrate CNS neurons for electrophysiological recordings and cell culture. B.A. Barres (1990) first used a procedure termed "tissue printing" to isolate and record from rat optic nerve glial cells. We now report a modification of this procedure to print vertebrate CNS tissue slices and obtain an organotypic distribution of identifiable, isolated cells without exogenous enzyme treatment.

Transverse 200 µm ELL slices were placed in cold, oxygenated sucrose-NaCl substituted aCSF. Slices were placed in a petri dish with 2ml L-15 medium and maintained overnight at 4°C. Slices were warmed to 23°C for 60 min, attached to nitrocellulose paper, and inverted onto a glass coverslip pre-coated with Concanavalin A, poly-L-lysine and laminin. Slices were surrounded by a low level of HEPES-buffered medium in which Na^+ was substituted with sucrose and Na Gluconate, KCl and KH_2PO_4 with K Gluconate, 0.1 mg/ml Kyn. acid, 10 µM picrotoxin, 1 mM Na Pyruvate, 0.1 mM $CaCl_2$, and 4.5 mM $MgCl_2$. Slices were centrifuged at 450 X g for 5 min, the slice removed from the coverslip and the dish filled with L-15 medium.

Isolated cells were distributed as an organotypic monolayer, with the boundaries of the slice and major cell laminae clearly delineated. Small diameter cells (ie glia, cerebellar granule cells) and axonal processes were isolated most often, followed by larger diameter somata and dendritic processes. In many cases, an excellent preservation of cell structure was obtained, with identifiable somata and attached dendrites up to 500 µm in length. All ELL cell classes have been identified, including pyramidal, polymorphic, granule and spherical cells. Tissue prints are prolific in culture, given the high density and organotypic distribution of isolated glial cells. This work was supported by the AHFMR and Canada MRC.

179.13

FEATURE EXTRACTION IN THE ELECTROSENSORY LATERAL LINE LOBE (ELL) OF WEAKLY ELECTRIC FISH. F. Gabbiani*,[1], W. Metzner[2], R. Wessel[3] and C. Koch[1], [1]Div. of Biol., Caltech 139-74, Pasadena, CA 91125, [2]Dept. of Biol., UCR, Riverside, CA 92521-0427 and [3]Dept. of Biol., UCSD, La Jolla, CA 92093-0357.

The ELL in the hindbrain of weakly electric fish is the first CNS nucleus of the electrosensory pathway. P-receptor afferents encode electric field amplitude modulations (AMs) and transmit this information to pyramidal cells in the ELL. Two functional groups of pyramidal cells are distinguished: E-cells respond to increases in amplitude of an externally applied electric signal and I-cells respond oppositely. The aim of this study was to characterize the temporal information processing of ELL pyramidal cells in *Eigenmannia* and relate it to the information carried by P-receptor afferents. The responses of P-receptor afferents and pyramidal cells were quantified by an estimation method (Wessel *et al.*, J. Neurophys. 1996) characterizing to what extent the neuronal response encodes the detailed time course of random AMs and a signal-detection method developed to identify features encoded in spike trains. Extracellular recordings from P-afferents revealed that they encode reliably the detailed time course of random AMs up to 40 Hz cut-off frequencies while specific features are not well encoded. In contrast, intracellular recordings from E- and I-cells revealed that, while the detailed stimulus time course is poorly encoded, up- and downstrokes of random AMs are reliably encoded. Up- and downstrokes were found to be encoded more reliably by short bursts rather than by isolated spikes. The results suggest that these features are extracted by the ELL circuitry from the detailed time-varying information provided by P-afferents. Analysis of the intracellular membrane voltage of pyramidal cells in response to the stimulus suggests that the feature extraction is most likely to occur in the dendrites of pyramidal cells. Support: NSF, ERC and UCR.

180.1

TASTE AND OTHER ORAL RESPONSIVITY TO A HIGH-FAT SWEETENED CHOCOLATE PRELOAD. P.J. Geiselman*, C.F. Smith, D.A. Williamson, R.A. Plum, N. Baker, C. Champagne, & D.H. Ryan. Pennington Biomedical Research Center and Dept. Psychol., Louisiana State University, Baton Rouge, LA 70808.

Our recent research has found that a high-fat, sweetened preload can provoke overeating in women. The present study was conducted to determine how the magnitude of overeating was associated with taste and other oral responsivity in women scoring high and low on disinhibition (tendency to lose control of food intake). We used the Three-Factor Eating Questionnaire to categorize women as high or low on the disinhibition scale. Women were randomly assigned to the preload or control condition. Subjects in the preload condition tasted and rated scales for intensity perception and hedonic responses to four chocolate puddings varying in sweetness (high and low) and fat (high and low) content. Preload subjects were then given a 350-kcal high-sugar/high-fat chocolate pudding to eat, following which they were presented with a pasta meal and told they could eat as much or as little as they wished. Subjects in the control condition were given only the pasta. Regardless of disinhibition level, subjects in the preload condition ate significantly more calories than did subjects in the control condition. Data will be presented showing how taste and other oral responsivity to the chocolate puddings are associated with overeating in each of the disinhibition groups.

USDA Funds.

180.2

FLAVOR-CUED ANTICIPATED SATIETY: CROSS-NUTRIENT COMPARISONS. Z.S. Warwick*, K. Jarkowiec, L.K. Grabill III. Dept. Psychology, Univ. of Maryland Baltimore County, Baltimore, MD 21228.

When trained with equal volumes of distinctively-flavored sucrose solutions differing in caloric density (and thus in total kcal), rats trained with relatively weak solutions (e.g. 1% and 5% wt/vol) preferentially consume in two-bottle tests (both flavors in identical solutions) the high-calorie-paired flavor. This "conditioned flavor preference" (CFP) is interpreted as reflecting the greater reinforcement previously provided by the more caloric solution. However, rats trained with concentrated solutions (5%, 30%) consume less of the high-calorie-paired flavor, presumably reflecting suppression of intake based on anticipated satiety. Since nutrients differ in their ability to reinforce a CFP (e.g. *Appetite* 14:29-44;1990, *Physiol. Behav.* 50:815-24;1991), the present studies investigated whether the magnitude of anticipated satiety was also nutrient-dependent. Rats were trained with distinctively-flavored solutions at 0.2 kcal/ml and 1.6 kcal/ml (one-bottle, overnight, 6 trials/solution), with nutrient type varied across groups. Nutrients tested thus far include the carbohydrates (CHOs) glucose, sucrose, and maltodextrin; and corn oil emulsion (FAT). Two-bottle tests (both flavors in 0.9 kcal/ml) assessed flavor-cued anticipated satiety. Anticipated satiety was evident in rats trained with CHOs: percent intake of the high-calorie-paired flavor averaged approximately 25% of total two-bottle intake for all CHOs. In contrast, FAT-trained rats consumed equal amounts of the high- and low-calorie paired flavors, indicating no anticipated satiety. Studies assessing anticipated satiety conditioned by other nutrients are in progress.

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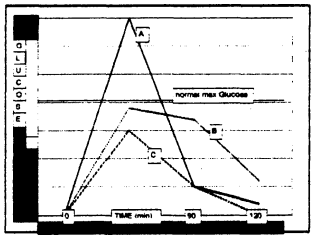
180.3

THE EFFECTS OF LOW GI FOODS AND FREQUENT EATING ON HUNGER, SATIETY AND FATTENING,

A. Alevizos* and K. Ageli.
IB program, Costeas - Gitonas School, Pallini, Athens, Greece

The pattern of blood glucose changes reflects insulin levels and determines satiety and the overall degree of fatness. Depending on the composition of meal and frequency of eating, 3 different blood glucose curves can be obtained.



Curve A, maintains hunger and promotes fattening.
Curve B, promotes satiety and fat reduction.
Curve C promotes extreme hunger.
Curve B, is obtained by using combinations of low glycemic index foods and frequent eating (every 2.5 - 3 hours)

180.4

FEEDING DYNAMICS: WHY RATS EAT IN MEALS AND WHAT THIS MEANS FOR FORAGING. B.S. Zanutto[1,2], J.E.R. Staddon[1], S. Schiffman[1]. [1]Deparment of Psychology: Experimental, Duke University, Durham, NC 27708, USA. [2]Facultad de Ingeniería, Paseo Colón 850, 1063 Buenos Aires, Argentina.

Feeding behavior in rats and other animals depends on both regulatory (homeostatic) processes and learning. In recent years, more and more aspects of feeding have been attributed to learning. We describe a very simple, real-time purely homeostatic model that can explain many aspects of feeding dynamics, including many hitherto attributed to learning. The model has two parts: a threshold, which is subject to stochastic fluctuations, and a satiation signal which is a delayed after-effect of eating. The output signal of a series of integrators simulates the temporal properties of the satiation signal, which is subtracted from the threshold to drive eating tendency. We consider that a meal is complete after the animal stops eating for a fixed amount of time. The predictions of this model, with a fixed set of parameters, are: i) animals eat in meals and, under free-feeding conditions, there is a significant correlation between meal size and the duration of the post-meal interval, but no correlation between the preprandial interval and meal size. When feeding is interrupted there is a positive correlation between the time of deprivation and the following meal size, up to a ceiling value at about 24-hours of deprivation. ii) Daily intake is regulated independently of the interpellet delay (smaller than 64 seconds). iii) In Collier-type procedures, with increasing prices for procurement or consumption of pellets, the frequency of eating is a decreasing function of the price, while the meal duration is an increasing function. The overall daily intake is a slightly decreasing function of the price. iv) When the price of access to food is alternated daily between low and high values (with a fixed low consumption price) animals eat much more at the low value. However, after a transient, animals eat almost the same amount of pellets at each price when the alternation is after two or more days. All these properties fit experimental data on feeding in rats. We conclude that most properties of feeding and foraging dynamics reflect regulatory rather than associative processes.

180.5

EFFECT OF DIETARY SATURATED FATTY ACID CHAIN LENGTH ON INSULIN, GLUCOSE TOLERANCE, AND MACRONUTRIENT SELECTION. **R.J. Kaplan, G.H. Anderson* and C.E. Greenwood. Dept. of Nutritional Sciences, Univ. of Toronto, Toronto, Ontario, Canada M5S 3E2.**

Dietary saturated fatty acid (SFA) content has been shown to mediate macronutrient selection and cognition. This study examined the influence of SFAs of varying chain length on macronutrient selection and the role of insulin sensitivity in mediating this behaviour. Groups of rats were fed, *ad libitum*, one of 4 single diets, adequate in essential fatty acids, differing only in fat source (40% energy from fat) for a 14-day stabilization period. The fat source was high in: 1) long-chain SFAs (C_{16} and above; fully hydrogenated soybean oil (HSB)), 2) intermediate-chain SFAs (C_{12} and C_{14}; hydrogenated coconut oil (HCO)), 3) medium-chain SFAs (C_8 and C_{10}; medium-chain triglyceride oil (MCT) or 4) polyunsaturated fatty acids (soybean oil (SBO)). The HSB, HCO and MCT diets contained similar concentrations of total SFAs. Each group selected for an additional 14 days between a high protein and a high carbohydrate (CHO) diet, each containing the identical fatty acid composition as the stabilization diets and dietary selection behaviour was monitored. Oral glucose tolerance and fasting insulin were determined 4 days after rats were returned to stabilization diets. While total energy did not differ, MCT rats consumed more energy as protein and less as CHO than rats in the other 3 groups. HSB rats did not select similarly to other, previously reported, long-chain SFA-fed rats. It appears, however, that HSB was not fully absorbed. Although group means did not differ with respect to plasma glucose or insulin levels, fasting insulin and the fasting glucose to insulin ratio significantly correlated with both percent of energy selected as protein and as CHO. These results suggest that SFA chain length mediates macronutrient selection, and a decrease in insulin sensitivity leads to an increase in protein and a decrease in CHO intake. HSB may be an anomalous fat source that acts more like a fibre or noncaloric substance than a normal dietary fat. (NSERC).

180.6

OBESITY PRONE RATS HAVE REDUCED SENSITIVITY TO THE SATIATING EFFECTS OF INTESTINAL FAT INFUSIONS COMPARED TO OBESITY RESISTANT RATS. D. Greenberg*, J. McCaffery, M.D. Fisch, G.A. Bray and D. York. Dept. of Psychiatry Cornell Univ. Med. College, White Plains, NY 10605 and Pennington Biomedical Research Center. Baton Rouge LA 70808.

The Osborne-Mendel (OM) rat strain shows elevated fat intake and is particularly susceptible to diet-induced obesity while the S5B/PL (S5B) rat specifically avoids fat intake and is highly resistant to diet-induced obesity (Schemmel et al 1970). We wished to determine if this difference in fat intake reflects an alteration in the satiating potency of fats in these rat strains. We tested the ability of duodenal infusions of Intralipid (IL) or linoleic acid to elicit satiety in sham feeding OM and S5B rats.

Male OM (n=9) and S5B (n=8) were fitted with gastric cannulas and duodenal catheters to allow intestinal infusions during sham feeding. Rats were overnight food deprived and sham fed liquid food (40% v/V L10007; Res. Diets Inc.). Intestinal infusion of IL (5kcal/10ml), linoleic acid (0.65 kcal/10 ml) or 0.15M NaCl (10ml, rate=0.44ml/min) began 12 min after food presentation. Intakes were measured every 5 min for 90 min. Intestinal infusions of IL and linoleic acid suppressed sham fed intakes in both rat strains but to a significantly greater extent in S5B than in OM rats (see table).

Infusate	OM	S5B	t
0.15M NaCl	58.1 ± 4.2	56.9 ± 6.8	
IL (5%)	24.5 ± 3.2	12.8 ± 1.7	3.2 $p<.01$
Linoleic acid (0.65)	26.0 ± 3.4	13.0 ± 3.7	2.5 $p<.03$

These results suggest that duodenally infused fats have elevated satiating potency in S5B rats and reduced satiating potency in OM rats which may underlie the altered fat intakes in these strains.

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180.7

CHANGES IN DIETARY SODIUM INFLUENCE DRINKING IN THE BILE DUCT LIGATED RAT. J. R. Lane*, E. M. Starbuck, & D. A. Fitts. Dept. of Psychology, University of Washington, Seattle, WA 98195.

We have.recently reported an increase in ad libitum water drinking at least two weeks following a ligation of the common bile duct (BDL) in rats maintained on a standard chow. In a separate group of BDL rats it was shown that the elevated water intake was accompanied by a reduced mean arterial pressure which generally results in increased renin secretion and high plasma levels of the dipsogenic hormone angiotensin II. In the present experiment, rats were given access to water and .3 M saline and maintained on a standard chow for approximately 6 days prior to a BDL or sham ligation and 11 days after the ligation. On day 12 post-ligation, all rats were given access to 5 pellets of sodium deficient diet in addition to their regular chow for approximately 9 days to habituate the rats to the diet. Rats were then given complete access to the sodium deficient diet for 3 more days. Rats receiving a BDL increased their consumption of water 7 days after the surgical manipulation compared to sham controls and this difference persisted throughout the remainder of the experiment. Reducing the sodium in the diet led to a reduction in water intake in BDL rats but less so in sham controls. Similarly, BDL rats with complete access to the low sodium diet drank less water than BDL rats with access to 5 pellets and standard chow or standard chow alone. The results suggest that the elevation in water intake following BDL is due partially to high dietary sodium intake. Supported by NS22274 and AA05390.

180.8

ION SPECIFICITY OF NEURAL TASTE RESPONSES TO ELECTROLYTES IN THE HAMSTER: RELATION TO TASTE QUALITY CODING. M.E. Frank*, B.I. MacKinnon, B.K. Formaker and T.P. Hettinger. Dept. BioStructure & Function, University of Connecticut Health Center, Farmington, CT 06030

Nerve fibers of the *Chorda tympani* nerve of the golden hamster (*Mesocricetus auratus*) respond to a variety of acids, salts and sugars applied to the tongue. Responses to electrolytes are found in N and H fibers, which show different sensitivities to cations and anions. N fibers are selective for sodium salts, are inhibited by amiloride and show small anion effects. H fibers are less selective for cations, are not affected by amiloride, and show greatly reduced responses to salts containing large anions such as D-gluconate. The average neural response of 6 H fibers to 0.1 M NaCl or NaBr was significantly larger than responses to 0.1 M Na acetate, Na benzoate or Na D-gluconate ($F(4,20)=5.41$, $p<0.005$). Hamsters show an innate aversion to sodium chloride that is partially blocked by amiloride (Hettinger and Frank, 1990). Behavioral responses of hamsters to anions are difficult to interpret because some anions, such as m-nitrobenzenesulfonate appear to be similar to sucrose in conditioned taste aversion experiments (Herness and Pfaffmann, 1986). N fibers presumably are involved in regulation of sodium intake in hamsters, as in rats (Bernstein and Hennessy, 1987). Human perception of saltiness (van der Klaauw and Smith, 1995) is not selective for sodium salts and parallels H-fiber more than N-fiber sensitivity.

Supported by NIH grant DC00058.

180.9

THE INFLUENCE OF THE ESTROUS CYCLE ON TASTE REACTIVITY RESPONSES IN RATS. S.N.D.A. Clarke* and K.-P. Ossenkopp, Neuroscience Program, University of Western Ontario, London, Ontario, Canada, N6A 5C2.

Hormones (e.g., estradiol) ostensibly modulate taste and palatability, in both humans and animals. This hypothesis is supported by documented changes in food intake during the reproductive cycle, which are in part predicated on changes in the gustatory system. Traditional investigations of changes in the taste system during the reproductive cycle have relied on level of intake as an indirect measure of taste and palatability. The present study adopted a direct measure, the taste reactivity test (TRT), to assess the influence of the estrous cycle on taste responsivity in rats. Male and female rats were fitted with intraoral cannulae which permitted the direct infusion of solutions into the mouth. Subjects then received a brief (1 min) infusion (0.78 ml/min) of one of three tastants: sucrose (0.3 M), quinine (0.0003 M) and a sucrose-quinine mixture. The frequency of ingestive responses (e.g., tongue protrusions) and aversive responses (e.g., chin rubs) elicited by exposure to these tastants was then analyzed to assess the influence of the estrous cycle on taste responsivity. Preliminary analyses revealed that both sex and stage of estrous had an effect on the frequency of taste reactivity responses elicited by the tastants. Thus, the TRT was able to identify sexual dimorphism in taste responsivity which appears to be modulated by the estrous cycle of the female rat. (Supported by NSERC of Canada).

180.10

SEX DIFFERENCES IN ASSOCIATIVE AND NONASSOCIATIVE AVOIDANCE OF A NOVEL TASTE IN RATS. M. Foy[1]*, S. Maren[2] and D. Mitchell[3]. [1]Dept. of Psychology, Loyola Marymount University, Los Angeles, CA 90045. [2]Dept. of Psychology, University of Michigan, Ann Arbor, MI 48109. [3]Dept. of Psychology, University of Southern California, Los Angeles, CA 90089.

Sexual dimorphism is commonly reported in tasks in which rats are offered a choice between novel and familiar alternatives, including taste neophobia and conditioned taste aversions (CTAs). Given theoretical assertions that delayed CTAs are mediated by nonassociative processes (Mitchell, Scott & Mitchell, 1977), and recent findings that basolateral amygdala lesions that disrupt taste neophobia also disrupt CTAs, we reasoned that both taste neophobia and delayed CTAs would also show significant sex differences. Accordingly, fluid deprived (30 min/day) male and female rats were compared in four different treatment conditions. Saccharin Poison Immediate (SPI) animals of both sexes were presented with a novel saccharin solution and made ill with lithium chloride (LiCl) injections administered immediately upon completion of the fluid session. Saccharin Poison Delay (SPD_4) animals were treated exactly as in condition SPI, except that LiCl administration was delayed 4 hrs after saccharin consumption. Water Poison Immediate (WPI) animals were also treated the same as SPI, except they were presented with familiar tap water immediately prior to LiCl administration. Water No Treatment (WNT) animals were presented with tap water and administered a needle poke immediately following the fluid session. Subsequent two bottle preference tests for the next 20 days revealed significant differences between the sexes in all treatment conditions. These results suggest that sex differences in CTAs are mediated by a common nonassociative learning process involved in both taste neophobia and delayed CTAs.

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180.11

LITTER SIZE AND ITS EFFECTS ON DAM MACRONUTRIENT SELF-SELECTION DURING LACTATION. S.H. Heil* and C.P. Cramer, Psychology Department, Dartmouth College, Hanover, NH 03755.

The existing literature on feeding during lactation suggest that there is a close positive correlation between a dam's food intake during this period and the number of pups she is nursing, i.e., dams with larger litters will consume more chow when compared to dams with smaller litters (Ota and Yokoyama, 1967; Isler et al., 1984). We have attempted to extend this finding by examining the effect of litter size on dam intake using a macronutrient self-selection paradigm.

On the day of birth, litters were culled to either six or fourteen pups (N = 6 litters/group). Throughout lactation, each dam's daily intake of isocaloric protein, carbohydrate, and fat diets, as well as dam bodyweight and cumulative litter bodyweight, were monitored. Contrary to previous findings, results indicate that dam bodyweight and total intake did not differ significantly between groups, although the litters of both groups grew at rates comparable to those reported in the literature. This may be a result of allowing the dams to self-select their diets, as the groups did differ significantly in their macronutrient intake over time. Specifically, dams with six pups had a higher protein:carbohydrate ratio when compared to dams with fourteen pups. Fat intake was comparable between groups. We are currently analyzing videotape recordings of these litters to determine whether different patterns of nursing (for example, bout duration or frequency) and/or different general activity levels may account for the distinct macronutrient patterns chosen by each group.

180.12

SERUM HORMONE LEVELS AND BODY WEIGHT GAIN IN NORMAL MALES DURING ONE-MONTH SULPIRIDE ADMINISTRATION. L. Tenoud*, T. Alastre, O. Contreras, J.L. Martinez, E. Araujo de Baptista, L. Hernández, and T. Baptista, Department of Physiology, Medical School, Universidad de los Andes, POB 109, Mérida, 5101-A, VENEZUELA.

Obesity is an undesirable side effect of neuroleptics, afflicting up to 50% of patients under a regimen on chronic administration. Two mechanisms have been proposed to explain the increased appetite and weight gain by neuroleptic drugs: a) direct stimulation of brain feeding-related areas; b) metabolic and endocrine abnormalities secondary to hyperprolactinemia. Few studies have correlated weight gain with the endocrine status in neuroleptic treated subjects. In the present study we administered Sulpiride (200 mg daily for 30 days, n =7) or placebo (n = 7) to healthy men, and correlated body weight gain with the serum levels of prolactin, luteinizing hormone, follicle-stimuling hormone, estradiol, free testosterone, thyrotropic hormone, free tetraiodothyroxine, cortisol, dehydroepiandrosterone sulphate (DHEA-S) and the ratios estradiol/testosterone and testosterone/DHEA-S. Body weight gain was significantly increased by Sulpiride ($p = 0.05$). The serum levels of prolactin and tetraiodothyroxine were significantly increased by Sulpiride ($p = 0.05$). A significant positive correlation was observed between prolactin levels and body weight gain in the Sulpiride group ($p = 0.01$). The results show that in healthy male subjects, body weight can be increased in the short term by neuroleptic administration. This effect can be related to hyperprolactinemia, independently from the usual changes in gonadal steroids observed in men with simple obesity.

Supported by the CDCH-T, ULA, Grant 493-94.

180.13

COMPARISON OF THE EFFECTS OF MIDAZOLAM AND MORPHINE ON THE MICROSTRUCTURE OF LICKING FOR INTRA-LIPID. Cooper, S.J. *, and Higgs, S. Department of Psychology, University of Durham, Science Laboratories, South Road, Durham, DH1 3LE, U.K.

There are many parallels between the effects of benzodiazepines and opioids on ingestive behavior. The benzodiazepine receptor agonist midazolam and the opioid receptor agonist morphine induce hyperphagia, enhance taste preferences and increase hedonic reactions to tastants in taste reactivity tests. The aim of the present studies was to use a microstructural approach to compare the effects of these compounds on the patterns of licking for a fat emulsion. The emulsion used was Intra-lipid (Pharmacia Ltd, Milton Keynes, UK) at concentrations of 1, 3 and 10%. A brief contact test was employed in which rats had 60s access to each concentration of Intra-lipid in a random order. The effect of pretreatment with either midazolam (0.3-3 mg/kg i.p.) or morphine (0.3-3 mg/kg s.c.) on licking behavior was then examined. Both midazolam and morphine a increased the total number of licks for Intra-lipid, but did so in different ways. Midazolam increased the number of licks by increasing bout duration, whereas morphine had a selective effect on bout number. The results indicate that the effects of benzodiazepines and opioids on ingestive behaviour can be differentiated using microstructural analysis of licking behaviour. They also indicate that these drugs affect ingestive responses for fat consumption.

180.14

FOOD INTAKE, BROWN FAT & PHYSICAL ACTIVITY ABNORMALITIES IN FAWN-HOODED RATS WITH CONGENITAL SEROTONIN ABNORMALITIES. M-T Maa, J. McCormick, T. Coon, S.R. Shenoy, C-T Hsu, B. Woynicz, D.C. Meyer* & P.F. Aravich. Eastern Virginia Med. School, Norfolk, VA 23501; VAMC, Hampton, VA 23667; Governor's School for Sci. & Tech., Hampton, VA 23666; Christopher Newport Univ., Newport News, VA 23606.

Fawn-Hooded (FH) rats are an animal model of depression & alcoholism and are characterized by, e.g., serotonin & glucocorticoid abnormalities. While both of these systems interact with ingestive behaviors, circadian rhythms & physical activity, little information is available on these parameters in FH rats under basal conditions. We evaluated these variables in female FH rats (5-mo old, n=6) & weight-matched Wistar controls (n=5) (0600:1800 LD cycle) (t-tests at $p<.05$). FH rats had decreased daily food intake under both resting & wheel-running conditions and increased brown adipose tissue weight relative to body weight, which suggests metabolic inefficiency. They ran less than Wistar controls but ate a normal amount of food per wheel revolution, indicating appropriate compensation for activity. FH rats had normal circadian distributions of food & water intakes that peaked between midnight & 0600 as well as a normal distribution of wheel running. They had decreased relative thymus weight, which is consistent with increased glucocorticoid secretion. Relative ovary, spleen and lung weights were normal. Daily water intake was normal but the water-to-food intake ratio & urinary output were elevated while relative posterior pituitary & kidney weights were decreased; these data are consistent with the presence of early kidney disease. It is concluded that 5-mo. old FH rats have food intake, metabolic & spontaneous wheel running abnormalities. They also have water balance abnormalities that are not shown by simple water intake measures. Finally, they have normal circadian distributions of ingestion & wheel running. Institutional Funds.

180.15

ADVERSE EFFECTS OF CHRONIC CAFFEINE EXPOSURE ON ANIMALS SUBJECTED TO EXERCISE STRESS & MALNUTRITION: IMPLICATIONS FOR ANOREXIA NERVOSA (AN). C-T Hsu, T. Coon, S.R. Shenoy, J. McCormick, M-T Maa, & P.F. Aravich*. Eastern Virginia Med. School, Norfolk, VA 23501; VAMC, Hampton, VA 23667; Governor's School for Sci. & Tech., Hampton, VA 23666; Christopher Newport Univ., Newport News, VA 23606.

Caffeine is the most widely used psychoactive substance. It can promote panic attacks & is abused by 15% of anorexics. AN is a devastating eating disorder associated with hyperactivity. Caffeine promotes brain serotonin (5HT), which may benefit AN. Thus, caffeine may be a way to self-medicate 5HT. However, it also promotes weight loss with sympathomimetics & has mixed effects on immunity. Hence, it might exasperate weight loss & immunosuppression in compromised, sympathetically activated anorexics. Animal modeling is needed to resolve these possibilities. We determined the effects of chronic caffeine on the weight-loss syndrome produced by exercise-stress in moderately food-deprived rats. Numerous authors have stressed the syndrome's relevancy to AN. Doses were used that produce plasma caffeine levels obtainable in humans (0, 10 & 40 mg/kg, ip, daily). The rats (female Sprague-Dawley; n/group: $\geq$10) were injected for a period that produces tolerance to several actions of caffeine (7 days) & then subjected to the syndrome. No food or body weight effects occurred during tolerance. However, chronic caffeine increased syndrome weight loss, worsened the stress triad (adrenal hypertrophy, immune organ atrophy) & worsened activity-stress ulcers. Posterior pituitary weight was also decreased. There were no differences in food intake or running on the terminal day of the experiment (i.e., syndrome maximum). It is proposed that the weight-loss effect is due to sympathetically mediated energy expenditure & the stress triad effect to excess glucocorticoids. These data raise concerns about the body weight & immunological effects of caffeine abuse in disorders related to exercise & malnutrition such as AN. Institutional Funds.

180.16

HYPERPHAGIA OF GENETICALLY OBESE ZUCKER RATS IS DEPENDENT UPON FORM OF DIET. A.J. Strohmayer* and D. Greenberg. Departments of Neurology and Psychiatry, Cornell Univ. Medical College, New York, NY 10021.

The meal patterns of genetically obese Zucker rats show increased daily food intake, increased meal size and decreased meal frequency when they are fed rat chow, Noyes pellets, or milk diet. This suggests a deficit in meal size regulation and satiety in obese Zucker rats. We wished to examine if meal patterns of Zucker rats are altered by the caloric density of the diet.

Male lean and obese Zucker rats (n=4/genotype) were tested in a meal pattern apparatus. Continuous recording was made of the intake of either solid lab chow (4.3 kcal/gm) or liquid (BioServ; 1 kcal/gm) diets. Body weights of both genotypes were maintained on both diets. With this apparatus we determined meal size (MS), daily food intake, intermeal intervals (IMI), and meal frequencies. Minimum IMI was 10 min.

Obese Zucker rats consumed more food per day, larger and less frequent meals compared to lean rats when feeding lab chow. When the less calorically dense liquid food was fed meal patterns of obese and lean rats did not differ.

Genotype	Diet	Daily Intake	MS	Meal Frequency	IMI
Obese	chow	134 ± 8.9	12.3 ± 0.9	10.9 ± 0.7	98 ± 6.4
Lean	chow	103 ± 2.4	8.4 ± 0.5	12.3 ± 0.9	89 ± 5.3
Obese	liquid	112 ± 3.7	5.2 ± 0.2	21.7 ± 1.4	55 ± 2.3
Lean	liquid	115 ± 2.9	7.3 ± 0.3	15.8 ± 1.1	76 ± 3.2

These findings could reflect differences in the gastric emptying rates of the two diets in lean and obese rats. The more rapid emptying of the liquid diet terminates meals sooner in the obese rats. The higher caloric density and slower gastric emptying of the chow may result in insufficient stimuli for satiation in the obese Zucker rat.

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INGESTIVE BEHAVIOR: PEPTIDE MEDIATORS

181.1

OPIOID RECEPTOR SUBTYPE AGONIST ENHANCEMENTS OF SUCROSE INTAKE ARE DEPENDENT UPON SUCROSE CONCENTRATION. H. Ruegg*, W.-Z. Yu, R. Fein and R.J. Bodnar. Dept. of Psychol. and Neuropsych. Doc. Prog., Queens Col., CUNY, Flushing, NY 11367.

Mu, delta and kappa agonists mediate palatable intake with enhancements typically occurring at higher concentrations of saccharin. Delta ($delta_1$, $delta_2$) and kappa ($kappa_1$, $kappa_3$) receptor subtypes have been described. The present study examined the pattern of enhancements of sucrose intake induced by central administration of selective mu ([D-Ala^2,MePhe^4,Gly-ol^5]-enkephalin, DAMGO), $delta_1$ ([D-Pen^2,D-Pen^5]-enkephalin, DPDPE), $delta_2$ ([D-Ala^2, Glu^4]-deltorphin, Delt II), $kappa_1$ (U50488H) or $kappa_3$ (naloxone benzoylhydrazone, NBzOH) agonists as a function of sucrose concentration (0.5,2.5,10%) in nondeprived rats. Sucrose intake following vehicle increased over 60 min as a function of concentration: 10%- 17.3 ml; 2.5%- 13.9 ml; 0.5%- 7.8 ml. DAMGO (500 ng: 17-66%) and DPDPE (1-20 ug: 17-46%) increased sucrose intake at 2.5% and 10%, but not 0.5%, concentrations. Delt II (1-20 ug, 27-50%) increased sucrose intake at 0.5% and 2.5%, but not 10%, concentrations. U50488H (1-20 ug) respectively increased (29%) and decreased (78%) sucrose intake at 10% and 0.5% concentrations. NBzOH (1-20 ug) sporadically increased (23-28%) sucrose intake across doses and concentrations. These data suggest that opioid receptor subtype agonists differentially enhance sucrose intake as a function of sucrose concentration. (Supported by NIDA 04194)

181.2

GENETICALLY-OBESE ZUCKER RATS DISPLAY DECREASED SENSITIVITY TO CENTRAL OPIOID RECEPTOR SUBTYPE ANTAGONIST WEIGHT REDUCTIONS. J.L. Cole*, N. Berman, W.D. Bowen, G.W. Pasternak and R.J. Bodnar. Dept. of Psychol. and Neuropsych. Doc. Prog., Queens Col., CUNY, Flushing, NY 11367.

Chronic antagonism of opioid receptor subtypes (mu, mu_1, $kappa_1$, $delta_1$, $delta_2$) significantly reduce weight and intake in rats. Genetically-obese Zucker rats (fa/fa) show enhanced sensitivity to naloxone's reductions of spontaneous and deprivation-induced intake relative to heterozygous lean litter mates (Fa/fa). The present study examined whether chronic (7 days) central microinjections of selective, long-acting mu (beta-funaltrexamine, 20 ug), mu_1 (naloxonazine, 50 ug), $kappa_1$ (norbinaltorphamine, 20 ug), $delta_1$ ([D-Ala^2, Leu^5, Cys^6]-enkephalin, 40 ug) or $delta_2$ (naltrindole isothiocyanate, 20 ug) antagonists differentially altered weight and intake in Zucker and lean rats relative to vehicle. Zucker rats displayed significantly greater basal weight and intake than lean rats. Opioid antagonism significantly reduced body weight after 7 days to a greater degree in lean (mu: 11.4%; mu_1: 18.9%; $kappa_1$: 8.0%; $delta_1$: 11.9%; $delta_2$: 9.3%) than in Zucker (mu: 8.9%; mu_1: 7.1%; $kappa_1$: 2.4%; $delta_1$: 4.1%; $delta_2$: 5.6%) rats. The reduced weight loss was noted over the 7 day paradigm, and occurred despite greater magnitudes of intake reductions in Zucker rats following mu, mu_1 and $delta_2$ antagonism. Thus, Zucker rats defend weight better than lean littermates following chronic opioid receptor subtype antagonist treatment. (Supported by DA04194)

181.3

MODIFICATION OF DEPRIVATION, GLUCOPRIVIC AND PALATABLE INTAKE FOLLOWING OPIOID RECEPTOR SUBTYPE ANTAGONISTS IN THE VENTRAL TEGMENTAL AREA OF RATS. A. Ragnauth*, H. Ruegg and R.J. Bodnar. Dept. of Psychol. and Neuropsych. Doc. Prog., Queens Col., CUNY, Flushing, NY 11367.

Selective mu, delta and kappa agonists in the ventral tegmental area (VTA) stimulate feeding. General and mu antagonism reduce deprivation, glucoprivic and palatable intake in either the hypothalamic paraventricular nucleus (PVN) or nucleus accumbens (NAcc). Kappa antagonism reduces all forms of intake in the PVN, and deprivation and glucoprivic intake in the NAcc. The present study examined whether general (naltrexone, 10-50ug), mu (beta-funaltrexamine, 1-4ug), kappa$_1$ (nor-binaltorphamine, 4ug), delta$_1$ ([D-Ala^2, Leu^5, Cys^6]-enkephalin, 4-8 ug) or delta$_2$ (naltrindole isothiocyanate, 1-4ug) opioid antagonists in the VTA alter intake under either deprivation (24h), glucoprivic (2DG: 500 mg/kg) or palatable (10% sucrose) conditions. Deprivation intake was significantly reduced by general (23%, 2-4 h) and delta$_2$ (28%, 2 h) VTA antagonism. Glucoprivic intake was significantly reduced by delta$_2$ (39-69%, 0.5-4 h) and kappa$_1$ (33-54%, 0.5-4 h) VTA antagonism, and transiently reduced by general (1 h) and mu (1-2 h) VTA antagonism. Sucrose intake was significantly reduced by general (35-46%, 10-60 min) and delta$_2$ (19-44%, 5-60 min) VTA antagonism. Opioid antagonism in the VTA appears most effective for glucoprivic intake, and less effective for deprivation and palatable intake. (Supported by DA 04194)

181.4

ANTISENSE OLIGODEOXYNUCLEOTIDES TARGETED AGAINST THE MOR-1 CLONE REDUCE HYPERPHAGIA INDUCED BY MU OPIOID AGONISTS IN RATS. L. Leventhal*, G.W. Pasternak, G.C. Rossi and R.J. Bodnar. Dept. of Psychol. and Neuropsych. Doc. Prog., Queens Col., CUNY, Flushing, NY 11367.

Antisense oligodeoxynucleotides (AS ODN) targeted against the MOR-1 clone selectively reduce analgesia elicited by mu opioid agonists. MOR-1 AS ODNs also significantly reduce body weight and spontaneous food intake. The present study evaluated whether central pretreatment over 5 days (Days 1, 3, 5) of AS ODNs (10 ug) directed against the 5' untranslated region of exon 1 of the MOR-1 clone altered hyperphagia induced by the selective mu opioid agonist, [D-Ala^2, Me-Phe^4, Gly-ol^5]-enkephalin (DAMGO: 0.5-5 ug) administered 24 h after the last AS treatment (Day 6). All DAMGO doses significantly increased food intake after 2 and 4 h. MOR-1 AS ODNs significantly reduced the magnitude of DAMGO-induced hyperphagia following 0.5 and 1 ug doses after 2 (71-82%) and 4 (87-100%) h, but failed to alter hyperphagia following a 5 ug dose. The specificity of these actions was confirmed using the selective mu antagonist, beta-funaltrexamine to eliminate DAMGO (0.5-1 ug)-induced hyperphagia. These data extend MOR-1 AS ODN effects upon mu-mediated opioid agonist actions to ingestive behaviors, and further validate the use of antisense approaches as pharmacological and molecular tools in specifying functional effects. (Supported by DA 04194)

181.5

CENTRAL INJECTION OF THE ORL-1 LIGAND, ORPHANIN FQ INCREASES FEEDING IN RATS. A.S. Levine*, J.D. Pomonis, and C.J. Billington. Graduate Program in Neuroscience, Univ. of Minnesota and VA Medical Center, Minneapolis, MN 55417

It has long been known that opioids play a role in feeding behavior and that opioid antagonists decrease feeding induced by a variety of factors. Recently, orphanin fq (nociceptin), the presumed ligand for ORL-1, the orphan opioid receptor, has been purified and has been shown to have structural similarities with dynorphin A. It has also been shown to have very little activity at the classic opioid receptors. Similarly, the classic opioid peptides show very low levels of activity at ORL-1. In this experiment, we tested whether central administration of orphanin fq had any effects on feeding. Six male, Sprague-Dawley rats were fitted with cannulas into the lateral ventricle and were injected with either saline, 1 nmol, or 10 nmol orphanin fq, and food intake was measured for the two hours following injection. Injection of orphanin significantly increased food intake in both the low and the high doses when compared to saline administration {$F(2,10)=5.019$, $p=0.0309$}. Administration of 3mg/kg naloxone 20 minutes prior to ICV administration of 10 nMol orphanin fq, blocked the orphanin fq-induced increase in food intake {$F(3,12)=4.515$, $p=0.0243$}. These data suggest that orphanin fq shares at least one functional aspect with the classical opioid peptides, that being the ability to increase food intake. (Supported by NIH DA03999)

181.6

EFFECT OF U50488, A HIGHLY SELECTIVE KAPPA-OPIOID AGONIST ON BAR PRESSING FOR A SUCROSE REWARD. **C. Barton*, K. Stokes, S. Bilyeu, and A. Armstrong. Department of Psychology, Tennessee Technological University, Box 5031, Cookeville, TN 38505.**

Previous studies have suggested that selective opioid agonists and antagonists may act to modulate dietary macronutrient selection in rats. Specifically, it has been demonstrated that the kappa-opioid agonist U50488 stimulates consumption of meals high in fat, while the kappa-opioid antagonist nor-Binaltorphimine produces the inverse effect. The objective of this study was to investigate if U50488, a highly selective kappa-opioid agonist would stimulate bar pressing behavior rewarded by sucrose pellets in sucrose-sated rats. Cannula were implanted into the lateral ventricle of 7 female Sprague-Dawley rats trained to bar press for 45 mg sucrose pellet rewards. Animals were injected with either U50488 (215, 21.5, or 2.15 nmoles/5 µl) or saline vehicle (0.9% w/v) using a randomized block, within subject design. Prior to testing animals were allowed unrestricted access to lab chow and were permitted to bar press for a 1 hr period prior to testing on a CRF schedule to receive sucrose pellets. Rats were then withdrawn from operant chambers, centrally injected, replaced into operant chambers, and bar pressing behavior as well as consumption of sucrose pellets were recorded for a period of 1 hr. The two highest doses of U50488 (215 & 21.5 nmoles) produced a significant increase in bar presses to obtain and consume sucrose pellets, as compared to saline controls. These findings suggest that the kappa-opioid receptor may not be specifically responsible for modulation of dietary fat macronutrient, but instead this receptor site may respond to agonists by stimulating rewarded behavior(s). **This research was supported by funds from Tennesse Technological University.**

181.7

MULTIPLE INJECTIONS OF CHOLECYSTOKININ FAIL TO INDUCE A CONDITIONED, AVERSIVE SHIFT IN TASTE REACTIVITY RESPONSES IN RATS. L.A. Eckel* and K.-P. Ossenkopp. Neuroscience Program, University of Western Ontario, London, Ontario, Canada, N6A 5C2.

The present study examined whether prolonging the pharmacological action of cholecystokinin (CCK) would promote aversive responses in rats exposed to a rapid gustatory conditioning paradigm. Over a 60 min test session, taste reactivity responses to 5 brief (30 s) oral infusions of a 0.30 M sucrose solution were monitored in rats following a single injection of lithium chloride (LiCl, 127 mg/kg, ip), or 5 injections of CCK (each 8 µg/kg, ip) or vehicle administered at 15 min intervals. Two days later, rats were tested for the development of a conditioned taste aversion (CTA). A conditioned, aversive shift in taste reactivity responses was observed in LiCl treated rats. In contrast, CCK treated rats showed an immediate, unconditioned decrease in ingestive responses that was not accompanied by an increase in aversive responses. Only the LiCl group showed evidence of a CTA. These results demonstrate that multiple injections of a moderate dose of CCK fail to produce the type of aversive internal cues responsible for conditioning a shift in taste reactivity responses and the subsequent production of a CTA. We conclude that these results are consistent with a satiety effect of CCK. (Supported by NSERC and MRC).

181.8

A 35 AMINO ACID FRAGMENT OF LEPTIN INHIBITS FOOD INTAKE. W.K. Samson*, T.C. Murphy, D. Robison, T.Vargas, E. Tau and J.-K. Chang. Physiology, UND School of Medicine, Grand Forks, ND 58202 and Phoenix Pharmaceuticals, Mt. View, CA 94043.

The 167 amino acid obesity gene related peptide, leptin, reverses hyperphagia in genetically obese animals and circumscribes feeding in normal animals. We attempted to identify the bioactive portion of the leptin molecule in feeding studies. Synthetic peptide fragments were injected either *ip* or *icv* into rats habituated to metabolic cages, after minimally three days of baseline data collection. Adminstration *ip*, once per day for two days, of the 116-167 fragment of leptin (15 µg) resulted in a slight but significant reduction in food intake at 24 and 48 hr. Central administration (3 µg) once per day for two days decreased food intake only after the second day of treatment. No receptor binding could be detected in CNS using I-125 labelled OBGRP 116-167. No significant effect on food intake was observed following injection of the 57-92 amino acid fragment of OBGRP. Similarly, injection of antiserum directed against the 57-92 aa fragment did not alter feeding suggesting that neither this fragment, nor a peptide fragment containing the 57-92 aa segment was bioactive. Finally we tested more N-terminally located fragments and observed significant, dose-related inhibition of food intake with the 22-56 aa fragment of OBGRP. A 25% decrease in intake was observed after *icv* 500 ng OBGRP 22-56 and a 45% decrease following 1000 ng. These data suggest that a small, readily synthesized fragment of the 167 aa peptide leptin can exert physiologically relevant satiety effects in brain.*Funding

181.9

CTAP REDUCES NPY- AND DEPRIVATION-INDUCED FEEDING AND THE ANTINOCICEPTIVE EFFECTS OF MU-OPIOIDS IN RATS. D.C. Jewett*, D.L. Totzkay, A.L. Shabazz, P. Sacchetti, R.R. Witte II and A.M. Young, Dept. of Psychol., Wayne State Univ., Detroit, MI 48202.

Opioid antagonists reduce deprivation- and NPY-induced feeding in a variety of species. Yet, the role of opioid receptor types in feeding regulation is unclear. The effects of CTAP, a peptidic antagonist selective for mu-opioid receptors, were studied in rats following 24-hr food deprivation [DEP] or intraventricular (i.c.v.) injection of NPY. DEP produced a 5-fold increase in food intake. CTAP (1-10 μg; administered 15 min prior to food access) dose-dependently reduced DEP-induced feeding. In non-restricted rats, NPY (5 μg) produced a 6-fold increase in food intake. CTAP also dose-dependently reduced NPY-induced feeding. The findings that CTAP is equipotent in reducing NPY- and DEP-induced feeding in rats is consistent with findings in other species (Jewett & Woods, 1995), and support further a role for mu-opioid receptors in NPY- and DEP-induced feeding. The ability of CTAP to antagonize the antinociceptive effects of mu-opioid agonists (morphine [MS] and DAMGO) was also assessed in the warm-water, tail withdrawal assay. Both MS and DAMGO dose-dependently increased tail-withdrawal latencies. The antinociceptive effects of fully effective dose of MS (10 mg/kg) or DAMGO (1 μg, i.c.v.) were reversed by CTAP (1 μg, i.c.v.). When administered as a pretreatment, CTAP (1 μg) reduced the potency of both morphine and DAMGO by 10- to 32-fold. Taken together, these studies demonstrate that CTAP is a very effective mu-opioid antagonist in rats. [Supported by USPHS Grants DA03796, K02 DA00132 and GM08167]

181.10

EFFECTS OF A NPY ANTAGONIST ON FEEDING IN RATS AND MICE. J.N. Wiley*, L.M. Georgic, J.L. Wright, R.G. MacKenzie, and T.G. Heffner, Parke-Davis Pharmaceutical Research, Division of Warner-Lambert Company, Ann Arbor, MI 48105.

Because brain neuropeptide Y (NPY) may exert important influences on the regulation of feeding, we examined the effects of a putative NPY-Y_1 antagonist, (R)-BIBP 3226 (Karl Thomae Co.), on food intake in rodents. (R)-BIBP 3226 was found to have high affinity for displacing [125]I-PYY from NPY Y_1 receptors expressed natively in SK-N-MC cells (Ki = 7.6 nM). When given IP at the start of the 12 h dark cycle, (R)-BIBP 3226 caused dose-related inhibition of food intake in non-deprived male Zucker obese rats. Doses of 3, 10, and 30 mg/kg IP reduced 2 h food consumption by 27, 57 and 88%, respectively, relative to vehicle-treated controls. The effect had diminished by 8 hours after dosing and was absent at 24 h. (R)-BIBP 3226 was also effective but less potent when given to normal male Sprague-Dawley rats: doses of 3, 10, and 30 mg/kg IP reduced feeding by 0, 41, and 73%, respectively at 2 h post dose. Only the 30 mg/kg dose continued to have an effect for 8 h. (R)-BIBP 3226 given ICV reduced feeding in CD-1 albino mice: total doses of 10 and 30 ug ICV inhibited consumption by 63 and 94%, respectively, at 2 h post dose, effects that diminished by 6 h. In an attempt to further link the effects on feeding to NPY receptors, the (S)-enantiomer of BIBP 3226, which lacks affinity for NPY Y_1 receptors (K_i >1,300 nM), was also tested. (S)-BIBP 3226 (30 mg/kg IP) reduced feeding in Zucker obese rats by 100% at 2 h, by 76% at 8 h, and by 83% at 24 h post dose. These data suggest that effects other than those on NPY receptors may account for the effects of the enantiomers of BIBP 3226 on feeding. (Supported by Warner-Lambert.)

181.11

GLUCAGON-LIKE PEPTIDE-1 (7-36) AMIDE (GLP-1) ACTIVATES THE HYPOTHALAMIC-PITUITARY-ADRENAL (HPA) AXIS OF RATS. T. Wang*, G. L. Edwards and C. A. Baile. Grad. Stud. Res. Ctr., The Univ. of Georgia, Athens, GA 30602.

GLP-1 was reported to reduce food intake (FI) of rats following injections into the 3rd cerebral ventricle (Nature 379:69-72, 1996). The objective of the present study was to investigate whether the same dose of GLP-1 that reduced FI influenced the HPA axis. Eighteen rats were surgically prepared with guide cannula into the 3rd ventricle. All rats responded by drinking at least 1.5 ml of water in 30 min following an injection of 100 ng angiotensin II in 5 µl artificial cerebrospinal fluid. In the first part of the study, FI and water intake were measured after 10 µg GLP-1 in 5 µl saline or 5 µl saline was injected into the 3rd ventricle at the beginning of the 12 hr dark phase. Cumulative FI of the GLP-1 treated rats (n=9), compared to that of the saline treated rats (n=9), was reduced from 1.0 g to 0.9 g ($p=0.85$), 2.2 g to 1.1 g ($p=0.05$), 4.3 g to 1.9 g ($p<0.001$), 5.9 g to 2.2 g ($p<0.001$), 22.4 g to 18.1 g ($p<0.02$), 28.4 g to 23.0 g ($p<0.01$) at 0.5, 1, 2, 3, 16 and 24 hr, respectively. Cumulative water intake was also decreased by GLP-1 treatment compared to saline treatment from 0.5 ml to 0.1 ml ($p=0.23$), 0.9 ml to 0.1 ml ($p=0.16$), 2.2 ml to 0.2 ml ($p=0.04$), 3.6 ml to 0.3 ml ($p=0.01$), 28.3 ml to 18.8 ml ($p=0.04$), 29.4 ml to 23.7 ml ($p=0.23$), respectively. In the second part of the study, the effect of GLP-1 on HPA axis activity was examined without food present. GLP-1 (10 µg in 5 µl saline), injected into the 3rd ventricle at the onset of the dark phase, increased plasma corticosterone concentration at 30 min post injection compared to saline treatment (347.5 ng/ml (GLP-1, n=7) vs. 247.6 ng/ml (saline, n=9) ($p=0.05$)). We conclude that GLP-1 decreases food intake and water intake and increases the activity of the HPA axis in rats. GLP-1 may have a role in control of ingestive behavior and regulation of the HPA axis. (Supported by The University of Georgia).

181.12

THE INHIBITORY EFFECT OF GLUCAGON-LIKE PEPTIDE-1 (GLP-1)(7-36) AMIDE ON FOOD AND WATER INTAKE IN RATS IS ABOLISHED AFTER NEONATAL TREATMENT WITH MONO-SODIUM GLUTAMATE (MSG). M TANG-CHRISTENSEN[1]*, S SHEIKH[2] and P.J. LARSEN[1] Medical Anatomy, section B and [2]Clinical Biochemestry, University of Copenhagen, Blegdamsvej 3, 2200 Copenhagen, Denmark.

Central administration of the gastrointestinal hormone Glucagon-Like-Peptide 1 (GLP-1) (7-36) amide inhibits food and water intake in hungry rats and the inhibitory effect upon water intake is also elicited by peripherally (ip) administered GLP-1.Furthermore GLP-1 inhibits angiotensin II (AII)(icv) induced drinking, both after ip and icv admnistration. By use of receptorautoradiography we have found wide distribution of central binding sites for I^{125}-GLP-1 including the subfornical organ (SFO) and the arcuate nucleus. These nucleii are involved in drinking and feeding behaviour in the rat. By far the majority of the SFO possesses no bloodbrain barrier, leaving this area accessible for hu-moral stimulation via the vascular system. It is our hypothesis that the SFO is in-volved in the GLP-1 induced inhibition of drinking elicited by thirst or AII whereas the arcuate nucleus (ARN) is involved in GLP-1 induced inhibtion of food intake in hungry rats.To test this hyppothesis we subjected neonatal wistar pups to excessive doses of subcutaneously administered mono-sodium-glutamate (MSG). Neonatal MSG-treatment infers profound lesions of the circumventricular organ, including part of the SFO and the ARN. The inhibitory effect of GLP-1 upon feeding was comple-tely abolished in MSG-rats. Furthermore we found that the AII induced drinking response was preserved in MSG rats. However, only icv injection of GLP-1 abolished AII induced drinking while peripheral administration (ip) of GLP-1 did not inhibit AII induced drinking behaviour. Thus, it is evident that GLP-1 can modulate feeding and drinking behaviour. Further, that the ARN could be responsible for the GLP-1 inhibition of foodintake and the SFO is a possbile site of action for the inhibitory effect of the drinking response.

181.13

CENTRAL INFUSION OF NEUROMEDIN B & GASTRIN-RELEASING PEPTIDE RECEPTOR ANTISENSE OLIGONUCLEOTIDES REDUCE ANXIETY, BOMBESIN (BN) BINDING, & BN-ELICITED BEHAVIORS. H. Plamondon*[1], T.W. Moody[2] and Z. Merali [1,3].

[1]Psychology & [3]Pharmacology, University of Ottawa, Ont. Canada K1N 6N5. [2]Biomarkers & prevention research branch, NCI, Rockville, MD 20850.

Central BN administration induces satiety and elicits intense grooming that can partially be reversed by gastrin-releasing peptide (GRP) receptor blockade. Furthermore, recent findings in our laboratory suggest a potential role of BN-like peptides in stress and anxiety. In the current study, antisense oligonucleotides to mRNA for GRP and neuromedin B (NMB) receptor subtypes were used to determine the physiological role of BN-like peptides. NMB and GRP antisense oligonucleotides were infused over 2 days into the 3rd ventricle via cannula attached to an osmotic pump, at the rate of 0.94 µg/0.5µl/h. Spontaneous food intake was monitored as was their response to an acute BN challenge (0.25 µg; i.c.v.) on palatable food intake and grooming response. Rats were also tested on an elevated plus maze and trough-tunnel oval maze, to assess their anxiety level. In both those mazes, the rats treated with the antisense oligonucleotides spent significantly more time on the open or exposed (anxiogenic) fields, reflecting an anxiolytic effect of the treatment, as compared to saline or scrambled antisense-exposed animals. The controls responded to acute BN challenge with a marked increase in scratching response and a suppression of the test meal consumption. In rats exposed to the oligonucleotides, acute BN failed to suppress food intake or to elicit scratching. In vitro receptor autoradiography revealed that antisense oligonucleotide treatment resulted in a reduction (15-35%) of BN binding site density in various brain regions. These results implicate physiological participation of BN-like peptides in the control of appetite and anxiety.
Supported by Medical Research Council of Canada.

181.14

MEAL-CONTINGENT INFUSION OF GASTRIN-RELEASING PEPTIDE $_{1-27}$ REDUCES MEAL SIZE AND INTERMEAL INTERVAL AND INCREASES THE SATIETY RATIO IN SPONTANEOUSLY FEEDING RATS. P. A. Rushing*, R. Henderson, and J. Gibbs. Bourne Laboratory, New York Hospital-Cornell Medical Center, White Plains, NY 10605 and Psychology Department, The Florida State University, Tallahassee, FL 32306-1051

We have recently reported that brief, meal contingent infusion of gastrin-releasing peptide$_{1-27}$ (GRP) reduces spontaneous feeding in rats. However, only one dose (5 nmol/kg) was investigated. The present study explored the effects of a range of doses of GRP using a new system designed to examine spontaneous feeding in rats consuming a liquid diet. Undisturbed, *ad lib*-fed (milk) male rats (n = 4) with chronic inferior vena caval catheters were infused with saline and each of 3 doses (5, 10, and 20 nmol/kg) of GRP in counterbalanced order. Infusions were activated at the onset of the first nocturnal meal and continued for 2 min (60 µl/min), with delivery of peptide during the first minute. Infusions and recording of meal data (licks) were fully automated and computer-controlled.

	0†	5	10	20
Meal Size (licks)	1337 ± 254	534 ± 174*	479 ± 150*	284 ± 66*
Meal Duration (min)	285 ± 27	107 ± 30*	105 ± 33*	58 ± 8*
Intermeal Interval (min)	72 ± 5	53 ± 7*	40 ± 4*	40 ± 5*
Satiety Ratio (min/licks)	.06 ± .01	.13 ± .04	.10 ± .03	.16 ± .02*

† nmol/kg GRP, * Significantly different from 0 nmol/kg, $p < .05$.

All doses of GRP significantly reduced the size and duration of the first nocturnal meal and shortened the interval from the end of the first meal to the onset of the next. Despite this, the satiety ratio (interval/meal size) tended to increase after GRP and was significantly increased by the highest dose. These results show that brief, meal-contingent intravenous infusion of GRP potently inhibits spontaneous feeding in undisturbed rats.
Supported by USPSH grants MH18390 (PAR), DK33248 (JG).

181.15

PREGASTRIC AND GASTRIC STIMULI OF PREFEEDING ARE SUFFICIENT TO ENHANCE THE SATIATING EFFECT OF GASTRIN-RELEASING PEPTIDE$_{1-27}$. J.M.Frennier, P.A.Rushing, C.Shamoian,* and J.Gibbs. Bourne Laboratory, Dept. of Psychiatry, Cornell Medical Center, White Plains, NY 10605.

Prefeeding enhances suppression of food intake by peripherally administered gastrin-releasing peptide $_{1-27}$ (GRP; Kirkham et al, 1995). To identify the site(s) for this effect, we used an inflatable pyloric cuff to block gastric emptying and postgastric sites. Five male Sprague-Dawley rats inplanted with cuffs were maintained on *ad lib* powdered chow and water. Rats received a 30-min test meal of high-carbohydrate liquid food under either Prefeed or No Prefeed conditions. In the Prefeed condition, the chow was removed, the cuffs were inflated, and rats were given a 3-min prefeed of a high carbohydrate diet (intake=7.45±0.5 ml). Fifteen minutes,after the 3-min prefeed, rats received a 30-min test meal of the same liquid food. The No Prefeed condition was similar, but the 3-min prefeed was omitted. Five minutes prior to the 30-min test, in both conditions, animals were injected IP with saline vehicle, 14 ug/kg, or 21 ug/kg GRP.

	Saline	14 ug/kg GRP	21 ug/kg GRP
No Prefeed			
30-min intake (ml ± SEM)	24.2 ± 2.3	24.4 ± 3.0	20.6 ± 1.4
% Suppression		-1.3 ± 4.4	14.7 ± 4.1
Prefeed			
30-min intake (ml ± SEM)	13.7 ± 1.2	6.2 ± 1.3*	7.0 ± 1.8*
% Suppression		43.6 ± 12.1†	52.8 ± 15.9†

* Significantly less than saline, † Significantly greater than No Prefeed

The enhanced suppression of intake produced by GRP after prefeeding with the cuff closed demonstrates that pregastric and gastric stimuli are sufficient to increase the satiating effect of GRP. [Supported by USPHS Training Grant MH18390 (PAR), and Research Grant DK33248 (JG)].

181.16

BEHAVIORALLY SPECIFIC ENHANCEMENT OF THE SATIATING POTENCY OF GASTRIN-RELEASING PEPTIDE$_{1-27}$ BY ESTRADIOL. A.M. Cuomo, P.A. Rushing, J. Gibbs*, and N. Geary. Bourne Laboratory, Dept. of Psychiatry, Cornell Medical Center, White Plains, NY, 10605.

Estrogen may cause the decrease in meal size during estrus by enhancing the actions of satiating peptides. To test this, 26 bilaterally ovariectomized, Sprague Dawley rats received either 0.1 ml 10 µg β-estradiol benzoate (EB) or the sesame oil vehicle subcutaneously at 0930h each Tues and Wed. The satiating potency of gastrin-releasing peptide$_{1-27}$ (GRP) vs saline (IP) was tested in crossover designs on consecutive Fridays. Test meals were 0.4M sucrose, and the prefeeding procedure of Kirkham *et al.* (Physiol Behav 58:1175, 1995) was used to maximize GRP's satiating potency.

Meal Size (ml)	Saline	5µg/kg	10µg/kg	21µg/kg
Oil	8.7 ± .09	6.8 ± 0.7*	5.8 ± 0.9	5.7 ± 1.2*
EB	8.4 ± .02	6.6 ± 1.3*	3.8 ± 0.8*+	3.9 ± 0.6*
% Inhibition				
Oil	-	14 ± 16*	3 ± 20	46 ± 8*
EB	-	25 ± 14*	52 ± 10*+	54 ± 8*

Values are mean ± SEM. + compared to saline, * compared to oil-treated, p<0.05.

In the 30 min between the prefeed and the test meal, EB-treated rats groomed significantly more (39 vs 25%; p≤0.02) and rested significantly less (2 vs 18%; p≤0.02) than oil-treated rats. GRP injection reduced meal size (Table), and 10 µg/kg GRP reduced meal size more in EB-treated than oil-treated rats. All rats displayed the full behavioral sequence of satiety after test meals. These data indicate that exogenous GRP has a specific satiating action on meal size in ovariectomized rats and that EB can increase the potency of this action. Increased satiating potency of GRP may be one mechanism mediating the effect of EB on meal size.

Supported by USPSH grants MH18390 (PAR), DK33248 (JG), MH51135 (NG).

181.17

ANGIOTENSIN RECEPTOR BINDING IN RELATION TO SODIUM APPETITE. *A. Morien* and N.E. Rowland. Psychology Dept., University of Florida, Gainesville, FL 32611*

Sodium appetite can be produced in rats by several procedures, including chronic low Na diet and administration of ANG I CEI (eg, enalapril, ENAL). These paradigms are known to produce changes in peripheral and central ANG II production. The purpose of the present study was to examine changes in ANG receptors in rat brain during these treatments. A second objective was to examine time-of-day effects. ANG II receptor binding was examined at four times: 2 & 8 hr after lights on and 2 & 8 hr after lights off. Three groups of male SD rats underwent two week treatment with (a) a mini-pump infusion of ENAL (3 mg/kg/day), or (b) a low Na diet (DIET, 0.03-0.05% Na), or (c) a normal diet (CONTROL, > 0.2% Na). Brains were sectioned, incubated with I^{125} SI-AII; alternate sections were blocked with losartan or ANG II. In the anterior circumventricular organs, ENAL increased binding and DIET tended to decrease binding, in relation to CONTROL. This may be related to the respective decrease and increase in plasma ANG II in these paradigms. Results from regions "downstream" of these organs showed greater binding in DIET and less binding in ENAL. A significant effect of TIME was evident in some regions. Grant: NIH-NRSA

181.18

EFFECT OF ANGIOTENSIN (AT_1 AND AT_2) ANTISENSE OLIGONUCLEOTIDES ON WATER DEPRIVATION INDUCED DRINKING. H.-B. Meng, E.-K. Yang, S.M. Galli, D.W. Walker* and M.I. Phillips. Dept. of Physiology, Neuroscience, College of Medicine, University of Florida, Gainesville, FL 32610-0274 and Department of Physiology, Kyungpook National University, South Korea.

Water deprivation induced drinking is partially regulated by Ang II. There are conflicting data on the roles played by AT_1 and AT_2 receptor subtypes in water deprivation induced drinking by using different AT_1 and AT_2 antagonists. To resolve this issue we tested 15 mer length antisense oligonucleotides (AS-ODNs) designed against AT_1 and AT_2 receptor mRNA. Male Sprague-Dawley (SD) rats were cannulated in the lateral ventricle one day before the treatment. After i.c.v. injection of 50 ug/2 µl of AS-ODN or SC-ODN, rats were returned to cages and subjected to a 24 hrs period water deprivation. Then water bottles became available and the drinking response was monitored for 14 hrs (overnight). The control level of water intake was 51.3 ± 12.2 ml of water. The AS-ODN against AT_1 receptor significantly decreased the drinking response (21.925 ± 6.37 ml, P<0.01). The AS-ODN for AT_2 decreased the drinking response, but not significantly (43.3 ± 16.4 ml). The SC-ODN injected group showed no difference compared to saline control. In a receptor binding assay for rat brain tissue, both AS-ODNs specifically decreased the corresponding receptor subtype. The data show that AT_1 but not AT_2 is important in the drinking to water deprivation. We conclude that AS-ODN are useful in discriminating the role of two Ang II receptor subtypes as an alternative to pharmacological antagonists. (Supported by NIH HL27334).

181.19

THE HYPOPHAGIC EFFECT OF CCK-8S DEPENDS ON THE FEEDING CONDITIONS AND DIFFERS IN ADULT AND AGED RATS. M. Voits*, J.-P. Voigt, J.P. Huston[1], H. Fink Institute of Pharmacology and Toxicology, Charité, Humboldt University, D-10098 Berlin, Germany; [1]Institute of Physiological Psychology I, Heinrich-Heine University of Düsseldorf, Universitätsstr. 1 D-40225 Düsseldorf, Germany.

The sulphated cholecystokinin octapeptide (CCK-8S) regulates food intake in many species including humans by peripheral and central mechanisms. The majority of studies reported a hypophagic action of exogenous CCK-8S, but the results of the effect of CCK on food intake in humans are inconsistent. Besides the hypophagic action, CCK was shown to be involved in several other behavioral processes e.g., analgesia, anxiety and memory.

This study was performed to investigate the effect of CCK-8S on food intake under a fixed feeding condition in comparison to a test meal taken after 16h food deprivation in young (8 weeks old) and aged (23 months old) male Wistar rats. In the fixed feeding condition rats were adapted to a 4h feeding schedule for 4 days.

CCK-8S (8 and 40µg/kg, i.p.) reduced the size of a test meal following 16h food deprivation significantly. This effect was independent of the age of the rats. However, under fixed feeding conditions neither of the doses used reduced food intake in the young adult rats, whereas the highest dose of 40µg/kg did so in the aged rats. These results suggest that the hypophagic effect of exogenous CCK-8S depends on experimental conditions, being effective after a period of food deprivation but not under a fixed feeding regimen in adult rats. Furthermore, the data suggest that age is a factor contributing to the complex behavioral actions of CCK, since only old animals were more susceptible to the hypophagic action of CCK-8S. This may be explained by an interaction of other behavioral effects of CCK with its satiating action under the fixed feeding schedule.

Supported by DFG (INK 231/A1-1).

181.20

ENDOGENOUS CHOLECYSTOKININ IN GASTRIC EMPTYING OF PEPTONE IN THE RAT, W. White, G. J. Schwartz, P. R. McHugh* and T. H. Moran. Dept. of Psychiatry, Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205.

The role of the brain/gut peptide cholecystokinin (CCK) in the control of gastric emptying of peptone solutions was studied. Awake male Sprague-Dawley rats were given intraperitoneal injections of 0, 10, 32, 100, 320, or 1000 µg/kg of the CCK A receptor antagonist L-364,718 (devazepide). Thirty minutes later they received, via gavage, a 5.0 ml intragastic infusion of 5.0% peptone solution, 12.5% peptone solution, or equimolar NaCl solutions. Ten minutes after infusion, stomach contents were withdrawn, and the volume of the remaining infused solution was measured. 5.0% peptone always emptied more quickly than 12.5% peptone. As the dose of the CCK A antagonist increased, the rate of emptying of both peptone concentrations appeared to increase in parallel and asymptoted at 100 µg/kg. A 0.9% saline solution emptied more quickly than either peptone concentration at any dose. Thus devazepide only partially reversed the slowed gastric emptying produced by peptone loads. Increasing the concentration (and so the osmolarity) of the NaCl solution also slowed gastric emptying, but solutions of saline emptied substantially more quickly than equimolar solutions of peptone, and devazepide had no effect on emptying of NaCl solutions. Peptone appears to delay gastric emptying via at least two mechanisms, a CCK-dependent mechanism that is sensitive to protein content, and a CCK-independent mechanism that is sensitive to osmotic concentration. Supported by DK 19302.

181.21

HUMAN OB PROTEIN INHIBITS NEUROPEPTIDE Y INDUCED FEEDING. F.J. Smith*, L.A. Campfield, M. Renzetti, J. Yu. Dept of Metabolic Diseases, Hoffmann-La Roche Inc., Nutley, N.J. 07110.

Administration of OB protein (or leptin), the *obese* gene product, caused a long-lasting reduction in food intake and body weight in obese *ob/ob* mice. A major role for neuropeptide Y (NPY), a potent central stimulator of feeding, as a mediator of OB protein actions has been suggested. NPY secretion in vitro and elevated arcuate nucleus NPY mRNA levels were reduced by OB protein injections in obese *ob/ob* mice. To test this hypothesis, the effects of sequential central administration of human OB protein and exogenous NPY on the feeding behavior of obese *ob/ob* mice were examined. Chronic lateral ventricle infusion cannulas were implanted and, following recovery, free-feeding mice received two ICV injections: recombinant human OB protein (0.001-1 µg/mouse) or CSF contained in a volume of 1 µl followed one hour later by single doses of NPY (0.1-5 µg/mouse) or CSF. Central administration of OB protein, in a dose-dependent manner, partially or completely inhibited the expected feeding induced by exogenous NPY administration. These results indicate that OB protein can functionally antagonize the actions of exogenous NPY by either modulating the binding of NPY to its receptors critical for feeding or/and inhibiting downstream signal transduction. These studies provide evidence that the post-synaptic, receptor-mediated actions of NPY on feeding are under the control of OB protein. These results also imply that mediators, other than NPY, may also be involved in the biological actions of OB protein. Supported by Hoffmann-La Roche Inc.

INGESTIVE BEHAVIOR: OTHER MEDIATORS

182.1

EFFECT OF FOOD INGESTION ON HYPOTHALAMIC SEROTONIN RELEASE IN NORMAL AND OBESE RATS E.B. Ribeiro*, R.C.T. Mori, R.B. Guimarães and C.M.O. Nascimento. Dept. of Physiology, São Paulo Federal Univ. (UNIFESP), São Paulo, SP 04023-900, Brazil.

Serotonin (5-HT) exerts an inhibitory effect on food ingestion and may participate of the central mechanisms controlling satiety. The present study was aimed at evaluating, in normal and obese rats, the hypothalamic serotonin response to the stimulus provided by food ingestion. Obesity was induced in newborn male Wistar rats by monosodium glutamate (MSG, 2 mg/g/day, days 2-10 of life, sc.). At 3-4 months of age Control (C, n=7) and MSG (n=8) rats were implanted with microdialysis probes in the lateral hypothalamus. On the next day, after an overnight fast, 20 min microdialysate samples were collected to establish basal extracellular 5-HT levels. These were (Means ± S.E.M.) 3.11 ± 0.88 and 4.81 ± 1.24 pg/50 µl for C and MSG, respectively, p >.0.05. Food (powdered chow + water, 1:2) was then introduced in the cage and six additional 20 min samples collected. During the two hours after the food was available C rats consumed 15.8 ± 3.8 g, 70% of this total within the first 40 min, corresponding to the two initial microdialysate samples. MSG rats consumed only 7.8 ± 0.9 g, 78% of which during the first 40 min. 5-HT levels in microdialysates were significantly elevated from basal levels in the first two samples in both groups. The increments were of 46 and 68% in group C and tended to be higher (80 and 85%, p > 0.05 vs. C) in MSG rats. No significant alterations occurred in 5-HIAA levels. The data suggest a higher serotonergic responsiveness to food in MSG rats, as indicated by the trend to a higher increment in 5-HT levels elicited by consumption of a significantly lower amount of food. Additionally, these proportionally high levels may have contributed to the lower food ingestion during the subsequent periods.
Supported by a grant from FAPESP (Brazil).

182.2

STZ-DIABETES DECREASES HYPOTHALAMIC ATROPINE-INDUCED ACETHYLCHOLINE INCREASE IN FREELY MOVING RATS. E. Murzi*, P. Rada, M. Puig de Parada, B. Valecillos and L. Hernandez. Laboratory of Behavioral Physiology. Medical School, Los Andes University. Mérida. Venezuela.

Behavioral diabetic abnormalities such as polyphagia have been related to dopamine alterations (1,2). But polydipsia could also be atributable to cholinergic alterations (3). Ip. injections of atropine block the dipsogenic effect of intrahypothalamic administration of acetylcholine (ACh) or carbachol in normal rats(4). We evaluated the effect of atropine-induced ACh release on lateral hypothalamus (perifornical area, pfa) of normal or STZ-diabetic rats. Extracellular levels of ACh were measured in the pfa using microdialysis in 20-min intervals after ip. atropine sulfate (0, 5, 15, 25 mg/Kg). Seven normal or diabetic rats were used in each dose. Neostigmine was used in the perfusate to improve ACh recovery. ACh was measured by a reverse phase, HPLC with electrochemical detection system.

Preliminary results show that atropine-induce ACh increase was significantly lower in diabetic than normal rats. ACh increase was linearly related to the dose of atropine, but the slope of the curve was greater in the normal than in the diabetic rats. This result could be an alternatively explanation for the polydipsia seen in diabetes.

1) Bitar, M., et al. J. Pharmacol Exp Ther 236: 432, 1986
2) Murzi, E., et al. Neurosci Lett 202: 141, 1996
3) Murzi, E., et al. 25th Annual Meeting. San Diego, 1995
4) Grossman, P. Science 132: 300, 1960

This research was supported by grant CDCHT-ULA M-536-95-03A

182.3

BEHAVIOURAL ANALYSIS OF THE HYPOPHAGIC EFFECT OF IVTH VENTRICULAR INFUSION OF THE 5-HT1B AGONIST CP-93,129. M. D. Lee* and K. J. Simansky. Dept Pharmacology, Medical Col. Pennsylvania and Hahnemann Univ., Philadelphia, PA 19129.

Previously we have shown that systemic administration of the pyrrolopyridine CP-94,253, a selective 5-HT1B agonist, reduces food intake and hastens the termination of meals (Lee and Simansky 1995) which suggests a role for 5-HT1B receptors in the control of ingestion. The structurally related compound CP-93,129, has no effect given systemically, but its administration into the area of the hypothalamic PVN causes anorexia (Macor et al. 1990). Rats implanted with 26g cannulae aimed to terminate more caudally, dorsal to the roof of the IVth ventricle 2.2mm anterior to the occipital crest suture, were treated with CP-93,129 (3µl/1min) at doses of 25 and 50 nmol. Consumption of a 10% sucrose solution was decreased by 32 and 44% with respect to saline controls ($p<0.05$). Time-sampling analysis revealed that non-feeding activity was reduced and standing increased, but meal duration was not affected. Microstructural analysis showed that lick number but not the efficiency of licking was reduced after the 50 nmol dose. These results using CP-93,129 as a probe, suggest that deep brainstem 5-HT1B receptors are involved in the inhibitory control of food intake. Currently we are performing autoradiographic binding studies of these receptors using ^{125}I-iodocyanopindolol in the presence of 8-OH-DPAT and isoproterenol, in order to localise sites into which CP-93,129 can be delivered directly. Supported by MH41987 to KJS.

182.4

ACUTE AND CHRONIC FLUOXETINE SELECTIVELY SUPPRESSES FAT INTAKE IN FEMALE RATS. L.K. Heisler and R.B. Kanarek.* Psychology Department, Tufts University, Medford, MA 02155.

Fluoxetine hydrochloride is one of the most frequently prescribed antidepressants and is taken by more women than men. Fluoxetine is associated with a reduction in food intake and body weight and is under investigation as a possible treatment for obesity. Fluoxetine acts by blocking the reuptake of serotonin into the presynaptic nerve terminal. It has been hypothesized that the increased availability of serotonin should lead to a selective suppression of carbohydrate consumption through a biobehavioral feedback mechanism. To examine the acute effect of fluoxetine on macronutrient selection, 14 female Long-Evans rats were provided with separate sources of fat, protein, and carbohydrate. After a 2-week acclimation period, animals were injected at the beginning of the dark cycle with 0.0, 5.0., 10.0, or 15.0 mg/kg fluoxetine (IP), and food and water intakes were recorded at 2, 4, 6, and 24 hours post-injection. Fluoxetine significantly suppressed fat intake in a dose-dependent manner at all time points. Protein and water intakes were also reduced in a dose-dependent manner (n.s.). The effect of fluoxetine on carbohydrate intake was minimal. To examine the chronic effect of fluoxetine, baseline macronutrient intakes were collected for 21 days, after which 12 rats were given 10.0 mg/kg fluoxetine and 7 rats were given distilled water (IP) daily at the beginning of the dark cycle for 28 days. Food and water intakes were recorded daily during baseline, drug, and a 28-day withdrawal period. Fluoxetine-treated animals significantly suppressed fat, protein, and water intake and gained significantly less weight than controls. Fluoxetine-treated animals actually increased carbohydrate intake, while control animals decreased carbohydrate intake during the drug and withdrawal phases, producing a significant interaction. Food intake was also suppressed when rats were in estrous; however, no interaction was found between cycle and fluoxetine treatment for macronutrient selection. The observed significant suppression of fat after acute and chronic fluoxetine questions the proposed selective effect of serotonergic drugs on carbohydrate intake.

182.5

ENDOCRINE EFFECTS OF NEUROLEPTICS AND BODY WEIGHT GAIN: A STUDY IN NORMAL FEMALE SUBJECTS. T. Baptista*, I. Calanche, M. de Quijada, O. Contreras, J.L. Martinez, L. Teneud, and L. Hernández. Department of Physiology, Medical School, Universidad de los Andes, POB 109, Mérida, 5101-A, Venezuela.

Weight gain and obesity is an important side effects of neuroleptic agents afflicting up to 50% of patients under chronic treatment. Drug-induced hyperprolactinemia has been proposed as the triggering factor of weight gain, by decreasing estradiol serum levels, which in turn impairs the functioning of satiety-related neurons in the ventromedial-paraventricular hypothalamus. Testing this hypothesis in psychiatric patients has been hampered by preexisting endocrine abnormalities independent from drug treatments. We administered Sulpiride (n = 17) or placebo (n = 13) to healthy women after a menstrual cycle in which serum levels of pituitary gonadotropins, gonadal and adrenal steroids, prolactin, cortisol, thyroid hormones and insulin under the curve of glucose tolerance were assessed during the follicular and luteal phases. During treatments, hormones were reevaluated the same day as in the first cycle. Body weight gain and appetite were also controlled. During Sulpiride treatment, a significant decrease was observed in estradiol and progesterone levels in the late follicular and luteal phases; follicle-stimulating hormone was significantly increased during the early folicular phase; prolactin was significantly increased during all the cycle; glucose was significantly increased and insulin tended to decrease. Except a mild increase in prolactin, none of these changes were observed in the placebo group. A mild, non-significant increment in body weight and appetite was observed during Sulpiride administration. The results suggest that changes in gonadal steroids observed during neuroleptic administration, if prolonged, may explain the excessive weight gain and obesity. Supported by CDCHT, ULA, Venezuela, Grant M-493-94.

182.6

CENTRAL UK-14,304 AND IMIDAZOLACETIC ACID AND HYDROSALINE INTAKE. T.T. Miguel, A. Renzi, J.V. Menani and L.A. De Luca Jr.*. Dept. of Physiological Sciences, School of Odontology - UNESP, Araraquara, São Paulo, Brazil.

Clonidine, an α_2-adrenergic agonist that also binds to imidazolic receptors, inhibits salt and water intake when injected into the brain. In the present work we test the effect of intracerebroventricular (ICV) injection of UK-14,304, a specific agonist of α_2-adrenergic receptors that does not bind to imidazolic receptors, and the ICV injection of imidazolacetic acid, which binds to only imidazolic receptors. UK-14,304 (5, 10, 20 and 40 nmol/µl) or imidazolacetic acid (40 nmol) was injected into the 3rd cerebral ventricle of adult male rats (n = 6/group) submitted to either sodium depletion (furosemide + 24-hour removal of ambient sodium) or 24 hour of water deprivation. Animals that received ICV vehicle (propyleneglycol) injection ingested 7 ± 1 ml/120 min of 3% NaCl or 16 ± 2 ml/120 min of water. UK-14,304 induced a dose-dependent inhibition (from 20 to 70%) of 3% NaCl and of water intake in sodium deplete and water deprived animals, respectively. Imidazolacetic acid did not alter 3% NaCl or water intake. The results are consistent with an inhibitory role of α_2-adrenergic receptors on hydromineral fluid intake and suggest that the inhibitory effect of clonidine is not through imidazolic receptors.

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182.7

EFFECTS OF CHRONIC CARBAMAZEPINE ON BEHAVIOR AND LIPID METABOLISM IN RATS. B. Culver*, D.C. Rule, and F.W. Flynn. Neurosci. Prog., Depts of Pharmacology, Animal Science, and Psychology, Univ. of Wyoming, Laramie, WY 82071.

Weight gain has been reported in patients following chronic use of carbamazepine (CBZ) and is a major cause of non-compliance. We evaluated behavioral and lipid metabolic effects of chronic administration of CBZ (100-200mg/kg, gavaged twice daily for up to 8 wks) to rats. Body weight was not significantly altered by CBZ. Locomotor activity of CBZ rats was reduced during the exploratory period but not during nocturnal or nonexploratory periods. Lipoprotein lipase activity in adipose tissue of CBZ-treated rats was 259 ± 77% of controls (62 ± 23 vs 24 ± 3 neq fatty acid released/ (g x min)). Glucose conversion to total triacylglycerols of CBZ-treated rats was 145 ± 12% and 152 ± 34% of controls in liver and adipose tissue, respectively. Lipolytic rate in adipose tissue was increased above basal by 1 µM isoproterenol to a greater extent in the control (3.9 ± 1.2 nmol glycerol released/(g x hr)) than in CBZ-treated rats (2.2 ± .5)). We conclude that CBZ treatment increases lipogenesis and may blunt the lipolytic response to catecholamine in adipose tissue.

182.8

Reversal of fenfluramine and fluoxetine anorexia by 8-OH-DPAT is attenuated following raphe injection of 5-7,dihydroxytryptamine. **P.J. Currie*, D.V. Coscina, and P.J. Fletcher. Section of Biopsychology, Clarke Institute of Psychiatry, Toronto, ON, M5T 1R8 and Department of Psychology, Wayne State University, Detroit, MI, 48202**

Injections of 5-HT, its agonists or uptake inhibitors have been shown to reduce food intake by directly or indirectly activating serotonergic receptors. In the present study we examined the effects of the 5-HT_{1A} agonist 8-OH-DPAT on the feeding suppressant action of the indirect 5-HT agonists fenfluramine (FEN) and fluoxetine (FLU), as well as the 5-$HT_{1B/2C}$ agonist TFMPP. 8-OH-DPAT was administered 5 min prior to FEN, FLU or TFMPP, injected 30 before access to a palatable wet mash diet. While FEN (0.625-2.5 mg/kg), FLU (2.5-10 mg/kg) and TFMPP (0.5-2 mg/kg) dose-dependently reduced 2 hr food intake, 8-OH-DPAT (62.5-250 µg/kg) stimulated eating. Pretreatment with 8-OH-DPAT also reversed the anorectic action of FEN and FLU but not TFMPP. Separate groups of rats were then treated with 5,7-dihydroxytryptamine (5,7-DHT), administered into both the dorsal and medial raphe, resulting in extensive 5-HT depletion in hypothalamus (80%), striatum (90%) and hippocampus (90%). In both sham injected and 5,7-DHT (3 µg free base) treated rats, FEN and FLU were found to suppress feeding. In 5,7-DHT treated rats, however, the ability of 8-OH-DPAT to either stimulate eating or block FEN and FLU induced anorexia was attenuated. These findings confirm that widespread depletion of brain 5-HT has little impact on the anorectic capacity of either FEN or FLU. In contrast, the reversal of FEN and FLU induced anorexia by 8-OH-DPAT is at least partially dependent on the integrity of brain 5-HT systems since their disruption compromises the ability of this 5-HT_{1A} agonist to antagonize the feeding suppressant action of both compounds. (Supported by MRC and NSERC).

182.9

HISTAMINERGIC ACTIVATION OF ENDOGENOUS ANGIOTENSIN II FAILS TO AFFECT ALCOHOL INTAKE IN RATS. F.S. Kraly* and K.M. Jones. Dept. of Psychology, Colgate Univ., Hamilton, NY 13346.

Subcutaneous (SC) injection of angiotensin II (Ang II) has been demonstrated to inhibit ingestion of alcohol in rats having daily access to ethanol (Grupp & Harding, Pharmacol. Biochem. Behav. 47: 385-392, 1994). We investigated the involvement of endogenous Ang II for ingestion of alcohol in adult male Sprague-Dawley rats maintained with limited (45-min) daily access to 3% ethanol, made available at the mid-point of the dark phase of a 12:12 light/dark cycle. Activation of the renal renin-angiotensin system (RRA) was accomplished with SC injection of histamine diphosphate (H): 0.3 mg/kg (threshold dose for decreased latency to initiate drinking of water) and 1.25 mg/kg (threshold dose for increased water intake) H elevated (ps<0.01) plasma renin activity (PRA) at the time that ingestion of water is initiated. Despite the ability of these doses of H to stimulate drinking of water and increase PRA, these doses of H failed to inhibit (ps>0.20) ingestion of alcohol in one-bottle tests. A 10 mg/kg dose of H increased (p<0.05) alcohol intake in a one-bottle test, but this effect of H appeared to be secondary to the dipsogenic effects of H because in two-bottle tests 10 mg/kg H decreased (p<0.05) intake of alcohol while increasing (p<0.001) water intake. A 10 mg/kg SC dose of the AT1 receptor antagonist losartan, sufficient to abolish drinking elicited by SC Ang II, failed to affect alcohol intake in one- or two-bottle tests (p>0.20). These findings do not support a role for endogenous Ang II for ingestion of alcohol in rats for two reasons: First, histamine-induced pharmacological activation of RRA was not sufficient to inhibit ingestion of alcohol. Second, losartan-induced pharmacological blockade of AT1 receptors for Ang II was not sufficient to increase ingestion of alcohol. (Supported by NIH grant NS19133.)

182.10

METABOLIC "MEMORIES": ENCODING BY THE RAT, SELECTIVE ACTIVATION BY LIPOPRIVATION, AND MEDIATION BY VAGAL AFFERENT FIBERS. T. L. Davidson, A. M. Altizer, E. K. Walls*, M.-C. Holst, S. C. Benoit, & T.L. Powley. Dept. of Psychological Sciences, Purdue Univ., West Lafayette, IN 47907.

Our research addressed three questions: (1) Can rats form distinct memorial representations of fats and carbohydrates? (2) Is activation of these metabolic *"memories"* selectively enhanced by lipoprivation or glucoprivation? (3) Does this selective enhancement involve vagal afferent pathways? In Experiment 1, rats (n = 8) learned that one conditioned stimulus (CS) signaled delivery of peanut oil and another signaled delivery of sucrose pellets. The identity of the CSs (10 sec tones or lights) was counterbalanced for each unconditioned stimulus (US). Following CS training, the rats received additional trials in which only the USs were presented. Ingestion of one US (oil for half the rats and sucrose for half) was followed by toxicosis induced by LiCl. Ingestion of the other US was followed by saline injection. Next, US delivery was suspended and the capacity of each CS to promote conditioned responses (i.e., approach to the food cup) was assessed. Conditioned responding was abolished only for the CS that signaled the poisoned US. This result indicates that the rats formed distinct representations of each US. In Experiment 2, all rats (n=24) were food sated following CS training. Then Group MA was injected with 400 umol/kg of the lipid anti-metabolite Na-2-mercaptoacetate (MA). Group 2-DG was injected with 350 mg/kg of the glucose anti-metabolite 2-deoxy-d-glucose. Group SAL received isotonic saline. During testing (without USs) Group MA showed reliably (p<.05) more responding to the CS that previously signaled oil than to the CS that signaled sucrose. No differences between CSs were observed for either Group 2-DG or SAL. Experiment 3 found that this capacity of MA to augment responding to a CS that signaled oil was abolished for rats (n=8) that received subdiaphragmatic vagal deafferentation but not for sham-lesioned controls (n=8). Vagal deafferentation had no reliable effect on performance during CS training. These results suggest that vagal afferents mediate the capacity of lipoprivic signals to selectively activate the memorial representations of fat USs. This research was funded by grants from the National Institutes of Health.

182.11

aODN TO THE MBK1 GENE CODING FOR K^+ CHANNEL STIMULATES FOOD INTAKE IN MICE A. Pecori Vettori, C. Ghelardini, N. Galeotti, °S. Capaccioli, °A. Quattrone and A. Bartolini Depts of Pharmacology and °Pathology, University of Florence, Italy, SPON: Eur. Neurosci. Ass.

Ashford et al. (1989, 1990) and Boden et al. (1989) have reported that the enhancement of plasmatic levels of glucose induced a depolarization of rat hypothalamic neurones by modulation of ATP-sensitive K^+-channels. To further investigate the role of K^+-channels as an intracellular effector in the regulation of food intake, the effects produced by antisense-mediated switching off of the K^+-channel expression were examined in mice. A specific aODN targeting the translation start region of the MBK1 K^+-channel mRNA was designed. Mice were randomly assigned to an antisense, degenerated or vehicle group. Each group received a single i.c.v. injection on days 1, 4 and 7. The effect of the aODN treatment was evaluated 72 h and 7 days after the last i.c.v. injection by measuring the food assumption in mice. The aODN (1-3 nmol per injection) produced, at 72 h, an increase in food intake whereas the dose of 0.5 nmol per injection did not produce any effect. The orectic efficacy of aODN was comparable to those produced by aurothioglucose (200 µg per mouse i.c.v.) and 2-deoxyglucose (200 µg per mouse i.c.v.) and greater than that induced by NPY (0.5 µg per mouse i.c.v.). The degenerated and vehicle treatments did not modify mouse feeding in comparison with naive and saline i.c.v. injected mice. A quantitative RT-PCR study demonstrated a reduction of mRNA levels only in the aODN treated group indicating the absence of an aspecific sequence-independent action on cerebral structures. Mice receiving the aODN treatment did not show any modification of spontaneous motility and inspection activity as revealed respectively by the Animex apparatus and the hole-board test. The integrity and functionality of central K^+-channels is, therefore, fundamental in the regulation of the mouse food intake.

This work was supported by grants from MURST.

182.12

POSSIBLE INVOLVEMENT OF NITRIC OXIDE IN GLUCOPRIVIC FEEDING IN THE MOUSE. D.A. Czech.* Department of Psychology, Marquette University, Milwaukee, WI 53201

Nitric oxide (NO) has been implicated in ingestive behaviors in several animal species. We recently observed that the NO synthase (NOS) inhibitor L-N^G-nitro arginine (L-NOARG) reliably attenuated chlordiazepoxide-induced food intake in mice in a dose-related manner. The current study probes an effect of L-NOARG on a feeding stimulatory action of insulin (INS) and 2-deoxy-D-glucose (2-DG).

In expt.1, male ICR mice were injected s.c. with several doses of L-NOARG or 0.9% NaCl vehicle (Veh). Forty-five min later, 5 U/kg of INS or 500 mg/kg of 2-DG was administered i.p. Nine groups were thus formed: Veh/Veh (baseline intake control) and eight groups given doses of L-NOARG (0 [Veh], 10, 25 or 50 mg/kg) along with INS or 2-DG. Mice were then placed in a test cage containing a preweighed rodent chow pellet held in a wall-mounted "food clip;" tap water was available *ad lib*. Pellet was weighed at 30, 60, 120 & 240 min, and cumulative food intake at each measurement point was separately evaluated with randomized one-way ANOVAs, along with Dunnett's and Student's *t*-tests; alpha level was set at $p<0.05$. A robust INS-induced hyperphagia was attenuated in a dose-related manner, reaching statistical significance at all doses of L-NOARG by 30 min. A marginal and transitory 2-DG feeding action was also attenuated.

In expt.2, mice were injected s.c. with 25 mg/kg of L-NOARG, along with 500 or 1000 mg/kg i.p. of the natural NOS substrate, L-arginine (L-arg), or 500 mg/kg of the inactive isomer, D-arginine (D-arg). INS (5 U/kg) was injected i.p. 45 min later. Food intake was monitored as in expt.1. Data were evaluated with Duncan's multiple range tests. L-arg partially, but significantly, reversed an L-NOARG attenuation of INS-induced hyperphagia; D-arg was without significant effect, reflecting a stereospecific action.

These data are consistent with and extend previous research reporting inhibitory action of L-NOARG on feeding behavior in several small animal models, and provide further support for some involvement of NO in feeding mechanisms.

182.13

EARLY ENVIRONMENTAL MANIPULATIONS AND/OR CAPSAICIN TREATMENT PERMANENTLY ALTER ENDOCRINE AND INGESTIVE RESPONSES TO BOMBESIN.

Z. Merali*[1,2], P. Kent[1], D. Michaud[1], & H. Anisman[3].
[1]School of Psychology & [2]Dept. of Pharmacology, University of Ottawa; [3]Institute of Neuroscience, Carleton University, Ottawa, Ontario, Canada.

Inasmuch as intra-individual variability exists in the ingestive patterns, and pharmacological responses to satiety peptides, this study was undertaken to elucidate whether neonatal handling and/or chemical deafferentation (using capsaicin) altered adult ingestive and endocrine responses to bombesin (BN). On day 2 of life, capsaicin (50 mg/kg; s.c.) was administered to rats (C) whereas the controls received vehicle (V). The groups were then subdivided into those that were handled (H) (for 1 min daily, for over 21 days) or those that were not handled (NH). The final group consisted of rats that were never injected, or handled (no treatment; NT). On day 40, the performance of rats on the elevated plus maze was assessed. The CH and VH groups spent significantly more time on the open arms, indicating that handled rats were less "anxious" than those not handled. Next, the rats were food deprived for 18 h, injected with BN (0, 4, and 8 µg/kg; i.p.) and their food intake monitored. BN dose-dependently suppressed food intake in NT group. The BN effect was slightly attenuated in VH and VNH groups. In the capsaicin treated rats, BN-elicited satiety was markedly blunted in the NH group and was completely blocked in handled rats. Handling also blunted BN-elicited ACTH increase and this effect was eliminated by capsaicin treatment. Finally, handled rats showed a potentiated corticosterone response to BN, which was attenuated in the CH group. It can be concluded that 1) both neonatal handling and capsaicin-treatment attenuate the satiety effects of BN, 2) these effects are additive, and 3) systemic BN mediates it's effects neuronally, through the capsaicin-sensitive myelinated A-δ and/or unmyelinated C-fibers of the primary afferent fibers. Supported by Medical Research Council of Canada.

182.14

CAPSAICIN TREATMENT INCREASES STIMULATED FLUID INTAKE BY RATS. K.S. Curtis*[1], J.G. Verbalis[2], and E.M. Stricker[1]. Departments of [1]Neuroscience, Univ. Pittsburgh, Pittsburgh, PA 15260 and [2]Endocrinology, Georgetown Univ. School of Medicine, Washington D.C. 20007.

These studies examined stimulated fluid intake by rats in which vagally-mediated signals of gastric distension were eliminated by systemic treatment with the neurotoxin capsaicin, as verified by the loss of cholecystokinin-induced inhibition of food intake. Administration of hypertonic NaCl ip stimulated greater water intake by capsaicin-treated rats than by intact rats (13.1 ± 1.3 ml/3 h vs. 8.0 ± 1.0 ml/3 h; n = 17, 8). Capsaicin-treated rats also drank more water than did intact rats when isosmotic hypovolemia was induced by sc administration of a hyperoncotic colloidal solution (22.4 ± 1.9 ml/3 h vs. 12.4 ± 1.1 ml/3 h; n = 10, 7). Similarly, after overnight food deprivation, intake of a 10% sucrose solution by capsaicin-treated rats was greater than that by intact rats (38.6 ± 2.5 ml/30 min vs. 26.7 ± 2.5 ml/30 min; n = 11, 7). Finally, during chronic administration of the mineralocorticoid deoxycorticosterone acetate, capsaicin-treated rats consumed more of a concentrated NaCl solution than did intact rats (18.7 ± 1.7 ml/3 h vs. 12.1 ± 2.4 ml/3 h; n = 11, 8). In all tests, intakes by capsaicin-treated rats were significantly greater than those by intact rats within 5-15 min. These results suggest that early signals of gastric distension, such as those that occur during food, water, or NaCl intake, may modulate ongoing ingestion, and that in the absence of such general inhibitory signals ingestion continues until later postgastric signals are detected. (MH-25140)

182.15

DIFFERENTIAL FEEDING-INHIBITORY EFFECTS OF PERIPHERAL AND CENTRAL CYTOKINE ADMINISTRATION IN ZUCKER OBESE (*fa/fa*) AND LEAN (*Fa/Fa*) RATS.

J.R. Vasselli, C.R. Plata-Salaman, H.R. Kissileff*, D. Casey, and G. Sonti. St. Luke's-Roosevelt Hosp., New York NY 10025 and Univ. of Delaware, Newark, DE 19716-2590.

The expression and release of TNF-α has recently been found in the adipose tissue of both rats and humans, and increases with increasing adipocyte size/mass. Also, central injections of this and other cytokines are known to potently inhibit feeding in normal rats. To test the feeding-inhibitory effects of cytokines in genetically obese rats, we injected rmTNF-α i.p. at the start of the dark cycle into 15-wk old male obese and lean rats at doses of 0 (saline), 50, 100, and 150 ug/kg body weight. Intake was monitored at 2, 4, 6, 8 and 24 hrs. Dose-dependent reductions of obese and lean intake were observed from hrs 6-24 ($p < 0.01$), with obese twice as responsive to the feeding-inhibitory effects of TNF-α as lean rats. We next tested the effects of intracerebroventricular infusions of IL-1 (1.0, 4.0, and 8.0 ng/rat), TNF-α (50, 100 and 500 ng/rat), and IL-1 (1.0 ng) + TNF (100 ng) in a similar feeding protocol. All doses of IL-1 and TNF-α, and their combination, inhibited feeding in obese and lean rats ($p<0.01$), with IL-1 significantly more potent than TNF-α. Obese rats were twice as responsive to the feeding-inhibitory effects of IL-1, and to the combination of IL-1 + TNF-α ($p<0.01$). We conclude that cytokines of peripheral origin may serve as feeding-inhibitory signals when elevated in associaton with the development and/or maintenance of the obese state. (Supp. by NIH P30 DK 26687)

182.16

CYTOKINE-INDUCED ANOREXIA: 1) CYTOKINE-CYTOKINE INTERACTIONS; 2) CYTOKINE-NPY INTERACTIONS; 3) ANOREXIA INDUCED BY ACTIVATORS OF THE SIGNAL TRANSDUCER GP 130 (USED BY IL-6 FAMILY RECEPTOR MEMBERS) THAT SHARES HOMOLOGY WITH A LEPTIN RECEPTOR. G. Sonti, S.E. Ilyin and C.R. Plata-Salamán*. Sch. Life and Hlth. Sci., Univ. Delaware, Newark, DE 19716-2590, USA.

Cytokine-cytokine interactions were studied with the ICV infusion of individual or multiple combinations (8 dyads and 5 triads) of IL-1beta, IL-8 and TNFalpha at doses that yield estimated pathophysiological concentrations in the CSF. Various cytokine combinations exhibited additive or synergistic activities inducing anorexia. Computerized analysis demonstrated that the most effective treatment (triad of 1.0 ng IL-1beta plus 20 ng IL-8 plus 20 ng TNFalpha/rat, n=11) decreased nighttime meal size (MS) by 42% and feeding rate (MS/meal duration) by 26%, while increased the satiety ratio (postprandial intermeal intervals/MS) by 80%; meal duration and meal frequency were not significantly affected. Heat-inactivated triad had no effect. Additive or synergistic cytokine-induced feeding inhibition may participate in the anorexia associated with pathological processes.

NPY (5.0 µg) blocked the anorexic effect induced by 1.0 (n=8), 4.0 (n=7) or 8.0 ng (n=7) IL-1beta when both compounds were infused (ICV) concomitantly. Central infusion of NPY also induced feeding in IL-1beta-pretreated rats exhibiting marked anorexia (n=9).

Various activators of the glycoprotein 130 (gp 130), a common signal transducer among receptors for members of the IL-6 family (IL-6, IL-11, CNTF, LIF, OSM), induce anorexia. The data show that ICV IL-11 and LIF are the most effective at 1.0, 20 and 100 ng (8 rats/group). A leptin receptor is most related to gp 130. Thus, gp 130 and related molecules may represent an interface associated with feeding control in health and disease. Supported by Univ. Del. Research Grants.

183.1

STRESS-INDUCED CHANGES IN PERIPHERAL BLOOD FLOW AND BEHAVIOR: A POTENTIAL FACTOR CONTRIBUTING TO NON-FREEZING COLD INJURY. D. Shurtleff*, C. L. Foreman, C.-M. Staschen; J.R. Thomas & J. Schrot. Naval Medical Research Institute, Bethesda, MD 20889-5607.

Non-freezing cold injury (NFCI) is a unique syndrome that results from damage to peripheral tissue, usually in the extremities, exposed to cold temperatures for prolonged periods of time. Although non-vascular components are involved in the development of NFCI, alteration in peripheral blood flow in cutaneous circulation is most often a salient feature. Previous research has shown that, in a rat tail model, brief exposure to cold water induces a protective pattern of blood flow -- vasoconstriction followed by vasodilation. This study determined whether exposure to a conditioned stressor disrupts peripheral blood flow. Rats' tail blood flow was measured with laser-Doppler flowmetry while they licked a tube for liquid. On separate days, rats experienced a CS+ condition (three 1-min long stimuli (tone (n=2) or light (n=2)) followed by a .1s foot shock) and a CSn condition (the alternate stimulus presented alone). Over sessions, the licking was suppressed in the presence of the CS+, an indication of a conditioned emotional response, but licking recovered following CS+ termination. Conditioned vaso-constriction also occurred during the CS+ but, unlike licking, continued long after CS+ cessation. The tone CS+ conditioned more vasoconstriction than light, suggesting that conditioned vaso-constriction, and not behavior, may be dependent on stimulus modality. CSn did not alter licking or blood flow. These data suggest that after cessation of a conditioned stressor, behavior recovers, while vasoconstriction continues. Therefore, conditioned stress could contribute to NFCI development by prolonging vaso-constriction, and potentially disrupting a cold-induced protective blood flow pattern. Supported by Naval Medical Research & Development 61153N.MR04101.007.1507.

183.2

EVIDENCE FOR LONG TERM BEHAVIORAL EFFECTS OF ACUTE DEFEAT. L.A. Lumley*, M.A. Hebert, M.L. Sipos, and J.L. Meyerhoff. Division of Neurosciences, Walter Reed Army Institute of Research, Washington, DC 20307-5100.

Acute defeat in male mice elicits prolonged avoidance of non-aggressive conspecifics but only transient decreased mobility in the forced swim test. Male DBA/2 mice received four 2 min trials of defeat, whereby the DBA mice were continuously attacked and consequently defeated by aggressive C57BL/6 mice. Unlike its reported effect in the Porsolt test, Fluoxetine (IP) administered immediately following defeat further decreased swim mobility 30 minutes after defeat. The altered physiological state of a defeated mouse, as well as different testing paradigms may account for these differences. Twenty-four hours after defeat, the mice avoided approaching a barrier separating them from a nonaggressive intruder placed within the home cage of the defeated mice. Previous fluoxetine administration had no observable effects on barrier avoidance. Without further defeat exposure, mice received daily injections of saline or fluoxetine and were tested in this modified resident-intruder paradigm four times over two weeks. In previous experiments, barrier avoidance persisted only for one week following acute defeat, indicating the effects of acute defeat on barrier avoidance are limited in duration. Two weeks following acute defeat, mice were placed in a novel cage with a filter paper floor and a barrier separating them from control DBA mice. Since urine fluoresces, urine marks were observed using a transparent grid and quantified under ultraviolet light. Previously defeated DBA mice failed to mark their territory, whereas many of the control DBA mice extensively marked their territory. These results from the urine marking test provide behavioral evidence of long term effects of acute defeat, lending further validation for a potential model of post-traumatic stress disorder. (Intramural research funded by U.S. Army Medical Research and Materiel Command.)

183.3

DIFFERENTIAL EFFECTS OF PSYCHOLOGICAL (FERRET EXPOSURE) & PHYSICAL STRESSORS ON NEUROPEPTIDE & ENDOCRINE RESPONSES OF 'SLOW' VERSUS 'FAST' SEIZURE SUSCEPTIBLE RATS.

P. Kent*[1], D. McIntyre[1], H. Anisman[3] & Z. Merali[1,2], [1]Sch. of Psychol. & [2]Dept. of Pharmacol., Univ. of Ottawa; [3]Institute of Neuroscience, Carleton Univ. Ont. Canada.

This study assessed the effects of psychological (predator (ferret) exposure) or physical (restraint) stressors (15 min. exposure) on plasma levels of ACTH and corticosterone (CORT) as well as regional levels of CRF and BN-like peptides in 'slow' and 'fast' seizure susceptible rats. Although these lines of rats were selectively bred for proneness to kindling effects, cursory observations suggested differential responsiveness to stress. Effects on CRF and BN were evaluated as they appear to play a role in mediation of stress response; administration of either peptide increases plasma ACTH and CORT levels. The results demonstrate that the strains differ in their CORT and ACTH responses to stressors; both strains showed a marked ACTH and CORT increase following restraint, however, 'slow' rats appeared to be more reactive to ferret exposure as reflected by a much larger ACTH and CORT response. In terms of basal peptide levels, the 'slow' rats had higher CRF levels at the hippocampus and lateral hypothalamus but lower BN levels at the cingulate cortex and nucleus of the solitary tract (NTS); basal peptide levels were comparable at all other regions examined. In the 'fast' rats, restraint increased levels of BN at the anterior hypothalamus, but decreased BN levels at the median eminence (ME) and CRF levels at the paraventricular nucleus (PVN). Exposure of 'fast' rats to ferret, decreased BN levels at the NTS, and both BN and CRF levels at the ME. In the 'slow' rats, restraint increased CRF levels at the PVN and ferret exposure decreased BN levels at the NTS. We can conclude that 1) Exposure of rat to a ferret represents a powerful psychological stressor, and 2) Inasmuch as marked inter-individual differences exist in response to stressors, these rat strains may provide useful animal models in the elucidation of mechanisms subserving such differences. Supported by NSERC.

183.4

CHRONIC STRESS INDUCES AN EARLY BUT TRANSIENT DISRUPTION OF ESTROUS CYCLING IN RATS. S.M. Anderson*, G.A. Saviolakis, R.A. Bauman, K.Y. Chu, S. Ghosh, and G.J. Kant. Division of Neurosciences, Walter Reed Army Institute of Research, Washington, D.C. 20307-5100.

Female Sprague-Dawley rats were chronically stressed using a behavioral paradigm of an around-the-clock signalled intermittent footshock in which some rats could pull a chain to avoid/escape shock (stress) while another group of rats was yoked to the first group (yoked-stress) and did not have control over shock termination. Control rats were never shocked but all groups leverpressed for food pellets on an FR1 schedule (one pellet per leverpress). Daily vaginal samples were obtained for several weeks prior to stress onset and throughout the chronic stress period. After 14 days of stress, the experiment was terminated. Stress transiently decreased leverpressing for food pellets and body weights, but both measures returned to prestress levels by day 14 of stress. Vaginal smears indicated a disruption of the previous pattern of sequential stages of the estrous cycle in both controllable stress and yoked-stress rats during the first few days after introduction of the stress. However, apparent adaptation to the stress resulted in no significant differences among experimental groups in estrous cycle length when averaged over the entire 14 days of stress.
(Intramural research funded by the U.S. Army Medical Research and Materiel Command)

183.5

STRESS-INDUCED DECREASE IN OUT OF NEST ACTIVITY IN THE MOUSE: A MODEL OF STRESS-INDUCED WITHDRAWN BEHAVIOR. Y. Zhang*, E.A. Stone and D. Quartermain Depts. Psychiatry & Neurology, New York University School of Medicine, New York, NY 10016

We have recently shown that various forms of stress reduce nocturnal out of nest activity in the mouse. The present experiments were undertaken to further characterize this behavioral change in terms of its probable cause and response to medication. Mice were housed singly in nest cages attached to outer cages in which nocturnal away from nest activity could be recorded by videotape. After baseline behavior was established the animals were subjected to daily aggression stress for 5 days during which time behavior was continuously recorded. It was found that the stress significantly reduced out of nest activity but did not affect food or saccharine consumption or risk assessment behavior in the nest. Plus maze behavior was unaltered. Plasma corticosterone levels were elevated immediately post stress, reduced at 5 hr post stress and no different at 24 hr post stress. Pretreatment with the antidepressant, DMI, attenuated the behavioral change whereas fluoxetine was ineffective. Acute treatment with librium on the night of the first stress exposure did not significantly affect the change. It is concluded that the reduction in out of nest behavior is a form of stress-induced withdrawn or avoidant behavior which does not appear to be prompted by an increase in anxiety but may be the result of a decreased motivation to leave the nest. Supported by NIH MH45265 and a Stanley Foundation Award.

183.6

CHRONIC SUBORDINATION STRESS ATTENUATES ACTIVE DEFENSES IN MALE SWISS WEBSTER MICE. M.A.Hebert*[1], H.H. Dangler[2], J.A. Henrie[1], J.K. Shepherd[2], D.C. Blanchard[1] & R.J. Blanchard[1,2]. [1]Bekesy Lab. of Neurobiology & [2]The Dept. of Psychology, Univ. of Hawaii, Honolulu, HI 96822.

The experience of social defeat in agonistic encounters with conspecifics is a potent naturalistic stressor that can have profound and lasting consequences. Even brief episodes of social defeat in mice have been shown to enhance anxiety-like behavior in such tests as the elevated plus maze. In a chronic model of social defeat in which male mice were defeated daily by aggressive counterparts, subordinate C57BL/6J mice developed depression-like behavior, such as increased immobility in the Porsolt swim test (Kudryavtseva et al. 1991). In the present study, pairs of male Swiss Webster mice (35-45 gm) were maintained in home cages that were divided into equal halves by a perforated partition that prevented animals from freely interacting but permitted non-tactile communication. On each of thirty consecutive days, the partition was removed to permit agonistic interactions between each pair. The encounters were terminated 4 min following the first attack or ten minutes, whichever came first. Dominance was quickly established, typically on the first encounter, and remained constant in almost every pair throughout the study. Single-housed males served as controls. During the final week, all mice were subjected to several behavioral tests of defensiveness ranging from the elevated "zero maze" (an improved variant of the elevated plus maze) to the Mouse Defense Test Battery (MDTB) in which the animals' reactions to a natural predator, the rat, were examined. Subordinate mice showed signs of reduced defensiveness in many of the tests: 1) they had significantly higher percent open "arc" times than dominants in the zero maze. 2) flight distance from the rat in the MDTB was significantly shorter in the subordinates relative to controls and dominants, 3) fewer subordinates displayed defensive attack (biting) toward the rat. The results appear to suggest that chronic subordination stress leads to shifts from active toward passive defensive strategies. Results are discussed with reference to the PAG. Sources of support: NSF IBN-11349, NIH RRO 8125, HHMI Undergraduate program, & NARSAD Young Investigator Award.

183.7

BEHAVIORAL CONSEQUENCES OF SUBORDINATION IN THE VISIBLE BURROW SYSTEM (VBS). E.B. Yudko*, D.C. Blanchard, M. Hebert, J.A. Henrie, R.J. Blanchard. Dept. of Psychology, University of Hawaii, Honolulu, HI 96822.

Subordinate male rats of groups maintained in semi-natural visible burrowing situations have higher mortality rates and higher plasma corticosterone and lower plasma testosterone levels than dominants. They show enhanced defensive behavior, but reduced social, sexual, exploratory and aggressive activity, within the habitat, and altered patterns of risk assessment in the open field, plus maze, and cat-odor test. They exhibit increased food and water consumption outside of the VBS but continue to lose weight. These findings indicate that while subordination is typically associated with heightened defensiveness it may produce specific changes in particular components of an animals defensive profile.

The present study attempted to better understand the nature of these changes by examining the behaviors of dominant and subordinate rats compared to controls in a variety of tests of defensive behavior. The tests were chosen to analyze defensive behavior over a continuum of threatening stimuli from human contact, to a novel environment, to the presentation of an anesthetized conspecific, a cat-odor, or a cat. In response to human contact subordinates exhibited less locomotion, less exploration, and longer latencies to right themselves after being inverted. In the same tests, dominants made fewer escape attempts, and hid less than controls showing more investigation of the experimenter. In the open field test, subordinates showed less activity than controls during VBS housing but not 7 days later. All animals showed marked reduction of activity when exposed to the cat. These data suggest an impact of subordination on defensive behaviors that is more prominently expressed in response to relatively low level threat stimuli.

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183.8

STRESS-RELATED ALTERATIONS IN AGGRESSION IN MICE SELECTIVELY BRED FOR HIGH AND LOW STRESS-INDUCED ANALGESIA. W.F. Sternberg*, C. Geneve, D. Goldstein, J.S. Mogil,Department of Psychology, Haverford College, Haverford, PA 19041.

The effects of stress on aggressive behavior were assessed in mice selectively bred for high and low analgesic (HA and LA, respectively) responses to stress. The selection procedure, based on analgesic responsiveness to a 3-min swim stress, resulted in large and reliable differences in opioid-mediated analgesic processes in succeeding generations; HA's display higher magnitudes of morphine, stimulation-produced, and stress-induced analgesia, and an increased density of brain opiate receptors compared to LA's. It is unknown, however, if HA's diverge from LA's with respect to other behaviors influenced by opioids, such as aggression. Opiate agonists decrease several forms of aggressive behavior; opiate antagonists increase aggression. HA and LA mice provide an interesting model in which to assess the effects of stress-induced opiate activation on aggression, since they exhibit such varied opiate responses to swim stress. Adult male mice of each line (n=8) participated as the residents in a territorial aggression procedure. Each subject established residence in the testing chamber for 24 hours, then was exposed to the swim stressor. Following recovery from the stress, number of intruder-directed aggressive attacks, latency to first attack and number of attacks per minute (APM) were recorded for the resident. Each animal also participated in a territorial aggression encounter under non-stressed conditions. Half of the subjects in each line received an injection of naloxone (10 mg/kg, i.p.) or saline before testing on each day. Analysis of variance revealed a significant inhibitory effect of stress on aggression. There was also a significant strain x drug interaction, with naloxone increasing APM in the HA's, but not in the LA's. A significant 3 way interaction indicated that in the baseline condition, naloxone decreased aggressive behavior in LA's, but increased APM in HA's. The results suggest that the profound stress-induced decrease in aggressive behavior is not opioid-mediated, however, naloxone increases the aggressive behavior seen in HA's under baseline conditions.

Supported by Haverford College Faculty Research Grant.

183.9

PLACE NAVIGATION ON THE OPEN FIELD IN MICE EXPOSED TO PRENATAL X-RAYS: REFERENCE AND SOUND STIMULI AS CONTRASTING AGENTS, D.M. Abramov (1), R.T. Vital (1), R.W.F. Vitral* (1,2) and S.L. Schmidt (1). (1)Dept°. Ciências Fisiológicas, UERJ, (2)IBCCF, UFRJ, Rio de Janeiro, 20551030, BRAZIL.

Previous studies suggest that mineralocorticoid receptors in the hippocampus may be related to the animal's tendency to move toward the center of the open-field when a stimulus is present in this region. Here we studied place navigation in irradiated mice on the open field with different backgrounds, since it has been shown that prenatal ionizing irradiation on sixteenth gestacional day (E16) with 3 Gy changes hippocampal lamination. Pregnant female mice were exposed to a source of X-rays at E16 receiving a total body dose of 3 Gy. At adulthood, 34 irradiated and 38 non-irradiated male mice were tested in an elevated open field (24 x 24 cm) with no walls divided into 8 rows and 8 columns. The open field was also divided in center and periphery (two more external rows/columns). The reference stimulus was a squared prism (4,25 x 4,25 cm), which was placed on its center. The sound stimulus had a frequency of 3500 Hz. For the behavioral testing, the animals were subdivided in four subgroups: ❶without reference stimulus/without sound (10 irradiated and 10 non-irradiated), ❷without reference stimulus/with sound (10 irradiated and 10 non-irradiated), ❸with reference stimulus/without sound (7 irradiated and 8 non-irradiated) and ❹with reference stimulus/with sound (7 irradiated and 10 non-irradiated). The animals were placed for 10 minutes in the open field where their movements were recorded with a video camera. The analysis of the place navigation showed that the irradiated and non-irradiated mice moved to the center in the presence of the reference stimulus. This was more pronunciated in the non-irradiated group. The presence of the sound stimulus abolished the differences between the two groups. We suggest that the differences seen in relation to the two background stimuli on the open field may be due to different mechanisms of behavioral reaction associated with the possible effects induced by the radiation upon the hippocampus.

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183.10

SOCIAL ROLES, TERRITORIAL AGGRESSION AND CENTRAL MONOAMINES IN WILD *Sceloporus jarrovi*.

Patrick J. Ronan , John M. Matter, S. Waller*and Cliff H. Summers. Dept. of Biology, University of South Dakota, Vermillion, SD 57069.

Central monoamines were quantified from wild mountain spiny lizards (*Sceloporus jarrovi*) during the breeding season by electrochemical HPLC. Monoamine profiles from territorial males, adult females, satellite males, young males and young females, as well as males following aggressive defense of territory, revealed significant differences in content and turnover between lizards from different social classes. Socially subordinate satellite males exhibited significantly higher telencephalic 5HIAA and 5-HIAA/5-HT, as well as enhanced diencephalic 5-HIAA/5-HT. Adult females had higher telencephalic 5-HTP and 5-HT. Additionally, immediately following a heightened agonistic interaction territorial males exhibited very rapid elevation of 5-HTP, 5-HIAA and 5-HIAA/5-HT. Following aggressive defense of territories, males exhibited higher elevated telencephalic and diencephalic DOPAC, telencephalic DOPAC/DA, and brainstem MHPG/NE compared to noncombative territorial males. Social stresses, experienced by satellites as they exist at the interstices of territories, and by males defending territories, activate serotonergic systems. Aggressive interaction, while stimulating serotonergic systems, also activates noradrenergic and dopaminergic systems in dominant males.

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183.11

EFFECTS OF POSTNATAL HANDLING IN SPRAGUE-DAWLEY, ROMAN HIGH AVOIDANCE/VERH AND ROMAN LOW AVOIDANCE/VERH RATS.

A. Tobeña[1], T. Steimer[2], R.M. Escorihuela[1], J.F. Núñez[1], P. Ferré[1], P. Driscoll[3], A. Fernández-Teruel*[1]. [1]Med Psychol Unit, Medicine School, Autonomous Univ Barcelona, Barcelona, Spain; [2]Psychopharmacology, IUPG, Chene-Bourg/GE, Switzerland; [3] ETH-Zentrum, Animal Science Inst, Zurich, Switzerland.

The present studies were devoted to evaluate postnatal handling (PH; during the first 21 days of life) effects on tests specifically measuring anxiety (i.e. tests involving conflict or conditioned fear) both in randomly bred Sprague-Dawley (SD) rats as well as in Roman high- and low-avoidance (RHA/Verh and RLA/Verh) rats. The Roman lines, which have been selected for rapid vs non acquisition of two-way active (shuttlebox) avoidance, present differential emotionality, as well as HPA-axis reactivity and prolactin response to stress (low in RHA/Verh and high in the RLA/Verh). A second objective was to evaluate whether those genetically-related differences could be enduringly affected by PH. The results show that both spontaneous and conditioned emotionality/fear were enduringly reduced in all PH-treated rats (as compared to control animals) in several situations. PH significantly decreased HPA-axis (ACTH and corticosterone) and prolactin responses to open field exposure in adult SD and RLA/Verh rats. Remarkably, PH eliminated the differences among the Roman/verh lines in most parameters, including self-grooming, defecation under fear-conditioning and prolactin response to open field exposure in RLA/Verh rats.

(Supported by FISS 95/1779).

183.12

MAINTAINING PREGNANT RATS ON A STANDARD VS. REVERSED LIGHT/DARK CYCLE: IMPLICATIONS FOR GESTATIONAL STRESS, A. E. McCrea* and M. T. Williams. Tobacco and Health Res. Inst., Lexington, KY 40506.

Stressing pregnant rats during the third trimester can produce offspring that themselves demonstrate an attenuated response to stress. There are, however, inconsistent results in the literature, which may be attributable to methodological variations, such as stressing females during the light or the dark phase of the light dark cycle. The present study addresses this issue. Pregnant females were randomly assigned to a colony room maintained on a standard (STAND) or reverse (REV) light/dark cycle and further to a gestationally-stressed (GS) or nonstressed (NS) condition. Offspring weights and anogenital distances (AGD) were measured on PN-1 and PN-20. On PN-21, one male and female from each litter was either isolated for 1 hour in a novel environment (ISOL) or sampled immediately upon removal from the home cage (BASE) during the dark phase of the cycle. Trunk blood was assayed for corticosterone (CORT), aldosterone (ALDO), ACTH, and in males, testosterone (T). The weights of GS offspring were reduced on all days. Furthermore, GS males had shorter AGDs than NS males; no effect was observed among females. As expected, CORT, ALDO and ACTH were elevated in ISOL pups. However, the elevations over basal levels for CORT and ALDO were smaller in STAND pups. Furthermore, plasma ALDO and CORT were greater in females, although CORT only approached significance. BASE and ISOL titers of ACTH were lower for GS vs. NS pups. Elevations in T levels over basal levels were greater in REV than in STAND males. ACTH was the only hormone observed to be affected by GS, however, the attenuation in endocrine responses in the STAND room suggests that maintaining rats under standard animal husbandry procedures may itself be an external source of stress.

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183.13

CORTISOL LEVELS AND SOCIAL COMPETITION IN THE MARMOSET (.CALLITHRIX JACCHUS). Woodall K.L, Domeney A.M.,[1] Kelly M.E*. Postgraduate Studies in Pharmacology, University of Bradford, Bradford, W.Yorks, BD7 1DP. [1]Battelle Europe, 7 Route de Drize, CH 1227 Carouge, Geneva.

Studies of dominant-subordinate relationships in terms of competition for palatable substances have been studied in rodents and non-human primates (Syme, 1974, *Anim. Behav.*, 22: 931-940). Hierarchical relationships have been proposed as being relatively more stressful for the subordinate animal and this has implications in the development of psychological disorders such as anxiety. The purpose of the present study was to determine if there were differences in plasma cortisol levels between dominant and subordinate marmosets during basal conditions and following social competition.

During a 5 min test period, pairs of marmosets (n=4) were given access to milk and the dominant-subordinate animal in each pair differentiated according to the time spent at the drinking spout; the dominant animal spending the most time. Blood samples (0.5 ml) were taken via the femoral vein, centrifuged and the plasma cortisol measured by radioimmunoassay. Data were analysed using two factor ANOVA followed by Dunnett's t-test.

Results from this study show that there was no difference in basal cortisol concentrations between subordinate and dominant marmosets, 117.3±14.4µg/dL and 83.5±7.1µg/dL respectively. Following 5 min competition for milk, the level of cortisol in the plasma of subordinate animals was significantly higher than in dominant animals, 167.8±12.0µg/dL compared to 105.5±18.8µg/dL ($p<0.05$).

These findings provide evidence that competition in pairs of marmosets induces a situation that is more stressful for the subordinate member of the social hierarchy.

(Supported by Costall Naylor Award, University of Bradford)

183.14

NEONATAL HANDLING INCREASES BEHAVIORAL DESPAIR AND ACOUSTIC STARTLE RESPONSES E.P. Zorrilla*, E. Redei. Depts.of Psychology and Pharmacology, U.Pennsylvania, Philadelphia, PA 19104.

Previously, we found that neonatal handling, especially when administered during the second postnatal week, increased immobility during the Porsolt forced swim test (FST) in adulthood (Zorrilla *et al. Soc. Neurosci. Abstr.* 21:1686). To replicate and extend these findings, we subjected 16 litters derived from Sprague-Dawley dams mated in our colony to 1 of 2 postnatal conditions: cage-cleaning control (C; n=7) or twice daily handling during the 2nd postnatal week (H2; n=9). At 95 days of age, behavior in the FST was assessed at the unit of litter (1-3 offspring/litter mean). Both H2 males and females were more immobile early in the posttest; males climbed less and both males and females swam less. Additionally, males climbed less and were more immobile during the initial test. Irrespective of rearing, marked sex differences were observed; females were more immobile than males, as they swam and climbed less. These differences replicate our prior findings. At 125 days of age, offspring were tested for habituation/sensitization of acoustic startle responses (ASRs) or acoustic startle threshold and prepulse inhibition. H2 females, but not males, showed exaggerated ASRs to 120 dB stimuli. Greater ASRs were due, in part, to a failure to habituate, as H2 females showed an initial and delayed sensitization. H2 offspring did not differ in their acoustic startle threshold, suggesting that differences were not due to perceptual differences. Furthermore, H2 offspring showed normal prepulse inhibition and unstimulated motor activity. Coupled with the well-established finding that handled animals have increased HPA negative feedback efficacy, the present findings of increased 'behavioral despair' and altered regulation of startle responsiveness suggest that neonatal handling should be pursued as a potential animal model of post traumatic stress disorder.

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183.15

DIFFERENTIAL EFFECTS OF BENZODIAZEPINE RECEPTOR AGONISTS AND INVERSE AGONISTS ON CARDIOVASCULAR AND SOMATIC DEFENSIVE AND STARTLE RESPONSES. S. Hart*, G. Berntson, M. Sarter. Dept. Of Psychology & Neuroscience Program, The Ohio State Univ., Columbus, OH 43210

Previous work from our laboratory has demonstrated that systemic administration of the benzodiazepine receptor (BZR) partial inverse agonist β-carboline FG 7142 (FG) augments the cardiovascular defensive response to non-signal stimuli. This effect mimics the cardiovascular effects of a conditioned aversive stimulus, and was found to depend on the basal forebrain cholinergic system. More recently, however, we have found that FG decreases somatic and cardiovascular startle responses. This dissociation between defensive and startle responses raises an important question as to what specific reactions are potentiated by the putative anxiogenic agent FG 7142. The present studies were designed to further explore this issue. Rats were trained with either contingent or random relation pairs of the light CS and a 0.6 mA shock. Following training, subjects systemically received either FG, chlordiazepoxide or vehicle. Somatic and cardiovascular responses to the startle stimuli, and cardiovascular responses to a novel probe tone were measured in the presence or absence of the light CS. Consistent with previous findings, results suggest that FG enhances cardiovascular reactivity to non-signal stimuli and light CS, but attenuates somatic startle in the same experimental context. These findings suggest that BZR partial inverse agonists may selectively enhance defensive-like responses. Supported by NIH/National Heart, Lung, and Blood Grant # 1 RO1 HL54428

183.16

THE ROLE OF CENTRAL NORADRENERGIC MECHANISMS AND FOS RESPONSES TO STRESS IN STRESS-INDUCED BEHAVIORAL DEPRESSION. E.A. Stone*, Y. Zhang and D. Quartermain Depts. Psychiatry & Neurology, New York University School of Medicine, New York, NY 10016

The noradrenergic system has long been thought to play a role in the genesis of depressed behavior. The fos response to stress, which is largely controlled by the noradrenergic system (Stone & Zhang, Brain Res. 694:279,1995) is also thought to contribute to the disorder or be an adaptation to stress. The present study investigated the role of these factors in depression evoked by stress. Mice were housed in specially designed cages that allowed recording of nocturnal activity. After behavior had stabilized the animals were subjected to restraint stress once daily for 2 days. Prior to receiving stress or control treatment the animals were administered saline, combined prazosin (alpha-1 antagonist) and betaxolol (beta-1 antagonist) or atipamezole (alpha-2 antagonist). Blockade of alpha-1 and beta-1 receptors reduced the fos response to stress whereas blockade of alpha-2 receptors augmented the response. Preliminary results indicated that by itself alpha-1 and beta-1 blockade produced a depression in nocturnal activity whereas alpha-2 blockade produced a variable stimulation. The interactions of these treatments with stress is currently under investigation. Supported by NIH MH45265 and a Stanley Foundation Award.

183.17

EFFECTS OF FLUMAZENIL ON RESPONSES TO YOHIMBINE IN HEALTHY HUMAN SUBJECTS.

M.Narayan, D.S.Charney, S.W.Woods*, G.M.Banks, G.R.Heninger, A.W.Goddard. Dept of Psychiatry,Yale U. Sch.of Med, New Haven, CT 06519.

Flumazenil (FLU) has been found to be anxiogenic in patients with panic disorder (PD). Also a subgroup of patients with PD have evidence of noradrenergic overactivity. This study investigated the hypothesis that acute administration of a specific benzodiazepine (BDZ) antagonist (FLU) would result in the potentiation of behavioral, biochemical and cardiovascular effects of the α2 adrenergic antagonist yohimbine (YOH). METHODS: Six healthy human subjects (4 males and 2 females; mean age ± SD = 25 ± 3.7 years) consented to participate in this protocol. FLU (2mg) IV and YOH (0.4 mg/kg) IV were administered to subjects in a randomized, placebo-controlled, cross-over fashion. Each subject received a combination test of either YOH /FLU, YOH/placebo, FLU/placebo, or placebo/placebo on 4 separate days, approximately 1 week apart. Within test measures included visual analog scale (VAS) (0-100 mm, self report) anxiety, nervousness, vital signs, plasma MHPG and cortisol. RESULTS:The mean peak change from baseline anxiety levels in subjects receiving a combination of YOH and FLU= 26 ± 27 mm on the VAS, compared to the score =9±11 mm on the YOH/placebo test (paired t-test, t=2.1, df=5, p=0.1). The YOH/placebo test was more anxiogenic than placebo (t=2.1, df=5, p=0.1). The combination test had no particular effects on either nervousness or blood pressure in comparison to controls. Additional physiological and neuroendocrine data will also be presented. DISCUSSION: The combination of FLU and YOH resulted in a trend towards increased anxiety in healthy human subjects. Interactions between the BDZ/γ-amino-butyric acid and noradrenergic systems may be involved in the mediation of human subjective anxiety.

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183.18

EFFECTS OF BENZODIAZEPINE AGONIST/ANTAGONIST ON CONDITIONED FEAR STRESS IN RATS. T.Izumi,* T.Inoue, T.Ohmori and T.Koyama . Dept. of Psychiatry., Hokkaido Univ. Sch. of Med., Sapporo 060, Japan.

We investigated the effects of benzodiazepine omega 1 and omega 2 agonist, lorazepam and benzodiazepine omega 1 antagonist, flumazenil on conditioned fear stress (CFS) in rats, an animal model of anxiety. CFS (exposure to an environment paired previously with footshock) 24 hours after single footshock induced marked freezing behavior. Administration of lorazepam (0.001-0.003 mg/kg, s.c.) 30 min before CFS significantly reduced freezing, suggesting that lorazepam inhibited expression of conditioned fear. Administration of flumazenil 30 min before CFS significantly reduced freezing only at a high dose (10 mg/kg, s.c.). This result suggests two possibilities. One is that high dose of flumazenil exerted agonist activity, and another is that flumazenil antagonized endogenous ligands for benzodiazepine receptor, such as DBI, that act as a negative allosteric modulator of GABA.

183.19

THE EFFECTS OF CAFFEINE IN THE DEFENSIVE WITHDRAWAL TEST. D.A. White* and D.L. Birkle. Department of Pharmacology & Toxicology, West Virginia University, Morgantown, WV 26506-9223.

The defensive withdrawal testing paradigm (DWT) is a behavioral test used to determine levels of anxiety, stress, and psychoactivity via the measurement of a number of behavioral parameters including: latency to the first exit, motor activity, rears per time in field, time in chamber, time in field, total exits, and time per visit in the chamber. Traditionally, increased latency, increased time per visit, and decreased rears per time in field have served as markers for anxious behavior. Corticotropin releasing factor (CRF) given i.c.v. and restraint stress have both been previously shown to cause changes in behavior in the DWT associated with increased levels of anxiety in rats. With this in mind, the effects of caffeine, a known anxiogenic agent, were tested in male Sprague-Dawley rats using the DWT. Caffeine at 0, 10, 20, and 40 mg/kg (i.p.) was given to rats, which were subsequently tested for 10 min. Their behavior was video taped for later analysis. It was found that there were no significant changes in the time per visit or latency at any dose of caffeine. However, there was a significant decrease in the rears per time in field at both the 20 and 40 mg/kg doses, which is similar to an effect observed after the administration of i.c.v. CRF. This suggests that CRF may be altering complex behavior via multiple pathways and that caffeine may exert at least some of its behavioral effects via a common pathway. This research was supported in part by NSF (IBN-9222263) and the WVU Foundation.

183.20

EFFECTS OF CHRONIC SUBORDINATION STRESS ON BETA-ADRENOCEPTOR BINDING IN THE BRAIN. G. Flügge*, O. Ahrens and E. Fuchs. German Primate Center, D-37077 Göttingen, Germany.

Psychosocial stress (PSS) is known to affect the functioning of the central noradrenergic system. In the present study, the consequences of recurrent stressful experiences on β_1- and β_2-adrenoceptors (ARs) were analyzed in male tree shrews (*Tupaia belangeri*). The animals were submitted to subordination stress for 2, 10, 21, and 28 days, and β-adrenoceptors were quantified by in vitro autoradiography using ^{125}I-iodocyanopindolol.

β_1-ARs were transiently down-regulated after 2 days of PSS in the prefrontal cortex and in the olfactory area, and were decreased after 28 days of PSS in the parietal cortex and the hippocampus. A transient up-regulation of β_1-ARs occured in the pulvinar nucleus after 10 days of PSS. β_2-ARs were transiently down-regulated after 2 days of PSS in the prefrontal cortex, and up-regulated in the pulvinar nucleus after 28 days of PSS.

These data demonstrate that chronic PSS stress leads to time dependent changes in the central nervous β-AR system. The high regional variability in receptor regulation may be due to the complex mechanisms of intracellular β-AR sequestration which enables the individual to balance the central nervous adrenoceptor number. (Supported by German Science Foundation; SFB 406 to G. F.)

NEUROPEPTIDES AND BEHAVIOR I

184.1

CORTICOTROPIN-RELEASING FACTOR ANTAGONIST INFUSED INTO THE LOCUS COERULEUS ATTENUATES IMMOBILIZATION STRESS-INDUCED INCREASE OF NOREPINEPHRINE IN THE PREFRONTAL CORTEX IN RATS G.N. Smagin*, J. Zhou, R.B.S. Harris, D.H. Ryan Pennington Biomedical Research Center, Louisiana State University, Baton Rouge, LA 70808 USA

Substantial evidence indicates that CRF mediates stress-induced responses. Neuroanatomical, neurophysiological and behavioral studies also suggest that brain stem nucleus locus coeruleus (LC) plays an important role in this response. The present study was designed to clarify, whether infusion of CRF antagonist, αhCRF into the LC could attenuate or block stress-induced changes in norepinephrine (NE) concentrations in microdialysates collected from the medial prefrontal cortex (PFM), which receives noradrenergic input primary from the LC. Rats were implanted with a bilateral cannulae assembly aimed in the LC and a microdialysis probe (4 mm active membrane length) in the PFM. Ten minutes before the onset of 30 min immobiliztion stress animals received bilateral infusion of αhCRF(1 µg per site) into the LC. Immobilization of animals significantly increased the concentration of NE in microdialysates from PFM to a maximum of 170.8 ± 12.8% of the baseline ten minutes after the onset of stressor. Concentration of NE in dialysates remained significantly elevated for the next 40 minutes. Infusion of αhCRF into the LC significantly attenuated stress-induced increase in PFM NE concentration in samples collected at 10, 20, 30, 40 and 50 minutes after the onset of immobilization. Infusion of αhCRF alone (no immobilization) did not change NE concentrations at any time during sample collection.

These results are consistent with other studies and suggest that stress can facilitate NE release in the PFM through the activation of the CRF system in the brain.

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184.2

BEHAVIORAL ACTIVATING PROPERTIES OF CORTICOTROPIC RELEASING FACTOR INFUSED INTO THE NUCLEUS ACCUMBENS SHELL. Matthew R. Holahan , Ann E. Kelley, and Ned H. Kalin* Department of Psychiatry, University of Wisconsin Medical School, Madison, WI 53706

Corticotropin releasing factor (CRF) has long been recognized as a neuropeptide involved in the response to stress. Although the behavioral effects of intraventricular CRF have been well characterized, little is known about its possible functions in the ventral striatum. The posterior shell of the nucleus accumbens has moderate levels of CRF fibers and receptors. In the first experiment, the effects of CRF (125, 250 ng) were evaluated following infusion into the core and shell regions of accumbens. CRF elicited a significant, dose-dependent increase in spontaneous motor activity after infusion into the shell region, but not the core. In a second experiment, CRF (250, 500 ng) induced behavioral activation when infused into the shell, but not the ventricle. This activation appeared to consist of increased movements within a small area, rather than locomotor activity. A third experiment utilizing observational methods examined the precise behaviors affected by CRF (250, 500 ng) injected into the shell. Treated rats displayed increased levels of grooming, sniffing, and oral behavior, with much lower levels of "still" behavior. In a fourth study, CRF (250 ng) infused into the posterior shell via angled cannulae that avoided the ventricle elicited the same behavioral pattern. These results suggest that CRF within the posterior accumbens modulates behavior; however, its relationship to motivational state, stress, or reinforcement awaits further study. Supported by grant DA04788, NIDA.

184.3

BEHAVIORAL AND PHYSIOLOGICAL EFFECTS OF LONG-TERM ADMINISTRATION OF CORTICOTROPIN-RELEASING FACTOR IN THE RAT BRAIN. B. Buwalda*, A.A. van Kalkeren, S.F. de Boer, J.M. Koolhaas. Dept. of Animal Physiology, University of Groningen, P.O.Box 14, 9750 AA Haren, The Netherlands.

In the present study we examined the effects of long-term, chronic and episodic administration of corticotropin-releasing factor (CRF) on a number of physiological and behavioral parameters. By implanting transmitters in male Wistar rats daily rhythms of body temperature and activity were biotelemetrically monitored in the home cages. CRF was administered for a period of 10 days intracerebroventricularly (icv) either chronically (5µg CRF/day) with the aid of osmotic minipumps or episodic (1µg/3µl saline as a bolus injection in the morning). For both groups controls were administered saline in a similar fashion. The chronic infusion of CRF increased both night and day temperature as compared to controls. However, the effect on temperature was strongest and most persistent (6 days) during the light, i.e. resting period. CRF reduced the daily amplitude in temperature for this period. The rats habituated to the end of the 10-day period. CRF, however, persistently reduced daily amplitudes of motor activity. In the episodic treatment design heart rate was also monitored. Immediately following CRF injection in the morning heart rate and temperature and motor activity increased. Compared to control treated rats the heart rate during the night period was reduced during the 10-day period. Temperature responses to the daily CRF injections gradually decreased. There was no habituation in heart rate and motor activity increases during daytime following administration of the peptide. Chronical and episodic CRF administration reduced weight gain during the treatment period. Organ weight of adrenals was increased and thymus weight reduced. Exposure to the elevated plus-maze 7 days after starting the chronical and episodic treatment showed an increased anxiety in the CRF treated rats in this test.

184.4

NEUROTENSIN AND ITS ANTAGONIST, SR48692 DIFFERENTIALLY MODULATE EXPLORATORY BEHAVIOR IN THE ELEVATED PLUS MAZE: A POTENTIAL ROLE FOR CORTICOTROPIN RELEASING HORMONE.

W. Rowe[1]*, J. Rochford[1], D. Gully[2], M. J. Meaney[1] and R. Quirion[1] [1]*Dept. of Psychiatry and Neurology/Neurosurgery, Douglas Hospital Research Center, McGill University, Montreal, Canada, H4H 1R3.* [2]*Sanofi Recherche, 31036 Toulouse Cédex, France.*

The neuroendocrine actions of centrally administered neurotensin (NT) on the hypothalamic-pituitary-adrenal (HPA) axis are mediated via the activation of hypophysiotropic corticotropin releasing hormone (CRH) release (Rowe et al., *J. Neuroendo.*, 1995, 7:109-117). CRH, acting in its capacity as a hypothalamic releasing hormone, stimulates the release of adrenocorticotropin (ACTH) and corticosterone (B) following NT administration. However, CRH in the central nervous system also appears to enhance behavioral responses to stressors. These effects are believed to occur independently of the HPA axis and can be reversed by selective CRH antagonists. Further, the localization of CRH and its receptors in extrahypothalamic brain regions indicates that CRH may act as a neurotransmitter. Thus, we were interested in determining whether the NT-induced increases in the HPA activity were also accompanied by alterations in CRH and CRH-related behavior. Male Long-Evans rats were treated with chronic NT for 14 days (1 pmol/hr) via Alzet Miniosmotic pumps. Immunoreactive CRH levels were found to be significantly higher in the median eminence, consistent with its role in mediating hypophysiotropic HPA activity. Similarly, these animals also displayed significant decreases in latencies and entries into the open arms in the elevated plus maze. A significant decrease in mean locomotor activity was also noted in the chronically-treated NT animals following exposure to a novel environment. Conversely, when a separate group of animals were treated with the newly developed NT antagonist, SR48692, prior to testing on the elevated plus maze, these animals spent significantly more time on the open arms than controls. As one of the behavioral characteristics of CRH hyperactivity is an increase in anxiolytic behavior elicited by novelty (Stenzel-Poore et al., 1994, *J. Neuroscience*, 1994, 14: 2579-2584) it is likely that a NT-induced increase in HPA activity and central CRH levels may in part be related to the behavioral response to novelty seen in these animals. Supported by MRC, FCAR and NSERC.

184.5

EFFECT OF CENTRAL ADMINISTRATION OF UROCORTIN, A NOVEL CRF-RELATED NEUROPEPTIDE, IN BEHAVIORAL STUDIES IN RATS. M. Spina, A.M. Basso, E. Merlo-Pich, R. Chan[1], J. Rivier[1*], W. Vale[1] and G.F. Koob. The Scripps Research Institute, Dept. of Neuropharmacology, and [1]Clayton Foundation Laboratories for Peptide Biology, The Salk Institute , La Jolla , CA 92037.

Corticotropin-releasing factor (CRF) is known to be secreted by the hypothalamus in response to stress. Administration of CRF intracerebroventricularly (ICV) produces a "stress-like" reaction of the hypothalamic-adrenal-axis including an increase in symphathetic activity, a decrease in parasympathetic outflow and a decrease in feeding behavior. Recently a neuropeptide called urocortin (Ucn) has been identified in mammals which shares similar biological activities with rat/human CRF (45% homology) and fish urotensin (65% homology). As already found with other non-mammalian CRF-like peptides as urotensin, Ucn binds with better affinity to CRF_2 receptors as compared to CRF. The aim of this study was to compare the effects of Ucn, CRF and urotensin in a series of behavioral tests. ICV administration of doses as low as 0.1µg of Ucn were effective in decreasing food-intake in food-deprived animals without producing "anxiogenic-like" effects on plus-maze, behavioral activation in locomotor activity and taste aversion. Thus, Ucn was significantly less potent than CRF in producing stress-like effects and activation but significantly more potent than CRF in suppressing food intake in fasted animals. This effect was attenuated or reversed by central administration of CRF antagonists. The results suggest that Ucn has a different central nervous system profile than CRF and may have an important role in controlling food-intake. (Supported by NIH DK 26741).

184.6

APPETITE-SUPPRESSING EFFECTS OF UROCORTIN, A NOVEL CRF-RELATED NEUROPEPTIDE, IN FREE FEEDING RATS. A.M. Basso, M. Spina, E. Merlo-Pich, P. Griffin, J. Rivier[1], W. Vale[1] and G.F. Koob*. The Scripps Research Institute, Dept. of Neuropharmacology, and [1]Clayton Foundation Laboratories for Peptide Biology, The Salk Institute, La Jolla , CA 92037.

Corticotropin-releasing factor (CRF) has been implicated in modulating behavioral and physiological responses to stress. Central administration of CRF increases arousal and produces "anxiogenic-like" and anorectic effects. These effects are independent of the pituitary-adrenal-axis activation, suggesting a direct action on brain CRF receptors. Until recently only endogenous CRF-like neuropeptide had been isolated from mammalian brain and had been implicated in stress-induced behavioral changes, including anorexia. Urocortin (Ucn), a novel 40 amino acid neuropeptide related to CRF which binds to CRF_2 receptor, has been shown to be less potent than CRF in inducing anxiogenic-like effects and behavioral activation, but more potent than CRF in suppressing food intake in meal deprived animals. The aim of this study was to evaluate the appetite-suppressing effect of Ucn in animals not submitted to food deprivation. Male Wistar rats were allowed to obtain 45mg pellets and 100 ul of water ad-libitum during the dark phase of the light/dark cycle using a nose-poke response. Ucn at doses as low as 0.1 µg administered intracerebroventricularly (ICV) produced a significant decrease in food and water intake for up to 12h. At doses of 0.01 - 1.0µg Ucn ICV decreased significantly the meal size and at 1.0µg Ucn decreased the number of meals. These results indicate that Ucn has a different profile than CRF showing a powerful anorectic effect without aversive or anxiogenic-like effects, suggesting that Ucn may be an endogenous CRF-like factor responsible for controlling appetite. (Supported by NIH DK 26741).

184.7

THE ROLE OF THE BED NUCLEUS OF THE STRIA TERMINALIS IN CRH-ENHANCED STARTLE: AN ANIMAL MODEL OF ANXIETY. Y. Lee* & M. Davis, Dept. of Psychiatry & Psychology, Yale Univ., New Haven, CT 06508

CRH given i.c.v. produces a profound, dose-related increase in acoustic startle amplitude in rats (CRH-enhanced startle). However the primary receptor sites for CRH given i.c.v. have not been identified. Our laboratory showed that electrolytic, but not chemical, lesions of the septum blocked CRH-enhanced startle, suggesting that fibers passing through the septum (e.g., the fornix) are critically involved in CRH-enhanced startle. Because the ventral hippocampus (VH) projects to the bed nucleus of the stria terminalis (BNST) via the fimbria/fornix, and to the amygdala via the BNST, the role of these structures in CRH-enhanced startle was investigated.

Chemical lesions of the VH or the BNST, or knife cut of the fimbria/fornix blocked CRH-enhanced startle, whereas chemical lesions of the amygdala did not. However, infusion of CRH into the VH did not mimic i.c.v. CRH, whereas CRH given to the BNST enhanced startle in a dose-dependent manner. Moreover, a CRH antagonist infused into the BNST blocked CRH-enhanced startle, suggesting the BNST may be a primary receptor site for i.c.v. CRH.

A functional double dissociation between the BNST and the amygdala was also found. Thus, chemical lesions of the BNST, but not the amygdala blocked CRH-enhanced startle, whereas lesions of the amygdala but not the BNST blocked fear-potentiated startle. A CRH antagonist infused into the amygdala did not block the expression of fear-potentiated startle, and there was perfect additivity between fear-potentiated and CRH-enhanced startle measured in the same animal.

The present studies strongly suggest that CRH in the CSF can activate the BNST via mechanisms different from those involved in conditioned fear. Currently we are using *c-fos* to assess whether the constellation of neural effects produced by i.c.v. CRH will be blocked by lesions of the BNST. [Supported by MH47840, MH00004 and AFOSR F49620-93-1-0293 DEF]

184.8

REGIONAL AND DEVELOPMENTAL DIFFERENCES IN ALTERATION OF CRF CONTENT IN BRAIN OF PRENATALLY STRESSED RATS. D.L. Birkle* and D.A. White. Department of Pharmacology & Toxicology, West Virginia University, Morgantown, WV 26506-9223.

The effects of prenatal stress were studied in offspring of pregnant rats subjected to daily 0.9% NaCl injection (0.1 ml, s.c.) from gestational day 14 to 21 (G14-G21). In adulthood, these prenatally stressed (PNS) offspring exhibit increased fearfulness, elevated content of CRF in amygdala, increased density of CRF-immunopositive neurons in amygdala and hypothalamus, adrenal hypertrophy, and elevated plasma corticosterone levels. In the present study the content of CRF was measured by RIA in brain regions from PNS rats perinatally. There was an age dependent increase in CRF content in all regions, but with varying time courses. In hypothalamus from PNS rats, elevated CRF content was detectable at all ages, including at embryonic day 18 (E18). In control offspring, CRF levels were stable in the hypothalamus over the stress-hyporesponsive period (129 pg/mg protein at P3 and P13), while in PNS rats, CRF increased markedly during this period (148 pg/mg protein at P3 increasing to 284 pg/mg protein at P13). This may explain elevated ACTH and corticosterone responses observed previously in preweanling PNS rats. In amygdala, PNS-induced increases in CRF content were not evident until P13 (21 pg/mg protein in control; 57 pg/mg protein in PNS). This suggests a posible role for post-natal factors in the manifestation of the effects of prenatal stress in amygdala. In anterior telencephalon (frontal cortex, striatum, hippocampus) and in brain stem (pons, medulla, midbrain), there were substantial age dependent increases in CRF from E18 to P13 but no effects of PNS at any age. Supported in part by NSF IBN9222263 and the WVU Foundation.

184.9

ELECTROPHYSIOLOGICAL AND BEHAVIORAL STUDIES ASK: IS THERE AN NPY_1-LIKE RECEPTOR INVOLVED IN FEEDING? T.A. Pitler*, D.G. Harden, J.V. Cassella, S.H. Lang, M.M. Bennett, P.Z. Gallipoli, and C.A. Blum. Neurogen Corporation, Branford, CT, 06405.

NPY is one of the most potent stimulators of feeding behavior in laboratory animals, and at least four subtypes of the NPY receptor exist. Various fragments of the NPY peptide differentially bind to these receptor subtypes. Use of the $NPY_{1\text{-}36}$ and $NPY_{2\text{-}36}$ fragments has led to some confusion regarding the involvement of the NPY_1 receptor or an NPY_1-like receptor subtype in feeding behavior. We investigated the effects of both human(H)- and porcine(P)-derived 1-36 and 2-36 fragments, as well as the potent and selective NPY_1 receptor antagonist, BIBP3226 ((R)-N^2-(diphenylacetyl)-N-[(4-hydroxyphenyl)methyl]-argininamide), in two functional assays. In *Xenopus* oocytes, peptide effects were assessed electrophysiologically by coexpressing the NPY_1 receptor with a G-protein activated inward rectifying K^+ channel (GIRK1). Despite large differences in potency reported in the literature, in oocytes $NPY_{1\text{-}36}$ was only three to ten fold more potent than $NPY_{2\text{-}36}$, and P and H forms of the peptides were equipotent. Currents induced by NPY fragments at the NPY_1 receptor were antagonized by BIBP3226 (IC_{50} ~1.5 nM). In behavioral studies, ICV infusion of H- or P-derived forms of both NPY fragments (2.5 and 10 µg) in rats housed in metabolic cages elicited robust and equipotent feeding responses while BIBP3226 significantly attenuated feeding elicited by $H\text{-}NPY_{1\text{-}36}$. Thus, using these fragments, electrophysiological studies on the NPY_1 receptor and behavioral studies are concordant supporting the involvement of the NPY_1 receptor in feeding behavior. (Funding provided by Neurogen Corporation.)

184.10

HYPOTHALAMIC cAMP RESPONSIVE ELEMENT BINDING PROTEIN, CREB, MEDIATES NPY-INDUCED FEEDING IN RATS. S. Sheriff, W. T. Chance*, J. E. Fischer and A. Balasubramaniam. Department of Surgery, University of Cincinnati and VA Medical Centers, Cincinnati, OH 45267

We have previously shown that NPY-induced feeding is mediated by a pertussis toxin sensitive G protein in rats [Peptides 10:1283-1286, 1989]. NPY receptors are coupled to cAMP and/or Ca^{2+}. Since these second messengers are known to activate cAMP responsive element (CRE) binding proteins, CREB, CREM or ATF-1, we investigated whether these transcription factors mediate NPY-induced feeding in rats. As compared to control injections of CSF (1 μl), intrahypothalamic (iht) administration of NPY (1 μg) increased CRE binding to rat hypothalamic nuclear extracts in a time-dependent manner, as detected by an electrophoretic mobility shift assay. In contrast, iht-administration of the anorectic neuropeptide, PACAP ($2\mu g$) strongly inhibited the CRE binding. Food deprivation for 48 hrs also increased CRE binding, while 8 hrs of refeeding normalized CRE activity. Preincubation of the hypothalamic nuclear extracts of NPY-treated and fasted rats with antibody specific to CREB blocked CRE binding, while preincubation with phospho-CREB antibody supershifted the CRE-CREB complex indicating that phospho CREB is involved in this process. Iht-NPY also increased hypothalamic CaM kinase II activity. These results suggest that CaM kinase induced phosphorylation of CREB regulates the feeding behavior induced by NPY, hunger and satiety in rats.

184.11

INCREASED PROGRESSIVE RATIO RESPONDING AFTER INTRAHYPOTHALAMIC NEUROPEPTIDE Y IS NOT MEDIATED BY DOPAMINE. C.M. Brown[*1], P.J. Fletcher[1], and D.V. Coscina[1,2], Section of Biopsychology, Clarke Institute of Psychiatry, Toronto, ON M5T 1R8[1] and Dept. of Psychology, Wayne State Univ., Detroit, MI 48202[2].

Neuropeptide Y (NPY) is a 36 amino acid pancreatic peptide that is distributed throughout the central and peripheral nervous systems. One of its best known behavioural effects is to induce feeding in previously satiated animals after injection into the hypothalamus, especially the perifornical region (PFH). NPY also appears to have rewarding properties of its own, as evidenced by its ability to produce a conditioned place preference following injection into the nucleus accumbens. This effect is seemingly mediated by dopamine (DA) since α-flupenthixol (FLU), a DA antagonist, blocks this effect. Another paradigm that has been used to assess reward is the progressive ratio (PR) operant schedule. To determine if intra-PFH NPY increases PR responding for sucrose and if DA might mediate this effect, four different experiments were conducted. In the first, PR output for sucrose reward was determined by injecting NPY (0, 78, 156, and 235 pmol) into the PFH of male rats. In the second study, FLU (0, 0.05, 0.1, 0.2 mg/kg, s.c.) was given 2 h prior to intra-PFH NPY (156 pmol) and PR responding was recorded. The third study tested FLU's effects alone on PR responding. The last experiment examined FLU's capacity to block intra-PFH NPY(156 pmol)-induced free-feeding. Although NPY reliably increased PR responding and FLU attenuated it, FLU had no greater inhibitory effect on NPY-stimulated output compared to drug-free output and also did not affect NPY-induced free-feeding. Collectively, the results suggest that distinct reward mechanisms are activated during NPY-induced feeding vs. PR responding since FLU disrupted the latter but not the former.

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184.12

ANXIOLYTIC EFFECTS OF NEUROPEPTIDE Y IN RATS WITH LESIONS OF THE LATERAL SEPTUM. E. Thomas* and J. Snellman. Dept. of Psychology, Bryn Mawr College, Bryn Mawr, PA 19010

The role of the lateral septum in the mediation of the anxiolytic properties of neuropeptide Y (NPY) was assessed using the elevated plus-maze. The design of the experiment was a 2x2 factorial with half of the animals receiving a bilateral electrolytic lesion of the lateral septum and the other half receiving a sham lesion. Half of each these groups received NPY (1 nmol) administered intracerebroventricularly The other half received vehicle alone. Thirty minutes later animals were placed in the plus-maze for a 5 minute session. Number of entries into the open arms and time in the open arms were measured.

Both the drug and the lesions were found to significantly increase the percentage of entries and the amount of time spent on the open arms. The effects of the lesion and the drug were additive with the greatest effect seen in the animals receiving both lesion and NPY.

The data suggest that the septum is not the primary site of action of the anxiolytic properties of NPY despite being relatively high in NPY receptor density. The results were interpreted as reflecting a combination of an anxiolytic effect of NPY with an impairment of a fear-relief mechanism normally provided by the septum.

184.13

IN-VITRO SECRETION AND IN-SITU EXPRESSION OF HYPOTHALAMIC NEUROPEPTIDE Y DURING ZINC DEFICIENCY C. Tovar-Palacio, A. Cole, H. Mangian, and N. Shay* Division of Nutritional Sciences; Department of Food Sci. & Human Nutrition; University of Illinois, Urbana, IL 61801.

Zinc deficient (Zn-) rats show marked anorexia and growth retardation compared to rats consuming a Zn-adequate (Zn+) diet. The mechanism causing this anorexia is not well understood. We have been investigating the changes in neuropeptide metabolism in the hypothalamus during zinc deficiency. Herein, we report findings from two studies: I - In vitro secretion of NeuropeptideY (NPY) from microdissected sections of the paraventricular nucleus (PVN); and II - In-situ immunohistological assays of NPY in the PVN. Male Sprague-Dawley rats were provided either a Zn+ or Zn- diet for 21 d. In study II, groups of Zn- rats were also repleted with Zn after the development of zinc deficiency. All rats were killed at the onset of the dark cycle and tissues were either microdissected for secretion studies or fixed in buffered formalin for embedding and sectioning. In study I, tissue was incubated in neurobasal medium (GIBCO) for two 30 min periods, followed by a third 30 min incubation in high K^+ to verify tissue viability. Secreted NPY in media was measured by ELISA. NPY was secreted at a higher rate from Zn- rats (0.41 vs. 0.22 ng/mL, $p < 0.05$). In study II, immunohistochemical staining was assessed through both blind ranking of immunostaining in photographs and by computer-based quantitation of immunostaining using image capture and "NIH Image" software. We found that NPY was present in the PVN at higher levels in the Zn- group. Data from studies I and II are consistent with data from our own lab indicating elevated NPY peptide and mRNA content in the PVN and ARC of Zn- rats. We conclude that although Zn- rats are anorexic, NPY content and secretion is higher, paralleling results observed in food-restricted rats, which eat reduced amounts of diet, but in contrast have a desire to consume greater amounts of diet.

184.14

THE EFFECTS OF INTRACEREBROVENTRICULAR INJECTIONS OF NPY, PYY, AND PP ON ACUTE FEEDING BEHAVIOR AND LOCOMOTOR ACTIVITY IN THE MOUSE. M.E.Judge*. Dept. of Behavioral Pharmacology, Novo Nordisk A/S, DK-2760, Måløv, Denmark.

Neuropeptide Y (NPY) is known to be involved in the central regulation of feeding. Central injections of NPY and the related peptides PYY and PP via indwelling cannulae in the rat have been shown to enhance feeding (PYY>NPY>PP), but the need for surgical pretreatment places severe limits on study designs. In this study, mice were given injections of the neuropeptides directly into the lateral ventricle utilizing a freehand technique requiring neither anaesthesia nor pre-implanted guide cannulae. This permitted the testing of relatively large groups of animals, allowing full dose response curves to be generated. Thirty minutes after ICV injection of .02-10 micrograms (μg) of human NPY, PYY, or PP, separate groups of 6 mice were given one of two tests, each lasting ten minutes. Infrared photocell activity chambers were used to record spontaneous locomotor activity. All three peptides depressed activity at high doses, but for NPY and PP the maximum effect was only marginal ($p<0.1$), while 5μg of PYY suppressed activity by 77% ($p<0.01$). NPY increased activity at low doses, with a maximum increase of 109% over controls at 0.5μg ($p<0.001$). No increases in activity were seen with PP or PYY. Effects on feeding were tested by measuring the total time spent consuming an infant milk formula during a 600sec test. In terms of the maximum response measured, PP gave the least enhancement of feeding (107sec / 5μg), NPY gave a somewhat greater response (127sec / 5μg), and the greatest response was obtained with PYY (311sec / 2μg), a 4 fold increase over controls. These data are similar to those obtained in rats, demonstrating intra-species similarities, some of the differences between the peptides, and the utility of the technique.

This research was supported by Novo Nordisk A/S

184.15

NEUROTOXIN-INDUCED TRANSIENT HYPERPHAGIA IS NEGATIVELY CORRELATED WITH HYPOTHALAMIC NEUROPEPTIDE Y M.G. Dube, B. Xu, S.P. Kalra and P.S. Kalra*, Depts. of Physiology and Neuroscience, Univ. Fla. Col. Med., Gainesville, FL 32610

The ventromedial hypothalamus (VMH) is known to play a role in the hypothalamic control of hyperphagia and obesity, however, its relationship with hypothalamic NPY, a neuropeptide involved in stimulation of feeding, remains to be ascertained. We employed colchicine (COL), a neurotoxin that interrupts axonal flow, to examine the relationship between food intake and NPY levels and gene expression. Microinjection of COL (4 μg/0.5 μl saline) bilaterally into the VMH of male rats produced a transient and robust hyperphagia; 24-hour food intake for the first 3 days was 2-3-fold higher than in saline injected control rats. COL-injected rats displayed marked body weight gain and weighed 50 g more than the controls by day 4. Thereafter, both food intake and rate of weight gain stabilized and were similar to controls. On days 1, 2 and 4 post-injection, we measured NPY levels in the paraventricular nucleus (PVN) and preproNPY mRNA levels in the arcuate region by RIA and RNase protection assay, respectively. Surprisingly, a negative relationship between hypothalamic NPY and hyperphagia was detected in COL-injected rats; On days 2 and 4, NPY gene expression was decreased by 75% and NPY levels in the PVN were reduced to 50%. These results show that a temporary inhibition of information flow from the VMH results in a marked decrease in NPY activity. Thus, the transient hyperphagia may be due either to increased release of NPY and/or to increased NPY receptor activation in the PVN target sites. (Supported by NIH NS32727).

185.1

THE ANTICIPATORY EFFECTS OF ETHANOL ON DOPAMINE RELEASE IN THE NUCLEUS ACCUMBENS OF P AND WISTAR RATS. S.N. Katner* and F. Weiss. Dept. of Neuropharmacology, The Scripps Research Institute, La Jolla, CA 92037.

It has been argued that alcohol-associated environmental cues can activate reinforcement substrates, thereby initiating ethanol-seeking behavior and maintaining ethanol intake until the direct reinforcing actions of the drug take effect. To test this hypothesis independent groups of alcohol-preferring (P) and Wistar rats were trained to orally self-administer either 10% (w/v) ethanol or saccharin (0.05%). Accumbal dopamine (DA) efflux was then monitored via microdialysis during self-administration of either substance. Self-administration of saccharin failed to elevate extracellular DA in both strains. However, when ethanol-trained P rats were tested by substituting saccharin for ethanol, the sweetener produced a significant elevation (150% of baseline within the first 15 minutes) in extracellular DA. In contrast, when ethanol-trained Wistar rats were tested by substituting saccharin for ethanol, no change in extracellular DA was observed. These results indicate that in P, but not Wistar rats, the expectation of ethanol promotes some DA release during the self-administration of a substance that is not normally associated with increased DA efflux. It is possible the "expectation-induced" stimulation of DA release in the P rat plays a role in the maintenance of ethanol-seeking behavior and that incentive stimuli enhance the neurochemical effects of the primary reinforcer in this line of rats. (Support: NIAAA AA08164, AA10531)

185.2

THE EFFECT OF NEFAZODONE ON ETHANOL-INDUCED ACCUMBAL DOPAMINE RELEASE, ETHANOL PREFERENCE AND INTAKE IN RATS. P. Olausson*, M. Ericson, A. Petersson, A. Kosowski, J.A. Engel and B. Söderpalm. Inst. of Physiology and Pharmacology, Dept. of Pharmacology, Göteborg University, Medicinaregatan 7, S-413 90 Göteborg, Sweden.

Most drugs of abuse, including ethanol, activate the mesocorticolimbic dopamine (DA) system, an effect considered to be involved in mediating drug reward. Evidence indicates that serotonin (5-HT) may influence rewarding mechanisms via modulation of mesocorticolimbic DA activity. The present study evaluated the effects of the antidepressant nefazodone, a combined 5-HT_2 receptor antagonist and 5-HT reuptake inhibitor, on ethanol reward mechanisms in the rat.

In microdialysis experiments in male Wistar rats, ethanol (2.5 g/kg i.p.) and nefazodone (50 mg/kg s.c.) both induced DA release, 40% and 30% respectively, above baseline in the nucleus accumbens (N Acc). Interestingly, nefazodone (50 mg/kg s.c.) injected 40 min before the administration of ethanol strongly attenuated ethanol-induced DA release (30% decrease compared to ethanol alone) in the N Acc. Nefazodone (25 mg/kg i.p.) did not affect the DA release per se and did not significantly alter the ethanol-induced DA release in the N Acc.

The effect of nefazodone (50 mg/kg s.c.) on voluntary ethanol preference and intake in high- (≥ 60% ethanol) and low-preferring (≤20% ethanol) Wistar rats was also evaluated. In high-preferring rats administration of nefazodone significantly decreased both ethanol preference (20%) and intake (50%), while the intake of water was unaffected. However, in low-preferring rats the water intake was significantly lowered but the ethanol preference and intake were not altered, probably due to the low baseline ethanol intake.

The present results show that nefazodone attenuates ethanol-induced DA release in the N Acc and suggest that this neurochemical effect may be of functional significance in terms of ethanol reward in high-preferring rats.

This study was supported by Bristol Myers Squibb, Scandinavia and by the Swedish MRC (grants no 4247 and 11583).

185.3

ACCUMBAL DOPAMINE-OVERFLOW AFTER ETHANOL; LOCALIZATION OF MECAMYLAMINE'S ANTAGONIZING EFFECT. M. Ericson*, O. Blomqvist, J.A. Engel and B. Söderpalm. Inst. of Physiology and Pharmacology, Dept. of Pharmacology, Göteborg University, Medicinaregatan 7, S-413 90 Göteborg, Sweden.

Ethanol, as well as several other drugs of abuse can activate the mesocorticolimbic dopamine system, which is regarded as an important neuroanatomical substrate for drug dependence. It appears that ethanol's activation of this system involves central nicotinic acetylcholine receptors (nAChR). Thus, we have previously shown that mecamylamine (1 mg/kg i.p.), a blood brain barrier penetrating antagonist at the nAChR, completely blocks ethanol-induced (2.5 g/kg i.p.) dopamine overflow in the nucleus accumbens in Wistar rats, as measured by *in vivo* microdialysis, while hexamethonium (10 mg/kg i.p.), a quarternary nAChR antagonist, does not.

In the present study, two microdialysis probes were placed in each rat for simultaneous perfusion in the ventral tegmental area (VTA) and the ipsilateral nucleus accumbens. When mecamylamine was perfused in the VTA, but not in the nucleus accumbens, ethanol-induced (2.5 g/kg i.p.) accumbal dopamine overflow; 30% above baseline levels, was completely blocked. Mecamylamine by itself produced no statistically significant effect neither in the nucleus accumbens nor in the VTA. Since mecamylamine, given systemically, previously was demonstrated to decrease voluntary ethanol-intake in high-preferring rats, we hypothesize that ethanol's dopamine-releasing effect, which may be of importance for its reinforcing effect, is mediated via activation of central nAChR preferably in the VTA.

In ongoing studies we examine whether high-preferring rats decrease their voluntary ethanol-intake when perfused with mecamylamine in the VTA.

This study was supported by Swedish MRC (no 11583 and no 4247)

185.4

SINGLE UNIT RESPONSES RECORDED IN THE NUCLEUS ACCUMBENS OF RATS DURING ETHANOL SELF-ADMINISTRATION ARE RELATED TO BOTH OPERANT RESPONDING AND REINFORCER DELIVERY. P.H. Janak* and D.J. Woodward. Dept. of Physiology and Pharmacology, Bowman Gray School of Medicine, Wake Forest University., Medical Center Blvd., Winston-Salem NC 27157.

A variety of studies, including single-unit recording studies, suggest that the nucleus accumbens (Nac) may be part of a neural circuit that regulates behavioral responding for drugs and other reinforcers. In the present study, we recorded neural activity within the Nac during ethanol self-administration to determine if neural activity in this region also is associated with ethanol reinforcement. Male Long-Evans rats were trained to bar press on a continuous reinforcement schedule for drops (.1 cc) of 10% ethanol, or 10% ethanol/2% sucrose, solutions. A tone preceded delivery of the reinforcer. Following training, rats were chronically implanted with one or two square arrays of 16 microwires each into the Nac. After surgical and behavioral recovery, simultaneous recordings of the extracellular spike activity of 6-30 single units per subject were made during ethanol-reinforced operant behavior. Neurons showed phasic changes in firing related to the operant response and to the delivery of the reinforcer. In addition, a small number of units showed either excitation or inhibition in relation to the tone stimulus that preceded reinforcer delivery. These data suggest that neural activity within the Nac may serve to integrate sensory stimuli and motor activity preformed to obtain ethanol, and support the hypothesis that the Nac is a component of a response initiating system that associates conditioned stimuli and reinforcers with operant behavior. *Supported by AA 07565, 10980. WWW: http://biogfx.bgsm.wfu.edu.*

185.5

ETHANOL ALTERS ACTION POTENTIAL SHAPE IN DOPAMINERGIC NEURONS OF THE VENTRAL TEGMENTAL AREA. M.S. Brodie* and S.A. Shefner, Dept. Physiology & Biophysics, Univ. Illinois at Chicago, College of Medicine, Chicago, IL 60612-7342.

The reinforcing effects of ethanol may be mediated by dopaminergic (DA) neurons of the ventral tegmental area (VTA). Intracellular recordings were made from VTA neurons in brain slices from male rats (Fischer 344 and Sprague-Dawley). All cells were identified as DA based on electrophysiological criteria. Ethanol (40 - 160 mM) excited 60% of 35 spontaneously active VTA neurons studied. Most cells (57%) were depolarized by ethanol. Mixed cationic, time-dependent inward rectification appeared to be increased by ethanol (40 - 160 mM) in 65% of cells tested ($n = 26$).

For cells excited by ethanol, action potential data were collected and analyzed with pClamp software. Measurements of spike shape were made on averaged spontaneous action potentials in the presence and absence of ethanol and compared by paired t-test. Spike amplitude was reduced by 80 mM ethanol from 59 ± 2.3 mV to 55 ± 2.4 mV ($n = 11$, $P < 0.01$), and by 160 mM ethanol from 66 ± 1.4 mV to 60 ± 1.7 mV ($n = 12$, $P < 0.001$). Spike duration at 1/2 amplitude was not significantly altered by either concentration of ethanol. The peak amplitude of the afterhyperpolarization was reduced by 80 mM ethanol from -15.7 ± 1.3 mV to -14.7 ± 1.3 mV ($n = 11$, $P < 0.05$), and by 160 mM ethanol from -16.4 ± 0.7 mV to -14.4 ± 0.9 mV ($n = 12$, $P < 0.001$). Of cells excited by ethanol, 80 mM ethanol caused a mean membrane potential change from -50 ± 1.9 mV to -49 ± 1.7 mV ($n = 11$, $P < 0.05$); 82% of these cells were depolarized by 80 mM ethanol (from < 1 mV to 4 mV). Ethanol (160 mM) also depolarized most cells tested (75%, from < 1 mV to 4.5 mV), but produced a non-significant change in mean membrane potential from -54 ± 1.6 mV to -53 ± 1.8 mV ($n = 12$, n.s., $P > 0.05$). These effects may underlie the ethanol-induced excitation of VTA neurons which is associated with the rewarding effects of alcohol.

Grant Support: PHS AA09125 and AA05846.

185.6

VOLTAGE-DEPENDENT ETHANOL EFFECT ON NMDA RECEPTOR-MEDIATED EXCITATORY SYNAPTIC TRANSMISSION IN BASOLATERAL AMYGDALA. J. L. Calton, S. D. Moore*, and W. A. Wilson. Duke University Medical Center and VAMC, Durham, NC 27705.

We are currently using the whole-cell recording technique to investigate the effects of ethanol on NMDA transmission in the cells of the basolateral amygdala, a brain region which may mediate anxiolytic effects of ethanol.

Coronal brain sections were prepared from rats (21-28 days old) and whole-cell current-clamp and voltage-clamp recordings were made of pyramidal neurons in basolateral amygdala, using a Cs^+ based internal solution. Bicuculline (20 μM) and DNQX (20 μM) were added to the bath. Stimulation near the cell produced large (15-45 mV) depolarizing events that were eliminated by bath application of APV (50 μM). Application of ethanol to the bath in anxiolytic concentrations (22-88 mM) resulted in a dose-dependent attenuation of these NMDA receptor-mediated events. We determined this effect to be voltage-dependent, with increased attenuation of the evoked responses at more hyperpolarized membrane potentials. The average attenuation at an ethanol concentration of 44 mM was 12.2% at 0 mV, 13.1% at -20 mV, and 23.1% at -40 mV. This voltage-dependent relationship was seen at all ethanol concentrations tested. Washout of the ethanol effect could be observed after 20 minutes.

We feel that a voltage-dependent ethanol effect will have significant consequences for overall functioning of the amygdala network.

Supported by grants from the NIH and the VA.

185.7

Effects of Chronic Ethanol Treatment on Glutamate Receptor Subunits in Rat Brain Regions. R.P. Yasuda[1,2]*, A.W. Dunah[1,2], J. Luo[1], Y.-H. Wang[1], J.G. Rudolph[3], and F.T. Crews[3]. [1]Department of Pharmacology, and [2]Interdisciplinary Program in Neuroscience, Georgetown University School of Medicine, Washington, D.C. 20007, and [3]Center for Alcohol Studies, University of North Carolina, Chapel Hill, N.C. 27599.

Chronic ethanol treatments lead to changes in glutamate receptor function and receptor densities. The glutamate ionotropic receptors are complexes of several different subunits. Therefore, chronic ethanol treatments could alter the function or the number of receptors by changes in one or all of the subunits of the ion channel. Recently, Trevisan *et al.* (J.Neurochem. 62:1635-1638, 1994) observed a 65% increase in the NMDA R1 receptor subunits in the rat hippocampus after 30 days of chronic ethanol treatment. This study investigates whether other NMDA and AMPA receptor subunits change after 2 weeks of an ethanol diet. Rats were pair-fed a liquid ethanol diet and rats consumed 4-5 grams of ethanol a day for two weeks. The rat frontal cortex and hippocampus were examined with antibodies that have been previously characterized as being selective for a given NMDA or AMPA receptor subunit. Preliminary studies have demonstrated a 19% increase in the NMDA 2D receptor subunit in the hippocampus but not the frontal cortex. The NMDA R1, NMDA 2A, NMDA 2B, GluR1, and GluR4 receptor subunit number were not different from control rats in either the hippocampus or frontal cortex. Other rat brain regions will be examined. NMDA receptors containing the NMDA 2D receptor subunit form a channel with a prolonged decay time and a decrease in the ability of magnesium to block the channel. Since the NMDA receptors in the hippocampus have been implicated in long-term potentiation , an increase in the expression of this receptor subunit may alter the normal function of the NMDA receptor complex in learning and memory.

185.8

WITHDRAWAL FROM CHRONIC ETHANOL EXPOSURE IN HIPPOCAMPAL EXPLANT SLICE CULTURES: EPILEPTIFORM EVENTS CORRELATE WITH ENHANCEMENT OF THE NMDAR COMPONENT OF SYNAPTIC TRANSMISSION. MP Thomas* and RA Morrisett. Dept. of Pharmacol., Univ. Nebraska Med. Ctr., Omaha, NE, 68198-6260.

Much evidence suggests an involvement of voltage-gated calcium channels in the induction and expression of ethanol withdrawal hyperexcitability (Whittington et al., 1993). A good deal of correlative evidence also implicates NMDA receptors in this alcohol-related disorder. We utilized the hippocampal slice explant preparation to simultaneously assess alterations in NMDAR-mediated synaptic responses and neural hyperexcitability following withdrawal.

Slice cultures were prepared from 9-10 day old rat pups and exposed to 35 or 75 mM ethanol for 6-11 days. Field potentials evoked by Schaffer collateral stimulation were recorded from the CA1 cell layer. Recordings were initially made in ethanol and for 7 hours following acute *in vitro* withdrawal; control slices were recorded in standard solution over the same time period.

Withdrawal from chronic exposure was associated with an enhancement of the slow, NMDAR-mediated synaptic potential (D-APV-sensitive) and the induction of epileptiform events. The NMDAR component increased by 74% and 132% in the 35 and 75 mM explant groups respectively over the entire 7hr WD period compared to pre-WD levels. Prolonged electrographic seizure events (>90 sec) occurred following ethanol withdrawal in 10 of 14 slices from both treatment groups. Bath application of the NMDAR antagonist, D-APV (25-50 μM) selectively inhibited the enhanced NMDAR synaptic responses and completely abolished epileptiform activity.

These data demonstrate a dose-dependent enhancement of NMDAR function following chronic ethanol exposure and suggest a direct correlation with the expression of seizure activity during withdrawal.(R29 9230 to RAM).

185.9

HIPPOCAMPAL SUPERSENSITIVITY FOLLOWING CHRONIC ETHANOL EXPOSURE. L. P. Gonzalez* and M. Wu. Univ of Oklahoma HSC, Dept of Psychiatry & Behavioral Sci., P.O. Box 26901, Oklahoma City, OK 73190.

The studies reported here were conducted to determine if chronic ethanol alters the responsiveness of hippocampal neurons to locally-applied acetylcholine. Extracellular single-unit activity was recorded with one barrel of a multibarrel glass micropipette containing 4M NaCl; additional barrels contained acetylcholine chloride (ACh), glutamate and vehicle control solutions. Brief applications of ACh (0.05-5.0 mM) or of glutamate (0.01-1.0 mM) were administered by micropressure-ejection. Acetylcholine application resulted in increases in single-unit activity in the hippocampal CA_3 field, but only slight changes in the CA_1 field. Glutamate increased single-unit activity in both the CA_1 and CA_3 fields Chronic exposure to ethanol by vapor inhalation, followed by withdrawal from ethanol for ten to 24 hrs resulted in significant increases in the response to ACh in the hippocampal CA_3 field, but not in the CA_1 field. Responding in the CA_3 field returned to control levels within 48 hrs after ethanol withdrawal. Responses to glutamate did not differ in either area after chronic ethanol exposure and withdrawal. These data suggest a significant increase in the cholinergic responsiveness of hippocampal CA_3 pyramidal cells after chronic ethanol exposure, and show that this effect is unique to this region of the hippocampus.

[Supported in part by NIAAA grants AA07578 and AA9959]

185.10

ETHANOL IMPAIRS DISCRIMINATION PERFORMANCE: RELATION TO HIPPOCAMPAL NEUROPHYSIOLOGY B. Givens* and D. Kent. Department of Psychology, The Ohio State University, Columbus, OH, 43210.

The disruption of hippocampal circuitry by ethanol may underlie ethanol-induced memory impairments. Rats were trained on a two tone auditory discrimination task, and then implanted with electrodes in the dentate gyrus, the perforant path and the medial septal area. Ethanol (0.0, 0.5, 0.75, and 1.0 g/kg i.p.) dose-dependently impaired performance on the discrimination task, and simultaneously affected hippocampal physiology. The slope and amplitude of the dentate evoked responses elicited by perforant path stimulation were dose-dependently suppressed by ethanol. In addition, the magnitude of paired-pulse inhibition was reduced by ethanol. In contrast to previous results in a working memory task, the auditory stimuli did not produce a resetting of theta activity. However, theta activity during the 2 sec period following tone onset was significantly greater for the relevant (rewarded) tone than for the irrelevant tone. Ethanol dose-dependently reduced this difference by selectively suppressing theta activity following the relevant tone. The data indicate that disruption of hippocampal neurophysiology correlates with decrements in discrimination performance following ethanol. Supported by PHS-AA09484.

185.11

CHRONIC ETHANOL ADMINISTRATION INCREASES TYROSINE HYDROXYLASE MRNA, BUT NOT NOREPINEPHRINE TRANSPORTER MRNA IN THE LOCUS COERULEUS Patricia Szot*, Sylvia S. White, Richard C. Veith and Dennis Rasmussen. Depts. Psy. Beh. Sci. and Med., Univ. Washington and VA Pugent Sound Health Care System, GRECC, Seattle, WA 98108.

Noradrenergic neurons have been implicated in the development of dependence and tolerance to ethanol's effects. Chronic ethanol (ETOH) consumption increases the rate of NE synthesis and turnover with no change in norepinephrine transporter (NET) sites. The following study was performed to determine the effects of chronic ethanol administration on two indices of noradrenergic function: the mRNA expressions of the rate limiting enzyme tyrosine hydroxylase (TH) and NET in the locus coeruleus (LC). Adult male Sprague-Dawley rats were given ETOH 6 % in a liquid diet for 5 weeks, followed by a slow withdrawal over the next week. To adjust for altered food consumption during chronic ETOH administration, both pair-fed (PF) and adlib fed control groups were added. After 5 weeks of ETOH and 1, 3 and 7 days following 0 % ETOH administration, animals were sacrificed by decapitation and the brains were rapidly removed and frozen. TH mRNA expression in the LC was significantly elevated compared to ad-lib and PF only in animals exposed to ETOH for 5 weeks (pixels over LC in ad-lib=824.6±69.8, PF=964.5±48.8 and ETOH=1423.8±132.3). The increase in TH mRNA expression could be attributed to a significant increase in the number of cells in the LC expressing TH mRNA (number in ad-lib=110±10.7, PF=111±4.3 and ETOH=140±6.6). TH mRNA expression in the LC was not significantly different following ETOH withdrawal. NET mRNA expression in the LC was not significantly different in the ETOH animals or withdrawn animals compared to ad-lib or PF. These data indicate that TH mRNA expression is elevated following a chronic 5 week administration of ETOH, while the expression of the neurotransporter mRNA is unchanged. The increase in TH mRNA in the LC may be responsible for the enhanced rate of NE synthesis observed with chronic ETOH.
[Supported by Epilepsy Foundation of America, NAAA RO1 AA10567 and Department of Veterans Affair]

185.12

ENDOGENOUS CATECHOLAMINES REGULATE ETHANOL SENSITIVITY ON CEREBELLAR PURKINJE NEURONS. **Michael R. Palmer*, Ronald K. Freund, and Yun Wang. Dept. of Pharmacology, Univ. of Colorado Health Sci. Ctr., Denver, CO 80262 and National Defense Medical School, Taipei, Taiwan, R.O.C.**

We and others previously reported that ethanol (EtOH) will potentiate the β-adrenergic modulation of GABA-induced depressions of cerebellar Purkinje neurons, and we have published evidence that isoproterenol, a β-adrenergic agonist, potentiates the depressant effects of EtOH on these neurons. During that study, we also found that on 20% of Purkinje neurons in Sprague Dawley rats A) the depressant EtOH effects can be decreased by timolol, a β-adrenergic antagonist, and B) the EtOH potentiation of unmodulated GABA responses can be blocked by timolol, suggesting that endogenous catecholamine transmission might interact with these EtOH actions in that population of neurons. We tested that possibility in the present investigation. 1) We found that a catecholamine reuptake antagonist, DMI, potentiated the depressant effects of EtOH on those cells. 2) We also observed that naloxone withdrawal from chronic morphine, which activates catecholamine circuits, caused EtOH-induced depressions to become larger. 3) Finally, we found that stimulation of the catecholamine input to the cerebellum by stimulation of the locus coeruleus facilitated the inhibitory actions of EtOH on spontaneous firing. We concluded that endogenous catecholamine input to the cerebellum can regulate the responsiveness of cerebellar Purkinje neurons to the depressant effects of EtOH.

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185.13

ABERRANT MEMBRANE AND SYNAPTIC PROPERTIES OF INFERIOR COLLICULUS DORSAL CORTEX NEURONS DURING WITHDRAWAL IN ETHANOL DEPENDENT RATS. Y. Li* and C.L. Faingold. Department of Pharmacology, Southern Illinois Univ. Sch. Med., Springfield, IL 62794-1222.

Ethanol withdrawal (ETX) after continued administration results in neuronal hyperexcitability. Previous *in vivo* results have shown that the inferior colliculus (IC) is critically involved in the network for audiogenic seizure (AGS), which is one of the most severe ETX signs. The present study investigated firing patterns, membrane properties and synaptic responses in IC dorsal cortex (ICd) during ETX. Intracellular recordings *in vitro* were made from IC slices during ETX in rats that were made dependent by ethanol administration three times daily for 4 days (9-15 g/kg/day). The data from ETX were compared with data from normal rats. In normal ICd, spontaneous action potentials (APs) were never observed (0 of 31). However, tonic or phasic spontaneous APs were recorded in ETX (5 of 16). Bath applications of an AMPA receptor antagonist DNQX (20 μM) and an NMDA receptor antagonist, AP-5 (50 μM), blocked spontaneous APs. Synaptic stimulation of the IC commissure can normally induce two IPSPs with different time courses in ICd neurons. These IPSPs may be mediated by $GABA_A$ receptors, since bicuculline (20 μM), a $GABA_A$ antagonist, can block them. During ETX as the intensity of the stimulus was increased the EPSP occurred between the IPSPs became more dominant and often developed into an AP (3 of 3), which was not seen in normals (0 of 4). This EPSP could be blocked completely in the presence of AP-5 and DNQX. Synaptically-evoked paired pulse facilitation was seen in ICd neurons during ETX (3 of 3), whereas paired pulse inhibition was observed in most normal ICd neurons (6 of 8). Epileptiform activity can be induced by electrical stimulation of the IC commissure during ETX in ICd neurons (4 of 10), but could not be evoked in normal ICd neurons (0 of 15) unless the slice was first perfused with bicuculline or NMDA. Thus, ICd neurons during ETX show aberrant firing patterns, hyperexcitable membrane properties and abnormal synaptic responses, which are likely to contribute importantly to AGS susceptibility during ETX. (Supported by NIAAA AA08591)

185.14

PARTIAL CHARACTERIZATION OF Ca^{2+} CHANNELS WHICH MEDIATE MUSCIMOL-INDUCED INCREASE IN CYTOSOLIC $[Ca^{2+}]$ IN GT1-7 NEURONS: EFFECTS OF ETHANOL. M.A. Javors*, T.S. King, and X. Chang. Departments of Psychiatry, Pharmacology, and CS&B, University of Texas HSC, San Antonio, Texas 78284.

GT1-7 hypothalamic neurons, which synthesize and secrete GnRH (gonadotrophin releasing hormone), were immortalized from transgenic mice and have been shown to express functional $GABA_A$ receptors. Our previous experiments showed that stimulation of these receptors with muscimol produced an efflux of ^{36}chloride, an increase of cytosolic $[Ca^{2+}]$, and the release of GnRH. Ethanol enhanced $GABA_A$-mediated increase in cytosolic $[Ca^{2+}]$, but not ^{36}chloride efflux, suggesting that the ethanol effect did not occur at the $GABA_A$ receptor. This previous work supports the hypothesis that the $GABA_A$ γ subunit, which is not expressed in GT1-7 neurons, may be required for low dose ethanol effects. The purpose of this study was to further characterize the calcium channels that mediate muscimol-induced increase in cytosolic $[Ca^{2+}]$ (M-ΔCa) and to test the effects of ethanol. GT1-7 neurons were grown to confluence on plastic coverslips and cytosolic $[Ca^{2+}]$ was measured using the fura-2 method and a Deltascan fluorometer. Our results indicate that nimodipine, an L-type Ca^{2+} channel inhibitor, inhibited M-ΔCa by approximately 70% with an IC50 of 1 μM. Conotoxin and agatoxin had minimal effects on M-ΔCa. (+/-)BayK8644 enhanced M-ΔCa with an ED50 of 0.05 μM. This enhancement was inhibited by ethanol. (-)BayK8644, an activator of L-type Ca^{2+} channels, enhanced M-ΔCa and its effect was inhibited by ethanol. (+)BayK8644, an inhibitor of L-type Ca^{2+} channels, inhibited M-ΔCa. Pentobarbital enhanced M-ΔCa, probably through an effect at the $GABA_A$ receptor. These results support the notion that L-type Ca^{2+} channels mediate a significant percentage of muscimol-induced, $GABA_A$ mediated increase in cytosolic $[Ca^{2+}]$ in GT1-7 neurons and that ethanol may interact with these channels. (Supported by NIAAA grant number 10112.)

185.15

EFFECTS OF ETHANOL ON SPONTANEOUS SYNAPTIC CURRENTS IN CULTURED CORTICAL NEURONS W. Marszalec* and T. Narahashi Dept. of Mol. Pharmacol. & Biol. Chem., Northwestern Univ. Med. Sch., Chicago, IL 60611.

Neurons dissociated from embryonic rat frontal cortex generate spontaneous synaptic currents after a week in culture. With the membrane potential held near -40 mV these currents could be differentiated into outward excitatory and inward inhibitory postsynaptic currents (EPSCs and IPSCs) by replacing some of the chloride in the pipette solution with the impermeant gluconate anion. IPSCs were inhibited by the $GABA_A$ receptor antagonist bicuculline, whereas EPSCs were blocked by the glutamate antagonist CNQX. No spontaneous activity occurred in the presence of tetrodotoxin or 10 mM $MgCl_2$.

The bath application of 100 mM ethanol dramatically inhibited the occurrence of spontaneous EPSCs in all cells tested. Concomitant with this effect was a small enhancement of IPSC amplitude. IPSC frequency in most experiments was also reduced. The reversal of these effects upon washout of ethanol was sometimes accompanied by a rebound enhancement of EPSC amplitude. Ethanol, at 30 mM, produced similar effects in some, but not all cells. Ethanol at 30-100 mM decreased the currents evoked by 3 μM glutamate by approximately 25%, whereas currents evoked by 3 μM GABA were unaffected. The data suggests that the depression of spontaneous synaptic currents in these neurons by ethanol cannot be explained by an effect on postsynaptic glutamate or GABA receptor channels alone. Supported by NIH Grant AA07836.

185.16

MODULATION BY ADENOSINE (ADO) OF ETHANOL-INDUCED MOTOR IMPAIRMENT (EIMI) IN THE RAT MOTOR CORTEX: POSSIBLE ROLE OF CYCLIC AMP. V. S. Barwick and M. S. Dar*. Department of Pharmacology, School of Medicine, East Carolina University, Greenville, NC 27858.

Earlier work from our lab showed that, when given systemically, ADO antagonists lessen and uptake-blockers enhance EIMI. Subsequently, we have shown that ADO agonists and antagonists accentuate and attenuate, respectively, EIMI when microinfused into the ventricles, striatum and the cerebellum of rats. Since then, we have shown that, via the ADO A_1 receptor, ADO agonists enhance and antagonists diminish EIMI when microinfused into the motor cortex. Participation of the motor cortex in the mediation of EIMI was established by the benzodiazepine partial agonist, Ro15-4513. Microinfused bilaterally into the motor cortex, Ro15-4513 significantly attenuated EIMI in a dose related manner. The dispersion patterns of a microinfused [^{3}H]-labelled ADO agonist and antagonist, followed by systemic injection of ethanol (EtOH, 1.5 g/kg) or saline, were similar. The dispersion was confined to a circumscribed area of the motor cortex and was not affected by EtOH or saline. It is well known that the A_1 receptor is coupled to the pertussis toxin (PT)-sensitive G_i/G_o-protein. Pretreatment of the motor cortex with PT significantly attenuated the enhancement of EIMI by ADO agonists. There was also an attenuation of EIMI which approached significance at 45 min post-EtOH ($p<0.07$). These data suggested a role for the PT-sensitive G_i/G_o protein in the modulation of EIMI by ADO. A significant decrease in motor cortical cAMP in response to EtOH as measured by RIA suggested the possible involvement of the AC/cAMP system and provided further insight into the signal transduction pathway in the modulatory role of ADO in EIMI. Further, behavioral data has indicated that forskolin, which stimulates AC, significantly lessens EIMI when microinfused into the motor cortex. We propose that the elements involved in the mediation of EIMI, and perhaps modulation by ADO, in the rat motor cortex include the G_i/G_o protein and the AC/cAMP system. Dept. of Pharmacology, ECU

185.17

FYN TYROSINE KINASE AND ETHANOL SENSITIVITY: RELATION TO NMDA RECEPTOR FUNCTION.

T. MIYAKAWA[1*], H. KITAZAWA[2], N. KAWAI[2], T. YAGI[3], M. YASUDA[3] and H. NIKI[1] [1]Dept. of Psychol., Fac. of Lett., University of Tokyo, Tokyo, 113, Japan, [2]Dept. of Physiol., Jichi Medical Sch., Tochigi, 329-04, Japan, [3]Dept. of Neurobiol. Behav. Genet., National Inst. of Physiol. Sci., Okazaki, 444, Japan.

The aim of the present study was to examine possible roles of Fyn in ethanol sensitivity. We produced Fyn-deficient mice by inserting the *lac* Z into the *fyn* gene. Fyn-deficient mice were found to be more sensitive to ethanol when we measured the duration of loss of righting reflex. Western blot analysis revealed enhanced tyrosine phosphorylation of the NMDA receptor in the hippocampus after administration of ethanol while such up-regulation was not seen in the Fyn-deficient mice. Extracellular recording of field EPSPs in the CA1 field of the hippocampal slices showed that acute tolerance to ethanol inhibition of NMDA-mediated EPSPs developed in the heterozygous Fyn-deficient mice while it did not in the homozygous Fyn-deficient mice. These results suggest a role of Fyn in the recovery process from the inhibition of the NMDA receptor function after application of ethanol. **Supported by Grant-in-Aid for Scientific Research 07451018.**

185.18

THE EFFECTS OF ETHANOL ON THE EXPRESSION OF BRAIN METALLOTHIONEIN AND CYTOCHROME P4502E1. G. Helal, M.L. Heidrick, F.M. Hamada, M.P. Leuschen, J. Hofert, J.F. Rodriguez-Sierra, P.L. Iversen and M. Ebadi. Depts. of Pharmacology, Biochemistry and Molecular Biology, and Cell Biology and Anatomy, Univ. of Neb. Coll. of Med., Omaha, NE 68198-6260.

Microsomal ethanol-oxidizing system (MEOS), a nonalcohol dehydrogenase (ADH) pathway of ethanol oxidation, plays a significant role in ethanol metabolism, and its activity increases after chronic consumption of ethanol. This system is dependent on cytochrome P450, and the ethanol-inducible form has been designated as cytochrome P4502E1 (CYP2E1), which exhibits no difference in its gene frequency in healthy subjects when compared to alcoholics. Ethanol induces single DNA breaks in brain cells, inhibits neurite extension by increasing N-myc and C-myc protein expression, and increases oxygen derived free radical generation, through reaction of CYP2E1. In view of the fact that: 1) zinc is a cofactor for alcohol dehydrogenase; 2) ethanol causes a zinc deficiency state; and, because 3) zinc is able to prevent the deleterious effects of ethanol, the association among metallothionein (MT), a low molecular weight zinc binding protein capable of averting oxidative stress, and CYP2E1 was studied in the liver and various brain regions of ethanol treated rats. Acute administration of ethanol dramatically induced the synthesis of MT in the liver but not in the brain. On the other hand, the chronic consumption of ethanol modestly and nonuniformly enhanced both the levels of MT and CYP2E1 in various regions of the brain. However, no direct or causal association among the effects of ethanol on the levels of MT and CYP2E1 were observed. (Supported in part by a grant from USPHS NS34566).

185.19

CONDITIONED TOLERANCE TO ETHANOL-INDUCED C-FOS EXPRESSION IN THE RAT BRAIN. M. F. Roitman, T. E. Thiele, G. E. Schafe, D. S. Ramsay*, and I. L. Bernstein. Dept. of Psychology, U. of Washington, Seattle, WA 98195.

Learned tolerance refers to the observation that environmental cues that have been repeatedly associated with delivery of a drug, such as ethanol, can, through conditioning, come to trigger responses which contribute significantly to the development of drug tolerance. Effects of ethanol which show clear development of tolerance are hypothermia and the induction of cFos expression in a number of brain regions including the paraventricular nucleus of the hypothalamus (PVN) and locus coeruleus (LC). To assess the contribution of conditioning to the development of tolerance to ethanol-induced cFos expression rats were tested to determine the extent to which tolerance development relies on exposure to an ethanol-paired-environment. Over a series of trials, rats received 12 ethanol injections (2.0g/kg) in the presence of one set of environmental cues and 12 saline injections in the presence of a different set of cues. Other rats received saline injections in both environments. On the test day, alcohol pretreated animals received ethanol injection either in the ethanol environment (Same) or in the saline environment (Different). Saline pretreated animals were given their first ethanol injection at that time. Relative to those receiving ethanol for the first time, cFos expression in PVN and LC was substantially lower. Although the Same and Different groups had received the same number, dose and spacing of prior ethanol treatments, tolerance was significantly greater in the Same group, tested in the ethanol-paired environment; much less tolerance was seen in the Different group, tested in the saline-paired environment. A similar pattern of results was seen for ethanol-induced hypothermia. These results support a significant role that conditioning plays in the development of tolerance to ethanol-induced cFos expression in PVN and LC.

This research was supported by AA07455 and DC-00248.

185.20

EFFECTS OF ACUTE ADMINISTRATION OF ETHANOL ON DOPAMINE, SEROTONIN, GLUTAMATE AND GABA IN THE MESOCORTICOLIMBIC PATHWAY. Q. S. Yan*, M. E. A. Reith, M. Y. Li, P. C. Jobe and J. W. Dailey. Department of Biomedical and Therapeutic Sciences, University of Illinois College of Medicine, Peoria, IL. 61656

The time course of the effects of acute administration of ethanol on the extracellular levels of dopamine (DA), serotonin (5-HT), glutamate (GLU), and r-amino butyric acid (GABA) within the nucleus accumbens (NACC) and prefrontal cortex (PFC) of freely-moving Sprague-Dawley rats was analyzed using microdialysis in conjunction with HPLC and electrochemical detection. Local infusion of ethanol (0.5, 1, 2%, v/v) through a microdialysis probe produced a significant reduction of GLU and increment of DA and 5-HT in dialysate output from the NACC, both in a dose-dependent fashion. No significant change of GABA in dialysate output from the NACC was observed. Perfusion with 0.5% of ethanol caused larger increases in extracellular DA but smaller increases in extracellular 5-HT in the NACC than it did in the PFC, suggesting that focal administration of ethanol preferentially affects DA transmission in the NACC and 5-HT transmission in the PFC, respectively. In a separate group of rats with two-probe implantation, systemic administration of ethanol (0.5 g/kg, i.p.) resulted in larger increases in extracellular DA in the NACC than in the ventral tegmental area (VTA) in the same animal suggesting the involvement of additional brain circuitries in the effects of systemic as opposed to focal ethanol. Local application of sulpiride (20 μM) into the NACC or VTA did not affect significantly the effects on DA transmission produced by systemic administration of ethanol in either the NACC or the VTA, consonant with our previous findings of a lack of dependence of the focal ethanol effects on impulse activity.

DRUGS OF ABUSE: ETHANOL, BARBITURATES, AND BENZODIAZEPINES I

186.1

ETHANOL DECREASES SYNAPTIC TRANSMISSION IN THE RAT SPINAL CORD. G. Cheng*, B.-X. Gao, H. Xie, and L. Ziskind-Conhaim. Dept. of Physiology and Ctr. for Neuroscience, University of Wisconsin, Madison, WI 53706.

Effects of acute ethanol exposure on synaptic transmission, repetitive firing, and voltage-gated Ca^{2+} channels were studied in spinal cords of 1-3-day postnatal rats. Dorsal root-evoked synaptic potentials were recorded intracellularly in motoneurons in the hemisected spinal cord. Bath-applied ethanol (70 mM) significantly reduced the amplitude of dorsal root-evoked synaptic potentials. The effect was reversible, and the amplitude of evoked synaptic potentials returned to its control level within minutes after ethanol washout. To study the action of ethanol on neuronal excitability, whole-cell current clamp recording were carried out in motoneurons in thick spinal cord slices. In control postnatal motoneurons, long-lasting intracellular current injection generated a sustained train of action potentials, and exposure to ethanol effectively reduced the number of action potentials. To test the possibility that ethanol-induced decrease in both synaptic transmission and motoneuron excitability resulted from a reduction in voltage-gated Ca^{2+} currents, the effect of ethanol on high-threshold Ca^{2+} channels was examined. Whole-cell voltage clamp recordings illustrated that the amplitude of inward Ca^{2+} currents, generated by a voltage step of 50 mV (from -50 mV to 0 mV), was significantly reduced by ethanol. These findings implied that ethanol-induced decrease in synaptic transmission and neuronal excitability might be related to its effect on voltage-gated Ca^{2+} currents. Supported by NIH grant NS23808 to L.Z-C.

186.2

NMDA RECEPTOR SUBUNIT COMPOSITION AND ETOH SENSITIVITY IN PRIMARY NEURONAL CULTURES. R. L. Popp*, R. L. Lickteig, M. D. Browning, and D. M. Lovinger. Dept. Mol.. Physiol. Biophsy., Vanderbilt Univ. Sch. Med., Nashville, TN, 37232.

We are studying how ethanol (EtOH) sensitivity relates to N-methyl-D-aspartate receptor (NMDAr) subunit composition using different primary neuronal cultures. We have characterized NMDAr responses from striatal (S) and cerebellar granule cell (CGC) cultures using ifenprodil, a selective antagonist to NR2B-containing complexes and potentiation induced by spermine, which results when NR2B is complexed with the NR1-1a splice variants. Whole-cell patch-clamp electrophysiological recordings were performed and the effects of ifenprodil, spermine or 10, 25, 50, 100 and 200 mM EtOH in the presence of 100 μM NMDA and 10 μM glycine were examined. Values presented are the means ± s.e.m. Western blots using antibodies directed against the C-terminus of the NR2A, NR2B or the N-terminus of the NR2C subunits were also performed. CGC were grown in high K^+ (25 mM KCL) for 3-5 wk. In CGC cultures, 7 out of 8 cells were insensitive to 10 μm ifenprodil (% inhibition =1.6 ± 4.4). The one cell showed 60% inhibition of NMDA-induced current. In all cells steady state (ss) current was inhibited by 100 μm spermine at -25 mV (% inhibition = 35.03 ± 2.9). Western blot analysis revealed the presence of NR2A but not NR2B in these cultures. Preliminary EtOH data suggested low EtOH sensitivity in that inhibition was less than 20% at 50 and 100 mM EtOH concentrations (8 out of 11 cells). In contrast, S cells exhibited a decrease in ifenprodil inhibition (58%, n=1) to a complete loss of inhibition (n=3) in 2-3 wk-old cultures (total n=8). This correlated well with the appearance of the NR2A subunit. Unlike CGC cultures, after 10 d in culture a population of S cells showed a potentiation (>10%) of ss current by 100 μm spermine at -25 mV (10 d: 2 of 5; 2 wk: 6 of 7; 3 wk: 3 of 4 cells). Preliminary EtOH data indicated an inhibition threshold between 10 and 25 mM and a mean inhibition for 100 mM of -31.88 ± 2.1. We will continue to examine the relationship between potentiation by spermine and EtOH sensitivity. Supported by NS30470, AA08986, AA09675 and AA03257.

186.3

THE EFFECTS OF PRENATAL ETHANOL ON BARREL FIELD CORTEX PLASTICITY ARE EXACERBATED BY POSTNATAL SENSORY DEPRIVATION. V. Rema* and F. F. Ebner. Institute for Developmental Neuroscience, John F. Kennedy Center, Vanderbilt University, Nashville, TN 37203.

Exposure of the fetal brain to ethanol throughout gestation results in long-term down regulation of NMDA receptor subunits in the adult rat brain (Rema and Ebner, NS abst, 1995). We have been testing the hypothesis that the long term effects of prenatal ethanol exposure and of sensory deprivation alone may have additive effects on cortical function impairment. Further, that these effects will be detectable by a test of cortical plasticity induced in the adult rat cortex by an non-invasive bias in sensory activity from the whiskers called "whisker-pairing" (WP) plasticity. WP is induced in SI cortex by trimming all but two whiskers on one side of the face; eg., D1 and D2 whiskers are left intact and the other whiskers are trimmed for 1 to 30 days at any age. In normal cortex when whiskers D1 and D2 are left intact, the neurons in the D2 barrel column develop a significant bias in their response to the paired D1 and the cut D3 whisker (D1 response up, D3 down) after <3 days (Armstrong-James, et al, J. Nsci., 1994). Sensory deprivation by itself, produced by trimming all of the whiskers from P-0 to P-30, delays the bias significantly past 3 days of WP in P-90 animals (Huang and Ebner, NS abst, 1995). Here we show that prenatal ethanol exposure alone delays the onset of bias for longer than 3 days of WP, although after 7 days of WP some animals do show significant changes. In contrast, none of the alcohol exposed, sensory deprived animals show any bias after whisker-pairing for more than 7 days. The results show that both the prenatal toxin and the deprivation alone produce long term modest, but significant, slowing in the rate of activity-dependent cortical synaptic modification. The important implication of the results is that the depressed postnatal activity levels in sensory systems significantly exacerbate the negative impact of prenatal alcohol on cortical synaptic plasticity mechanisms throughout life. (Supported in part by NS-13031 and HD-15052.)

186.4

TIMING AND DOSE EFFECTS OF FETAL ALCOHOL EXPOSURE ON CORPUS CALLOSUM DEVELOPMENT IN RATS. A.J. Elberger*, S.E. Maier and J.R. West. Depts. of Anatomy and Neurobiology, The University of Tennessee, Memphis TN 38163 and Texas A&M University, College Station, TX 77843.

Humans with fetal alcohol exposure show abnormalities of the corpus callosum (CC), and we have previously found that rats exposed to alcohol *in utero* also show CC abnormalities. The goal of this study is to evaluate independently the effects of different doses and different time periods of fetal alcohol exposure on the rat CC.

Pregnant rats were given alcohol (EtOH), or an isocaloric equivalent of maltose dextrin (PF/INT) as a control, by intragastric intubation. A third group of pregnant rats served as normal controls (CHOW). Different doses of EtOH during gestation day (G) 1-20 caused peak EtOH blood alcohol concentrations (BACs) ranging from 81-497 mg/dl. Different timing of EtOH during G1-10, G11-20 or postnatal day (P) 4-7 in a narrow dose range caused peak BACs of 192-325 mg/dl. All pups were transcardially perfused with 4% paraformaldehyde at P10. Brains were sagittally bisected and crystals of DiI were applied to the anteroposterior extent of the midsagittal CC bundle. Tissue was stored in the dark at 37° C during DiI diffusion.

Sections 100μm thick were examined with a confocal laser scanning microscope. CC connections were identical in CHOW and PF/INT controls. However, CC connections were differentially abnormal in each EtOH dose and timing group. CC abnormalities included reduction in number of somata, laminar displacement of somata, tight clustering of somata, overextension of apical and basilar dendrites to one side, and misorientation of apical dendrites. These results suggest that the timing of alcohol exposure may be a significant factor in abnormal human CC development. Supported by UT Medical Group Research Grants Program (AJE) and NIAAA10090 (JRW).

186.5

EFFECTS OF PRENATAL ETHANOL EXPOSURE ON THE DEVELOPMENT OF DENDRITIC SPINES IN CULTURE. T. Wu*, S. Antonios, M.J. Maguire, F.F. Ebner. Institute for Developmental Neuroscience, John F. Kennedy Center, Vanderbilt University, Nashville, TN 37203

Prenatal ethanol exposure has been shown by others to affect the development of the density and morphology dendritic spines in vivo. We have investigated whether prenatal ethanol interferes with dendritic spine formation in vitro through its direct influence on neurons, or indirectly through its influence on glial cells. Pregnant rats were given a liquid diet containing 6.5% ethanol between day 1 and day 19 of gestation. At birth, the rat somatosensory cortex was removed to either harvest neurons which were then grown on normal glial cells, or to produce monolayers of ethanol-exposed glial cells on which normal neurons could be plated at a later time. Thus, three combinations of cells were examined in the present experiment: (1), normal neurons on normal glial cells (controls); (2), ethanol-exposed neurons on normal glial cells; (3), normal neurons on ethanol-exposed glial cells. After 28 days in vitro, neurons were injected with Lucifer yellow and dendritic spines of labeled neurons were imaged with a confocal microscope. The spine densities (#/100 µm dendrite) and spine lengths for mushroom, stubby, thin and irregular-shaped spines were analyzed. We found that dendritic spine densities on the ethanol-exposed neurons on normal glia showed significant reductions in thin and stubby types of spines, but an increase in irregular-shaped spines which resemble the appearance of small growth cones. Spine lengths were significantly longer except for mushroom spines. Spine densities on the neurons plated on ethanol-exposed glial cells were not significantly different from those of control neurons. However, spine lengths were significantly shorter than those of control neurons, except for stubby spines. These results suggest that prenatal ethanol exposure interferes with spine formation through its direct influence on neurons. Ethanol-exposed glial cells may result in impairing mechanisms that adjust the efficacy of spines through modifying spine length. (supported in part by NS-13031 and HD-15052).

186.6

"BINGE" ETHANOL-INDUCED BRAIN DAMAGE IN RATS: PROTECTION BY FUROSEMIDE. **E.J. Neafsey*, J.Y. Zou, and M.A. Collins. Neuroscience Program and Depts. of Cell Biology, Neurobiology & Anatomy; and Molecular and Cellular Biochemistry, Loyola Univ. Med. Cntr., Maywood, IL 60153.**

Modifying our severe (3xdaily) binge ethanol model (Collins et al., 1996), ethanol administered *once* daily to male adult rats for 6–10 days produces obvious neuronal degeneration (de Olmos cupric silver stain) in the entorhinal cortex (EC), dentate gyrus (DG), and olfactory bulb (OB). The effective dose is about 5g/kg in 0.9% saline, yielding a blood ethanol level (BEL) of approx. 250mg/dl at 2h. Since cytotoxic edema has been linked to other forms of brain damage, we examined whether it was involved by administering furosemide, a loop diuretic, (10-15mg/kg, IP) at 1h and 8h after each of the 10 daily ethanol doses (EF rats); a second group (E) received ethanol alone. Mean daily blood ethanol levels were similar in both groups. Brain damage was measured by cell counts (±SEM) of degenerating, argyrophilic neurons in EC or DG on 6–10 horizontal brain sections from each animal at levels 6–8mm below bregma; OB damage was measured as the percentage of glomeruli containing degeneration.

Grp(n)	Mn BEL (mg/dl)	%EC degen.	Entorhinal Cx		Dentate Gyrus		OB % Degen.
			Max	Mean	Max	Mean	
E(9)	252±8	100	29.4±10	9.2±3	16.8±4	5.9±2	19.3±6
EF(12)	253±8	58	*4.3±2	*1.4±1	*5.3±2	*1.5±1	20.9±6

Furosemide significantly reduced ($p < .005$,Mann-Whitney U) the mean and maximum counts/region/section of degenerating neurons in both EC (-84%) and DG (-74%) and completely prevented degeneration in the EC in 5/12 (42%) of the rats. The results support brain cell swelling as an essential step in ethanol-induced cortical neurodegeneration. Disturbed vasopressin secretion could be involved. Interestingly, brain overhydration has been hypothesized to underlie brain damage in alcoholics (Lambie, *Med. Hypoth.*, 1985). (LUMC NSAI)

186.7

DELAY-DEPENDENT MEMORY DEFICITS IN FETAL ALCOHOL EXPOSED RATS: RELATIONSHIP TO D1 AND D2 DOPAMINE RECEPTOR BINDING IN THE BRAIN. J.A. Dowd, R.I. Handa, and A.H. Nagahara*. Department of Cell Biology, Neurobiology, and Anatomy. Loyola Medical Center, Maywood, IL 60153.

Previous results in our laboratory have shown that fetal alcohol exposed (FAE) rats show a reduced induction of c-fos, jun-b, and zif268 mRNA in the prefrontal cortex and caudate following testing in a t-maze alternation task. These rats demonstrated a delay-dependent memory deficit in this task, suggesting that changes in the prefrontal cortex or caudate of FAE rats may contribute to this memory deficit. Since the dopaminergic system is prominent in these regions, we investigated the relationship between alternation performance and D1 and D2 receptor binding in these brain regions of FAE rats. Subjects were female offspring of Sprague-Dawley dams fed either a 35% ethanol derived-calories diet, pair-fed with sucrose, or control-fed with lab chow. Rats (104 days old) were food-deprived prior to training and trained in the T-maze for food reward. Rats were then tested at 0-s, 30-s, 60-s, and 180-s delays. On the day of sacrifice, rats were tested at the 60-s delay for 12 trials and were sacrificed 30 min after testing. Dopamine binding was examined by receptor autoradiography using [3H]-SCH23390 (D1) and [3H]-YM-09151-2 (D2). FAE rats showed a memory deficit at both the 60-s and 180-s delays, but not at the 0-s or 30-s delays. However, no differences in D1 and D2 receptor binding were observed in the prefrontal cortex, caudate, and other regions of the FAE rats compared to the control groups. These findings suggest that delay-dependent memory deficits observed in these FAE rats are not due to changes in D1 or D2 receptor binding in these brain regions. Supported by USPHS AA08696 (RH) and K21AA00192 (AHN).

186.8

Prenatal Exposure to Alcohol Decreases BDNF mRNA and Granule Cell Number in the Main Olfactory Bulb in Rats. S.E. Maier*, J.A. Miller, J.R. West & F. Sohrabji. Human Anatomy and Neurobiology, Texas A&M University, College Station, Texas, 77843.

Developing forebrain structures such as the neocortex, hippocampus and olfactory bulb are significantly affected by prenatal exposure to alcohol. Since many forebrain neurons are sensitive to the neurotrophin family of growth factors, we hypothesized that alcohol-induced developmental anomalies may results from decreased availability of neurotrophins. In the present study, we examined the expression of the brain derived neurotrophin factor (BDNF) mRNA in the olfactory bulb following prenatal exposure to alcohol. Timed pregnant rats were intubated daily with alcohol from embryonic day 1 (E1) to E20 (mean BAC ≈365 mg/dl). Pairfed controls received isocaloric maltose dextrin solution, chow controls were fed *ad libitum*. Olfactory bulbs from E21 fetuses were rapidly dissected and harvested for RNA. BDNF mRNA was analyzed by quantitative RT-PCR. Alcohol exposure significantly reduced total mRNA yields. Analysis of equivalent amounts of total RNA indicated that BDNF mRNA was significantly reduced compared to pairfed controls. To test whether the same prenatal alcohol paradigm would result in concomitant decreases in olfactory bulb neurons, brains from offspring at postnatal day 10 were processed for histological analyses. Serial sections through the olfactory bulbs were used to count granule and mitral cells using stereological methods. The prenatal alcohol treatment resulted in a significant decrease in the number of granule cells, but not mitral cells. These data suggest that a decrease in granule cell number may be related to alcohol-induced decreases in BDNF availability. Ongoing research will investigate the effect of alcohol-induced decreases in BDNF mRNA and granule cell number target size. *(Supported by NIAAA 10090 to JRW).*

186.9

Alcohol Exposure During the Brain Growth Spurt Period in Rats Altered the Responsiveness of Neurotransmitter Systems to Acute Cocaine Challenge. W.-I.A. Chen* & I.R. West, Human Anatomy and Medical Neurobiology, Texas A&M University HSC, College Station, Texas 77843

Alcohol exposure during the brain growth spurt (BGS) induces various detrimental effects on brain development. This study examined the influences of alcohol exposure during the BGS period on specific neurotransmitter levels following acute cocaine challenge. Two distinct brain regions, cerebellum (CB) and nucleus accumbens (NAc), were selected due to the CB's vulnerability to alcohol insults during BGS and NAc's sensitivity to acute cocaine challenge. Sprague-Dawley rat pups were reared artificially from postnatal day (PD) 4 to 10. Alcohol was administered at doses of either 3.3 or 4.5 g/kg from PD 4 to 9; appropriate controls also were included. On PD 10, animals were sacrificed 20 min after acute cocaine challenge (i.p.). The results were summarized as follows. (1) Alcohol exposure during the BGS period altered the basal content of dopamine (DA) in NAc of the 4.5 g/kg group, but not in the 3.3 g/kg group. (2) Acute cocaine administration significantly elevated the levels of DA, dihydroxyphenylacetic acid (DOPAC), serotonin (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) among control animals in NAc, but not in CB. (3) The treatment of 4.5 g/kg alcohol significantly retarded the elevation of DA, DOPAC, 5-HT and 5-HIAA, however, 3.3 g/kg alcohol treatment only inhibited the elevation of DA and DOPAC. Taken together, these data suggest that alcohol exposure during the BGS period significantly altered the responsiveness of various neurotransmitter systems, and these alterations appeared to be brain region and neurotransmitter system specific. *(Funded in part by NIAAA grant AA05523)*

186.10

SURVIVAL AND DEVELOPMENT OF ETHANOL-TREATED RAT CORTICAL NEURONS GROWN IN TISSUE CULTURE. L.M. Mudd*, J. Katz and T. F. Lopez. School of Natural and Health Sciences, Barry University, Miami Shores, FL 33161.

Prenatal exposure to ethanol leads to a variety of effects known collectively as fetal alcohol syndrome. A number of toxic effects occur in the central nervous system, involving the decrease in survival or development of multiple subpopulations of neurons. We undertook a study to determine which aspects of neurite development might be affected by ethanol at the same concentration as effects on neuronal survival. Cortical neurons from embryonic day 16 rats were grown in defined medium with a glial plane at a distance of 1mm from the neurons. More than 96% of the cells in the neuronal layer were immunostained for neurofibrillary protein, while more than 97% of the cells in the glial plane were immunostained with an antibody to glial fibrillary acidic protein. Ethanol was administered on day in vitro 1 (DIV 1) and every 48 h, thereafter. Ethanol concentrations diminshed rapidly, being less than one-tenth of the initial concentration at the 100mM dose after 24 h. In control cultures 70±6% (mean±SEM) of neurons were alive at DIV 4 while 53±8.1 and 35±7.4% were present at concentrations of 10 and 100mM ethanol, respectively. While treatment with 10mM or 100mM ethanol caused no significant effect on the numbers of primary neurites after 6 DIV, there were significant decreases in the numbers of secondary neurites and the ratio of secondary to primary neurites at both ethanol concentrations and there were significant decreases in the length of the longest neurite and the total neurite length in the 100mM ethanol group.

(L.Mudd and T.Lopez are supported by NIH-NIGMS MBRS Grant, GM 45455, Barry University)

186.11

THE EFFECTS OF PRENATAL ETHANOL TREATMENT ON REPRODUCTIVE BEHAVIOR AND NEURONAL EXPRESSION OF C-FOS. R.E. Leipheimer*and R. Vivo. Dept. Biological Sciences, Youngstown State University, Youngstown, OH 44555

These experiments were designed to examine the effects of ETOH exposure in utero on the reproductive behavior and neuronal expression of c-fos in adult male rats. Pregnant rats were divided into three groups and given diets of either 35% ETOH, pair-fed liquid control diets, or solid food. Offspring from the ETOH-treated females had higher mortality rates and shorter body lengths at birth. When mature, the offspring were tested for sexual behavior and for neuronal expression of c-fos in separate experiments. 1. Naive males from each treatment group were tested with stimulus females for sexual behavior in three separate test sessions. Results demonstrated no significant differences between groups for mount latency, intromission latency or ejaculation latency. Thus, it appears that ETOH treatment had no significant effects on the sexual learning of the offspring. 2. Males were exposed to soiled bedding gathered from either estrous or ovariectomized females prior to immunocytochemical localization of fos. An increase in fos was found in the nucleus accumbens, diagonal band of Broca and corpus striatum in ETOH-treated rats suggesting that prenatal exposure to ETOH increases the expression of fos in response to stimulation.

Supported by NICHHD grant HD26903 to REL

186.12

THE EFFECTS OF BUTHIONINE SULFOXIMINE ON THE OUTCOME OF THE IN UTERO ADMINISTRATION OF ALCOHOL ON FETAL DEVELOPMENT. E. Reyes*, G. Marquez, J. C de Baca, and S. Ott. Dept. of Pharmacology, UNM, Sch. of Med., Albuquerque, NM 87131

Fetal Alcohol Syndrome (FAS) is characterized by pre and postnatal growth retardation, mental retardation, behavioral deficits and facial deformities. In spite of numerous animal studies, the biochemical mechanism(s) by which alcohol produces teratogenic effects on the developing fetus are not well understood. Several studies have shown that administration of alcohol to adult rats produces a decrease in hepatic levels of glutathione (GSH). The *in utero* administration of alcohol has also been shown to produce a decrease in GSH levels as well as prenatal growth retardation and intrauterine death. In an effort to determine if GSH may have a vital role in protecting the fetus against the teratogenic effects of alcohol, buthionine sulfoximine (BSO) was utilized to deplete GSH levels in the mother and fetus. Timed pregnant Sprague-Dawley rats were placed on a liquid BioServ diet containing either 0%, 11%, 23%, 29%, 31%, 33%, or 35% Ethanol Derived Calories (EDC), with or without BSO (888 mg/Kg/24h), starting on day one of pregnancy. The mothers were maintained on the diet until gestation day 21 when they were anesthetized with sodium pentobarbital and the pups delivered by Cesarean Section. The offspring were counted, weighed , sacrificed and the brain and liver weighed. The effects of BSO on the alcohol dose response curves (body weights, brain weights and litter number) were then determined in order to ascertain if a depletion in GSH potentiated the effects of alcohol. The *in utero* administration of BSO, aside from the depletion of GSH in liver and brain in the developing fetus, produced a shift to the left in the alcohol dose response curve. Supported by NIGMS/MBRS 08139 & NIAAA 08072

186.13

MOTOR SKILL TRAINING ALTERS RAT CEREBELLAR MORPHOLOGY FOLLOWING POSTNATAL ALCOHOL EXPOSURE. A.Klintsova*, J.. Matthews, C.Goodlett[1], R.Napper[2] and W.Greenough. Depts. of Psych. and Cell & Struct. Biol., Neurosci. Prog. and Beckman Inst. Univ. of IL, Urbana IL 61801, [1]Dept. of Psych., IUPUI, Indianapolis, IN 46202 and [2]Univ. Otago, Dunedin, New Zealand.

Developmental exposure to alcohol can produce permanent behavioral deficits and morphological damage in the CNS. The cerebellum is particularly sensitive to the effects of alcohol during the period of the "brain growth spurt" (West, *Alcohol Drug Res.*, 1987). The permanent loss of Purkinje cells (PC) and granule cells was shown to correlate with performance deficits on motor tasks (e.g. Goodlett et al., *Neurotox. Teratol.*, 1991). This study examined whether motor skill training, that has been demonstrated to increase synapse number, vasculature and glial cell processes in the cerebellar cortex, can compensate for the effect of alcohol exposure. Rats were assigned to one of the following treatments on days P4-P9: alcohol exposure (AE) of 4.5g/kg/day (using artificial rearing); an artificially reared control (GC); or suckle control (SC). At the age of 6 months, half of the rats from each group underwent the 10-day motor training; the other half stayed in individual cages. Although the three groups did not differ in the time to complete the obstacle course at the end of the training period, the acquisition time for the task was significantly higher in GC and AE animals on days 2, 5 and 6 ($p<0.05$) in female rats and on days 1, 2 and 3 ($p<0.05$) in males. The number of PC in the paramedian lobule (PML) and the number of parallel fiber synapses in the molecular layer of the PML were estimated using the physical disector. There was a significant loss of PC in the AE female ($F_{2,24}=4.19$, $p<0.03$) and male ($F_{2,22}=5.37$, $p<0.01$) rats. The number of synapses per PC in the PML was significantly higher in the AE animals trained on the motor skill tasks than in non-trained AE, GC and SC rats.

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186.14

INCREASED p53 AND TUNEL STAINING IN ETHANOL INDUCED APOPTOTIC CELL DEATH IN SCHWANN CELLS. J.M. Collier and M.I.Johnson*. Dept. of Pediatr., Steele Mem. Childr. Res. Cntr., Univ. of Arizona, Tucson, AZ 85724-5073.

Fetal alcohol exposure may result in CNS dysfunction and microcephaly, possibly with reduced numbers of neurons and glia known to be secondary to increased cell death in the CNS of developing mice. Studies in our laboratory have shown that ethanol increases axonal elongation but decreases the total number and percent dividing rat Schwann cells with concentrations as low as 2-3 mM and exposure of less than 24 hours. Studies using SEM and TEM show that dying cells have multiple cytoplasmic vacuoles and pseudopodia with chromatin condensation, which are characteristic of cell death via apoptosis. In these experiments, we use the TUNEL method of detecting nicked DNA as well as MAb staining to apoptosis-associated proteins. Rat sympathetic ganglia explants were cultured for 5 days in vitro and treated for 22-24 hours with ethanol prior to fixation, followed by TUNEL and p53 staining. The results show a dose-related increase of apoptotic cells with ethanol, especially with concentrations over 150 mg% and distances in excess of 1.0 mm from the explant edge. In one experiment the percent of total Schwann cells that were TUNEL positive at 1.5 mm was 12.4 ± 5.1 for controls, and 20.7 ± 5.6, 25.6 ± 11.4, 31.2 ± 11.1, 30.3 ± 7.0 and 32.0 ± 6.5 for 50, 100, 150, 300 and 750 mg% ethanol respectively (mean ± SEM; $p<.04$ for both 300 and 750 mg%). TUNEL positive cells stain positively for p53, with staining observed in both the cytoplasm and nucleus. In summary, dying Schwann cells in culture have the characteristics of apoptosis, the percentage of cells with TUNEL positive staining is dose related and TUNEL positive cells show p53 staining. (Supported by USPHS-NIH R01 AA08950 and Flinn Foundation 029100-171-92).

186.15

EFFECTS OF ETHANOL (EtOH) ON DIFFERENT LAYERS AND SUBTYPES OF NEURONS IN THE SOMATOSENSORY CORTEX. T.N. Felder, F.-C. Hsu, J. Zhai, R.C.-S. Lin, A.E.K. Kosobud, S.J. Wieland, M.C. Kennedy* and F.M. Sessler. Dept. of Anat. and Neurobiol., Med. Col. of Pennsylvania and Hahnemann Univ., Philadelphia, PA 19102, and Dept. of Psychol., Indiana Univ. IN 47405

In this study, we characterized the effects of EtOH on postsynaptic potentials (PSPs) and membrane properties of somatosensory neurons. The hypothesis tested was that EtOH exerts differential effects on cortical layers and cell types within somatosensory circuits. Two selection schemes were formed to test the impact of EtOH: 1) A comparison of EtOH effects on layer II-III vs. Layer V and 2) A comparison of EtOH effects on 4 characterized subtypes of neurons. Intracellular recording of neurons in slices of rat somatosensory cortex revealed a concentration dependent (EtOH 10-100mM) suppression of PSPs and a decrease in cell excitability which were both stronger in L5 cells. In most of the adapting regular spiking cells, EtOH 10mM decreased input resistance (IR) and increased rheobase (Rh) resulting in a reduction of cell excitability. Similar observations were made in non-adapting regular spiking cells with the exception of a small group which was facilitated by EtOH. In both, intrinsic bursting and doublet spiking neurons, EtOH also produced changes in excitability. Other parameters are under examination. These preliminary observations support the hypothesis of a differential impact of EtOH on layers and cell components of the somatosensory cortex. In addition, it is suggested that mechanisms involved in EtOH depression of cell excitability may be distinct from those disrupting synaptic transmission. (Supported by NIDA DA08405 and Allegheny-Singer Research Institute Award to FMS).

186.16

ETHANOL DECREASES DENDRITE NUMBER AND TOTAL LENGTH IN DEVELOPING RAT HIPPOCAMPAL PYRAMIDAL NEURONS *IN VITRO*. P.A. Clamp[1] and T.L. Fletcher[1,2]*. Depts. of Pharmacology & Neuroscience[1], and Psychiatry[2], Albany Medical College, Albany, NY 12208

Some of the neuropathological effects of prenatal alcohol exposure may result from disruption of normal neuronal development. Rat hippocampal pyramidal neurons in low density culture (Goslin and Banker, 1991), which develop axons and dendrites in a stereotypical sequence of events, can be used to assess the effects of ethanol on the establishment of neuronal polarity. We recently reported that ethanol accelerates the rate at which hippocampal neurons develop axons. The current study focuses on the effects of ethanol on dendritic outgrowth. Hippocampi from E18 Sprague-Dawley rats were dissociated and plated onto poly-L-lysine coated glass coverslips in serum-containing medium. After 3 hours, coverslips were transferred into dishes containing a monolayer of astroglial cells in defined medium with or without the addition of ethanol (300mg/dL or 800mg/dL). Cultures were maintained in modular incubator chambers saturated with water or ethanol (300mg/dL or 800 mg/dL) to prevent evaporation of ethanol from the medium. Neurons were fixed after 7 days *in vitro*, and somata and dendrites were stained by immunofluorescent cytochemistry using an antibody against MAP2. Stained cells were randomly selected and photographed. Dendrite length was measured using a digitizing pad and Jandel Sigmascan software. Ethanol treatment decreased both the number of dendrites per cell and total dendrite length per cell in a dose-dependent fashion ($p<.001$). These findings suggest that ethanol may disrupt the mechanisms by which neurons establish distinct axonal and dendritic processes. Such altered molecular compartmentation may contribute to the neuropathologies observed in Fetal Alcohol Syndrome. Supported by NIH R03AA09624

186.17

CHANGES IN BRAIN CYTOSKELETAL PROTEINS FOLLOWING CHRONIC ETHANOL TREATMENT E. Fifkova*, H. Eason, A. McReynolds, J. Pooh. Dept. of Psychology, Univ. of Colorado, Boulder, CO 80309.

Ethanol-sensitive LS/IBG mice were treated with ethanol for 15 days and compared to control mice. Triton solubilized brain homogenates were fractionated under microtubule-stabilizing conditions (Black et al., Brain Res., 295:255, 1984), which yielded polymerized cytoskeletal components in the pellet (P1). After resuspension of P1 in a Ca buffer at 4 C, subsequent centrifugation yielded a Ca/cold-insoluble cytoskeletal component in the pellet (P2). The amounts of α-tubulin and actin were assessed with quantitative immunoblotting (Black et al., J. Neurosci, 9:358, 1989). Fifteen days of ethanol treatment resulted in a significant increase of α-tubulin in P2 without any changes in the actin pool. Comparison of these results with those of 4 months on ethanol show that the initial increase of α-tubulin in P2 is followed by a decrease in P1. Such a bifasic response suggests activation of compensatory mechanisms to the initial insult which breaks down after a prolonged exposure to the noxious stimulus. No bifasic response was ob-served in actin which was reduced in P1 after 4 months of ethanol. Changes in both α-tubulin and actin could be caused by acetaldehyde which may be produced in the brain by at least two alcohol metabolizing enzymes: the alcohol-induced cytochrome P450 II E1 (Anandatheerthavarada et al., Brain Res., 601:279, 1993) and peroxide-dependent catalase (Gill et al., Alcoholism, 16:910, 1992). Acetaldehyde by forming stable adducts with both α-tubulin (Tuma et al., Ann. NY Acad. Sci. 625:786, 1991) and G-actin (Xu et al., Alcohol & Alcoholism 24:281, 1989) will remove these proteins from the functional pool. The loss of α-tubulin and G-actin results in a loss of microtubules and in a rigid actin network, respectively. Loss of microtubules will impair transport mechanisms of the neuron and a rigid actin network will reduce plastic properties of synaptic contacts.

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186.18

EFFECT OF ETHANOL EXPOSURE DURING GESTATION ON SPATIAL MEMORY IN ADULT/JUVENILE RATS. P.J. Best, D.B. Matthews, P.E. Simson*. Miami Univ., Cen. for Neuro. Res. & Dept. of Psy., Oxford OH, 45056.

Exposure to ethanol during gestation causes damage to several brain sites, including the hippocampus. Damage to the hippocampus includes decreased number of CA1 pyramidal neurons (Barnes & Walker, 1981), aberrant mossy fiber projections from the dentate gyrus to the CA3 region (West, Hodges & Black, 1981) and decreases in dendritic spines and branches (Ferrer, Galofre, Lopez-Tejero & Llobera, 1988). However, many of these changes, such as decreases in dendritic spines and branches, may not be permanent (Ferrer *et al.*, 1988).

Exposure to ethanol during gestation also impairs cognitive processing that is dependent on the hippocampus. For example, both juvenile and adult rats prenatally exposed to ethanol show impaired learning on spatial tasks (Blanchard, Riley & Hannigan, 1987; Gianoulakis, 1990).

In the present study, prenatal exposure to ethanol produced deficits in adult rats' use of spatial memory. However, these deficits were only observed after a long (3 day), but not a short (1 day) time delay between training and testing. Additional studies are underway to determine if similar results are obtained in juvenile rats prenatally exposed to ethanol.

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186.19

SECOND GENERATION EFFECTS OF MATERNAL ALCOHOL CONSUMPTION DURING PREGNANCY IN RATS. J. Homewood, M.K.-P. Lam, and E.J. Mazurski* School of Behavioural Sciences, Macquarie University, Sydney, NSW, Australia, 2109.

Twenty four pregnant Hooded Wistars (FO) consumed either a nutritionally complete liquid diet containing 5% (v/v) ethanol or sucrose, or standard rat chow. The male and female offspring (F1) were subsequently bred with drug-free partners. The next generation (F2, total n=122), was assessed for neuromotor development and spatial learning ability. Weight and latency to right following placement on the dorsal surface were recorded on postnatal days 1,5, and 7. Lower weights were recorded, particularly in males, whose dams were exposed to alcohol in utero (maternal line). Latency to right differences were seen on day 5. Pups descended from the alcohol-consuming group through the maternal line were the slowest to right. Rats did not differ on activity levels at 30 days of age. The spatial task measured alternation in a water-filled T-maze with a 1 min delay between trials. The number of errors (incorrect arm choice) decreased across the four days in all groups. Fewer errors were seen in the rats derived from alcohol-treated animals. These data demonstrate cross generational effects of in utero exposure to alcohol, which appear to be mediated by line of descent and sex of subject.

Funded by Macquarie University Research Grant to JH.

186.20

RADIAL ARM MAZE PERFORMANCE IS ENHANCED IN ADULT RATS CHRONICALLY, BUT NOT ACUTELY EXPOSED TO ETHANOL. E.S. Steigerwald, and M.W. Miller*. Departments of Psychiatry and Pharmacology, University of Iowa College of Medicine, Iowa City IA 52242 and Research Service, Veterans Affairs Medical Center, Iowa City IA 52246.

The effect of ethanol exposure on learning and memory was assessed in adult rats. Adult male rats were required to learn versions of a radial arm maze task that depended upon extramaze cues (visual and somatosensory spatial) or intramaze cues (various odors). Rats were fed *ad libitum* on a liquid diet containing ethanol, pair-fed an isocaloric/isonutritious diet, or fed chow and water *ad libitum*. These diets were provided for 0, 14, 20, or 28 wk prior to maze testing. Each rat was tested twice daily for 26 d while being maintained on the diet. The latency required to successfully navigate the maze and the number of reference and working memory errors were recorded. Rats maintained on the ethanol diet performed significantly better on the reference memory task in the odor condition of the maze task. This enhancement was transient (observed only after 14 or 20 wk of ethanol exposure). The performance of the ethanol-fed rats in the spatial task was similar to that for the two control groups. Thus, after an extended period (e.g., 28 wk), alcohol had no effect on learning and memory. On the other hand, under certain conditions, alcohol can transiently (14 or 20 wk) enhance memory.

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DRUGS OF ABUSE: AMPHETAMINES I

187.1

THE EFFECT OF 2,3-DIHYDROXY-6-NITRO-7-SULFAMOYL-BENZO(F)QUINOXALINE ON METHAMPHETAMINE- AND COCAINE-INDUCED BEHAVIORAL SENSITIZATION, K. Akiyama*[1], H. Ujike[1], K. Sakai[2] T. Ishihara[1], Y. Shimizu[1] and S. Kuroda[1], Departments of Neuropsychiatry[1] and Neurology[2], Okayama University Medical School, Okayama 700, Japan.

The present study investigated the effect of pretreatment with 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo(f)quinoxaline (NBQX), a novel antagonist of α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor antagonist of quinoxalinedione derivative on methamphetamine- and cocaine-induced behavioral sensitization, respectively. 7 -week-old male Sprague Dawley rats were randomly assigned to four groups which received daily either NBQX 20 mg/kg + methamphetamine 3 mg/kg, NBQX 40 mg/kg + methamphetamine 3 mg/kg, methamphetamine 3 mg/kg, or saline during the 10-day pretreatment session. In the other experiment, methamphetamine was replaced by cocaine 15 mg/kg. NBQX (20, 40 mg/kg) inhibited both acute methamphetamine- and acute cocaine-indcued rearing and locomotion, but not activity/stereotypy score. Pretreatment with NBQX (20, 40 mg/kg) did not affect augmentation of activity/stereotypy scores by repeated administration of methamphetamin or cocaine for 10 days. There was no significant difference in the activity/stereotypy scores following a challenge injection with methamphetaine (2 mg/kg) or cocaine (15 mg/kg) alone among all treatment groups 7 days after the last dose of repeated pretreatment session, although rearing induced by this challenge was significantly inhibited in rats which had been pretreated with NBQX 40 mg/kg prior to each methamphetamine or cocaine injection. Blockade of AMPA receptors inhibits some behaviors induced by acute psychostimulants, but AMPA receptors are unlikely to be involved in formation of behavioral sensitization induced by repeated administration of methamphetamine and cocaine. Supported by the research grant (5A-7) for Nervous and Mental Disorders from the Japanese Ministry of Health and Welfare.

187.2

SENSITIZATION TO LOCOMOTOR EFFECTS OF AMPHETAMINE IS INFLUENCED BY THE TIME OF ADMINISTRATION O. Gaytan, A. Swann, and N. Dafny* Dept. of Neurobiology and the Dept. of Psychiatry, UT Medical School at Houston, P.O. Box 20708, Houston, TX 77225

Chronic administration of stimulants like amphetamine (Amp) have been reported to produce behavioral sensitization to its effects on locomotor an stereotypic activity. This phenomena may be related to the pathophysiology of recurrent psychiatric disorders. Disturbances in various physiologic circadian rhythms are found in many psychiatric disorders. This study was designed to investigate the effect of repeated treatment with a low dose of Amp on the circadian pattern of locomotor activity, and whether the development of sensitization differs if Amp is given at different times of the light/dark cycle. 96 male Sprague-Dawley rats were housed in activity monitoring system test cages after 7 days of acclimation to a 12:12 light/dark cycle (lights on at 07:00) and continuos recording for 16 days was begun as follows: Baseline (Day 1- 2), Saline Injection (Day 3), Amp Challenge Doses (Day 4) - either 0.3, 0.6, or 1.2 mg/kg s.c., followed by Maintenance dose of 0.6 mg/kg Amp for five days (Day 5-9), Withdrawal (Days 10-14), and rats were Re-Challenged on Day 15 with the same Amp doses as day 4. This regimen was run at 4 times (0800, 1400, 2000, 0200). In general, sensitization occurred at all the time points with the largest augmentation in effect occurring with the lower doses (0.3 and 0.6 mg/kg). The index of total distance (cm) was more affected than horizontal activity. There were slight differences between the time points with the 08:00 displaying the weakest sensitization. There were no significant changes in the levels of diurnal activity with repeated administration. In conclusion, the development of sensitization to the locomotor effect of Amp is not greatly affected by the time of drug administration.

187.3

ONTOGENY OF METHYLPHENIDATE-INDUCED SENSITIZATION AND PROTEIN KINASE A ACTIVITY. T.L. Meier, R. Collins, S. A. McDougall, J. B. Watson[1], M. S. Levine[1], C. A. Crawford[1]*. Department of Psychology, CSUSB, San Bernardino, CA 92407. [1]Department of Psychiatry, UCLA, Los Angeles, CA 90024.

Repeated exposure to the psychostimulant amphetamine, produces augmented behavioral responses (i.e., behavioral sensitization) accompanied by dopamine receptor mediated changes in signal transduction. However, it is unclear if repeated treatment with the widely used psychostimulant, methylphenidate (MPH) or Ritalin, produces sensitization. Moreover, it is not known to what extent development plays in these behavioral or neurochemical changes. To test this hypothesis, 16- and 90-day-old rats were given 5 daily injections of saline or MPH (5, 10, or 20 mg/kg, ip). After a 2 day washout period, behavior was recorded for 40 min., rats were sacrificed, and cAMP-dependent protein kinase A (PKA) activity was measured. Results showed that repeated MPH treatment (10 & 20 mg/kg) enhanced the sniffing of young and adult rats while the same doses of MPH depressed the locomotor activity and rearing of both age groups (relative to controls). Interestingly, acute treatment with MPH produced a dose-dependent increase in the PKA activity of adult rats, whereas repeated treatment with MPH produced a dose-dependent decline. PKA activity of young rats showed a very different response, in which neither repeated nor acute treatment with MPH (10 & 20 mg/kg) affected the low baseline levels of PKA activity. We concluded from these results that MPH will induce behavioral sensitization in both young and adult rats, but that the downstream regulation mediated via PKA signalling pathways may vary according to age.

187.4

THE EFFECTS OF CCK_A AND CCK_B RECEPTOR BLOCKADE ON THE ACQUISITION AND EXPRESSION OF BEHAVIORAL SENSITIZATION TO AMPHETAMINE. G.R. Wunderlich*[1], N.J. DeSousa[1], and F.J. Vaccarino[1,2]. [1]Dept. of Psych., Univ. of Toronto, Toronto, Canada, M5S 1A1; and [2]Clarke Inst. Psychiatry, Toronto, Canada, M5T 1R8.

Numerous studies implicate the mesolimbic dopamine (DA) system in both the acquisition and expression of behavioral sensitization to psychostimulants. The neuropeptide, cholecystokinin (CCK), is co-localized with DA in a significant subpopulation of mesolimbic DA neurons and modulates DA neurotransmission. These experiments investigated the possibility that endogenous CCK modulates the acquisition and/or expression of locomotor sensitization to amphetamine (AMPH). In exp. 1, rats were treated in their home cage with AMPH (0, 1.5 mg/kg i.p.) once daily for seven days (acquisition phase). Following a 10 day withdrawal period, animals were injected with AMPH (0, 0.75 mg/kg i.p.) and placed in a test environment and their locomotor activity was recorded (expression phase). Exp. 2 followed a similar protocol except that AMPH-treatment was preceded by i.p. injection of either a CCK_A antagonist (devazepide; 0, 1, 10 and 100 μg/kg i.p.) or a CCK_B antagonist (L-365,260; 0, 1, 10 and 100 μg/kg i.p.) during the 7 day acquisition phase. In exp. 3, rats were pretreated with either devazepide or L-365,260 (0, 1, 10 and 100 μg/kg i.p.) prior to AMPH challenge during the expression phase. Results from exp. 1 showed that prior exposure to AMPH resulted in a sensitized AMPH-induced locomotor response. Results from exp. 2 replicated these findings and further showed that treatment with devazepide and L-365, 260 attenuated the acquisition of this effect. Results from exp. 3 also replicated those in exp. 1, and showed that devazepide, but not L-365,260, attenuated the expression of the sensitized response to AMPH. These results suggest that endogenous CCK modulates both the acquisition and expression of behavioral sensitization to AMPH. (Funded by a Medical Research Council of Canada grant to FJV).

187.5

COMPONENTS OF BOTH THE ACUTE AND SENSITIZED BEHAVIORAL RESPONSE TO AMPHETAMINE ARE DIFFERENTIALLY BLOCKED BY HALOPERIDOL. L.H. Conti*, D.S. Segal and R. Kuczenski. Dept. of Psychiatry, Univ. California, San Diego, La Jolla, CA 92093

Following amphetamine (AMPH) administration, extracellular concentrations of dopamine (DA) decline from peak levels while behavioral stereotypy continues to be maintained. Thus, the initial extracellular DA peak may act as a "trigger" for both the induction and maintenance of stereotypy. To address this hypothesis, saline or haloperidol (HAL; 0.1 mg/kg, ip) was administered either prior to AMPH (4.0 mg/kg, sc), or after the induction of stereotypy. Expression of stereotypy was precluded by prior HAL treatment, and was interrupted by HAL administration during the stereotypy response. Instead of stereotypy, a large-magnitude locomotor response was displayed under both conditions. These results suggest that DA does not simply act as a "trigger" for the expression of stereotypy, and indicate that the response remains sensitive to DA receptor blockade even when extracellular DA levels are declining. The fact that a locomotor response was displayed in place of stereotypy indicates that not all acute effects of AMPH are equally sensitive to HAL. With repeated AMPH administration, sensitization of both the stereotypy and locomotor components of the response develops. To test the hypothesis that the development of sensitization of both responses is also differentially dependent on stimulation of HAL-sensitive DA receptors, responses to AMPH challenge were assessed in rats that had received either saline or HAL prior to each daily injection of AMPH. HAL/AMPH-treated rats did not display stereotypy during repeated treatment, and showed no evidence of sensitization of stereotypy during AMPH challenge in the absence of HAL. Instead, locomotion was exclusively displayed during the repeated treatment phase and, this response was sensitized. Thus, sensitization failed to develop only for the aspects of the AMPH response which were acutely sensitive to HAL. [Supported by NIDA]

187.6

cAMP IN THE NUCLEUS ACCUMBENS IS INVOLVED IN THE ENHANCED LOCOMOTOR RESPONSE TO AMPHETAMINE IN A PERTUSSIS TOXIN MODEL OF SENSITIZATION. S. Narayanan*, A. Dalia, J. Battisti, L.J. Wallace and N.J. Uretsky. Division of Pharmacology, College of Pharmacy, The Ohio State University, Columbus, OH 43210.

The administration of pertussis toxin (PTX) into the ventral tegmental area (VTA) mimics some of the salient features of sensitization seen after repeated administration of psychostimulant drugs. This model of sensitization is characterized by a markedly enhanced responsiveness to activation of dopaminergic D_1 agonists in the nucleus accumbens (N.Ac). The purpose of the present study was to determine whether altered responsiveness of second messenger systems regulated by D_1 receptors exists in this sensitized state. Ten to 14 days after treatment with PTX in the VTA, rats were challenged with amphetamine either in the presence or in the absence of SQ22536 (0.2 μg/side), an adenylate cyclase inhibitor, administered directly into the N.Ac. SQ22536 inhibited amphetamine induced activity in PTX-pretreated animals but not in controls. In addition, administration of 8-Br-cAMP (2 μg/side), a membrane permeable analog of cAMP, into the N.Ac. produced an enhanced locomotor response in PTX-treated but not control animals. These observations suggest that the administration of PTX into the VTA results in a sensitization of the adenylate cyclase-protein kinase system in the N.Ac. Amphetamine induction of c-fos, an event thought to be mediated by D_1 receptor stimulated increases in protein kinase A activity, was also augmented in PTX-treated animals. Taken together, these results suggest that the injection of PTX into the VTA results in postsynaptic changes in the N.Ac., involving the D_1 receptor and cAMP. (NIH grants DA 06776 and DA 7722)

187.7

NORMAL PSYCHOSTIMULANT SENSITIZATION IN LTP-IMPAIRED *FYN* MUTANT MICE. Bryan K. Tolliver, Seth G.N. Grant, and S. Paul Berger* Department of Psychiatry, Univ. of Calif. San Francisco and SFVAMC, San Francisco, California and Center for Genome Research, Univ. of Edinburgh, Edinburgh UK

Mice with a null mutation in the nonreceptor tyrosine kinase gene *fyn* are known to be significantly impaired in their development of long-term potentiation (LTP) and some forms of spatial learning. Because the augmented behavioral response which develops with repeated stimulant treatment shares several common features with LTP, we hypothesized that *fyn* mutant mice may be deficient in the development of behavioral sensitization and conditioned place preference (CPP), a process known to require contextual learning. No differences in baseline locomotion or in the acute locomotor response to d-amphetamine (3 mg/kg i.p.) were observed between homozygous mutant, heterozygous, and wild-type (C57BL/6J-129/SV hybrid) mice. The locomotor response to amphetamine challenge was significantly enhanced relative to the initial response in all three groups of mice after 7 daily amphetamine injections, with the greatest sensitization found in homozygous mutants. *Fyn* mutants treated daily with amphetamine for one week were significantly more responsive to amphetamine challenge than mutants administered daily saline. When given a choice between two chambers previously paired with saline or amphetamine, mice of all three *fyn* genotypes preferred the drug-paired chamber. These results indicate that the *fyn* gene is not required in the development of stimulant sensitization or place preference conditioning, suggesting that not all of the mechanisms necessary for the induction of LTP are involved in behavioral sensitization. (Supported by USPHS Award DA-07376)

187.8

ELECTROLYTIC LESIONS OF THE NUCLEUS ACCUMBENS IN RATS ENHANCE LOCOMOTOR SENSITIZATION TO 0.4 MG/KG NICOTINE. J. E. Kelsey* and E. P. Johnson. Dept. Psychology, Bates College, Lewiston, ME 04240.

To examine the hypothesis that the locomotor activating and sensitizing effects of nicotine are mediated by activation of the mesolimbic DA pathway, we examined the effects of electrolytic lesions of the n. accumbens on locomotor responses to nicotine. In two experiments using different lesion placements, 60 male Long Evans rats were placed in 58- x 58- x 45-cm activity chambers immediately after an i.p. injection of isotonic saline or 0.4 mg/kg nicotine (base weight) for 7 1-hr sessions on alternate days. Distance travelled was measured by a video tracking system. The lesions tended to increase locomotion. Nicotine had no overall effect on the sham-operated animals, either on the first session or across sessions. Although nicotine also had no overall effect on the lesioned animals on the first session, repeated injections increased locomotion across sessions, producing locomotor sensitization. Moreover, a subsequent challenge dose of 0.1 mg/kg nicotine increased activity only in the previously sensitized lesioned rats. There was no evidence of conditioned sensitization. Subsequent amphetamine injections (1.0 mg/kg) increased activity equally in all four groups. Thus, in contrast to the prediction of the mesolimbic DA hypothesis, electrolytic lesions of the n. accumbens enhanced, rather than retarded, locomotor sensitization to nicotine. These data indicate that the n. accumbens contains neurons that act to inhibit locomotor sensitization produced by nicotine. (Support was provided by Bates College.)

187.9

METHAMPHETAMINE-INDUCED REVERSE TOLERANCE PHENOMENON IN STEREOTYPY AND IMPAIRED CLIFF AVOIDANCE REACTION IN THREE STRAINS OF RATS.

S. Yoshida*, Y. Numachi#, H. Matsuoka, Y. Fuse and M. Sato. Dept. Psychiatry, Tohoku Univ. School of Med., Sendai, 980-77 Japan. #Mol. Neurobiol. Br., NIH/NIDA/IRP, Baltimore, MD 21224, U.S.A.

To elaborate an index behavior reflecting the late-onset methamphetamine (MAP) psychosis, MAP-induced reverse tolerance (MAP-RT) in the behavioral stereotypy was compared with impairment of cliff avoidance reaction (CAR). Three strains of rats (Fischer 344: F, Lewis: L, and Sprague-Dawley: SD) were received daily MAP administrations (4 mg/kg/day, i.p.) for 30 days. MAP-RT developed within 10, 14 and 21 days in L, SD and F, respectively. On the other hand, incidence of impaired CAR was unchanged during daily MAP administrations for 4 weeks in L, while it was increased significantly after 14 and 21 days in F and SD, respectively. Thus, MAP-RT developed earliest in L without accompanying any late-onset impairment of CAR even after 4 weeks of MAP administration. Contrary, F showed slowest development of MAP-RT with earliest impairment of CAR. As 4mg/kg of MAP did not affect motor function including ataxia, impaired CAR might reflect a cognitive dysfunction. It is suggested that later increase in CAR impairment, rather than MAP-RT, appeared during chronic MAP administrations may be a new model for the late-onset MAP psychosis (ICD-10).Supported in part by grant-in-Aid for Scientific Research (C) (#07671045).

187.10

CORRELATION OF DOPAMINE TRANSPORTER AND D_2 RECEPTOR BINDING WITH BEHAVIORAL AMPHETAMINE SENSITIZATION

J.-C. Chen*, H.-J. Su, L.-Y. Huang and M.-C. Hsieh. Dept. Pharmacology, Chang-Gung College of Medicine & Technology, Tao-Yuan, Taiwan, R.O.C.

Repeated intermittent administration of amphetamine (AMPH) HCl (5 mg/kg/d, i.p. for 14 days) in male SD rats produce behavioral sensitization. The AMPH-induced stereotypy behaviors, such as sniffing, licking or head nodding appeared earlier and thus suppressed the subsequent affected locomotor activity. In order to understand the subcellular changes of DA neuron underlying behavioral AMPH sensitization, we examined the dopamine transporter (DAT) and DA D_2 receptor binding in both dorsal and ventral striatum at AMPH withdrawal periods. Using [^{3}H]BTCP to label DAT binding site, we found there was an approximate 2-fold increase in Kds in the ventral striatum at withdrawal day 7. The binding affinity to [^{3}H]BTCP in the dorsal striatum as well as Bmax values in both dorsal and ventral striata did not change up to withdrawal day 10. On the other hand, the binding of DA D_2 receptors, defined by [^{3}H]raclopride, exhibited a progressive decrease in Bmax values in both dorsal and ventral striata after withdrawal day 7. The binding affinity of DA D_2 receptors in both striata remained unchanged up to withdrawal day 10. We further tested the behavioral activity to D_2 receptor agonist, bromocriptine (5 mg/kg, i.p.) in both saline- or AMPH-withdrawal animals. The result showed that AMPH-sensitized animals had greater locomotion response than controls during first 2 hrs, whereas all the animals reached the plateau level at 5-hr recording period. It appears that behavioral D_2 up-regulation was in contrast to the D_2 receptor binding in the striatum during AMPH sensitization. It is possible that there is a differential regulation on pre- vs. post-synaptic D_2 receptor during the development of AMPH sensitization, the issues are currently under investigated. (NSC85-2331-B-182)

187.11

ALTERED ENERGY METABOLISM ASSOCIATED WITH BEHAVIORAL SENSITIZATION INDUCED BY QUINPIROLE.

D.V. Coscina*, P.J. Currie, J.W. Chambers and H. Szechtman. Dept. of Psychol., Wayne State Univ., Detroit, MI 48202; Sect. of Biopsychol., Clarke Inst. Psychiat., Toronto, ON M5T 1R8; and Dept. of Biomed. Sci., McMaster Univ., Hamilton, ON L8N 3Z5

Repeated exposure to drugs classed as psychostimulants can produce behavioral sensitization, defined as augmented motor responses to fixed doses of such compounds. For example, chronic treatment of rats with the D2/D3 dopamine agonist, quinpirole (QUIN), can increase motor activity up to several times normal levels. To determine if such enhanced locomotor output is associated with changes in nutrient utilization, one group of rats received 10 injections of QUIN (0.5 mg/kg every 2nd day) while a second received equal numbers of saline (both ns = 16). Immediately after each injection, rats were placed in one of eight closed plastic chambers the size of their home cages in which O_2 consumption, CO_2 output and motor activity were measured every 5 min for the next 2 hr. Locomotor responses to QUIN doubled during the course of this treatment even in such a relatively confined space, thus confirming the establishment of behavioral sensitization. The magnitude of energy metabolism and motor activity were closely coupled in control rats but not in QUIN-treated rats. Of particular interest, QUIN reliably reduced rats' Respiratory Quotient (CO_2/O_2), suggesting that the substrate for overall energy utilization had shifted towards the use of lipids for metabolic needs. The capacity to utilize such fuel may represent a physiological adaptation that causes or sustains the dramatic increases in locomotion and exploration normally seen in rats tested in more spacious environments after QUIN-induced behavioral sensitization.

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187.12

Influence of practice and environment-drug pairing on the expression of sensitization induced by the dopamine agonist quinpirole.

Haim Einat* and Henry Szechtman. Dept Biomedical Sciences, McMaster University, 1200 Main St. West, Hamilton, Ontario, CANADA L8N 3Z5

The relative influence of the behavior practised during chronic drug treatment on the expression of sensitization to quinpirole was compared to the influence of environment-drug pairing. Four groups of rats received 10 injections of quinpirole (0.5 mg/kg) and saline, according to a two factor design. One factor was the presence or absence of cage bedding (to increase the likelihood of mouthing and locomotion respectively). The other factor was the explicit pairing or unpairing of quinpirole with the test environment. All groups were tested for the expression of sensitization in a common environment that permitted the display of both locomotion and mouthing. Results showed that locomotor sensitization to quinpirole is greater in rats pretrained in the cages without bedding than with bedding. Mouthing showed tolerance but it was retarded by the presence of bedding. The effect of environment-drug pairing on the locomotor or mouthing response to chronic quinpriole was not statistically significant. It is suggested that the behavior practised during chronic drug treatment is a non-associational mechanism influencing locomotor sensitization and tolerance of mouthing, and that the role of this mechanism at the 0.5 mg/kg dose of quinpirole is relatively greater than that of an associational process due to environment-drug pairing. (HS is a Research Associate of the OMHF. Supported by MRC).

187.13

Chronic quinpirole treatment induces a switch from locomotor inhibition to excitement in the drug-paired environment.

Karen K. Szumlinski, James R. Blackburn* and Henry Szechtman. Dept Biomedical Sciences, McMaster University, 1200 Main St. West, Hamilton, Ontario, CANADA L8N 3Z5

The repeated administration of the D2/D3 receptor agonist, quinpirole (QNP), results in a progressive augmentation in the behavioural response to subsequent injections (behavioural sensitization). However, the relationship between QNP dose and locomotor behaviour in the chronically treated animal remains undescribed. Such a description is provided by this study. To induce behavioural sensitization, animals received either 19 injections of QNP (0.5 mg/kg, s.c., twice weekly) or saline, followed by dose-response tests for the expression of behavioural sensitization (0, 0.01, 0.04, 0.05, 0.06, 0.07, 0.08, 0.2 and 1.0 mg/kg). Chronic QNP-treated animals displayed a robust behavioural sensitization as indicated by an approximately 10-fold increase in locomotor behaviour across injections. Compared to the acute dose-response curve, chronic QNP treatment increased the efficacy and potency of QNP. In addition, some doses which produce behavioural inhibition in the acute QNP animals induced maximal locomotor activation in animals repeatedly treated with the drug. The results demonstrate that chronic QNP treatment not only enhances sensitivity to this drug but also alters the drug's biphasic profile. (HS is a Research Associate of the OMHF. Supported by MRC).

187.14

DOPAMINE TRANSPORTER MRNA IS UP-REGULATED IN THE VENTRAL TEGMENTAL AREA OF BEHAVIORALLY SENSITIZED RATS AFTER ONE WEEK WITHDRAWAL FROM AMPHETAMINE TREATMENT.

P.D. Shilling*, J.R. Kelsoe, D.S. Segal. Dept of Psychiatry and Neurosciences Program, Univ. Calif., San Diego, CA 92093.

Converging evidence supports a significant role for dopamine in the development of behavioral sensitization. There have been several reports of changes in dopamine transporter (DAT) mRNA in behaviorally sensitized rats (Xia et al. J Neurochem. 59:1179;1992, Cerruti et al. Mol. Brain Res. 22:132;1994). To determine if changes in DAT mRNA are long lasting and parallel the time course of amphetamine-induced behavioral sensitization we performed the following experiment. Four groups of adult Sprague Dawley rats were used. Two groups were given daily injections of saline (SAL) for 5 days and two groups were treated with AMPH (2.5mg/kg). After a 1 week withdrawal period, one SAL pretreated group was challenged with SAL and the second SAL pretreated group was challenged with AMPH (2.5mg/kg). The two AMPH pretreatment groups were also challenged with either AMPH or SAL. Locomotor activity was measured for 4 hours after AMPH challenge. All animals were sacrificed 6 hours after the last treatment and *in situ* hybridization was performed with a DAT antisense probe. Message was detected by film autoradiography. Quantification of mRNA was accomplished by computer analysis of digitized images of the ventral tegmental (VTA) area using Image 1.42. Rats pretreated with AMPH and challenged with AMPH exhibited robust behavioral sensitization. In addition, rats pretreated with AMPH and challenged with SAL exhibited a significant up-regulation of DAT mRNA in the VTA compared to the baseline group (SAL/SAL) ($p < .05$). These data support a role for DAT in the expression of amphetamine-induced behavioral sensitization. [Supported by PHS grants DA-01568 (DSS), 5 T32 MH19934-02 (PDS) and RSA MH-70183 (DSS)]

187.15

LONG-LASTING SENSITIZATION TO THE ACCELERATING EFFECTS OF AMPHETAMINE ON THE SPEED OF A BIOLOGICAL "STOPWATCH." A. Badiani * [1] and J. Stewart [2]. [1]Biopsychology, The University of Michigan, Ann Arbor, MI 48109; [2]Center for Studies in Behavioral Neurobiology, Concordia University, Montréal, PQ, Canada H3G 1M8.

Drinking in the rat occurs in bursts of rapid licking (mean within-burst inter-lick interval, ILI, of about 150 ms) driven by a neural pacemaker, or "stopwatch," located in the brain stem. This neural "stopwatch" is thought to be similar to those that control more complex psychological processes such as time discrimination and time estimation. There is some evidence that the speed of neural "stopwatches" can be modulated by drugs acting on dopaminergic transmission. Since long-lasting sensitization of central dopaminergic systems has been observed in animals repeatedly exposed to amphetamines, we speculated that enduring changes in the activity of a neural "stopwatch" could be induced by repeated amphetamine. We studied the effect of repeated i.p. injections of 3.0 mg/kg of *d*-amphetamine on licking behavior in the rat. Amphetamine reduced the mean ILI on the first day of treatment and this effect increased progressively over test sessions (i.e., sensitized). This sensitization was long-lasting, being clearly evident when amphetamine was re-administered after almost two weeks of withdrawal. The reduction in the mean ILI was accompanied by a shift to the left in the frequency distribution of the ILIs. The present findings might be of importance for an understanding of the psychotropic effects of amphetamine, and possibly even for its addictive properties. (MRC#MT6678)

187.16

CLONAZEPAM PREVENTS THE DEVELOPMENT OF SENSITIZATION TO METHAMPHETAMINE.

K. Ito*, T. Ohmori , and T. Koyama. Department of Psychiatry, Hokkaido Univ. Sch. of Med., Sapporo 060 Japan.

The GABA-benzodiazepine neurotransmission has been implicated in various forms of plasticity such as kindling and learning. The present study examined the effects of clonazepam, a GABA-benzodiazepine agent, on the development of behavioral sensitization to methamphetamine (MA). Rats (Male Wistar-King rats) treated with MA (1 mg/kg, sc.) for 10 days /lshowed significantly enhanced motor activity compared to those treated with saline when tested with MA (1 mg/kg) after a 7-8 day withdrawal, indicating the development of behavioral sensitization. Pretreatment with clonazepam (0.5mg/kg) prior to MA administration prevented the development of the phenomenon. Rats treated with clonazepam alone showed no difference in the motor activity compared to those treated with saline. These results suggest that stimulation of GABA-benzodiazepine receptors plays a role in the development of behavioral sensitization. This study was supported in part by Grant-in-Aid No 05670796 and No 07671042 for Scientific Research from Ministry of Education, Science and Culture, Japan.

187.17

REPEATED ADMINISTRATION OF AMPHETAMINE INDUCES SENSITIZATION OF ACOUSTIC STARTLE IN RATS. D.-Y. Chen, S. Hsiao*, & K. C. Liang. Dept. of Psychology, Natl. Taiwan Univ., Taipei, Taiwan, 10764, R.O.C.

Repeated administration of amphetamine (AMPH) renders its effects sensitized to later AMPH challenge. This sensitization seems to involve conditioning to the testing environment. AMPH given systemically enhances acoustic startle. Evidence shows that this enhancement is further augmented by repeated AMPH injections in mice. In view of these findings, this study addressed the role of conditioning in development of AMPH sensitization of acoustic startle in male Spargue-Dawley rats.

Startle was elicited by a noise burst (115 dB, 40 ms) under a background noise of 55 dB. Each session consisted of 180 trials with 30 s inter-trial intervals. After the 60th trial, AMPH was injected (i.p.). Differences between pre- and post-AMPH scores were used to evaluate the enhancement. Consistent with previous findings, 5.0 mg/kg AMPH enhanced startle but 1.0 or 3.0 mg/kg did not. This effect was not abolished by adrenalectomy and intra-cerebroventricular infusion of 50 μg AMPH produced similar potentiation, suggesting that the effect is central. Sensitization was developed by daily injections of 5.0 mg/kg AMPH consecutively for 7 days. One or two days later, rats were challenged with 3.0 mg/kg AMPH which was previously ineffective. Rats receiving chronic AMPH in their home cages did not show sensitization. Rats receiving chronic AMPH in the startle chamber but without being tested daily developed small and marginally significant sensitization. Rats receiving chronic AMPH in the testing chamber and being tested daily for startle under AMPH influences developed robust sensitization. When rats were tested daily and then given AMPH afterwards, sensitization was barely discernible. These findings, taken together, suggest that repeated elicitation of the target response under the influence of AMPH in the specific context may be crucial for rapid development of amphetamine sensitization.

(Supported by funds from Dept. of Psychology, Natl. Taiwan Univ., Taiwan, R.O.C.)

187.18

SEX DIFFERENCES IN BEHAVIORAL SENSITIZATION TO AMPHETAMINE. R.H. Mills [1]*, T. Slade [1], J. Meiners [1], L.W. Johnson [1], G. R. Hanson [2], G.J. Bloch [1]. [1]Dept. of Psychology, BYU, Provo, UT 84602 and [2]Dept. of Pharmacology & Toxicology, University of Utah, SLC, UT 84112

Camp & Robinson ('88) have shown that intact male rats show less sensitization to d-amphetamine (d-AMPH) than intact females, adult-Gx males, and adult-Gx females; adult-Gx male and female rats, however, were not significantly different from each other. Forgie & Stewart ('93), however, reported that adult-Gx male rats showed less sensitization to d-AMPH than adult-Gx females. To further study this issue, males and females were Gx or sham-Gx at 6-30 hr after birth; all rats that remained gonadally intact were Gx at 75-80 days of age. Animals were acclimated to the activity chambers on days 5 & 6 after adult-Gx. On days 7-10, animals were acclimated, then injected i.p. with 1.0 mg/kg d-AMPH. Locomotor behavior was analyzed by recording both ambulatory and stereotyped behavior for 35 min. In addition, each animal was given a behavioral sensitization rank based on the combined ambulatory and stereotyped behavior scores (Kohlert & Bloch, '94). Adult-Gx males showed significantly less behavioral sensitization than the other groups ($p < 0.04$, ANOVA; $p < 0.001$, Kruskal-Wallis). No significant difference was detected in striatal d-AMPH concentrations following the first or after the 4 daily injections of d-AMPH, indicating that differences in brain metabolism of d-AMPH cannot explain the sex difference. These results suggest that testosterone acts after the neonatal period to decrease the behavioral sensitization response to d-AMPH. Since this response was observed in the adult-Gx male, this effect on neural structures may be an organizational one. Supported by HD27334

187.19

SENSITIZATION AND TOLERANCE TO DIFFERENT EFFECTS OF AMPHETAMINE ON SEXUAL BEHAVIOR IN THE MALE RAT. M.F. Wilkins* and J.G. Pfaus. CSBN, Department of Psychology, Concordia University, Montréal, QC, Canada H3G 1M8.

The present study was conducted to examine whether sensitization or tolerance to amphetamine's disruptive effects on male rat sexual behavior is dependent upon attempting sexual activity during drug intoxication. *D*-amphetamine sulfate (2.5 or 5 mg/kg) was injected either 30 minutes prior to (drug-before) or immediately following (drug-after) tests of sexual behavior conducted every 4 days in bilevel chambers. Males in all groups received 8 drug trials. On the final drug trial, all rats were injected prior to the test. Results obtained in the drug-before group replicated previous findings, with a dose-dependent decrease in the proportion of rats that mounted, intromitted, and ejaculated. Conditioned level-changing was also disrupted, secondarily to the induction of stereotypy. In rats that continued to copulate, latencies to initiate mounts and intromissions increased whereas the number of intromissions and the latency to ejaculate decreased. In contrast, all males in the drug-after groups copulated to ejaculation and latencies to mount and intromit declined. Anticipatory level changing was not affected. However, as with the drug-before rats, both the number of intromissions and the latency to ejaculate declined. On trial 8, drug-after rats showed greater increases in mount and intromission latencies and a larger decrease in ejaculation latencies compared to the drug-before group, indicating the development of tolerance in drug-before rats. Thus, although sensitization develops to some of amphetamine's effects on sexual behavior, tolerance can accrue contingently to those effects. *Supported by NSERC of Canada (OGP-0138878).*

188.1

ACTIONS OF LITHIUM AND VALPROATE ON GENE EXPRESSION. H. K. Manji, P. X. Yuan, W. Z. Zeng, J Granneman* and G. Chen, Dept. of Psychiatry & Behavioral Neurosciences, Wayne State Univ. School of Medicine, Detroit, MI 48201

Lithium (Li) and Valproic acid (VPA) are widely used, clinically effective antimanic and mood-stabilizing agents, but despite extensive research, their molecular mechanism(s) remain to be elucidated. Interestingly, the therapeutic effects of Li and VPA are only observed after chronic (days to weeks) treatment; such delayed effects suggest that the therapeutic effects may be modulated by the long-term modulation of gene expression. We have examined the effects of lithium and VPA on gene expression since both Li and VPA have marked effects on protein kinase C (PKC) isozymes, and since PKC is known to play a major role in the regulation of many transcription factors. Our initial studies have shown that VPA increases AP-1 DNA binding activity *in vitro* and *ex vivo*. The ability of both drugs to turn on gene expression has been further demonstrated in C6 glioma and neuroblastoma cells which were either transiently or stably transfected with a reporter (luciferase) gene driven by SV40 or MMTV-LTP promoter/enhancers. Both Li and VPA increased luciferase gene expression in a time and concentration dependent manner. Using mRNA differential display methodologies, we have identified a gene which is markedly turned on by both Li and VPA treatment in rat brain. Taken together, the evidence indicates that both lithium and VPA modulate of gene expression in brain; these effects may play a major role in the long term therapeutic effects of these agents.

188.2

COMPARISON OF VALPROIC ACID AND LITHIUM ON THE EXPRESSION OF THE PKC SUBSTRATE MARCKS IN IMMORTALIZED HIPPOCAMPAL CELLS. D.G. Watson[1]*, J.M. Watterson[2], and R.H. Lenox[1,2,3]. Departments of Psychiatry[1], Pharmacology[2], and Neuroscience[3], University of Florida, Gainesville, FL 32610.

While lithium has been the drug of choice for the acute treatment of mania and the prophylactic management of manic-depressive illness, the broad spectrum anticonvulsant valproic acid (VPA) has recently emerged as an efficacious alternative and adjunctive treatment for this disorder. Studies in our laboratory and others have provided evidence for a role of PKC in mediating the effects of chronic lithium in the brain. We previously reported that in both the rat hippocampus and in immortalized hippocampal cells in culture, chronic lithium down-regulates a major PKC substrate, MARCKS (Myristoylated Alanine-Rich **C-K**inase Substrate), a protein known to be critical for normal CNS development, and found in regions of the brain which exhibit a high degree of plasticity. The lithium-induced regulation of MARCKS is modulated by inositol availability and receptor-mediated signaling through the IP_3/DAG pathway. We present evidence that, like lithium, chronic exposure of HN33 cells to VPA, produces a dose- and time-dependent reduction in the expression of MARCKS protein. The reduction in MARCKS protein expression was observed following chronic, but not acute, exposure to VPA. Maximal reduction in MARCKS levels were observed following 3 days of VPA exposure at or above the therapeutic level (0.6-1.0 mM). The reduction of MARCKS produced by lithium or VPA was enhanced when the two drugs were combined. However, in contrast to lithium, where the reduction of MARCKS is both prevented and reversed by the addition of inositol, the down-regulation observed following VPA exposure was unaffected by the addition of *myo*-inositol (up to 1 mM) to the medium, suggesting an alternative mechanism for VPA's effect. These data suggest that regulation of the MARCKS protein may represent a pharmacological property shared by drugs with therapeutic efficacy in the prophylactic treatment of manic-depressive illness. (Supported in part by NIMH grant MH50105).

188.3

EFFECTS OF SEROTONIN-1A AGONISTS AND ANTAGONISTS ON ULTRASONIC VOCALIZATIONS IN ADULT RATS. R. Lopez and A. Frazer. Univ. of Texas Health Sci.Ctr., Dept. of Pharmacology, and Audie L. Murphy Memorial Veterans Hospital, San Antonio, TX 78284.

Rats and other small rodents will emit ultrasonic sounds (20 to 40 kHz) when confronted with aversive stimuli, or when anticipating the occurrence of an aversive event (e.g., electric foot shock). These ultrasonic vocalizations may be a useful tool for studying neurochemical mechanisms underlying anxiety. We have developed a system that quantifies the duration of ultrasonic vocalizations on-line for any given period of time. The output of a condenser microphone is fed to a Pentium minicomputer outfitted with an analog-to-digital card. A software package developed by us monitors and analyzes (using Fast Fourier Transforms) the output of the analog-to-digital card to determine if there are ultrasonic vocalizations present and their durations. In the present experiments, male Wistar rats (100-200 g) were exposed to 20 foot shocks (0.3 mA, 2 sec duration, distributed across 20 min) once daily for 4 days. On Days 5 and 6, rats were exposed to 5 foot shocks over 42 sec and then immediately monitored for ultrasounds for 5 min. On subsequent days, rats were treated with drugs or vehicle, tested again for 5 min, and ultrasound durations were compared to the average duration of Days 5 and 6. Agonists at the serotonin-1A receptor (gepirone, 0 to 0.3 mg/kg, s.c.; 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT), 0 to 3 mg/kg, s.c.) caused dose-dependent reductions in ultrasonic vocalizations. Administration of the new serotonin-1A antagonist 4-(2'-methoxyphenyl)-1-[2'-[N-(2"-pyridinyl)-p-iodobenzamido] ethyl]piperazine (p-MPPI 10 mg/kg, i.p) 10 min prior to gepirone completely antagonized the effect of gepirone. These results suggest that this ultrasonic vocalization system is capable of detecting pharmacological manipulations affecting this measure of anxiety, in particular those involving the serotonin-1A receptor. (Supported by USPHS grant MH48125 and Research funds from the VA).

188.4

DEXFENFLURAMINE ELEVATES C-FOS EXPRESSION IN HYPOTHALAMIC CRF AND OXYTOCIN NEURONS VIA A SEROTONERIC MECHANISM. T.S. GRAY*, A. JAVED, M. KAMRADT, C. BARTHOLOMEW, AND L.D. VAN DE KAR. Neuroscience Program and Dept. of CBNA, Loyola University Medical Center, Maywood, Il. 60153

Dexfenfluramine (DFEN), a serotonin (5-HT) releaser, has been shown to have anorectic effects in both clinical and experimental studies. It is used as an appetite suppressant in Europe, and was recently approved for use in the United States. DEFEN increases the expression of c-fos in discrete brain regions, including the hypothalamus and amygdala (Li and Rowland, 1993). In addition, it stimulates the secretion of corticotropin-releasing factor (CRF), oxytocin, and vasopressin (VP). The present study was conducted to test the hypothesis that DEFEN increases c-fos expression in CRF, oxytocin, and VP neurons. The subjects of this study were male adult rats that were handled for twice daily to reduce background c-fos expression. DEFEN was given i.p. at 5 mg/kg. Some rats received fluoxetine 10 mg/kg i.p. 12 hours prior to DEFEN injection to competitively block the access of DEFEN into the serotonin terminals. Two hours after DEFEN administration, rats were deeply anesthetized and perfused. Brains were removed and processed according to standard immunohistochemical procedures. Sections from the hypothalamus were immunohistochemically stained for c-fos and then labeled for CRF, oxytocin, or VP. DEFEN caused a significant increase in c-fos expression in the medial parvocellular division of the PVN and in other regions as previously described. Numerous CRF containing neurons were observed to contain c-fos. A small percentage of oxytocin cells also contained c-fos. No c-fos expression was observed in neurons expressing VP. Fluoxetine blocked c-fos expression in CRF and oxytocin cells, and overall in the medial parvocellular PVN. This study suggests that DEFEN activates CRF and oxytocin cells in the hypothalamus through a serotonergic mechanism. Supported by NIH NS20041 and NS 34153.

188.5

PHARMACOKINETICS (PK) AND PHARMACODYNAMICS (PD) OF BMS-180048 IN MIGRAINE N.R. Cutler (1)*, I.E. Fulmor (2), D.E. Salazar (2), J.J. Sramek (1), N. Ford (2). (1) California Clinical Trials, Beverly Hills, CA; (2) Bristol-Myers Squibb Pharmaceutical Research Institute, Princeton, NJ

BMS-180048 is a novel 5-HT_{1D} receptor agonist currently in development for the treatment of migraine. This double-blind, placebo-conirolled, randomized, parallel group study was designed to evaluate the PK, safety, and preliminary efficacy of BMS-180048 in migraine patients during migrainous and pain-free states. Patients who participated met IHS criteria for migraine with or without aura, and suffered between one and six migraines per month for at least one year. Patients were randomized to receive BMS-180048 75 mg, 150 mg, 200 mg, or placebo. Blood samples were obtained just prior to and 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 5, and 6 h after dosing. Headache intensity was rated prior to and up to 6 h after dosing. Seven to 30 days after completion of the in-clinic treatment, patients returned to the clinic in a pain-free state and were administered a repeat of the original study medication. All PK and safety measures were then repeated. Forty-eight patients (9 males, 39 females) were randomized and received BMS-180048 75mg (n=12), 150mg (n=12), 200mg (n=12), or placebo (n=12). Peak plasma concentrations of BMS-180048 were achieved 1 to 2 h following study drug administration in migraine and pain-free states for all doses. PK parameters of BMS-180048 were proportional to dose after administration during a migraine attack over the 75-200 mg dose range. The 150 mg and 200 mg doses of BMS-180048 demonstrated a greater decrease in headache intensity scores and a higher percentage of patients reporting "Any Relief" at 2 h post-dose. The most common adverse event was paresthesia. Thus, BMS-180048 was rapidly absorbed, well tolerated, and demonstrated preliminary efficacy in this population.

This study was supported by Bristol-Myers Squibb Company

188.6

EFFECTS OF MILACEMIDE AND CGP 37,849 ON PUNISHED RESPONDING IN A MODIFIED GELLER-SEIFTER PROCEDURE IN RATS. A.D. Compton[1]*, J.D. Holcomb[2], S.E. Strong[2], J.L. Wiley[2], J. H. Porter[2], and R.L. Balster[3]. Dept. of Psychology, Univ. of Richmond[1], Dept. of Psychology, VA Commonwealth Univ.[2], and Dept. of Pharmacology & Toxicology, Medical College of VA[3], Richmond, VA.

A number of NMDA antagonist compounds have produced promising results in animal models that assess anxiolytic potential. In the present study, 15 male, Sprague-Dawley rats were trained to barpress for food reward on a modified Geller-Seifter operant procedure (MULT FI 30 s, FR 10 + shock). Dose/response data were first collected with chlordiazepoxide (VEH, 1.25, 2.5, 5.0, 10.0, & 20.0 mg/kg), which produced modest and selective significant increases in punished responding at the 10 and 20 mg/kg doses. The pro-glycine compound, milacemide (VEH, 100, 200, & 400 mg/kg), significantly decreased response rates in the unpunished schedule component at the 200 mg/kg dose, and in both schedule components at the 400 mg/kg dose. The competitive NMDA antagonist, CGP 37,849 (VEH, 0.3, 1.0, 3.0, 5.6, & 10 mg/kg) increased punished responding to a similar degree as did CDP at the 1.0 mg/kg dose; however, the effect was not selective, as unpunished responding was also significantly elevated at this dose. CGP 37,849 produced a similar (but selective) increase in punished responding in a previous study that used the Vogel lick model, suggesting that the anxiolytic effects of NMDA antagonists may be task specific. Further, the present results suggest that milacemide would not share anxiolytic effects with NMDA antagonists. (Research supported by NIDA grant DA-01442.)

188.7

COMBINATION OF OPEN FIELD AND ELEVATED PLUS-MAZE: A SUITABLE TEST BATTERY TO ASSESS STRAIN AS WELL AS TREATMENT DIFFERENCES IN RAT BEHAVIOR. ULRICH SCHMITT, KLAUS MANN* AND CHRISTOPH HIEMKE. *Department of Psychiatry, University of Mainz, 55131 Mainz, Germany.*

A test battery consistent of an standard open field, an enriched open field and elevated plus maze was used to study rat behavior. The first part of the study was aimed to evaluate interstrain differences with and without pre-test handling. The study included male rats of the strain PVG/OlaHsd (PVG) and Sprague-Dawely-Hsd (SPRD) (150-200g body wt). SPRD rats displayed higher motor activity levels in each of the tests, and also higher levels of exploratory behavior than the PVG rats. In contrast plus-maze activity indicated more anxiety of SPRD than PVG rats. One week pre-test handling increased the activity of both strains but it increased explorative behavior in the enriched open field only in SPRD rats. Moreover, the behavior in the elevated plus-maze was differentially affected by the handling procedure. The second part of the study based on the results of the first study in the way that male PVG rats (150-200g body wt) were used and was aimed to study rat behavior after acute treatment with diazepam or zolpidem. The behavioral pattern in the 3 different tests indicated a substantial anxiolytic effect of diazepam whereas zolpidem enhanced the explorative activity but had only minor anxiolytic properties. In conclusion, a test battery in contrast to a single test has been shown to be a neccessary tool to evaluate differences in pharmacological activity and also displayed that strain differences in behavior of rats are not only determined genetically, it may also be the result of preceding handling procedures. Since the rat strains responded differentially to the pre-test handling, a well-defined handling procedure is recommended to establish the "spontaneous" behavior of control animals before start of treatments like psychopharmacological tests that require handling procedures.

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188.8

EFFECTS OF KETOCONAZOLE ON BENZODIAZEPINE PHARMACOKINETICS, RECEPTOR BINDING AND FUNCTION. G.A. Pritchard*, J.S. Pratt, J.M. Grassi and D. J. Greenblatt. Department of Pharmacol. and Exp. Ther., Tufts University Sch. of Med., Boston, MA 02111.

Ketoconazole (KET) is an antifungal agent which has been shown to inhibit benzodiazepine metabolism in humans. We have recently demonstrated this effect in a clinical study. Despite elevations in triazolam (TRZ) plasma levels, the degree of pharmacodynamic change (EEG effects) of KET plus TRZ was lower than TRZ alone despite equivalent plasma TRZ levels. The present study examines the effect of KET on TRZ pharmacokinetics and pharmacodynamics as well as its effect on benzodiazepine receptor binding in vitro in male CD-1 mice. TRZ alone inhibited [^{3}H]FNTZ binding with an IC50 of 0.85 nM and a Ki of 0.46 nM. In the presence of KET (1,3 or 9 μM) the IC50 of TRZ was increased to 1.11 nM, 1.58 nM and 5.73 nM respectively while maximal binding was reduced by 36, 69 and 89%, respectively. This disruption of TRZ displacement of [^{3}H]FNTZ binding could be explained by KET ability to dose dependantly antagonize [^{3}H]FNTZ binding in a competitive manner with an IC50 of 1.56 μM and corresponding Ki of 0.98 μM. Consistent with clinical findings, ketoconazole (50 mg/kg, i.p.) significantly elevated mouse serum, brain and liver TRZ levels versus TRZ alone. KET or vehicle (VEH, PEG400 100 μl i.p. final volume) was administered to mice at time 0 and TRZ (0.05, 0.1, 0.2 and 0.3 mg/kg) or VEH (100 μl i.p.) were administered 60 minutes later. Open-field activity was monitored for 50 min following TRZ or VEH injection and blood, brain and liver samples were collected to determine KET and TRZ levels. KET administration had no effect open field activity (horizontal activity, rears and stereotypy) but significantly potentiated TRZ's ability to decrease open field activity on all three parameters analyzed. Preliminary evidence in a pentylenetetrazole (PTZ) seizure susceptibility assay indicates TRZ (0.1 mg/kg) had a significant protective effect versus VEH but was not significantly altered by KET (50 mg/kg) coadministration.

188.9

THE MOUSE CHROMOSOME-4 *BIS1* GENE IS INVOLVED IN BOTH SEIZURE AND ANXIOGENIC PROCESSES. B. Martin*†, Y. Clément‡, P. Venault‡ and G. Chapouthier‡. (SPON: European Brain and Behaviour Society) †URA CNRS 1294 - CDTA, 3b rue de la Férollerie, 45071 Orléans Cedex 02- France. ‡URA CNRS 1957, 47 boulevard de l'Hôpital, 75651 Paris Cedex 13 - France.

Two genes were recently identified, *Bis1* on chromosome 4 and *Bis2* on chromosome 13, as specifically involved in ß-CCM-induced seizures and not in pentylenetetrazol- (PTZ) or strychnine-induced seizures. The aim of the present work is to show whether *Bis1* is also involved in anxiogenic and/or mnesic processes. To this end, we used JE/Le mice carrying the *je* marker very close to the *Bis1* locus; the *je/je* genotype predicting with a very strong probability the *Bis1/Bis1* genotype, and alternatively, the *je/+* genotype predicting the *Bis1/+* genotype. The preceding work on the JE/Le strain, had shown that *je/je* mice are sensitive to ß-CCM-induced seizures whereas *je/+* mice are highly resistant to ß-CCM-induced seizures, reflecting that *Bis1/Bis1* is a ß-CCM-drug-sensitive genotype whereas *Bis1/+* is a ß-CCM-drug-resistant genotype. We applied a T-maze learning task to *je/je* and *je/+* mice under saline, ß-CCM or PTZ treatments, at anxiogenic doses. An increased learning performance is interpreted as a learning process whereas a decreased is interpreted as an anxiogenic process. The statistical analysis confirms the preceding hypotheses concerning the involvement of the *Bis1* locus in anxiogenic processes.

188.10

SYMPATHONEURONAL AND BRAIN GLUCOSE METABOLIC RESPONSES TO α2 ADRENERGIC BLOCKADE DURING ELECTROCONVULSIVE TREATMENT OF DEPRESSION. Mark E. Schmidt*, Michael Henry, Bradley Folley, John Matochik, Hyung-Gun Kim, William Z. Potter. Section on Clinical Pharmacology, NIMH, Bethesda,MD, 20892.

Convulsive treatments are highly effective for ameliorating severe mood episodes, although the mechanism of therapeutic effect is not known. Current theories of the mechanism of action include changes in central catecholamine receptors such as α2 adrenoceptors. We compared sympathetic and brain metabolic responses to acute α2 blockade by idazoxan (IDX) given before and after electroconvulsive therapy (ECT). 6 patients (2 men, 4 women) with a Major Depressive Episode underwent 6-12 ECT treatments. IDX (200 μg/kg) challenge studies were conducted before and 1 week after the course of ECT. FDG PET scans were obtained before and after infusion of IDX; plasma norepinephrine (NE), epinephrine (EPI) and blood pressures were measured. Brain metabolism images were analyzed voxel by voxel following stereotactic normalization and adjustment for global rate (SPM). Systolic pressure (SBP), NE, and EPI increased after IDX. SBP, NE and EPI responses were slightly, but not significantly larger after ECT. In brain, IDX resulted in relative increases in metabolism in primary visual cortex. Relative metabolic rate decreased in left dorsolateral prefrontal cortex (PFC), only after ECT. While preliminary, our findings of slightly larger sympathetic responses and relative decreases in left PFC are consistent with greater sensitivity to α2 blockade following ECT.

188.11

BRAIN GLUCOSE METABOLIC AND SYMPATHETIC RESPONSES TO ACUTE ALPHA-2 BLOCKADE BY ETHOXYIDAZOXAN IN MALE VOLUNTEERS R. J. Oshinsky*, Mark E. Schmidt, Jennifer Schouten, Bradley Folley, William Z. Potter Section on Clinical Pharmacology, NIMH, Bethesda, MD, 20892.

Alpha-2 adrenoceptors in brain modulate the release of a number of neurotransmitters thought to influence arousal, attention, anxiety, and mood. Intravenous challenge with idazoxan(IDX), an α2 antagonist, results in increased activity in primary visual cortex and decreased activity in right prefrontal cortex in healthy males. IDX also has affinity for imidazoline (I_2) binding sites in brain. To determine whether previous findings can be accounted for solely by α2 blockade, we measured the sympathetic and brain glucose metabolic(CMRglu) responses to ethoxyidazoxan (ETX), an IDX analogue with no affinity for I_2 sites. Seven healthy male volunteers (20-38 y/o) underwent serial F*18-fluorodeoxyglucose (FDG) PET scans, before and after infusion with ETX, 9μg/kg. Brain metabolism images were analyzed by Statistical Parametric Mapping. A transient increase in systolic BP occurred after ETX, without a significant change in diastolic BP or heart rate. In brain, the post-ETX FDG scan revealed relative increases in CMRglu in the mesial occipital and left parietal-occipital cortices. Relative CMRglu decreased in right prefrontal cortex after ETX. These findings parallel those reported with IDX infusion, and lend credibility to the concept that IDX-mediated changes in CMRglu in these regions are mediated by α2 receptor blockade.

188.12

TREATMENT OF POSTOPERATIVE NAUSEA/VOMITING(PONV): OLD DRUGS, NEW USE, Y.F.Sung*, The Emory Clinic and Emory Univ.Sch. Of Med., Atlanta, GA 30322

One of the most distressful syndromes that presents in post anesthesia care units (PACU) besides pain is PONV. As day care surgery gains in popularity, this syndrome must be prevented or treated to get the patient (Pt) home-ready, prevent hospital admission, and decrease medical cost.

The aim of this study was to compare the combination of ephedrine(E) and promethazine (P) with saline (S) placebo and with each drug individually in treating patients who had a previous history (Hx) of PONV or severe motion sickness.

One hundred sixteen female pts who had laparoscopic procedures participated in the study. All pts had similar anesthesia. Pts had intramuscular injection of one of 5 treatments 15 min prior to emergence in 1.5 mL volume: Group S; E (E25mg + 1mL S); EP1 (E25mg + P12.5mg + 0.5mL S); EP2 (E25mg + P25mg); P (P25mg + 0.5mL S).

Anesthesia times were similar among 5 groups (ANOVA - NS). Average PACU time range was from EP1, 122 min, to S, 144 min (ANOVA = NS). PONV in PACU among 5 groups were: S 14/21, E 11/23, EP1 7/26, EP2 5/24, P 12/22 (chi square test, P = 0.008). Individual treatment group compared with saline group (Fisher's exact test): EP1 vs. S (P=0.009), EP2 vs. S (P=0.003), E & P were NS.

The Emory Clinic/Emory Univ. School of Medicine

188.13

DISSOCIATION OF NORADRENERGIC AND CHOLINERGIC EFFECTS ON ALERTING AND ATTENTIONAL ORIENTING IN RHESUS MONKEY.

M.C. Davidson* E.B. Cutrell, and R.T. Marrocco

Institute of Neuroscience, University of Oregon, Eugene OR. 97403

This study combined a cued target detection (CTD) task with systemic drug treatments to explore the relationship between neurotransmitters and attentional behavior. Increases in cholinergic activity following low dose nicotine injections increased the size of the validity effect (invalid cue RTs - valid cue RTs) by decreasing the invalid cue trials relative to saline controls. The cholinergic antagonist scopolamine reduced the size of the validity effect by increasing valid cue RTs. In contrast, the α-2 agonist clonidine reduced the size of the alerting effect (double cue RTs- no cue RTs) by increasing RTs in double cue trials relative to saline controls. These data are consistent with previous results using yohimbine, an α-2 antagonist, which also altered the alerting effect. Neither adrenergic drug affected validity scores. Thus, our data suggest a dissociation between attention and alerting behaviors and the systems that presumably mediate these processes.

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188.14

EFFECTS OF SMOKING ON EEG AND THE VEP ARE INDEPENDENT. M. S. John and E. R. Ervin*. Dept. of Psychiatry, McGill Univ., Montreal, QC H3A-1A1.

Changes in the EEG after smoking were examined both at rest and under mental load as induced by playing the game TETRIS and a computer version of Reverse Mirror Drawing Task. Visual evoked potentials were also recorded both before and after smoking. Under mental load, approximately half the subjects produced frontal midline theta (FMT) as has been previously reported. In addition to FMT, mental load produced an increase in delta, most pronounced posteriorly. In half of the subjects, post-nicotine EEG was characterized by a 300 percent narrow band increase of beta2 power. The beta2 response started 2 minutes after termination of the smoking period, and lasted for 7 minutes. Overall, the peak frequency of delta, theta, and alpha was increased by nicotine, and the duration of the VEP P1-N2 response was decreased by nicotine. However, while a negative correlation (-.72) was found between the post-nicotine alpha rhythm amplitude and P1-N2 amplitude, no correlation was found between the latency of any VEP component and the increase in alpha rhythm peak frequency. Results suggest the posterior alpha rhytm and VEP are independently modulated by nicotine thereby contradicing the model proposed by Liberson.

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188.15

FUNCTIONAL MAGNETIC RESONANCE IMAGING (FMRI) OF NICOTINE EFFECTS ON BRAIN ACTIVATION IN HUMANS. E.A. Stein, A.S. Bloom*, J. Pankiewicz, S.A. Fuller, H.H. Harsch, and J-K. Cho. Departments of Psychiatry and Pharmacology, Medical College of Wisconsin, Milwaukee, WI 53226.

Nicotine is the most commonly abused psychoactive substance producing a high level of tolerance and phsical dependence. However, the sites and mechanisms within the human CNS responsible for these properties are poorly understood. fMRI provides excellent spatial and temporal resolution able to resolve the anatomy and physiology of drug effects in the CNS. Whole brain imaging was obtained with single shot GE-EPI on a 1.5 T Signa scanner. Experienced smokers received IV saline and nicotine (0.75, 1.5 or 2.25 mg administered over 1 min) in a cumulative dosing paradigm every 30 min first in a hospital setting to test for drug safety and subsequently while FMR data were acquired for 20 min (TR=6 sec; TE=40 msec; 8 mm slice thickness). Subjects all displayed consistent increases in heart rate, arterial blood pressure and generally reported positive subjective effects to the drug. Plasma drug levels peaked at 2 min and decreased to about 50% by 5 min. A waveform recognition algorithm was used to identify activated voxels. Areas of activation included dose-related increases in the inf., med. and sup. frontal gyrus, anterior and posterior cingulate, visual and insular cortex, cerebellum, colliculi and caudate n. Signal activation peak occured between 2.5-3 min, and declined by 50% within 3.5 min; mean signal intensity was 2-3 % above baseline. These data suggest that nicotine produces a regionally selective, dose-related increase in brain activity consistent with the observed behavioral and physiological effects of the drug. Supported DA09465 to EAS.

188.16

BLOCKADE OF APOMORPHINE-INDUCED CONDITIONED TASTE AVERSIONS IN RATS BY THE NK_1 ANTAGONIST GR205171A. K.H. M. McAllister and J.A.Pratt. SPON: Brain Research Association. Dept. of Physiology and Pharmacology, University of Strathclyde, Glasgow, G1 1XW, U.K.

Neurokinin$_1$ (NK_1) receptors have been implicated in nociception, migraine and emesis. The aim of this study was to determine if the NK_1 receptor is involved in the phenomena of conditioned taste aversion (CTA), which has been hypothesised to recruit similar neural systems to those involved in emesis. CTA responses to apomorphine (0.25 mg kg^{-1}; sc) and the NK_1 antagonist GR205171A (0.1 - 1.0 mg kg^{-1}; sc) were investigated in rats using a 2-trial conditioning procedure. Water deprived rats were presented with either a sodium saccharin (0.1%) or a sodium chloride solution (0.9%) and immediately afterwards injected subcutaneously with apomorphine or vehicle. During the conditioning trials pretreatment with GR205171A was given 15 minutes prior to flavour presentation. The drug-paired and the vehicle-paired solutions were then presented simultaneously in a two-stimulus test to assess CTA and the fluid intake recorded. Following treatment with apomorphine (0.25 mg kg^{-1}) the percentage of the drug-paired fluid intake was 26.2 ± 1.1%. GR205171A (0.1 - 1.0 mg kg^{-1}) blocked apomorphine-induced CTA in a dose dependent manner. At doses of GR205171A (0.3 & 1.0 mg kg^{-1}) apomorphine induced CTA was blocked with drug-paired fluid intakes being 43.9 ± 1.0% and 44.8 ± 1.3% respectively. Conversely GR205171A alone (0.1 - 1.0 mg kg^{-1}) did not evoke CTA. These data suggest an involvement of NK_1 receptors in the CTA phenomena. However it remains to be established whether this occurs through an action of GR205171A on NK_1 receptors located in brain stem emetic circuitry or in forebrain structures involved in aversion learning. We would like to thank S.H.E.R.T. and Glaxo Wellcome for supporting this work.

188.17

THE EFFECTS OF NPA ON U50488-INDUCED BEHAVIORS AND FOS IR IN THE YOUNG RAT. M. Duke, T. Meier, C. Bolanos, C. Crawford[1] & S. McDougall*, Dept. of Psych., CSUSB, San Bernardino, CA 92407; [1]NPI, UCLA, Los Angeles, CA 90024.

The ability of NPA, a direct DA agonist, to modulate U50488-induced behaviors and Fos IR was assessed in young rats. In the first experiment, 17-day-old rats were given the *k*-opioid agonist U50488 (5.0 mg/kg) followed, 30 min later, by NPA (0.001-1.0 mg/kg, IP). Activity and sniffing were assessed for 60 min. Rats were killed 1 h later for analysis of Fos IR. In a second experiment, the ability of U50488 to cross-sensitize (CS) with NPA was determined. To that end, 11-day-old rats were given NPA (1.0 mg/kg) for 5 days, with CS being tested 2 days later after an injection of U50488. The results showed that U50488 dramatically enhanced the activity of the rats, with NPA depressing this activity in a dose-dependent manner. Conversely, sniffing was increased by NPA, an effect blocked by U50488. Fos IR in the nucleus accumbens, olfactory tubercles, septal area, striatum, habenula, and preoptic area was also enhanced by U50488. NPA attenuated U50488-induced Fos in all regions except the habenula and preoptic area. Although NPA-induced sensitization was evident, there was no CS between NPA and U50488. When considered together, these results suggest that the *k*-opioid agonist U50488 and the DA agonist NPA have a generally antagonistic relationship, with NPA depressing U50488-induced activity and Fos IR.

188.18

LIPID-MEDIATED GENE TRANSFER IN THE RAT BRAIN, A. Thorsell[1], E. Fox[2], M. Heilig*[1], Karolinska Institute, Department of Clinical Neuroscience, Section for Clinical Alcohol and Drug Research, Magnus Huss Clinic, Karolinska Hospital, S-171 76 Stockholm, Sweden, [2]Megabios Corp.863A Mitten Rd. Burlingame CA 94010

Gene transfer and expression in the central nervous system (CNS) has to date mainly employed viral vectors as carriers of genetic material. Disadvantages to the use of viral vectors, such as e.g. immune reactions, may limit the usefulness of this strategy. A novel, alternative strategy for gene transfer is based on cellular uptake of expression-vectors bound to cationic lipids. This approach has been developed and proven efficient in cell-culture systems, and its potential has previously been demonstrated *in vivo* outside of the CNS. Here, its potential in the CNS was examined.

In initial experiments, an expression construct encoding prepro-neuropeptide Y (NPY) cDNA complexed to lipofectin or lipofectamine was administered i.c.v. to rats. This resulted in low-level but long term (up to 1 month) expression of a vector-derived NPY-transcript detected with RT-PCR within most brain regions. To optimize expression levels, in order to yield them useful for functional studies, we turned to a proprietary biodegradable lipid, which can be administered repeatedly without apparent toxicity, and a reporter construct encoding chloramphenicol-acetyltransferase (CAT). Expression of a functional CAT-protein was obtained after i.c.v. administration of complexes. Expression levels were assayed after 2, 7, 28 and 56 days. In most brain regions, expression was found to increase over the first 4 weeks. By 8 weeks, expression remained at high levels in cortex, but started to decline in other brain regions. It was demonstrated that the lipid:DNA-ratio is a critical parameter for effective lipofection, since only one of three lipid:DNA ratios studied mediated a robust gene transfer and expression.

Long-term expression of transferred genetic material is presently being evaluated. Different means of delivering complexes to the lateral ventricles and to local structures are being examined, as are ways to increase expression levels.

These results have been obtained in the apparent absence of adverse effects. Thus, in summary, cationic lipid-mediated gene transfer appears to have a considerable potential as an efficient, safe and accesible experimental method for the study of genes and their products in the normal adult living brain.

189.1

OLANZAPINE VS. CHLORPROMAZINE IN TREATMENT-RESISTANT SCHIZOPHRENIA. R. R. Conley*, C. A. Tamminga, W. Satterlee, and the Maryland Study Group. MD Psychiatric Research Center, Baltimore, MD 21228.

Since the demonstration of the superior effect of clozapine in treatment-resistant schizophrenia (Kane, 1988) there has been an ongoing search for other drugs with the same effect. Although there have been multiple hypotheses about the mechanism of clozapine's efficacy, none have yet clearly explained clozapine's differential effect. This is largely because there are no other antipsychotics that have shown clozapine's effect in a similarly designed trial. Olanzapine is an excellent candidate drug for study. It has a very similar binding profile to clozapine (e.g., Ki @ D_1-31 nM, D_2-11 nM, alpha-1 19 nM, H_1-7 nM, S_2-5 nM, M1-1.9 nM), but has a higher dose potency. It has a high affinity at D_4 receptors (Beasley, 1993) which may be a critical clozapine property (Lahti, 1992). Olanzapine is an effective antipsychotic (Satterlee, 1995). We have studied the effect of olanzapine vs. chlorpromazine in a ten-week, double-blind clinical trial. All subjects had a DSM-III-R diagnosis of schizophrenia and were classified as treatment-resistant by failing to have a 20% or greater response to at least two different neuroleptics, exclusive of haloperidol (at 1000 mg/ day CPZ equivalents for six weeks each), been persistently ill with no periods of good functioning for at least five years, and had failed to show a 20% improvement in a six-week prospective trial of haloperidol. All subjects signed informed consent. Subjects had a two-week single-blind neuroleptic washout, followed by ten weeks of treatment either with 25 mg/day of olanzapine or 1200 mg/ day of chlorpromazine in a fixed-dose design after a one week titration period. Subjects were rated weekly. To date, 81 subjects have entered the trial, 10% have responded. We will report unblinded data at the meeting. This is, to our knowledge, the first large trial of olanzapine in this population.

Supported by NIMH MH 47311, Lilly Pharamaceutica provided olanzapine.

189.2

MOOD STABILIZING ACTION OF SM-13496, A NOVEL ATYPICAL ANTIPSYCHOTIC AGENT, IN CONDITIONED FEAR STRESS-INDUCED FREEZING BEHAVIOR MODEL IN RATS. Y. Ohno*, K. Ishida-Tokuda, H. Sakamoto, T. Ishibashi, R. Tojima, J. Yasui, T. Morita and M. Nakamura. Research Center, Sumitomo Pharmaceuticals Co., Ltd., Konohana-ku, Osaka 554, Japan.

SM-13496 is a novel 5-HT2 and dopamine D2 receptor antagonist with negligible extrapyramidal and CNS depressive side effects. To evaluate the action of SM-13496 on the dysphoric mood disturbances in animals, we compared its effects on the conditioned fear stress (CFS)-induced freezing behavior in rats with those of other antipsychotics. Exposure of rats to the environment previously paired with the foot shock induced marked freezing behavior, which was reduced by the anxiolytic diazepam or the antidepressants, desipramine and imipramine. SM-13496 (0.3-6 mg/kg, p.o.) significantly attenuated the CFS-induced freezing behavior in a dose-dependent manner. Other serotonin-dopamine antagonists (SDA), clozapine and risperidone, also reduced the freezing behavior showing a U-shaped dose-response curve but the improvement rate was lower than that with SM-13496. By contrast, neither the conventional antipsychotic, haloperidol, chlorpromazine, thioridazine, mosapramine nor tiapride reduced the CFS-induced freezing behavior. In addition, the selective 5-HT2 antagonists, ritanserin and ketanserin, also reduced the induction of the freezing behavior by CFS. These findings suggest that SM-13496, like other SDA-type antipsychotics, is effective for the treatment of mood disturbances (e.g., anxiety, tension and depression) associated with schizophrenia and have a broader efficacy profile as compared with the conventional antipsychotics. The 5-HT2 blocking action of SM-13496 seems to at least partly be involved in its mood stabilizing action in the CFS-induced freezing model.

189.3

RESTORATION OF PREPULSE INHIBITION IN SOCIALLY ISOLATED RATS BY SEROQUEL AND OLANZAPINE. M.A. Geyer*, V.P. Bakshi, D.L. Braff and N.R. Swerdlow. Depts. of Neuroscience and Psychiatry, UCSD, La Jolla CA 92093.

Intense auditory or tactile stimuli elicit involuntary startle responses that are attenuated when a weak stimulus (a prepulse) is presented immediately prior to the startling stimulus. This phenomenon of "prepulse inhibition" (PPI) is thought to be an operational measure of sensorimotor gating and is disrupted in schizophrenia (SZ) patients. The early developmental manipulation of rearing animals in social isolation results in a deficit in PPI that models the impairment seen in SZ. The present investigation evaluated the ability of two new putative atypical antipsychotics, seroquel and olanzapine, to reverse the deficit in PPI produced by isolation rearing. Upon weaning, all animals were assigned randomly to one of two housing conditions for the duration of the studies: isolation rearing (IR, singly housed) or social rearing (SR, housed in groups of 3). Eight weeks later, all animals were treated with either saline or seroquel (5.0 mg/kg) 30 min before being tested with 120 dB startling stimuli presented either alone or 100 msec after non-startling prepulses that were 3, 6, or 12 dB above the background noise (65 dB). Two weeks later, animals were treated with either saline or olanzapine (2.5 mg/kg) and tested for PPI 30 min later. IR animals exhibited markedly lower levels of PPI than did SR animals. Pretreatment with either seroquel or olanzapine significantly improved PPI in IR animals without affecting PPI in SR rats. These results indicate that IR-induced deficits in PPI are sensitive to atypical antipsychotics.

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189.4

EFFECTS OF TYPICAL AND ATYPICAL NEUROLEPTICS ON DOPAMINE REUPTAKE IN THE STRIATUM: AN ELECTROCHEMICAL STUDY. D. S. Rothblat*, and J. S. Schneider. MCP and Hahnemann University, Department of Neurobiology and Anatomy, Philadelphia, PA 19102-1192.

Typical neuroleptics, such as Haloperidol (HAL), have high affinity for dopamine (DA) D2 receptors, enhance DA release and have a high incidence of motor side effects while atypical neuroleptics, such as Clozapine (CLOZ), have low affinity for DA D2 receptors, do not enhance DA release and have reduced likelihood of producing motor side effects. While DA D2 receptor affinity may underlie some of the side-effect potential of neuroleptics, it is possible that these drugs may effect DA release and/or reuptake which could also influence production of motor side effects. The present study investigated neuroleptic effects on DA-reuptake in the dorsal and ventral striatum of urethane-anesthetized (1.25mg/kg) adult Sprague Dawley rats. High-speed chronoamperometry utilizing nafion-coated carbon fiber electrodes was used to measure DA reuptake in the striatum. Dopamine (200μM) was pressure ejected from a pipette (2-15 psi) attached to the recording electrode (100-300μm tip separation). Once a reproducible signal was achieved, CLOZ or HAL (10μM) was pressure ejected from a second pipette and effects on DA clearance were measured. Pressure and duration of DA ejections were varied to reproduce peak levels recorded prior to neuroleptic application. Reuptake rate (μM/sec) was reduced by 28% in the dorsal striatum and 46% in the ventral striatum after CLOZ application, while HAL reduced the reuptake rate by 55% in the dorsal and only 19% in the ventral striatum. Similar trends were also observed in animals that received systemic HAL (10mg/kg) or CLOZ (5mg/kg). These results suggest that while both typical and atypical neurolpetics can slow DA-reuptake, HAL's effect was much greater in the dorsal (sensorimotor) striatum. Increased extracellular DA levels, particularly in the dorsal striatum, could contribute to the generation of motor side effects by several mechanism including augmentation of glutamatergic neurotransmission. Such mechanisms will be the focus of future studies. Supported by the F.M. Kirby Foundation.

189.5

GLUTAMATERGIC MECHANISMS OF ANTIPSYCHOTIC DRUG ACTION. T.I. Lidsky*, E. Yablonsky-Alter, L. Zuck, S.P. Banerjee. NYS Institute for Basic Research, Staten Island, NY 10314

Previous work from this laboratory indicated that some antipsychotic drugs (APs) possess unique action at NMDA receptors that could contribute to antipsychotic efficacy. Both functional neurochemistry and *in vitro* electrophysiology showed that APs, at concentrations similar to that found in schizophrenics' CSF, augment NMDA activity while, at higher concentrations, NMDA activity is suppressed. Using similar analysis, the present paper reports that this pattern of response, consistent with partial agonism, is shown by haloperidol, chlorpromazine, thioridazine, pimozide and clozapine. In contrast, sulpiride and metoclopramide, drugs with high affinity for D2 dopamine receptors but with weak or no antipsychotic efficacy, lack effects at the NMDA receptor. The present results indicate that D2 activity is neither necessary nor sufficient to achieve antipsychotic potency. Conversely, the drugs with clinical efficacy that were tested in the present study all share unique influence on NMDA receptors. Further work with other APs will be necessary to determine if partial agonism at NMDA receptors is the defining chacteristic of antipsychotic effectiveness.

189.6

SEROTONIN AND DOPAMINE RECEPTOR AFFINITIES PREDICT ATYPICAL ANTIPSYCHOTIC DRUG (AAD) ACTIVITY, HY Meltzer*, B Roth, P Thompson. *Ctr for Psychobiology, Case Western Res Univ, Cleveland, OH 44106

Atypical antipsychotic drugs (AAD), e.g. clozapine, differ from typical antipsychotic drugs (TAD), e.g., haloperidol, in producing low extrapyramidal side effects (EPS) and have apparent advantages for improving some types of psychopathology. A hypothesis offered by us to explain the biological basis for the differences in these classes of drugs has been weak D_2 relative to 5-HT_{2A} receptor blockade. However, a discriminant function analysis (DFA), using only the affinities for these two receptors, classified 4 of 37 drugs incorrectly. We have now examined whether the inclusion of other receptor affinities can improve the classification. Affinities of 11 and 9 AAD and TAD, respectively, were determined for D_1, D_2, D_4, 5-HT_{2A}, 5-HT_{2C}, 5-HT_8, and 5-HT_7 receptors, using cloned cell expression systems or brain membranes. With DFA, in order of importance, D_2, 5-HT_{2A}, 5-HT_{2C}, and 5-HT_7 receptor affinities correctly classified all compounds, including loxapine and amoxapine, which had been incorrectly classified previously. The squared canonical correlation was 0.77, indicating 77% of the variance in atypicality is explained by this model. The parameter estimates were: (1) D_2, 0.53, p=0.001; (2) 5-HT_{2A}, -0.41, p=0.01; (3) 5-HT_{2C}, 0.25, p=0.02; and, (4) 5HT_7, -0.18, p=0.06, indicating AAD had weak D_2 and 5-HT_{2C} and potent 5-HT_{2A} and 5-HT_7 affinities. These results suggest that multiple receptor affinites are important to the pharmacology of the APD and that compounds highly selective for the receptors studied here are unlikely to be of comparable value in the treatment of schizophrenia. The differences between serotonin-dopamine antagonists such as clozapine, risperidone, and olanzapine with regard to the affinities for these four receptors may account for some of the clinical differences between these compounds.

189.7

COMPARATIVE ANTIPSYCHOTIC PROFILES OF NEUROTENSIN AND A RELATED SYSTEMICALLY ACTIVE PEPTIDE AGONIST. S. Sarhan, J.M. Hitchcock, C.A. Grauffel, R.M. Pruss* and J.G. Wettstein. Hoechst Marion Roussel, Department of Pharmacology, 16 rue d'Ankara, 67080 Strasbourg, France.

Several lines of evidence have shown that neurotensin can modulate dopamine neurotransmission. It has been suggested that neurotensin has potential antipsychotic activity because it reduces dopaminergic activity preferentially in the nucleus accumbens. In the present study, the effects of neurotensin and NT1 (N^{α}MeArg-Lys-Pro-Trp-Tle-Leu or Eisai hexapeptide), a metabolically stable and systemically active neurotensin agonist, were examined in several models of antipsychotic activity and side effect liability in mice. Analgesic and hypothermic effects of both compounds also were determined. Even at high doses, neurotensin (5.0 and 10.0 µg, i.c.v.) and NT1 (10.0 and 20.0 mg/kg, i.p.) did not produce catalepsy. A much lower dose of neurotensin (0.03 µg, i.c.v.) significantly reduced amphetamine-stimulated locomotor activity; NT1 also diminished amphetamine- and phencyclidine-stimulated locomotion with ED_{50} values of 0.3 and 0.4 mg/kg, i.p., respectively. In addition, neurotensin (0.01 - 0.3 µg, i.c.v.) and NT1 (0.1 - 1.0 mg/kg, s.c.) produced dose-dependent analgesia in the paw pressure test and decreased body temperature; these effects were insensitive to pretreatment with naloxone (10.0 mg/kg, i.p.). Together, the results support the hypothesis that neurotensin agonists have antipsychotic and analgesic activity. Moreover, use of such compounds may not produce extrapyramidal side effects.

189.8

EFFECTS OF ANTIPSYCHOTIC DRUGS AND OTHER CNS-ACTIVE AGENTS ON THE BEHAVIORAL ACTIONS OF THE HALLUCINOGEN DOI (1-[2,5-DIMETHOXY-4-IODOPHENYL]-2-AMINOPROPANE). J.G. Wettstein,* M. Host and J.M. Hitchcock. Hoechst Marion Roussel, Department of Pharmacology, 16 rue d'Ankara, 67080 Strasbourg, France.

It has been proposed that antagonists at 5-HT_2 receptors, in particular 5-HT_{2A} sites, could have antipsychotic activity in humans. In an effort to understand the contribution of 5-HT_2 activity to the effects of antipsychotic drugs and to enhance screening for such compounds, a series of conventional, atypical and purported antipsychotics were assessed as antagonists of DOI in rats. DOI is an hallucinogen having high affinity and selectivity as an agonist at 5-HT_2 receptors. Over a 30-min period after injection, DOI (0.3 - 10.0 mg/kg; i.p.) produced dose-related behavioral effects including head-and-body shakes, forepaw tapping and skin-jerks. The effects of antipsychotics and other compounds (30 min pretreatment; i.p.) were examined against a fixed dose of DOI (3.0 mg/kg). In a dose-dependent manner, risperidone, haloperidol, clozapine, MDL 100,907, iloperidone, olanzapine, amperozide, ritanserin and the neurotensin agonist N^{α}MeArg-Lys-Pro-Trp-Tle-Leu attenuated the behavioral effects of DOI. The following compounds had little or no effect on the DOI-induced behaviors: citalopram, CP 99994, diazepam, fluoxetine, ondansetron and SKF 97541. These data show that, as a drug class, antipsychotics block the effects of DOI and those with notable 5-HT_2 affinity (e.g., risperidone, MDL 100,907 and iloperidone) are quite potent in this regard; such effects are selective as a series of non-antipsychotic, centrally-acting drugs were inactive in the procedure.

189.9

PREVENTION OF DOI-INDUCED DISRUPTION OF LATENT INHIBITION BY MDL 100,907, CLOZAPINE, HALOPERIDOL AND RISPERIDONE. J.M. Hitchcock*, S. Lister, T.R. Fischer and J.G. Wettstein. Hoechst Marion Roussel, Department of Pharmacology, 16 rue d'Ankara, 67080 Strasbourg, France.

Latent inhibition (LI), a measure of the ability to learn to ignore irrelevant stimuli, is disrupted in schizophrenics and in rats treated with amphetamine; antipsychotics prevent amphetamine-induced disruption of LI in rats. The 5-HT_2 agonist DOI [1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane HCl] has hallucinogenic properties in humans, and evidence suggests that 5-HT_2 antagonism is an important component of antipsychotic activity. In the present study, the ability of DOI to disrupt LI in rats was tested, and the effects of clinically-used and putative antipsychotics were assessed in this procedure. The method consisted of four phases. After *habituation* to the apparatus, thirsty rats underwent *pre-exposure* to a tone 24 hrs prior to two tone-shock *conditioning* trials. LI was demonstrated at *testing* (an additional 24 hrs later) by reduced lick suppression during tone presentation. When administered at both pre-exposure and conditioning phases, DOI disrupted lick suppression itself, obscuring measurement of LI. However, when administered at the pre-exposure phase only, DOI dose-dependently disrupted LI. Clozapine, haloperidol, risperidone and the selective 5-HT_{2A} antagonist MDL 100,907 prevented DOI-induced disruption of LI. These results indicate that antagonism of DOI-disrupted LI has potential as a method for detection of antipsychotic activity and further support the involvement of 5-HT_{2A} antagonism in antipsychotic activity.

189.10

LOCALIZING DRUG EFFECTS ON SENSORIMOTOR GATING IN A PREDICTIVE MODEL OF ANTIPSYCHOTIC POTENCY. S. Hart, M. Zreik, R. Carper and N.R. Swerdlow*. UCSD Dept. of Psychiatry, La Jolla, CA 92093-0804

The degree to which a startle response to a loud noise is inhibited by a weak prestimulus is an operational measure of sensorimotor gating. "Prepulse inhibition" (PPI) can be measured across species, and is reduced or absent in schizophrenia patients, and in dopamine (DA)-activated rats. Preclinical and clinical data suggest that PPI is regulated by neural circuitry connecting prefrontal and limbic cortex with subcortical structures that innervate the pontine tegmentum. The loss of PPI in apomorphine (APO)-treated rats is reversed by DA antagonists; the ability of drugs to restore PPI in APO-treated rats correlates highly with their clinical antipsychotic potency.

We compared the ability of systemic- vs intracerebrally (ic)-administered haloperidol (HAL) to restore PPI in APO-treated rats. Consistent with previous studies, systemic (sc) administration of HAL completely restored PPI in rats treated with APO (0.5 mg/kg sc), with an ED50 of approximately 0.05 mg/kg. In an otherwise identical paradigm, HAL failed to fully restore PPI after infusion into either the nucleus accumbens (NAC), caudate nucleus (CN), ventral subiculum (VS), dentate gyrus, medial prefrontal cortex, ventral tegmentum or dorsomedial thalamus. A subtotal, but statistically significant restoration of PPI was achieved after HAL infusion into the NAC, CN and VS. ED50's for the effects of ic HAL tended to be approximately 5-fold lower than those detected after sc administration. Data from the effects of simultaneous multi-site HAL infusions will be presented. The results suggest that systemically-administered HAL may restore PPI in APO-treated rats via its action at multiple levels of PPI-regulatory circuitry.

189.11

CLOZAPINE BUT NOT HALOPERIDOL INDUCES C-*FOS* IN CERTAIN BRAIN REGIONS IMPLICATED IN SCHIZOPHRENIA. D. K. Rush*, D. E. Bregna, S. E. Hanak, M. R. Szewczak, and M. G. Cilio. Hoechst Marion Roussel, Inc., CNS Therapeutic Area, Bridgewater, NJ 08807-0800.

Much like the 2-deoxyglucose method for examining regional changes in metabolism induced by specific stimuli, induction of c-*fos* mRNA and protein has been utilized to examine regional changes in cellular activation induced by a variety of stimuli including drugs. In the current study, we examined the effect of haloperidol (1 mg/kg) and clozapine (30 mg/kg) on c-*fos* protein in a wide variety of forebrain regions. The drugs or vehicle (n=3-4, Sprague-Dawley rats) were administered ip 2 h prior to transcardial formaldehyde perfusion. c-*fos* was detected with a rabbit polyclonal antibody (Oncogene, PC05, 1:3000) with biotin-avidin amplification (Vector Elite) and DAB-nickel intensification in 30 µm cryostat sections taken every 150 µm from the beginning of the caudate-putamen to the posterior part of the hippocampus (+1.7 to -4.16 mm, Paxinos & Watson atlas).

A qualitative examination of all brain regions was followed by quantification of the number of c-*fos* positive cells in digitized video images using image analysis (MCID M4, Imaging Research Inc.). Haloperidol but not clozapine induced c-*fos* in the medial and lateral caudate-putamen. Both haloperidol and clozapine induced c-*fos* in the n. accumbens; clozapine increased c-*fos* in both shell and core, haloperidol was only effective in the core. Clozapine but not haloperidol induced c-*fos* in the lateral septum, thalamus, amygdala, hypothalamus and bed nucleus of the stria terminalis. Many of these latter areas, especially the thalamus, have been implicated in both the negative and positive symptoms of schizophrenia and may be sites of action of this atypical neuroleptic agent.

189.12

EFFECTS OF CLOZAPINE IN SHR AND WKY RAT PUPS CHALLENGED WITH AMPHETAMINE: STEREOTYPY, CATALEPSY, AND ACTIVITY DATA. M.K. Murphy, P.M. Bettino, and A.C. Santucci. Dept. of Psychology, Manhattanville College, Purchase, NY 10577.

Previous studies have suggested the use of spontaneously hypertensive rats (SHR) as an animal model for Attention Deficit/Hyperactivity Disorder (ADHD). However, the literature is uncertain as to the cause of the hyperactivity seen in these animals. Therefore, in an attempt to better characterize this animal model, the present study examined the effects of dopamine blockade on measures of activity, stereotypy, and catalepsy. SHR and normotensive WKY controls (17-21-day-old pups) were injected with one of three doses of clozapine (0, 12.5, & 25 mg/kg), a putative selective D_4 limbic antagonist. All animals were then tested 30 min. later for open-field and wheel-running activity, stereotypy, and catalepsy in response to a d-amphetamine challenge (0 and 0.75 mg/kg). Results indicated that SHR animals were hyperactive as reflected in an increased number of square crosses, rears, and wheel turns ($p < .05$). When tested under amphetamine, a paradoxical reduction in activity was not observed in SHR animals ($p < .05$). In addition, SHR subjects took longer to initiate exploration in the open-field ($p < .05$). This suggests that these animals were hyper-reactive when placed in a novel environment. In general, activity in all clozapine-treated subjects was severely diminished. Interestingly, however, clozapine differentially increased measures of catalepsy and stereotypy in SHR subjects. These data suggest that SHR animals may be hypodopaminergic. In addition, this hypodopaminergia may be related to the behavioral profile displayed by SHR animals.

189.13

EFFECTS OF ATYPICAL AND TYPICAL NEUROLEPTICS ON SINGLE-UNIT RECORDINGS OF SUBSTANTIA NIGRA RETICULATA NEURONS. W. Timmerman*, R. Bruggeman and B.H.C. Westerink. Department of Medicinal Chemistry, University Center for Pharmacy, 9713 AV, Groningen, The Netherlands.

Risperidone has proven to be effective as an antipsychotic drug and has fewer extrapyramidal side-effects than classical neuroleptics. Besides its D2 antagonistic properties, the antipsychotic agent is a potent serotonin-5HT2 antagonist. The atypical antipsychotic clozapine also posseses both, D2 and 5-HT2 receptor affinity next to affinities for other receptors. To gain insight in the consequences of treatment with these atypical neuroleptics vs typical neuroleptics for basal ganglia activity, effects of cumulative doses of risperidone, clozapine and haloperidol on substantia nigra reticulata (SNR) single-unit activity were studied in chloralhydrate anaesthetized male Wistar rats. Both, risperidone (0.05-3.2 mg/kg iv) and clozapine (0.1-6.4 mg/kg iv) dose-dependently decreased SNR activity maximally to 60% and 75% of basal activity, respectively. In contrast, haloperidol (0.012-0.8 mg/kg iv) induced a slight increase in SNR activity over time, which was identical to the SNR activity after saline treatment. Thus, these results indicate that typical and atypical neuroleptics differentially affect the output of the basal ganglia at the level of the SNR. The inhibition of SNR neuronal activity is hypothetized to be mediated via 5HT2 receptor blockade. [Supported by a grant from the Royal Dutch Academy of Sciences].

189.14

A NOVEL SERIES OF 1-ARYLOXY-3-(4-ARYLOXYPIPERIDINYL)-2-PROPANOLS AS POTENT, SELECTIVE DOPAMINE D4 RECEPTOR ANTAGONISTS. J.L. Wright, T.F. Gregory, T. G. Heffner, R.G. MacKenzie, T.A. Pugsley, S.J. Vander Meulen and L.D. Wise.* Parke-Davis Pharmaceutical Research, Division of Warner-Lambert Company, Ann Arbor, MI 48105

Both the antipsychotic efficacy and neurological side effects of dopamine (DA) antagonists have been correlated with their affinity for DA D2 receptors which are widespread in limbic and striatal regions of the brain. These receptors now include D2, D3 and D4 subtypes. The D4 subtype is expressed at relatively higher levels in limbic and cortical brain regions than in the basal ganglia. This suggests that a selective DA D4 antagonist might provide antipsychotic efficacy without causing neurological side effects. High volume screening of our chemical library and subsequent studies have uncovered a novel series of 1-aryloxy-3-(4-aryloxypiperidinyl)-2-propanols with high affinity for cloned human DA D4 receptors expressed in CHOp-5 cells. For example, the analog in which both aryls were phenyl has a Ki value of 4 nM at DA D4 receptors with >100 fold less affinity at DA D2 and D3 receptors. Substituents on the aryl groups did not significantly improve affinity but the (R)-enantiomers had slightly higher DA D4 affinity than the (S)-enantiomers. Several members of this series appeared to be antagonists; they showed no stimulation of mitogenesis in CHOp-5 cells expressing D4 receptors and blocked the stimulation of mitogenesis caused by the DA agonist quinpirole in such cells. These compounds modestly increased DA synthesis in rat hippocampus and striatum but, unlike traditional DA D2 antagonists, did not alter locomotor activity in rodents. Such DA D4 antagonists represent potential atypical antipsychotic agents.
Supported by Warner-Lambert

189.15

TYPICAL AND ATYPICAL ANTIPSYCHOTIC DRUGS UPREGULATE RAT CEREBROCORTICAL D_2 AND STRIATOLIMBIC D_4 DOPAMINE RECEPTORS. R.J. Baldessarini*, F.I. Tarazi, S.K. Yeghiayan, and J.L. Neumeyer[1]. Psychiatry & Neuroscience Depts, Harvard Medical School; Mailman Research Center, Belmont, MA 02178, [1]Research Biochemicals International (RBI), Natick, MA 01760.

We examined changes in dopamine receptor binding following 21d treatment of rats with a typical neuroleptic, fluphenazine (FLU; 1.0 mg/kg/d), an atypical antipsychotic, clozapine (CLZ; 20 mg/kg, twice daily) and a novel potential antipsychotic agent, S(+)-N-*n*-propylnorapomorphine ([+]-NPA; 2.0 mg/kg, thrice daily) using *in vitro* quantitative receptor autoradiography. FLU treatment significantly increased binding of the D_2-like ($D_2/D_3/D_4$) ligand ^{3}H-nemonapride in medial prefrontal cortex (MPC; 46%), striatum (STR; 22%) and nucleus accumbens (ACC; 67%). In contrast, CLZ (59%) and [+]-NPA (48%) treatment increased D_2-like receptor binding only in MPC. This pattern suggests that the MPC may be an important common site of action for typical and atypical antipsychotic drugs. D_4 receptors were quantified with ^{3}H-nemonapride plus excess S(−)-raclopride (300 nM) to occlude D_2/D_3 receptors, as well as 100 nM pindolol and 500 nM 1,3-ditolylguanidine (DTG) to mask $5HT_{1A}$ and sigma sites. FLU, CLZ and [+]-NPA treatments significantly increased D_4 receptor binding in ACC (124%, 71%, and 68% respectively) and STR (62%, 43%, and 48%) but not in the MPC. These results support the hypothesis that cerebrocortical D_2 and striatolimbic D_4 receptors may mediate the antipsychotic actions of both typical and atypical neuroleptic drugs, and encourage further consideration of [+]-NPA as a possible atypical antipsychotic agent.
Supported by MH-34006, MH-31154, MH-47370, and B.J. Anderson Foundation.

189.16

EFFECT OF CHRONIC ANTIPSYCHOTIC DRUG TREATMENT ON CYTOCHROME OXIDASE ACTIVITY IN THE RAT BRAIN P.D. Lambert*, T.D. Ely, R.E. Gross and C.D. Kilts Dept. of Psychiatry and Behavioral Sciences, Emory Univ. Sch. of Med., Atlanta, GA 30322

The therapeutic effects of antipsychotic drugs are mediated by gradually developing, adaptive effects occurring as a consequence of long term receptor actions. An understanding of the relationship between such patterns of altered brain activity and the distinct clinical effects of different antipsychotics may provide neural system targets for novel drugs. Cytochrome oxidase (CO) is a mitochondrial enzyme that catalyzes the terminal transfer of electrons to molecular oxygen in cell respiration. CO mapping defines localized changes in energy demand resulting from sustained changes in neuronal activity and represents a powerful technique for identifying enduring patterns of altered brain function. We have used a histochemical assay of CO activity to map changes in neuronal functional activity following neuroleptic and atypical antipsychotic administration. Male Sprague-Dawley rats were dosed with clozapine (20 mg/kg i.p.), haloperidol (1mg/kg i.p.) or vehicle (tartaric acid 0.3%) once daily for 21 days. 18 hours following the final injection, brains were collected, sliced (30μm) and stained for CO using the methods of Gonzalez-Lima. The repeated administration of antipsychotic drugs was associated with significant alterations in CO activity in structures of the limbic, motor and prefrontal basal ganglia-thalamocortical circuits. These patterns were distinct for clozapine and haloperidol. Significant bilateral increases in CO activity compared to control were seen following 21d clozapine treatment in the central nucleus of the amygdala (R 31%, L 36%), globus pallidus (R 73%, L 49%), dorsolateral caudate (R 22%, L 30%), anteroventral thalamic nucleus (R 22%, L 26%) and mediodorsal thalamic nucleus (R 25%, L 22%). Thus, CO activity maps define patterns of changes in neuronal activity in response to antipsychotic drug administration and by pathway mapping may allow the neural system targets for individual antipsychotic drugs to be identified.
Supported by MH39967.

DEVELOPMENTAL DISORDERS II

190.1

OLIGODENDROCYTE-RELATED ENZYMES IN HYDROCEPHALIC RAT BRAINS. M.R. Del Bigio* and J.N. Kanfer, Departments of Pathology and Biochemistry, University of Manitoba, Winnipeg, MB Canada R3E 0W3

Hydrocephalus, a dilatation of the cerebral ventricles, can damage white matter. We induced hydrocephalus by injecting kaolin into the cisterna magna of 3 week old rats. After 1, 2, or 4 weeks, rats were killed or treated by shunting of CSF. Samples of corpus callosum/ supraventricular white matter, fimbria, and medulla were dissected from each brain. The myelin enzymes 2',3'-cyclic nucleotide 3'-phosphodiesterase (CNP) and p-nitrophenylphosphorylcholine phosphodiesterase (PNP), and the oligodendrocyte enzyme UDP-galactose: ceramide galactosyltransferase (CGalT) were assayed. In corpus callosum, CNP was reduced by 25% ($p<.0001$) and PNP was reduced by 47% ($p<.0001$), compared to controls. CGalT, which showed a maturation-related decline, was unaffected by hydrocephalus but showed a 43% increase ($p=.0019$) following early shunting. In the medulla, CGalT was reduced by 27% ($p<.0001$) in the 1 wk hydrocephalic rats. No changes were observed in the fimbria. Immunohistochemical labeling of myelin basic protein and 011 was unchanged in the corpus callosum, striatum, and fimbria. Labeling of oligodendrocytes in hydrocephalic brains by 04 resembled that of older control rats. The incidence of brain cell death, as determined by labeling of fragmented DNA, progressively increased from 1-3 weeks ($p<0.01$). Summary: Damage to white matter by ventriculomegaly is reflected by reduced activity of myelin-related enzymes. Conclusion: Early reduction of ventricular size allows re-activation of oligodendrocytes, as indicated by CGalT activity in the corpus callosum. (Funded by the P.H.T. Thorlakson and Manitoba Medical Services Foundations.)

190.2

EFFECTS OF VALPROIC ACID ON THE EXPRESSION OF NEURAL CELL ADHESION MOLECULE DURING MOUSE NEURAL TUBE CLOSURE. K.K. Terry*, S.L. Dial, R.D. Streck, and D.K. Hansen. Div. Reproductive & Developmental Toxicology; National Center for Toxicological Research, Jefferson, AR 72079.

During neurulation, neural cell adhesion molecule (N-CAM) plays an important role in neural tube closure. Valproic acid (VPA), an anticonvulsant drug, increases the incidence of neural tube defects in humans and elicits exencephaly in mice. This study examined quantitative changes in embryonic N-CAM expression following exposure to VPA. On gestation day (GD) 8, pregnant CD-1 mice were subcutaneously injected with saline or 600 mg VPA/kg. Embryos were examined for gross morphology on GD 9.25, and whole embryo heads were processed for Western blot analysis. Anterior neural tubes failed to close in 63 ± 8% (mean ± S.E.M.) of the VPA-treated conceptuses compared to 6 ± 5% of those exposed to saline. Preliminary Western blot analyses suggest that VPA induced no significant alterations in the overall amount of either 180 or 140 kD N-CAM isoforms in whole embryo heads. VPA may have effects on N-CAM expression in highly localized regions; therefore, concurrent *in situ* hybridization studies are underway to examine VPA-induced changes in the distribution of N-CAM transcripts within the closing neural tube. Supported by NCTR/FDA/DHHS.

190.3

CHRONOLOGICAL CHANGES OF CELL PROLIFERATION AND REGENERATION CAPACITY OF SURGICALLY INDUCED OPEN NEURAL TUBE DEFECTS IN CHICK EMBRYOS. K.C. Wang*, M.S.Lee, Y.J. Lee, K.B. Sim, J.G. Chi and B.K. Cho Div Ped Neurosurg & Ped Path, Seoul Natl Univ Sch of Med, Seoul 110-744, Korea

To investigate the correlation between the regeneration capacity of surgically induced neural tube defect and its cell proliferation activity, The chronological changes of the size of the NTDs and proliferating cell nuclear antigen (PCNA) positivity in norma developing chick embryos were correlated. Neural tubes were incised at Hamberger & Hamilton stage 17-20 at the wing bud level. The incision was of 3- or 6-somite length. The healing was active in early postoperative days. It gradually became less active until the postoperative day 10, when the curves reached plateau. The chronological changes of healing of NTD correlated well with those of PCNA positivity in normal chick embryos. Though these data are not conclusive in the causal relationship, high possibility of correlation between the two processes is suggested.

Partly supported by Seoul National University Hospital Research Grant.

190.4

ISCHEMIA-INDUCED OLIGODENDROGLIAL DAMAGE IN THE ONE-WEEK-OLD RAT PUP S.E. Jelinski, J.Y. Yager†, and B.H.J. Juurlink*. Departments of Anatomy & Cell Biology, Pediatrics† and the Saskatchewan Stroke Research Centre, University of Saskatchewan, Saskatoon, SK, CANADA, S7N 5E5.

Periventricular leukomalacia, a predominant antecedent of cerebral palsy, is the most common hypoxic-ischemic (HI) lesion of developing white matter in premature infants. A suggested pathogenesis is that the glial cells in the periventricular region are undergoing active differentiation resulting in an intrinsic vulnerability to an HI injury (Volpe, 1989). In vitro work has shown that oligodendroglial precursors cells are more readily damaged by oxidative stress brought on by hypoxia than are astrocytes (Husain and Juurlink, 1995). To examine the effects of an HI lesion on oligodendroglia in vivo, 1week-old rat pups, developmentally equivalent to premature infants born between 34 and 36 weeks of gestation, were subjected to a 10 min bilateral carotid ligation in the presence of 8% oxygen. Over the next 24 hr, during the reperfusion period, cells in the germinal zones and corpus callosum underwent pycnosis. Immunocytochemistry demonstrated that the affected cells bound O4 and/or BRDU antibodies, thus demonstrating the oligodendroglial nature of the cells. We suggest that cell death may have been caused by an inability to cope with oxidative stress since it was coincident with both lipid peroxidation, as demonstrated by the TBAR reaction, and induction of hsp32. We also suggest that the increased vulnerability of oligodendroglial precursors following an HI insult may contribute to the pathogenesis of periventricular leukomalacia. *Supported by the Heart and Stroke Foundation of Saskatchewan.*

190.5

A SINGLE EXPOSURE MODEL FOR ETHANOL-INDUCED TERATOGENESIS: CONVERGENT REGULATION OF MAPK? Margaret I. Davis[1] and William Shain[1,2] [1]University at Albany School of Public Health and [2]Wadsworth Center Albany, NY 12201.

Hippocampal damage can be caused by acute and chronic ethanol exposure during development. Wistar rat pups were given a single dose of ethanol (4.7 g/kg; 20% solution in normal saline) by intraperitoneal injection at 5 days of age (human third trimester equivalent) resulting in peak blood ethanol concentrations of 350 mg/dL 30 min after injection. One control group received isocaloric maltose and were isolated at 35°C for a period equivalent to the period of intoxication in experimental animals. A second control group remained with their dam. This exposure produced mild hyperactivity in males at 25 days of age. Ethanol can alter signal transduction through protein kinases and G proteins. Western blot was used to measure G protein levels. cAMP-dependent (PKA) and protein kinase C (PKC) activities were measured using kemptide and myelin-basic protein (4-14), respectively, as substrates. Mitogen-activated protein kinase phosphorylation state was detected by gel-shift. A single dose of ethanol produced biphasic changes in PKA activity. The initial increase peaked at 2 hours. $G_{\alpha s}$ levels were also increased in experimental animals 2 hours after exposure. A steady decline in PKC activity was observed 0-6 hours after exposure. MAPK (p42) phosphorylation was decreased in ethanol-exposed animals and increased in injection control animals. Western blot analysis using phosphotyrosine and phosphothreonine antibodies indicates that this decrease in phosphorylation occurs on both tyrosine and threonine residues. This decrease in phosphorylation is correlated with an increase in PKA activity ($r=-75$, $p<0.025$). These data indicate that binge-type exposure to ethanol may inhibit growth factor signaling through convergent regulation of MAPK by PKA and PKC. (Partially supported by RR-10957, AA-05381)

190.6

INCREASE OF POLISIALOGANGLIOSIDES BY NEUROTOXIC 2,4-DICHLOROPHENOXIACETIC ACID DURING CNS DEVELOPMENT. R.O. Duffard*, S. Rosso and A.M. Evangelista de Duffard. Lab. Toxicologia Experimental. Fac. de Cs. Bioquimicas y Farmacéuticas. Universidad Nacional de Rosario. República Argentina.

Phenoxyacetic acid, its esters and salts are herbicides widely used in Argentine and in the world. In the last years maternal exposure to environmental chemicals has increased and fetuses as well as neonates may be at greater risk than adults. There is concern that exposure to phenoxyherbicides would impliy toxicological risk, since teratogenic, embryotoxic and neurotoxic effects were observed. In order to determine whether the exposure to 2,4-Dichlorophenoxyacetic acid (2,4-D), during a specific period of the development, produce alterations in. DNA, Protein content and Gangliosides pattern, a neurochemical study was performed. Pups rat exposured to 2,4-D at different doses and times were used.There were neither body weight nor Protein changes at any level of 2,4-D or different period of treatment. However, a decrease brain weight and an increase of DNA brain content were observed in all the treated groups. An increase of total gangliosides and GT1b, GD1a and GD1b in 2,4-D treated pups was observed. These increases were, doses and exposure time dependent and would be an expression of neuronal plasticity after a chemical injury.
(Supported with grants from CONICET, Argentina)

190.7

FEW NEUROBEHAVIORAL ALTERATIONS RESULT FROM EARLY POSTNATAL LEAD (Pb) TREATMENT IN RATS. S.A. Ferguson*, R.J. Houston, P.H. Siitonen, R.A. Gazzara, R.R. Holson. National Center for Toxicological Research, Jefferson, AR 72079.

To investigate the neurobehavioral effects of low-level Pb treatment, Sprague-Dawley rats were treated with 300 ppm Pb or sodium acetate from birth to weaning via the dams' drinking water. Food and water intake were not altered by Pb treatment. Blood lead levels sampled from female offspring at weaning (postnatal {PND} 21) averaged 62 μg/dl for the Pb group. Offspring body weight through PND 120 was not affected by Pb treatment. At PND 21, whole brain weight was decreased $\approx$4% in female Pb offspring. At PND 119, there were no differences in whole brain weight; however, frontal cortex was $\approx$5% heavier in the Pb female offspring. Male offspring were assessed for play (PND 38 & 45), burrowing (PND 49-53), dominance (water competition test) (PND 58 & 65), activity (running wheel and Figure 8) (PND 68-82), maze (PND 83-94), and acoustic startle behavior (PND 98). There were no Pb-related differences on any behavior. Pb treatment may alter behaviors other than those assessed here, such as complex learning or attention/vigilance. Supported by DHHS/FDA/NCTR.

190.8

INFANTILE POSTURAL ASYMMETRY IN RATS γ-IRRADIATED *IN UTERO*. A. Gigi, M. Mintz, S. Ben-Eliyahu, & M. Myslobodsky* Psychobiology Res. Unit, Dept. of Psychology, Tel-Aviv Univ., Ramat Aviv, 69978 Israel

Pregnant Sprague-Dawley rats were exposed on days 15, 17, and 19 of gestation (E15, E17, and E19) to a single dose of γ-radiation (dose - 1.5 Gy, a dose rate of 0.15 Gy/min; 6040 Ci ^{60}Co source). The present study is based on 603 pups, 369 exposed and 234 sham-irradiated controls. At Day 2, animals were tested for head-to-tail position using a procedure modeled after Ross et al. (*Proc. Natl. Acad. Sci. USA*, 78:1958-1961, 1981). In a sample of control and exposed pups ($n = 603$), axial asymmetry was encountered in 68.5% of cases ($\chi 2 = 82.47$; $df = 1$; $p< 0.001$). This result was contributed by E17, and to a lesser extent by E19 pups, who manifested maximal rates of axial asymmetry (79% and 72%, respectively). By contrast, among E15, asymmetry was encountered in 63% ($\chi 2 = 7.42$; $df = 2$; $p = 0.02$). Sham-irradiated pups showed axial imbalance in 66% which was not significant from the rate of exposed pups ($\chi 2 = 1.27$; $df = 1$; $p< 0.26$). The role of gender was explored, but found not to play a statistically significant role. Likewise, no side "preference" appeared significant in either of the exposed or sham-irradiated groups. In summary, prenatal exposure caused no sharpening of the directionality of axial asymmetry. Only when all exposed and control asymmetry data were pooled, a slight (58.1%) and significant ($\chi 2 = 10.87$; $df = 1$; $p = 0.02$) left-side imbalance emerged. **Supported by Theodore and Vada Stanley Foundation Research Award*

190.9

STRESS AND IMMUNE MEASURES IN RATS GAMMA-IRRADIATED IN UTERO. S. Ben-Eliyahu*, A. Gigi, M. Mintz, E. Rosenne and M. Myslobodsky. Psychobiology Res. Unit, Dept. of Psychology, Tel-Aviv Univ., Ramat Aviv, 69978 Israel.

Pregnant Sprague-Dawley rats were exposed to a single dose of gamma-radiation (1.5 Gy, a dose rate of 0.15 Gy/min; 6040 Ci 60Co source) on days 15, 17, or 19 of gestation (E15, E17, and E19), or were sham irradiated. We assessed possible effects of irradiation on baseline and stress levels of two indices measured in the offspring: a) activity of the hypothalamus pituitary adrenal (HPA) axis, and b) resistance to the development of experimentally induced MADB106 tumor metastasis. This latter measure depends on natural killer (NK) cell activity and is markedly affected by activation of the sympathetic nervous system (SNS). The results indicated no differences in corticosterone levels at baseline and following 15 or 60 minutes of restraint stress in both 30 and 60 days old offspring. On the other hand, irradiation reduced the susceptibility to tumor metastasis in E15, but increased it in E17 and E19 in rats tested at the age of 2-3 month. Following 16 min of intermittent white-noise stress (3 burst/min, 40 mesc122 dB), control and all groups of irradiated adults displayed similar levels of significant increase in tumor metastasis. Compared to adults, prepubescent offspring (30 days old) demonstrated a 10-fold increase in tumor metastasis, but displayed no effects of irradiation or noise stress. This high tumor susceptibility of prepubescent rats may be related to low levels of NK activity found in our previous studies in such rats. The lack of effects of stress and irradiation in prepubescent rats may reflect delayed impact of irradiation, immature SNS, or a ceiling effect at this high level of tumor metastasis. Supported by Theodore and Vada Stanley Foundation Research Award.

190.10

CORRECTION OF NEUROCHEMICAL ABNORMALITIES IN THE ORNITHINE TRANSCARBAMYLASE (OTC) DEFICIENT SPARSE FUR (Spf/y) MOUSE GENE THERAPY. C.S. Pabin, X. Ye, M.L. Batshaw*, J.M. Wilson, and M.B. Robinson. Children's Seashore House, Depts. of Ped., Pharm., and Mol. and Cell. Eng., Univ. of Penn., Philadelphia, PA 19104.

OTC deficiency is an X-linked disorder of urea synthesis which is associated with elevations in plasma ammonium (NH_4^+) and resultant mental retardation. The neurodevelopmental disabilities are associated with increases in brain glutamine (Gln) and tryptophan (Trp). We have recently shown *in vivo* correction of the peripheral biochemical abnormalities in OTC deficient spf/y miceusing gene transfer with an adenoviral vector containing the OTC cDNA and deleted in E1 with a ts125 mutation in E2a. To determine if this approach also corrects the neurochemical abnormalities, animals were injectedby tail vein with the adenoviral vector ($1x10^{11}$ particles) and 14 days later brain tissue was harvested for analysis. Cortical Gln levels in untreated Spf/y mice were 9.4 ± 3.6 compared to 4.8 ± 1.2 (nmole/mg tissue) in control C3H mice. Gene transfer resulted in complete correction of this abnormality in the Spf/y mice (4.8 ± 0.8). Cortical Trp levels were partially corrected in OTC-treated mice (31.8 ± 6.3 pmoles/mg tissue) compared to C3H and Spf/y controls (21.8 ± 6.5 and 43.2 ± 8.3 pmoles/mg tissue, respectively). These data suggest that gene therapy may have the potential to correct neurochemical as well as metabolic abnormalities. (HD32649)

190.11

Neural Progenitor Transplantation into Newborn *Reeler* Cerebellum May Rescue Certain Aspects of Mutant Cytoarchitecture, K.I. Auguste[1], K. Nakajima[2], T. Miyata[2,3], M. Ogawa[3], K. Mikoshiba[2,4], & E.Y. Snyder[1*]. [1]Depts. Neur. & Pediatr., Children's Hosp., Harvard Med.Sch., Boston, MA 02115, USA, [2]Mol. Neurobiol. Lab, RIKEN, 305 Ibaraki, [3]Dept. Physiol., Kochi Med. Sch., Kochi 783, [4]Dept. Mol. Neurobiol., IMSUT, Tokyo 108, JAPAN.

Reeler, a recessive mutation in mice, results in tremors, impaired motor coordination and ataxia. The *reeler* mouse displays cytoarchitectonic abnormalities in the cerebral and cerebellar cortices, as well as other laminated regions of the brain. The *reelin* gene has recently been cloned and its absence has been associated with the pathology and phenotype associated with the *reeler* mutation. However, it remains unproven whether the absence of *reelin* is sufficient to account for the *reeler* phenotype. Complementation studies would help resolve this question. By supplying the developing homozygote *reeler* cerebellum with an exogenous source of *reelin*, rescue of this phenotype might be possible if its absence alone accounted for the pathology. We sought to test this hypothesis through transplantation studies. The *reelin* protein is presumably expressed by wild-type cerebellar cells, in particular the multipotent neural progenitor C17-2 cell line, initially derived from the external granular layer (EGL) of normal newborn mouse cerebellum. Following transplantation of C17-2 cells into the EGL of the developing cerebellum of newborn *reeler* mutants, granule cell migration and layering as well as granule cell survival (of both host and donor cells) appeared to improve. Purkinje cell layering also seemed to be subtly improved. (The efficiency of *reelin* expression by C17-2 cells *in vitro* and *in vivo* is presently being assessed using the recently generated CR-50 monoclonal antibody, which was generated by immunizing *reeler* mice with normal embryonic brains and is likely to recognize a *reelin* protein itself.) These suggestions of successful complementation help support the role of *reelin* in promoting normal lamination and the sufficiency of *reelin's* absence for engendering the reeler histogenetic phenotype. These findings may also suggest a strategy for the treatment of CNS diseases characterized by abnormal cellular migration, lamination and cytoarchitectural arrangement.

190.12

PURSUIT INITIATION IN DYSLEXIC CHILDREN WW Ting[1]*& SJ Heinen[2], Northwestern University Medical School, Chicago, IL[1] and the Smith-Kettlewell Eye Research Institute, San Francisco, CA[2].

Previous results concerning smooth pursuit eye movements of dyslexics have been mixed. We set out to determine if predictive smooth pursuit could provide a more sensitive indicator of dyslexia in children. Eye movements of two dyslexic and two normal children were measured using a dual-Purkinje-image eyetracker. The head was stabilized with a chin-rest and a head-rest. Ss first fixated, and then tracked a small bright spot that moved either left or right at 20°/s. The fixation period was either always 1000 ms (predictable), or random at 500 or 1000 ms (unpredictable). Saccades were removed from the velocity records using an acceleration criterion, and analyzed separately. Consistent with other studies, dyslexics exhibited more saccades during smooth pursuit in all conditions than did controls. However, dyslexics were more prone to make either smooth or saccadic eye movements *opposite* the direction of target motion at pursuit initiation. We defined reverse tracking intrusion (RTI) as a smooth or saccadic movement opposite target motion that occurred within ±50 ms of when the eye started to follow the spot. RTI was evident in 33.3% and 35.3% of trials for each of the dyslexic children , compared to 7.5% and 9.5% for normals. In the 500/1000 ms condition, normal Ss (and adult normals also) were "primed" by the 500 ms condition, i.e., they show as good as or better anticipation for 1000 ms as in totally predictable trials. However, the dyslexics did not show this trend. Finally, early initial eye acceleration when target motion was totally predictable (10-50 ms after pursuit onset) was greater than later eye acceleration (70-110 ms after pursuit onset) in dyslexics but not in controls. The results suggest that dyslexic children are more uncertain about initiating smooth pursuit, and cannot rely as easily on internal signals that specify when predictable object motion will occur; rather they depend more on the visible motion of the target to accelerate the eyes.

Supported by The Smith-Kettlewell Eye Research Institute.

190.13

DEVELOPMENTAL DYSLEXIA AND INCREASED CELL PACKING DENSITY IN LEFT TEMPORAL CORTEX: A CASE STUDY. D. L. Kigar*[1], S. F. Witelson[1] and I. I. Glezer[2]. [1]Dept. of Psychiatry, McMaster Univ., Hamilton, ON, L8N 3Z5; [2]Dept. Cell Biol. Anat. Sci., CUNY Med. School, N.Y.C.

The brain specimen of a 62-year-old man with documented difficulty in learning to read and write from Grade 1 on was studied. In spite of years of remedial education, good intelligence and financial success, he never learned to read or write. He was consistently right-handed (CRH). Death was due to non-neurological cancer. Brain weight and overall morphology were well within normal limits. Study was focused on gross and microscopic aspects of the planum temporale (PT) in the posterior region of the superior temporal gyrus which, on the left side, is part of the posterior language region. Left and right PT areas were within normal limits for both horizontal and vertical components of PT compared to a control group of 13 CRH males (Witelson & Kigar, *Soc NSc Abstr*, 1991). Hand preference was controlled for because Sylvian fissure and consequently PT anatomy are different in CRH vs nonCRH men. The pattern and degree of asymmetry were also typical (L > R). These results are similar to an MRI study of PT length in dyslexics (Leonard *et al., Arch Neurol*, 1993), but different from a postmortem study of optically reconstructed PT areas (Galaburda *et al., Ann Neurol*, 1985). Microscopic assessment revealed no evidence of ectopic cells or cytoarchitectonic dysplasia (of Galaburda *et al.*). Numerical density of neurons (N_v) was obtained for von Economo's cytoarchitectonic area TA_1 and compared to our sample of 4 CRH men with no reading difficulty (Witelson, Glezer & Kigar, *J NSc*, 1995). Mean cortical depth in each hemisphere was similar to that of the control group. N_v for the left hemisphere of the dyslexic case was more than 5 S.D. ($p < .05$) greater than the controls (57,909 vs 42,299, respectively). N_v values for the right hemisphere were comparable to the control group. Since PT morphology and neuron number are established early in life, these results support a neurobiological factor in the etiology of developmental dyslexia. Supported in part by grants NS18954 and MRC (CA) MA-10610.

190.14

TACTILE PERCEPTION IN DEVELOPMENTAL DYSLEXIA

A. C. Grant*, A. Zangaladze & K. Sathian

Dept. of Neurology, Emory Univ. Sch. Med., Atlanta, GA 30322.

Abnormal processing of visual and auditory stimuli has been reported in developmental dyslexia (e.g. Ann. N.Y. Acad. Sci., vol. 682, 1993). It has also been suggested that similar abnormalities involve many other aspects of neural function, including the tactile system. We therefore investigated, psychophysically, tactile perception at the index fingerpad in individuals with developmental dyslexia. We used gratings of alternating ridges and grooves, which have proven useful in psychophysical and neurophysiological characterization of the tactile system (Sathian, TINS, 12:513-519, 1989).

We assessed discrimination thresholds, corresponding to 75% correct performance, in two-alternative, forced-choice paradigms. In one task, spatial acuity was measured as the groove width required to discriminate the orientation of gratings impressed statically into the immobilized fingerpad. Many but not all dyslexic subjects performed poorly on this task, compared to normals. Another task assessed subjects' ability to discriminate gratings differing in ridge width, that were scanned actively. Thresholds of dyslexic subjects were in the upper part of the normal range on this task. Thus, abnormal sensory processing in dyslexia may not be exclusively in the temporal domain. *Supported in part by a grant from the Orton Dyslexia Society.*

190.15

NEURONAL SIZE IS ALTERED IN THE VENTROBASAL COMPLEX OF NEWBORN MICE WITH CORTICAL ECTOPIAS A. R. Jenner*, A. M. Galaburda, and G. F. Sherman, Dyslexia Research Laboratory, Beth Israel Hospital; Harvard Medical School, Boston, MA 02215

Smaller cells in the LGN and MGN may play a role in the perceptual deficits seen in dyslexics. These thalamic changes may originate from neocortical layer I ectopias (Livingstone at al., *PNAS*, 88:7943, 1991; Galaburda et al., *PNAS*, 91:8010, 1994). Animal models have been used to test this correlation between thalamic changes and ectopias. For example, adult male New Zealand Black mice (NZB/BINJ) with barrel field ectopias have fewer small neurons and more large neurons in the ventrobasal complex (VB) than male mice without ectopias (Sherman et al., *Soc. Neurosci. Abstr.*, 21:1712,1995). The present study examined neuronal size in VB of newborn NZB mice with and without ectopias to determine when thalamic changes are visible.

After immersion fixation in 4% paraformaldehyde and cryo-protection with sucrose, the brains of newborn (P0) NZB mice were cut coronally on a sliding freezing microtome at 30µm, mounted, dehydrated, and stained with thionin. Two sections (150µm apart) containing both VPM and VPL nuclei were selected from 14 ectopic and 16 non-ectopic mice. Cross sectional neuronal areas were measured in two fields of each nuclei in each hemisphere using a stereology-derived system (Williams and Rakic, *J. Comp. Neurol.*, 278:334, 1988). The cells were grouped into 10 consecutive bins increasing by approximately $7\mu m^2$. As in the adult, the cell size distributions at P0 differed between mice with and without ectopias. However, at P0 ectopic male mice had fewer large neurons and more small neurons in both VPM (χ^2=122.78, df=9, p<0.001) and VPL (χ^2=112.22, df=9, p<0.001). This difference was the most pronounced in the VPM nuclei ipsilateral to the ectopia (χ^2=20.02, df=9, p<0.05). Unlike VPL this nucleus directly connects with the barrel field (Jenner et al., *Soc. Neurosci. Abstr.*, 21:1712, 1995). These findings show that the prenatal onset of ectopias in somatosensory cortex affects the organization of related thalamic nuclei early in development and further more indicates that this effect changes during development. (Supported by NICHD grant HD20806)

190.16

CEREBROCORTICAL MICRODYSGENESIS IS ENHANCED IN C57BL/6J MICE EXPOSED *IN UTERO* TO ACETAZOLAMIDE. G.F. Sherman* and L.B. Holmes, Dyslexia Research Laboratory, Beth Israel Hospital; Genetics and Teratology Unit, Massachusetts General Hospital; and Departments of Neurology and Pediatrics, Harvard Medical School, Boston, MA 02215.

C57BL/6J mice spontaneously develop collections of neurons in neocortical layer I (Sherman et al, *Acta Neuropathol*, 74:239-242, 1987) similar to the "ectopias" seen in 40-50% of NZB and BXSB autoimmune mice (Sherman et al, *Brain Res*, 532:25-33, 1990). In the autoimmune strains, ectopias are present before birth, probably occur because of a breach in the pial-glial membrane during neuronal migration, and is a recessively inherited trait (Sherman et al, *NeuroReport*, 5:721-724, 1994). The present study was designed to determine whether environmental factors can alter the expression of the ectopia trait. Because carbonic anhydrase may play a role in glial development, we prenatally exposed C57BL/6J mice to acetazolamide, a carbonic anhydrase-specific inhibitor and teratogen, to determine whether the prevalence or severity of ectopias is affected.

C57BL/6J time-mated mice were injected i.p. on embryonic day 9 (plug date=E0) with sodium acetazolamide (750 mg/kg maternal weight) and control mice were injected with water (0.2 ml). On day 30 the mice were transcardially perfused with 0.9% saline followed by 10% formalin. The brains of 105 acetazolamide-exposed and 89 control mice were embedded in celloidin, cut in 30µm coronal sections, and stained with cresyl violet. The presence and size (based on an estimate of the number of neurons in layer I) of ectopias were noted under a light microscope .

There was no difference in the overall prevalence of cerebrocortical ectopias between the acetazolamide and control groups: 34% of the acetazolamide and 28% of the control mice had ectopias. Most were present in the prefrontal, motor, or somatosensory areas. There was a striking difference between the control and acetazolamide group in ectopia shape and size: 69% of the ectopias were large in the acetazolamide group, whereas only 32% of the ectopias in controls were (χ^2=6.89, df=1, p<0.01). Although most affected brains contained one ectopia, the acetazolamide group had more brains with multiple ectopias (36% vs 8%; χ^2=4.86, df=1, p<0.05). Thus, there may be a genetic predisposition for developing ectopias in this strain, however, epigenetic factors such as prenatal acetazolamide-exposure, can affect the severity of the malformations. Supported by NIH grant HD 20806.

190.17

EFFERENT AND AFFERENT CONNECTIVITY OF INDUCED NEOCORTICAL MICROGYRIA. G.D. Rosen* and A.M. Galaburda, Beth Israel Hospital and Harvard Medical School, Boston, MA 02215.

Cerebrocortical microgyria in humans is the result of injury during late neocortical neuronal migration, and microgyria can be induced in otherwise normal rodent neocortex by focal damage to the developing cortical plate (Humphreys et al., *J. Neuropath. Exp. Neurol.* **50**:145, 1991).Disorders of late neuronal migration are seen in developmental dyslexia (Galaburda et al., *Ann Neurol* **18**:222, 1985), and similar induced and spontaneous malformations in rats and mice are associated with a variety of behavioral alterations (e.g., Schrott et al., *Physiol Behav* **52**:1085, 1992; Fitch et al., *Cereb Cortex* **4**:260, 1994). It was hypothesized that these small focal malformations may cause connectional reorganization which might explain the widespread behavioral disturbances associated with them.

We induced microgyria in the somatosensory cortex (Par1) of the right hemisphere by placing a freezing probe on the skulls of rats on the first day of life for 10 s. For each lesioned animal, sham surgery was performed on another animal. In adulthood, some rats received biotinylated dextran amine (BDA) injections into the microgyria, while others were injected in the homologous region of the left hemisphere. Their matched shams received identically placed injections. The brains were subsequently processed for BDA visualization and the retrograde and anterograde labeling noted.

In comparison to controls, there was a decrease in efferent projections from the microgyric cortex to the left hemisphere. Par1 microgyria had abnormal efferent connections to secondary somatosensory cortex (Par2) of the left hemisphere. Although cortico-thalamic projections appeared normal, thalamo-cortical projections were markedly decreased. Injection of BDA into the homologous area of the left hemisphere highlighted aberrant projections into frontal and Par2 cortex of the affected hemisphere. The results support the hypothesis that early focal injury to the neocortex results in restructuring of connectivity.(Supported, in part, by HD20806).

190.18

VISUALIZATION OF SMALL FOCAL DISORDERS OF LATE NEURONAL MIGRATION IN THE CEREBRAL CORTEX OF THE RAT BY MAGNETIC RESONANCE IMAGING (MRI). A.M. Galaburda*, D. Burstein, and G.D. Rosen, Departments of Neurology and Radiology, Beth Israel Hospital and Harvard Medical School, Boston, MA 02215.

Disorders of late neuronal migration are associated with a wide variety of neurologic disorders. Magnetic resonance imaging (MRI) has proven useful in the visualization of some of the more severe anatomic disturbances (e.g., Kuzniecky, *Epilepsia* **35**:S44, 1994), but has yet to be successfully used to visualize the more restricted anomalies, such as focal microgyria and molecular layer ectopias, that are seen in conjunction with certain types of epilepsy and developmental dyslexia. By exploiting an animal model for these neocortical malformations, the current experiment sought to determine if these lesions could be visualized with MRI.

We induced focal malformations unilaterally by placing a freezing probe on the skulls of rats on the first day of life (P1) for 5-10 seconds. In adulthood, these rats were anesthetized and placed in a 4.7 T 30 cm bore small animal MRI. A sagittal scout image was obtained in 30 seconds, from which of a multislice coronal data set was set up with an in-plane resolution of 250 µm and a section thickness of 500 µm. After completion of the MRI studies, the location and extent of damage was assessed histologically.

Using a T2 weighted imaging sequence, we were able to detect a lesion in each animal imaged, ranging from a large porencephalic cyst to a relatively small region of microgyric cortex. The speed of image acquisition (<30 minutes) and the tight correlation with subsequent histology supports the use of MRI techniques for the visualization of minor focal neocortical malformations *in vivo*, permitting for the first time longitudinal assessment following interventions. Ongoing research is aimed at imaging smaller developmental neocortical malformation and extending these techniques to humans. (Supported, in part, by HD20806).

190.19

RADIAL-ARM MAZE LEARNING IN BXSB MICE: EFFECTS OF NEOCORTICAL ECTOPIAS. L. A. Hyde*, G.F. Sherman and V. H. Denenberg. Biobehavioral Sciences Graduate Degree Program, University of Connecticut, Storrs, CT 06269; Beth Israel Hospital and Harvard Medical School, Boston, MA 02215.

Learning behavior in the autoimmune BXSB mouse has been extensively studied in our laboratory. A major reason is that approximately 40-60% of these mice have neocortical ectopias, which are clusters of neurons abnormally located in layer 1 of cortex. Ectopias are formed prenatally, as early as E15, and are usually located in the prefrontal/motor region in the BXSB strain. This area of cortex has been shown to be important for working memory. One of our recent findings is that ectopic BXSB mice appear to be deficient in spatial working memory. However, we did not test the mice in a radial-arm maze, which is generally accepted as one of the better measures of working memory. We have recently developed a water version of the radial-arm maze and have tested a group of BXSB mice in the maze.

Mice were given one session per day for 11 days following 1 day of training. Ectopic mice exhibited more working memory errors in sessions 7-11 than non-ectopics, with the working memory errors of the non-ectopics dropping to low levels, while ectopics continued to make working memory errors through the end of testing. The two groups did not differ on the number of reference memory errors. These findings lend further support to our hypothesis that ectopic BXSB mice are impaired in working memory tasks. Supported in part by NIH grant H-20806.

191.1

RESPONSES TO AMYLOID BETA PEPTIDES IN XENOPUS OOCYTES INJECTED WITH BRAIN RNA. R. Blitzer, T. Wong, M. Pangalos, N.K. Robakis, A.J. Serby, and E.M. Landau*. Dept. of Psychiatry, Mt. Sinai School of Medicine and Bronx VAMC, New York NY 10029

To determine whether receptors for amyloid beta peptides (Aβ's) might be present in brain, Xenopus oocytes were used to express whole-brain RNA derived from adult rats. Two membrane responses were studied, both of which are produced by receptors coupled to the phosphatidyl inositol (PI) pathway and reflect intracellular Ca^{2+} release: (1) at V_H = -70 mV, an inward, fluctuating Cl^- current, and (2) the enhancement of T_{out-2}, an outward Cl^- current evoked by a voltage step from -100 to 0 mV. Responses were observed using Aβ(1-40) and Aβ(1-42) in RNA-injected oocytes, but not in uninjected cells or RNA-injected cells exposed to either Aβ(25-35) or the reverse peptide Aβ(40-1). The enhancement of T_{out-2} was the more sensitive response, with a K_D for Aβ(1-40) of 26 nM. Freshly-dissolved peptide was fully effective, indicating that expressed membrane receptors may recognize monomeric Aβ peptides. Both responses were intact in the presence of spantide and were therefore not mediated by substance P receptors, in contrast to earlier findings (*PNAS* 90, 7508-12, 1993).

These results suggest that specific Aβ receptors may be present in brain. The activation of the PI pathway by such receptors may play a role in neurotoxic and neuroprotective effects of Aβ's via the IP_3 and PKC pathways, respectively.

Supported by the Tow Foundation.

191.2

SOLUBLE Aß FORMS STABLE DIMERS AT LOW CONCENTRATIONS. W. Garzon,[1] M. Sepulveda,[2] S. Milton[3] and C. G. Glabe,[3]*. [1]Univ. Nacional Autonoma de Mexico, Facultad de Quimica, [2]Centro de Invest. Biomedica del Sur Xochitepex Mor IMSS, [3]Dept. of Mol. Biol and Biochem. Univ. of Calif. Irvine CA 92717.

Previously published data indicates that the smallest Aß1-40 species in aqueous solution elutes from a Superdex HR75 column with an apparent MW of a dimer. This data alone does not permit identification of this peak as a dimer because of uncertainties about the shape of Aß in solution that can influence its elution behavior. We have utilized Fluorescence Resonance Energy Transfer (FRET) to study Aß dimer formation in solution. We synthesized a series of fluorescent variants of Aß to be used as a donors and acceptors in FRET experiments. We compared the aggregation of the fluorescent derivatives to wild type Aß at pH 7.4, where Aß is largely soluble and at pH 5.0 and pH 7.4 in the presence of 10 mM Zn^{++}, where Aß is largely aggregated. We chose fluorescent peptides that displayed the same aggregation behavior as wild type Aß. Using several different donor-acceptor pairs, we confirmed that Aß is well solvated and largely monomeric in DMSO. When donor and acceptor Aß derivatives are mixed together in DMSO and then diluted into aqueous Tris buffer at pH 7.4 (10 uM total Aß), efficient FRET is observed immediately, indicating that a donor-acceptor dimer exists in solution. The observed efficiency of the energy transfer varied from 0.17 to 0.1 depending on the nature and location of the dyes. Gel filtration confirms that the fluorescent peptide mixture elutes at the same dimeric position as wild type Aß. Sub-uM concentrations of ^{14}C-labeled Aß1-40 also elute as a dimer. When homodimers of both donor and acceptor species are mixed at pH 7.4, no FRET is observed initially and after 24 hr incubation, indicating that the dimers are relatively stable once formed. Supported by NIH NS-31230.

191.3

APPICAN, THE PROTEOGLYCAN FORM OF AMYLOID PRECURSOR PROTEIN, PROMOTES NEURAL CELL ADHESION TO THE EXTRACELLUAR MATRIX. A.Wu*, M.N.Pangalos, S.Efthimiopoulos, J.Shioi, and N.K.Robakis. Department of Psychiatry and Fishberg Research Center for Neurobiology, Mount Sinai School of Medicine, New York, NY 10029-6574.

Appicans are secreted and cell-associated brain chondroitin sulfate proteoglycans produced by glia cells and containing Alzheimer's amyloid precursor (APP) as their core proteins. Rat C6 glioma cells transfected with appican displayed a dramatic change in their phenotypic appearance compared to control cells (non-transfected C6 cells or C6 cells transfected with APP). Appican-transfected cells lost the round shape of the control C6 cells, acquired a flat appearance, and elaborated more processes than control cells. Control cells were completely dissociated after 40 min of treatment with a cell-dissociation solution, while only 20% of the appican-transfected cells were detached. Extracellular matrix (ECM) prepared from appican-transfected cells contained high level of appican and was a significantly better substrate for the attachment of C6 cells than ECM prepared from control cells. Furthermore, cell adhesion to ECM was independent of the level of appican expression of the plated C6 cells. ECM prepared from appican-transfected C6 cultures stimulated adhesion of other neural cells including rat primary astrocytes, mouse N2a neuroblastoma and rat PC12 pheochromocytoma, but not fibroblast cells. Conditioned medium from appican-transfected C6 cells failed to promote cell adhesion. These results suggest that secreted appican incorporates into ECM and promotes adhesion of neural cells.

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191.4

FACSCAN ASSAY FOR Aß RECEPTORS: SATURABLE BINDING TO CELL SURFACE PROTEINS S. Bodovitz*, M.P. Lambert, A.K. Barlow, J. Lin, G.A. Krafft, and W.L. Klein Neurobiology & Physiology, Northwestern Univ., Evanston, IL 60208

Aß is a 39-43 amino acid peptide that accumulates as extracellular aggregates in Alzheimer's disease-afflicted brain tissue. Contact between synthetic aggregates and neurons is cytotoxic, although little is known about the nature of this interaction. We have investigated the binding of synthetic aggregates to the surface of intact CNS neuroblastoma cells using whole mount electron microscopy and flow cytometry. Electron microscopy revealed that aggregates bound to neurites and growth cones and were occasionally associated with actin stress fibers and/or local dystrophy. Binding to cells appeared to be predominately via cell surface fibrils and, even at high doses of aggregates, the cell surface was largely uncovered. Flow cytometry verified that binding to intact cells was saturable. Half maximal binding occurred at approximately 1-2 μM and plateaued at approximately 5μM. Binding could be selectively inhibited by glycosaminoglycans, including heparan, heparin sulfate, and chondroitin sulfate, but not by general soluble proteins such as bovine serum albumin or fetal calf serum. Gentle trypsinization completely blocked binding. This demonstrates that binding is exclusively to cell surface proteins and, because trypsinization is a selective proteolysis, that aggregates bind to a subset of cell surface proteins. Following trypsinization of the cells, binding began to recover in 30 minutes and was completely recovered after 90 minutes. The recovery was not blocked by the protein synthesis inhibitor cycloheximide, indicating a reserve of binding proteins. Pre-treatment with cycloheximide, however, in order to measure turnover of binding proteins, resulted in a 40% decrease in binding after 24 hours. The long half-life plus rapid recovery suggested that there was a large pool of binding proteins. Nonetheless, binding could be blocked with relatively low doses of either secreted proteins or cell surface polypeptides released by trypsinization. Isolation and identification of these potent inhibitors is currently under investigation.

Supported by grants to W.L.K from N.I.H. and the Alzheimer's Association.

191.5

INHIBITION OF NEURONAL CELL ADHESION TO FIBRONECTIN: A RAPID INDICATOR OF NEURONAL CELL INTERACTION WITH AMYLOID ß-PEPTIDE A.K. Barlow, W.S. Wade, H. Yu, G.A. Krafft*, & W.L. Klein Neurobiology & Physiology, Northwestern Univ., Evanston, IL 60208

Accumulating evidence implicates amyloid β-peptide (Aβ) as an important neurotoxic factor in the development and progression of Alzheimer's disease (AD). Association of toxic forms of Aβ with the neuronal cell surface is likely to be involved in the initial stages of neurotoxicity and as such provides an early opportunity for therapeutic intervention. We have therefore focused on cell-surface interactions with Aβ. Several studies have shown that aggregated Aβ prevents cell adhesion and neurite extension; Aβ contains putative integrin recognition site and HSPG binding domain which may interact with cellular adhesion receptors. We have developed a quantitative cell adhesion assay to determine the effect of aged $Aβ_{(1-42)}$ on human SH-SY5Y and rat B103 cell adhesion to fibronectin (Fn). Both cell lines show reduced adhesion to Fn in the presence of $Aβ_{(1-42)}$. The effect is dose-dependent and rapid; anti-adhesive effects are quantifiable within 2 h, establishing this as a rapid *in vitro* neuronal response to Aß, well upstream of neurotoxicity. No anti-adhesive effect was observed when non-amyloid peptides or non-aggregating $Aβ_{(1-42)}$ variants were used. The anti-adhesive effect of $Aβ_{(1-42)}$ in both cell types was markedly reduced when polylysine was used as substrate, indicating specificity of the effect of $Aβ_{(1-42)}$. Removal of putative HSPG and integrin binding sites from the $Aβ_{(1-42)}$ sequence did not prevent anti-adhesive effects, indicating that aged $Aβ_{(1-42)}$ does not impact directly on these cell surface receptors. We examined several compounds for their ability to protect against the anti-adhesive phenomenon; G-protein agonists Mas-7 and compound 48/80 increased SH-SY5Y cell adhesion in the absence of $Aβ_{(1-42)}$ and protected against $Aβ_{(1-42)}$ effects. Similar effects were obtained using pertussis toxin and cAMP or Forskolin. Compounds which impact cellular signaling molecules therefore can prevent the anti-adhesive effects of $Aβ_{(1-42)}$, suggesting that disadhesion may result in part from $Aβ_{(1-42)}$ induced signaling. We have demonstrated that $Aβ_{(1-42)}$ is capable of inducing Fak tyrosine phosphorylation and paxillin translocation in B103 cells (Zhang 1994, 1996, Berg 1996). Anti-adhesion may be a rapid indicator of Aβ-induced signaling and as such allow for screening of compounds able to prevent Aβ-mediated impact on neuronal cells.

Work supported by grants from NIH to GAK and Buehler Center on Aging to AKB

191.6

RAPID IMPACT OF THE ALZHEIMER'S Aß PEPTIDE ON FOCAL ADHESION SIGNALING PROTEINS M.M. Berg and W.L. Klein*. Dept. Neurobiology and Physiology, Northwestern Univ., Evanston, IL 60208.

This laboratory has previously established that treatment of nerve cell lines with aggregates of the Alzheimer's Aß peptide induces rapid membrane blebbing and disadhesion, followed by tyrosine phosphorylation of focal adhesion kinase (FAK), and cell death. Here we have further investigated the molecular mechanism(s) by which Aß may exert its effects on cell adhesion, morphology and viability. In immunoblot experiments, aggregates of Aß were found to induce the association of FAK, as well as the focal adhesion protein paxillin with the triton-insoluble cytoskeleton of B103 cells. Paxillin exhibited a 10-fold increase in cytoskeletal association after treatment with Aß, while FAK association with the cytoskeletal fraction increased 2-10 fold. The paxillin and FAK responses were relatively rapid, nearly maximal at 1 hour. The response was also specific, as the focal adhesion protein vinculin showed no increase in cytoskeletal association after treatment with Aß. In subsequent experiments, it was determined that only cytoskeletal-associated paxillin was phosphorylated on tyrosine. The rapid and specific effects of Aß on focal adhesion signaling proteins illustrates a novel mechanism by which Aß may impact processes linked to neuronal cytoskeletal architecture, synaptic plasticity, gene expression and cell viability.

Supported by grants from the NIH (to MMB and WLK), and the Alzheimer's Association and Boothroyd Foundation (to WLK)

191.7

MUSCARINIC M3 RECEPTOR-MEDIATED AMYLOID PRECURSOR PROTEIN (APP) PROCESSING IS CORRELATED WITH TYROSINE PHOSPHORYLATION OF PAXILLIN B.E. Slack*, Dept. of Pathology and Laboratory Medicine, Boston Univ. School of Medicine, Boston MA 02118.

Secretory processing of APP was examined in human embryonic kidney (HEK) cells expressing m3 muscarinic receptors. Activation of these receptors by the muscarinic agonist carbachol stimulates release of soluble APP derivatives (APPs) into the medium (Nitsch et al., Science 258:304, 1992) via a mechanism that exhibits both protein kinase C- and tyrosine phosphorylation-dependent components (Slack et al., JBC 270:8337,1995). In addition to stimulating APPs release, carbachol increased tyrosine phosphorylation of several proteins in HEK m3 cells. Two of these were identified as paxillin and focal adhesion kinase (FAK). Both proteins are components of focal adhesions (complexes of cytoskeletal-associated proteins that form at sites of attachment of cells to extracellular matrix) and both are known to exhibit increased tyrosine phosphorylation concomitant with focal adhesion formation. Tyrosine phosphorylation of paxillin reached maximal levels 5 to 10 minutes after exposure to carbachol, and remained elevated for at least 15 minutes. The increase in APPs release induced by carbachol was previously shown to exhibit a somewhat slower time-course, reaching a maximum within 30 minutes. Tyrosine kinase inhibitors caused concomitant reductions in APPs release and tyrosine phosphorylation of paxillin. In view of prior evidence that APPs release from adherent cells is greater than that from poorly-adherent cells (Mönning et al., JBC 270:7104,1995), a possible causal relationship between the formation of focal adhesions during cell attachment, and increased secretory processing of APP is proposed. (Supported by NIH grant NS-30791)

191.8

NEURONAL LOCALIZATION OF COMPONENTS OF CLASSICAL COMPLEMENT CASCADE IN ALZHEIMER'S DISEASE BRAIN. G. Bao*[1], R. Li[1], P. McGeer[2], and Y. Shen[1]
1Departments of Psychiatry & Medicine, University of Louisville School of Medicine, Louisville, Kentucky 40292, USA. 2Kinsmen Lab of Neurological Research, University of British Columbia, Vancouver, BC V6T 1W5, Canada.

Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by neuronal loss and the presence of extracellular senile plaques and intracellular neurofibrillary tangles. Studies have shown that classical complement proteins and membrane attack complex (MAC) can be identified within senile plaques and dystrophic neurites of AD brain, suggesting complement-mediated cell lysis may be present in AD. We have recently reported that *in vitro*, neuronal cells can make all of the major complement components. Under deficiency of endogenous complement regulators (CD59), neuronal cells can assemble the components to form MAC. To examine if neurons in human brain express complement components *in vivo*, we used in situ hybridization histochemistry technique to localize the distribution of classical complement component mRNAs (C1q, C2, C3, C4, C5, C6, C7, C8 and C9) in AD brains. While these components were expressed in hippocampus, frontal cortex and temporal cortex, the expression of components in cerebellum was low. Furthermore, in AD brains, messages for these complement components were detectable in neuronal populations. Expression of C1q, C3, C6, C7, C8 and C9 has been found in higher percentage of neuronal population in hippocampus and frontal cortex compared to expression of C2, C4, and C5 in these areas. These results further support that neurons can synthesize all the complement components, indicating that the source of complement activation in AD may be, in part, derived from neuronal cells in the brain.

191.9

ACTIVATION OF BOTH COMPLEMENT PATHWAYS BY THE ALZHEIMER'S DISEASE Aß PEPTIDE GENERATES ESTER-LINKED Aß-C3 COMPLEXES.
B.M. Bradt*, T.S. Bixby, and N.R. Cooper. Department of Immunology, The Scripps Research Institute, La Jolla, CA 92037

Among the proteins associated with the Aß peptide in neuritic plaques (NP), there are markers of inflammation including many complement components and regulatory proteins. The complement system, consisting of more than 30 proteins, has the well recognized ability to damage cells and alter cellular functions. We earlier showed classical complement pathway (CCP) activation by the Aß peptide which is consistent with the presence of C1q, C2 and C4 which have been immunohistochemically localized to the area of NP in AD patients. Hitherto, alternative complement pathway (ACP) activation has been presumed not to be relevant to AD since ACP components (Factor B, properdin) have been immunohistochemically undetectable. Novel capture ELISAs demonstrate that aggregated Aß directly and independently activates both the CCP and the ACP, in the absence of antibody, leading to the formation of Aß-C3 complexes, a finding confirmed with purified complement component mixtures. The sensitivity of these ELISA assays for detection of complement activation by Aß is considerably greater than other assays and detects complement activation by nanomolar concentrations of Aß. Such activation generates covalent Aß-C3 complexes which are capable of being dissociated with alkaline hydroxylamine, and thus are ester-linked. The covalent Aß-C3dg complexes co-migrate on reduced SDS PAGE. Such complexes in NP could mediate chronic complement activation with associated tissue injury.
Supported by NIH grant.

191.10

THE ALZHEIMER'S DISEASE Aß PEPTIDE GENERATES C5a AND THE MEMBRANE ATTACK COMPLEX VIA ACTIVATION OF EITHER COMPLEMENT PATHWAY
**N.R. Cooper* and B.M. Bradt
The Scripps Research Institute, Dept. of Immunology, La Jolla, CA 92037**

Neuritic plaques (NP), a hallmark of the Alzheimer's Disease (AD) brain, have been shown to co-localize with various inflammatory markers, including complement components. Also closely associated with NP are abnormal neuronal processes and increased numbers of reactive astrocytes and microglia. Clearly, complement activation in the vicinity of NP could potentially be responsible for damaging neuronal membranes and for activating nearby glial cells. In this regard, we have recently shown that synthetic Aβ peptides directly activate the alternative and classical complement pathways (ACP and CCP) in the absence of antibody (Ab) or Aβ binding proteins, leading to the formation of ester linked Aβ-C3 complexes. These findings provide a possible explanation for the presence of activation pathway components in NP from AD patients. In the present studies, we evaluated the ability of Aβ-mediated complement activation to activate the terminal, biologically important portion of the complement system. Aβ-mediated triggering of either the ACP or CCP generated C5a and led to assembly of the membrane attack complex. The ACP was far more efficient in this regard. ELISA analysis using multiple combinations of Abs to late complement components unequivocally showed that low nanomolar doses of Aβ 1-42 activated complement through the C9 step. These results provide a likely explanation for the association of the C5b-9 complex with damaged neuronal processes in the vicinity of NP from AD brains. They also suggest a possible mechanism for the accumulation of activated microglia and astrocytes around NP, since both of these cell types respond to C5a with activation and/or chemotaxis.
Supported by NIH grant .

191.11

ACTIVATION OF THE CLASSICAL COMPLEMENT CASCADE BY THE AMYLOID ß PEPTIDE (Aß) IS A FUNCTION OF THE AGGREGATION STATE OF Aß.
S. Webster*[1], J. Rogers[1], B. Bradt[2], Todd Bixby[2] & N. Cooper[2].
[1]L.J. Roberts Alzheimer's Center, Sun Health Research Institute, Sun City, AZ 85372. [2]Department of Immunology, The Scripps Research Institute, La Jolla, CA 92037.

There have been a number of hypotheses put forth in recent years to explain the neuronal loss and neuritic dystrophy characteristic of Alzheimer's disease (AD) pathogenesis. Many of these have focused on Aß-mediated neurotoxicity, and although no single hypothesis has gained universal acceptance, recent evidence suggests that correlates of Aß aggregation are crucial to AD pathogenesis. Activation of the classical complement pathway has been widely investigated as a mechanism of neurodegeneration in AD. Previous studies have shown that in the diffuse plaques of the cerebellum complement activation does not go to completion, in contrast to aggregated neuritic plaques where full activation of the classical complement pathway occurs. In this report we demonstrate that although both forms of Aß bind complement subcomponent C1q equivalently, fibrillar Aß is a more potent activator of complement than is nonfibrillar Aß. This suggests that it is the regular spacing of Aß moieties in fibrillar Aß that leads to activation of complement component C1, as has been demonstrated for other nonimmune activators of complement. In contrast, nonfibrillar or amorphous Aß is not characterized by regular, repeating structure and is therefore expected to exhibit random arrangements of Aß moieties. This allows amorphous Aß to bind C1q in a fashion that does not lead to significant activation of C1. These results suggest that the classical complement cascade is a mechanism whereby neuronal degeneration occurs in the proximity of fibrillar but not diffuse Aß deposits in the AD brain.

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191.12

STRUCTURAL FEATURES OF THE COMPLEMENT ACTIVATION DOMAIN OF β-AMYLOID.
A.J. Tenner, P. Velazquez and D. H. Cribbs*, Dept. Molecular Biology and Biochemistry and *Dept. of Neurology, University of California, Irvine, California, 92717

β-amyloid is a 39-43 amino acid peptide that accumulates in the brain of individuals afflicted with the neurodegenerative disorder Alzheimer's Disease (AD). β-amyloid has been implicated in the pathogenesis of AD via its toxicity to neurons and its ability to activate the classical complement (C') pathway. The ability to activate C' has been previously demonstrated to directly correlate with the ability of this peptide to form fibrils, and the interaction site on C1q, the recognition component of the classical C' pathway, mapped to the N-terminal region of the C1q A chain. A molecular model of this C1q region has now been constructed, and experiments designed to further define the structural elements of β-amyloid required for C' activation. Synthetic peptides that were either truncated or synthesized with altered amino acid sequences were evaluated for their ability to activate C'. Circular dichroism (CD) and electron microscopy (EM) were used to characterize the secondary and macromolecular structure assumed by these peptides. The results demonstrate that the region limited by amino acid residues 4 and 11 is essential for C' activation. Furthermore, the presentation of a negative charge at residue 7 was found to be critical, as substitution of asparagine for aspartic acid at residue 7 resulted in the complete abrogation of C' activating capacity. In addition, even more subtle changes such as the commonly detected isoaspartate modification of this residue disrupted C' activation. This was not due to a lack of β-sheet structure as discerned by CD, or to the lack of fibrillar assembly as detected by EM. While additional β-peptide-C1 interactions may be critical in regulating the activation of C', these studies provide structural information which may be useful in the design of potentially therapeutic inhibitors of C'activation by β-amyloid.
Supported by the Markey Foundation.

191.13

BETA AMYLOID PEPTIDE (1-42) INHIBITS EVOKED ACETYLCHOLINE RELEASE VIA A MECHANISM INDEPENDENT OF EFFECTS ON NEURON VIABILITY, CHOLINE UPTAKE AND ACH SYNTHESIS. N. Jacobsen, C. Cogswell and D. B. Gray*, Department of Biology, Simmons College, 300 The Fenway, Boston, MA 02115.

Last year this lab presented evidence that ACh release from cultured avian ciliary ganglion (CG) neurons is significantly inhibited by exposure to 25 uM 1-42 beta amyloid peptide (BAP). This peptide fragment is implicated in the pathogenesis of the neuritic plaques found in the brains of Alzheimer's patients. In this study, the beta amyloid peptide fragment 1-42 was 'aged' for at least 3 days at a concentration of 250 um before dilution ten-fold and addition to cultured stage 40 CG neurons for 24 hours. At this concentration, aged 1-42 peptide solution clearly contained insoluble crystals which decorated the neurons. After 24 hours in culture, potassium-evoked radiolabeled ACh release from CG cells is inhibited by 75-80 %. These neurons however, possess normal morphology and remain viable for at least 8 days, and labeled choline uptake is unaffected. Acetylcholine (ACh) synthesis rates in cells exposed to BAP 1-42 for 24 hrs were measured and were not significantly different from control levels. To determine if the peptide was affecting total ACh levels or loading of synaptic vesicles or exocytotic mechanisms, ACh release was next induced by adding 1 uM calcium ionophore (A23187), causing inward Ca^{++} flux., bypassing endogenous calcium channels. 25 uM BAP 1-42 was unable to inhibit ionophore-induced ACh release. These results support the hypothesis that the initial interaction between neurons and Alzheimer's associated β-amyloid peptides may cause a disruption of excitation-secretion coupling. Supported by The Simmons Fund for Research.

191.14

THE AMYLOID-β PROTEIN REDUCES THE SYNTHESIS OF ACETYLCHOLINE IN A MURINE SEPTAL CELL LINE, SN56. W.A. Pedersen, M.A. Kloczewiak, and J.K. Blusztajn*. Department of Pathology, Boston Univ. School of Medicine, Boston, MA 02118.

We previously reported that Aβ peptides reduce the intracellular levels of acetylcholine (ACh) in a murine cell line, SN56, derived from basal forebrain cholinergic neurons. After 48 hours of treatment, the ACh content of the cells was reduced up to 33% by Aβ 1-28 (50 pM), up to 43% by Aβ 1-42 (100 nM), and was unaltered by Aβ 1-16 (100 nM). None of these treatments caused toxicity to the cells nor altered their morphology in any way. We have further characterized the reductive effect of Aβ on the levels of ACh in these cells. Treatment of the cells with Aβ 25-28 (100 nM; 48 hours) caused a statistically significant decrease in ACh content, suggesting that the sequence GSNK is responsible for the effect. The reduced levels of ACh caused by Aβ 1-42 and Aβ 1-28 were accompanied by proportional decreases in choline acetyltransferase activity. In contrast, acetylcholinesterase activity was unaltered, indicating that Aβ specifically reduces the synthesis of ACh in SN56 cells. The ACh-reducing effect of Aβ 1-42 in SN56 cells could be prevented by cotreatment with all-*trans*-retinoic acid (10 nM) or with the tyrosine kinase inhibitor, genistein (75 μM). These results demonstrate a non-toxic, suppressive effect of Aβ on ACh synthesis, an action that may contribute to the cholinergic deficit in Alzheimer's disease basal forebrain.
(Supported by AG09525)

191.15

CHARACTERIZATION OF A NUCLEAR FACTOR THAT BINDS TO THE APBβ ACTIVATOR DOMAIN IN THE AMYLOID β-PROTEIN PRECURSOR PROMOTER.
A.A. Vostrov and W.W. Quitschke*, *Department of Psychiatry, State University of New York, Stony Brook, NY 11794-8101.*

The promoter of the amyloid ß-protein precursor [APP] gene directs high levels of cell-type specific transcription with 94 base pairs 5' to the main transcriptional start site. This region contains two nuclear factor binding domains designated APBα and APBß. The factor that binds to the APBα sequence was identified as USF. However, the primary activator domain in the proximal APP promoter is APBβ, which contributes approximately 70-90% of the total transcriptional activity. The recognition sequence of the APBβ domain is contained within the sequence GCCGCTAGGGGT.

The protein factor that binds to the APPβ site was partially purified by ion exchange and affinity chromatography. Using UV crosslinking and SDS polyacrylamide gel electrophoresis, its molecular mass was determined to be approximately 150 kDa. By comparison, gel filtration of the native protein complex resulted in an estimate of the molecular mass of about 350 kDa. Thus, under native conditions the protein exists either as a dimer or in a complex with additional proteins. [supported by NS30994]

191.16

MICE TRANSGENIC FOR A HUMAN AMYLOID PRECURSOR PROTEIN PROMOTER-lacZ REPORTER CONSTRUCT. K. Wright, M.N. Gordon, D.G. Morgan*, C. Zehr, X. Yu, J. Salbaum and K. Duff, Depts. of Pharmacology & Therapeutics and Biochemistry & Molecular Biology, Univ. of South Florida College of Medicine, Tampa, FL 33612-4799.

To further explore factors regulating the expression of the amyloid precursor protein (APP) in vivo, transgenic mice carrying the human APP promoter were constructed. A construct consisting of human APP 5' sequence (from +104 to -2045) was fused to the coding region for the lacZ reporter gene. Transgenic mice were generated by pronuclear injection into FVB mice. Known promoter elements contained within the construct include consensus sequences for heat shock, AP1, AP4 and SP1 elements, as well as GC rich regions. Transgenic founder, F1 or F2 mice (identified by tail biopsy) were anesthetized with pentobarbital, and perfused with 0.9% saline, followed by 4% paraformaldehyde in 100 mM phosphate buffer. Brains were postfixed for 1h, then sectioned at 100 μm in the horizontal plane using a vibratome. Sections were histochemically stained for β-galactosidase activity for 24h at 32°C. Many neurons were intensely stained throughout the hippocampus, cerebral cortex and thalamus in two independent transgenic lines. In the hippocampus, pyramidal cells were labeled, but granule cells and glial cells were not. Labeled cortical neurons were localized in deeper layers, and were most abundant in entorhinal cortex. These mice will be used to identify factors regulating APP expression, and their mechanisms of action. Supported by the Alzheimer's Association IIRG93-083 (DGM) and P01 AG13952 (DGM and KD).

191.17

A COMPARATIVE STUDY OF THE ßAPP GENE PROMOTERS FROM PRIMATES AND RODENTS. W. Song* and D.K.Lahiri. Laboratory of Molecular Neurogenetics, Institute of Psychiatric Research, Program in Medical Neurobiology, Indiana University School of Medicine, Indianapolis, IN-46202

To study the transcriptional control of the gene encoding Alzheimer's beta-amyloid precursor protein (ßAPP), we have cloned a 7.9 kb of the 5'-upstream regulatory region of the monkey ßAPP gene. The proximal region of the promoter lacks both a TATA box and a CAAT box, has a high G+C content (65%) and contains multiple transcription start site (tss). Two copies of the GGGCGC sequence are present in the monkey promoter. It has a consensus sequence for binding of the AP-1 protein. A minimum region of 76 base pairs from tss was found to be sufficient to drive the basal promoter activity which has been tested by transfecting PC12 cells with different deletion promoter plasmids. Serial deletion analysis starting from -3.4 kb of the promoter reveals interesting transcriptional regulatory elements.

We compared sequences in the promoter region of the monkey with the human (LaFauci et al., 1989), mouse (Izumi et al., 1992) and rat (Chernak, 1993). On comparing the promoter sequence, up to ~ 1100 bp upstream from the tss, among human, monkey and mouse, we observed that the human promoter sequence is 82.4 % identical to the corresponding region of the monkey ßAPP gene. On the contrary, the promoter sequences between the primate and rodent are only 26.1% identical. If we compare the promoter sequence of up to ~400 bp, we find a stronger identity (42.0%) between primates and rodents. Thus our results indicate that the basal level of promoter activity may be dependent on domains which lie within the first 400 bp of the promoter.

We thank NIH for supporting this research (PHS R01-AG10297).

191.18

CHANGES IN AMYLOID PRECURSOR PROTEIN mRNA ISOFORMS DURING OLFACTORY NEURON TURNOVER. N. Zaidi and B.R. Talamo* Dept. of Neuroscience, Tufts Medical School, Boston, MA 02111.

Amyloid precursor protein (APP) is the source of abnormal deposits of β-amyloid peptide in Alzheimer's disease. Although APP has been suggested to play a role in neuronal differentiation, neurite outgrowth and repair, its normal role is not understood. Evidence for the normal role of APP was examined by measuring expression of alternatively spliced mRNA transcripts during turnover of rat olfactory neurons. Quantitative RT-PCR was used for the three major isoforms, APP 695, 751 and 770, during experimentally accelerated olfactory neuron degeneration, neurogenesis and axon outgrowth at 3, 5, 7 and 14d following unilateral removal of the olfactory bulb, the synaptic target. Changes in GAP-43 and olfactory marker protein mRNA in the epithelium also were monitored as indicators of neurite outgrowth and mature, differentiated neurons, respectively. Initially, a 75% decrease was observed in the levels of all three APP mRNA isoforms, comparable to the loss in olfactory marker protein mRNA and consistent with our previous immunocytochemical and *in situ* data showing localization restricted to neurons. Recovery of levels of APP 695 lagged behind those of APP 751 and 770 during early stages of neuronal proliferation. By 7d, APP 695 had increased to pre-bulbectomy levels, while APP 751 and 770 isoforms already surpassed levels in unperturbed epithelium. During the phase of active neurite outgrowth at 14d, amounts of all three isoforms were almost double those of the control steady-state epithelium, comparable to the increase and continued elevation of GAP-43 mRNA. These results are compatible with an early role for APP 751 and 770 in initial phases of proliferation and differentiation, while APP 695 may be involved in subsequent stages of neuronal differentiation and neurite outgrowth. Supported in part by R01-AG09200.

191.19

EFFECTS OF SYSTEMIC MK-801 ADMINISTRATION ON THE EXPRESSION OF AMYLOID PRECURSOR PROTEIN (APP) mRNA. T. Kakigi*[1], M. Yasuda[2], Y. Yamamoto[1], K. Maeda[2] and C. Tanaka[2]. [1]Dept. of Psychiatry and Neurology, Kobe Univ. School of Med., 7-5-2 Kusunoki-cho, Chuo-ku, Kobe 650, Japan and [2]Neuropharmacology Lab., Dept. of Neuroscience, Hyogo Institute for Aging Brain and Cognitive Disorders, 520 Saisho-ko, Himeji 670, Japan.

Glutamate is an excitatory amino acid involved in corticocortical association pathways, and the key change in Alzheimer's Disease (AD) has been considered to be shrinkage or loss of corticocortical pyramidal neurons, which use glutamate as a transmitter. The *N*-methyl-*D*-aspartate (NMDA) receptor is a marker for glutamate activity. There is evidence suggesting that these changes play a role in pathogenesis of AD: glutamatergic corticocortical neurons degenerate in the disorder, glutamatergic neurons are prominent in the hippocampus, which degenerates consistently in AD, and animal studies suggest that the NMDA receptor complex is involved in neuronal networks that underlie memory and behaviour. β-amyloid forms the core of senile plaques in AD brain and is broken down from much larger proteins, the amyloid precursor proteins (APPs). MK-801 is one of the dissociative anesthetics and acts on the ion channel as a potent noncompetitive antagonist of the NMDA receptor. In the present study, to investigate the effect of MK-801 on the expression of APP mRNA, we used *in situ* hybridization technique to determine the APP mRNA levels in rat brain. MK-801 (1.0mg/kg) was injected subcutaneously, at 5 time points (0 hour before injection and 1, 4, 10 and 24 hour after injection) rats were killed by decapitation. Oligodeoxynucleotide probes labeled by ^{35}S-dATP were used for *in situ* hybridization. Densities of the interested regions were measured by BAS2000 image analyzing system. Results were shown as % compared to the value of 0 time point and were analyzed by ANOVA. Results were as follows: at 1 hour after injection, the densities in the medial prefrontal cortex, sensorimotor cortex and hippocampus were significantly reduced, after 4 hours, the significant reduction was observed in the medial prefrontal cortex and the elevation in the anterior and posterior cingulate cortex. After 10 hours, the density was significantly reduced in the medial prefrontal cortex and was elevated in the posterior cingulate cortex, and at 24 hours following the MK-801 administration, the APP mRNA expression was significantly reduced in the parietal cortex, hippocampus and anterior and posterior cingulate cortex. Our results showed that noncompetitive NMDA receptor antagonist, MK-801, altered the expression of APP mRNA especially in the posterior cingulate cortex, where MK-801 produces reversible injury, vacuoles, throughout the cytoplasm. (Supported by Hyogo Institute for Aging Brain and Cognitive Disorders-HIABCD, Hyogo, Japan)

MONDAY AM SYMPOSIA

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SYMPOSIUM. CENTRAL NERVOUS SYSTEM AUTOIMMUNITY IN HUMAN DISEASES. P. De Camilli, Yale Univ./HHMI (Chairperson); L. Steinman, Weizmann Inst. and Stanford Univ.; J. Posner, Memorial Sloan-Kettering Cancer Centr.; M. Solimena, Yale Univ.; S. Rogers, Univ. of Utah.

The objective of the symposium is to review, for a broad audience of neuroscientists, our present knowledge about autoimmune disorders of the central nervous system (CNS). The initial speaker, L. Steinman will provide a general description of the mechanisms of immune surveillance in the brain and of how breakdown of tolerance to self-antigens of the nervous system may occur following microbial infections. Examples will be shown with microbial sequences that mimic myelin antigens and which may either induce demyelinating diseases or suppress ongoing inflammatory processes within the brain. The second speaker, J. Posner will illustrate the link between small cancers originating outside the CNS, the onset of brain paraneoplastic syndromes and the occurrence of autoantibodies against neuronal antigens. He will center his attention on the "onconeural" antigen HuD, which appears to be important in the development and maintenance of neuronal function. The third speaker, M. Solimena, will focus on autoimmunity against the presynaptic antigens glutamic acid decarboxylase and amphiphysin in Stiff-Man syndrome, a neurological disorder characterized by continuous rigidity of the body musculature that is often associated with autoimmune diabetes or, less frequently, with breast cancer. The last speaker, S. Rogers, will report on the recent discovery of autoantibodies directed against non-NMDA glutamate receptors in a variety of neurological disorders, including Rasmussen's encephalitis, paraneoplastic neurodegenerative syndrome and olivocerebellar atrophy, and provide insights into how these antibodies could interfere with the normal receptor activity.

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SYMPOSIUM. MITOCHONDRIAL INVOLVEMENT IN NEURONAL DEGENERATION J.M. Dubinsky, Univ. of Minnesota (chair), I.J. Reynolds, Univ. of Pittsburgh (cochair), A.N. Murphy, George Washington Univ., J.T. Greenamyre, Emory Univ.; M.F. Beal, Massachusetts General Hospital; S.M. Rothman, Childrens Hospital-Washington Univ. Sch. Med.

The contribution of mitochondrial function and dysfunction in neuronal death will be addressed in relationship to apoptosis, excitotoxicity, long term neurodegenerative diseases and mental retardation.

A.N. Murphy will discuss the consequences of Ca^{2+} sequestration upon mitochondrial respiration and free radical production. Overexpression of bcl-2, which protects neurons from ischemia/reperfusion injury, potentiates mitochondrial Ca^{2+} uptake and provides resistance to Ca^{2+}-induced respiratory inhibition. **J.T. Greenamyre** will discuss mitochondrial dysfunction in Parkinson's disease. *In vivo* and *in vitro* studies demonstrate that bioenergetic defects lead to secondary excitotoxicity, apparently by disrupting calcium homeostasis. **M.F. Beal** will review the bioenergetic defects and oxidative stress associated with Huntingtons and Alzhemirs disease. Huntingtin binds to and inhibits glyceraldehyde-3-phosphate dehydrogenase. Lesions produced by mitochondrial toxins closely mimic Huntingtons disease. In AD there is evidence for mitochondrial defects encoding cytochrome oxidase. **S.M. Rothman** will discuss mutations of the mitochondrial genome resulting in hearing loss, blindness, epilepsy, myopathy, and neuronal degeneration. While ATP deficiency may be the primary explanation, there are alternatives, such as defective buffering of intracellular calcium. **I.J. Reynolds** will conclude by reviewing evidence from several laboratories regarding mitochondrial calcium changes associated with excitotoxicity.

VISUAL CORTEX: STRIATE II

198.1

THE MICROCIRCUITRY OF COMPLEX CELLS IN CAT STRIATE CORTEX *José-Manuel Alonso**. Laboratory of Neurobiology, The Rockefeller University, New York, NY 10021.

According to the original model, complex cells are generated by the convergent input of simple cells with similar orientation preference (Hubel and Wiesel, *J Physiol* **160**: 106, 1962). Alternatively, later studies have demonstrated that some complex cells receive direct input from the lateral geniculate nucleus (LGN) (Hoffman and Stone, *Brain Res* **32**: 460, 1971). The aim of this study was to reconcile these two sets of findings and learn more about complex cell microcircuitry. Single unit activity from simple and complex cells, and multiunit activity from the LGN were recorded simultaneously, and their connectivity measured by cross-correlation analysis. Small reversible blockades were made in layer A of the LGN (pressure injections of GABA) to study the contribution of this input to complex receptive fields.

Preliminary results demonstrated, as expected, the existence of excitatory connections from layer IV simple cells to superficial complex cells with similar orientation preference. In addition, geniculate blockades were able to suppress the activity of both simple and complex cells. Among the complex cells affected, some appeared to receive excitatory connections from simple cells but not from layer A of the LGN. Other complex cells were less affected by the geniculate blockade (Malpeli, *J Neurophysiol* **49**: 595, 1981) although some of them received direct geniculate input from layer A.

In summary, the mechanism of complex cell generation appear to be multifaceted. Some complex cells are apparently dominated by excitatory connections from simple cells. Other complex cells, however, might be generated by different mechanisms. (Supported by the C.H. Revson Foundation and NIH EY05253).

198.2

COMPLEX CELLS IN LAYER 6 PROJECT TO THE SUPERFICIAL LAYERS IN THE CAT STRIATE CORTEX. *Judith A. Hirsch*, José-Manuel Alonso, R. Clay Reid and Christine A. Gallagher*. Laboratory of Neurobiology, The Rockefeller University, New York, NY 10021.

Receptive field properties vary systematically across the different layers of the striate cortex. Understanding how these functional differences arise requires a precise description of the interlaminar connections. By making whole-cell recordings with dye-filled electrodes *in vivo*, we have been able to correlate the visual responses of cells in layer 6 with the pattern of their intracortical projections. The five complex cells we labeled projected to the superficial layers, a pathway not previously described in the cat. Two simple cells projected to layer 4, as first reported by Gilbert and Wiesel (*Nature* **280**: 120, 1979).

We recorded from complex cells at different depths of layer 6. Patterns of dendritic branching ranged widely, apical arbors ended in layer 5, 2+3, or 1. The somas of two neurons lay near the white matter and sent axons to only the lower half of layer 2+3. The other cells were located in middle or upper layer 6 and extended collaterals that traveled the full extent of the superficial layers. The densest projections were in close vertical register with soma and sparser collaterals formed at least one nearby cluster. In addition to the terminal fields in the superficial layers, there were various degrees of arborization in the other laminae; all cells projected to the white matter, presumably to subcortical targets.

Our conclusion is that simple cells in layer 6 direct their output to simple cells in layer 4, while complex cells in layer 6 contact complex cells in layer 2+3. This parallel functional and anatomical division of layer 6 in the cat is reminiscent of patterns of connectivity in the primate striate cortex (Lund, *Ann Rev Neurosci* **11**: 253, 1988). (Supported by NIH grants EY09593 and EY05253, the Klingenstein Fund and the C.H. Revson Foundation)

198.3

VISUAL ADAPTATION HYPERPOLARIZES CELLS OF THE CAT STRIATE CORTEX

M. Carandini* and D. Ferster
Dept. of Neurobiology, Northwestern University, Evanston, IL 60208

Prolonged exposure to visual stimuli decreases the responsiveness of striate cortical cells, as measured by their spike rates (Maffei *et al.*, 1973).

To investigate the cellular basis of this phenomenon we recorded intracellularly from cells in the striate cortex of anesthetized cats using the whole cell patch technique. Visual stimuli were optimal drifting sinusoidal gratings. In "ramp" experiments, stimuli of increasing contrast were followed by stimuli of decreasing contrast (Bonds, 1991). In "top-up" experiments, the order of contrasts was randomized and adapting stimuli were presented before each test stimulus (Blakemore & Campbell, 1969).

The mean membrane potential of most cells increased with stimulus contrast. Adaptation to high contrasts antagonized this increase, reducing the mean membrane potential by 2-8 mV. The effect of adaptation on the spike responses was, as expected, a 2- to 5-fold reduction in contrast sensitivity (Ohzawa *et al.*, 1979). The strongest effects of adaptation on mean membrane potential, however, were not reflected in the spike responses since they occurred at low-contrast test stimuli that did not elicit spikes. In simple cells the increase in the mean of the membrane potential responses caused by increasing contrast was accompanied by a large increase in their first harmonic component. The first harmonic component, however, was not as much affected by adaptation as the mean. We conclude that the decrease in contrast sensitivity of the spike responses caused by adaptation is largely due to hyperpolarization, which increases the distance between membrane potential and spike threshold.

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198.4

SPONTANEOUS FLUCTUATIONS IN MEMBRANE POTENTIAL OF COMPLEX CELLS IN VISUAL CORTEX

D. Ferster* and M. Carandini
Dept. of Neurobiology, Northwestern Univ., Evanston, IL 60208.

Arieli *et al.* (J. Neurophysiol. **73**:2072, 1995) have reported optically-recorded spontaneous waves of activity traveling across the cortical surface that compare in size to visually evoked responses. We have observed intracellularly recorded fluctuations in membrane potential that may underlie these optically-recorded signals.

Whole-cell patch intracellular recordings were obtained from the striate cortex of pentathol-anesthetized cats. In many complex cells and almost all simple cells, a moving bar evokes reproducible peaks of depolarization time-locked to the stimulus. In the absence of a stimulus in these cells, the membrane potential remains near rest with small spontaneous noise of a few mV in size. In the remaining complex cells, however, even in the absence of a stimulus the membrane potential rapidly and repeatedly rises by 10 to 20 mV and stays in this depolarized state for anywhere from 50 to 300 ms. These large depolarizing events occur with an average rate of 3-5 per second, but their timing seems largely random. Visually-evoked responses do not sum with the spontaneous events, since the size of these events is comparable to the size to the evoked response. Instead, visual stimuli increase the average time that the membrane remains depolarized. Nevertheless, the averaged responses of these complex cells still resemble those of complex cells that do not show spontaneous events.

The spontaneous fluctuations in membrane potential are well correlated with the extracellularly-recorded local field potential, as are the optically-recorded surface waves of activity of Arieli *et al.* This correlation and the similar dynamic properties of the fluctuations in membrane potential suggest that the intracellular and optical phenomena are related.

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198.5

THE CONTRIBUTION OF FEEDFORWARD PATHWAYS TO THE GAIN OF CORTICAL MICROCIRCUITS.

J. G. Mancilla* and P. S. Ulinski.
Comm. Neurobiol. and Dept. Org. Biol. and Anat., U. Chicago, Chicago, IL 60637.

Current models of cortical microcircuitry (e.g. Douglas and Martin, 1991; Suarez et al., 1995) suggest that relatively small thalamic inputs to the cortex are amplified by recurrent, excitatory pathways within the cortex. Since these models are based on experiments using electrical stimulation of the white matter, it seemed important to examine them using natural visual stimuli. Intensity-response functions were obtained from intracellular recordings of pyramidal cells in an *in vitro*, geniculocortical preparation of the turtle, *Pseudemys scripta*. One second, square light flashes of varying intensities produced ON responses composed of depolarizing potentials that increased in amplitude with increases in light intensity. The mean ± SEM maximal amplitude of the responses was 16 ± 5 mV (n = 5). Action potentials were only produced at intensitities corresponding to the maximal response and at higher intensities. The responses at intensities higher than that producing the maximal response demonstrated a clear decrement from the maximal. Sigmoid functions were fit to plots of response amplitude as a function of light intensity to obtain the slopes of the sigmoids at half maximal amplitude. The slopes yield a measure of the gain of the cortical responses (270 ± 20 $\mu V/photon \cdot \mu m^{-2} \cdot sec^{-1}$), which is significantly higher than the gain of single turtle cones (50 $\mu V/photon \cdot \mu m^{-2} \cdot sec^{-1}$; Burkhardt, 1995). The absence of action potentials at the low light intensities used to obtain the gain measurements suggests that the gain at these intensities is solely a product of the feedforward pathways, while the production of action potentials at higher intensities indicate the involvement of feedback pathways. Thus, the data suggest that natural visual stimuli produce high gains in the feedforward components of cortical microcircuits, and that feedback pathways apparently function in controlling the gain of cortical circuits, rather than amplifying thalamic inputs. JGM is supported by the Training in Neural Systems Grant T326MO7839

198.6

FEEDBACK CONNECTIONS FROM AREA V2 FAVOR ACTIVATION OF NMDA RECEPTORS IN NEURONS OF RAT PRIMARY VISUAL CORTEX.

A. Burkhalter[1], J. M. Nerbonne[2]* and Z. Shao[1]. [1]Dept. of Anatomy and Neurobiology, [2]Dept. Molec. Biol. and Pharmacology, Washington Univ. Sch. of Med., St. Louis, Mo 63110.

Feedback inputs from V2 have been shown to facilitate visual responses in V1. Such gain changes might be due to a potentiation of excitatory inputs mediated by NMDA receptors. We have tested this hypothesis in slices of rat visual cortex by studying the effects of the selective NMDA receptor blocker, AP-5, on synaptic potentials (PSPs) evoked by forward and feedback inputs.

The results show that in the presence of AP-5 the majority (5/8, 62.5%) of monosynaptic EPSPs evoked by feedback inputs were reduced in amplitude and exhibited an accelerated decay. In contrast, monosynaptic EPSPs elicited by forward inputs were affected only in 28.5% (2/7) of the cells tested. When inhibition was eliminated by intracellular blockade of Cl^-channels or extracellular application of bicuculline, ESPSs of all cells tested in both pathways were sensitive to AP-5.

In the majority of cells, feedback input of any strength evoked polysynaptic EPSPs (13/15, 87%). In in the forward pathway, in contrast, late EPSPs were seen only at lower stimulus strengths (5/6, 83%) and were infrequent at high stimulus strengths (1/12, 8%). The loss of the late EPSP was correlated with the appearance of a late IPSP that was evoked by stronger stimuli. Blockade of this late IPSP revealed late EPSPs that were blocked completely by AP-5.

These findings suggest that NMDA receptors play a more important role in feedback than in forward pathways and that this difference is determined in part by distinct inhibitory mechanisms present in each circuit (Johnson and Burkhalter, 1996).

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198.7

$GABA_B$ MEDIATED INHIBITION IN FEEDBACK CIRCUITS IS WEAKER THAN IN FORWARD CIRCUITS OF RAT VISUAL CORTEX.

Z. Shao[1], G.W. Harding[2]* and A. Burkhalter[1]. [1]Dept. of Anatomy and Neurobiology, [2]Dept. Otolaryngology, Washington Univ. Sch. of Med., St. Louis, MO 63110.

Mechanistic models of cortical networks have shown that tuning of selective receptive field properties of cortical neurons requires amplification of monosynaptic inputs by local excitatory circuits (Douglas and Martin, 1991; Somers et al., 1995). Evidence suggests that the gain of this amplification is controlled by inhibition of polysynaptic excitation which may be mediated by $GABA_B$ receptors (Morrisett et al., 1991). To better understand excitatory gain control in different cortical circuits we have compared the mechanisms of GABAergic inhibition in forward and feedback circuits of rat visual cortex.

Intracellular recordings in slices of rat visual cortex showed that in most cells tested (10/13, 77%) forward input from area V1 to LM (V2) generated early EPSPs followed by early (latency to peak ~10ms) and late IPSPs (latency to peak ~160ms). Few cells responded with late, polysynaptic EPSPs. In contrast, at all strengths tested, feedback input from LM to V1 rarely elicited fast (Shao and Burkhalter, 1996) or slow IPSPs (1/12, 8%). However, most cells showed early and late EPSPs. In both pathways early IPSPs were blocked by the $GABA_A$ receptor antagonist bicuculline. Late IPSPs evoked by forward inputs were abolished by the $GABA_B$ receptor blocker saclophen. With $GABA_B$ receptors blocked forward inputs typically revealed late EPSPs.

These results suggest that in forward circuits the excitatory gain is regulated by $GABA_B$ receptor mediated inhibition, which may play a role in contrast invariant tuning. Because feedback circuits lack this late inhibition, amplification of feedback signals by local excitatory networks must rely on different mechanisms. Supported by NIH Grant EY05935.

198.8

PAIRED-PULSE DEPRESSION OF UNITARY PYRAMID TO INTERNEURON EPSPs IN CAT VISUAL CORTEX *IN VITRO*.

E.H. Buhl*, G. Tamas, T. Szilagyi, O. Paulsen and P. Somogyi.
MRC Anatomical Neuropharmacology Unit, Oxford University, Oxford OX1 3TH, UK

Dynamic properties of unitary EPSPs were investigated using the paired-pulse paradigm in conjunction with dual intracellular recordings of synaptically coupled pyramid to GABAergic cell pairs (n=4; intervals 10 - 70 ms) in slices of the cat visual cortex. For each connection, we employed correlated light and electron microscopy to determine the numbers of *all* synaptic release sites (n = 1, 2, 2 and 7, respectively) as well as their exact placement, ranging from very proximal to distal. On average, all unitary EPSPs showed a modest degree of paired-pulse depression, ranging between 2 and 31% (mean reduction = 16%) of the averaged conditioning 1st EPSP (mean amplitude 1st EPSP = 0.79 mV; 2nd EPSP = 0.66 mV), whereas the degree of amplitude fluctuation remained virtually the same (mean SD 1st EPSP = 0.38 mV; mean SD 2nd EPSP = 0.38 mV). Even for those preselected 2nd EPSPs which followed a small 1st response (<20% of averaged 1st EPSP) there was no significant increase of their amplitude above the level of the averaged conditioning 1st EPSP. Likewise, there was only a weak tendency of paired-pulse depression to be more pronounced at short interspike intervals. In conclusion, transmission at cat cortical pyramid to interneuron connections (see also Paulsen, Stricker, Tamas, Szilagyi, Somogyi and Buhl, this meeting) is relatively robust, with unitary EPSPs showing only a modest degree of paired-pulse depression, which is relatively little affected by either amplitude of the conditioning event or the interspike interval.

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198.9

PYRAMIDAL CELL MODULES AND DOUBLE-BOUQUET CELL AXONS IN MONKEY PRIMARY VISUAL CORTEX. A. Peters* and C. Sethares. Dept. of Anatomy and Neurobiology, Boston Univ. Sch. of Med., Boston, MA 02118.

In earlier studies we showed that the apical dendrites of layer V pyramidal cells in rhesus monkey area 17 are clustered together into distinct and regularly spaced groups that have an average center-to-center spacing of 23 μm, and it was proposed that these clusters of apical dendrites represent the axes of vertically oriented modules of pyramidal cells. It was subsequently shown that the myelinated axons that extend from the pyramidal cells to the white matter as discrete bundles have a similar center-to-center spacing. Since use of tracers shows that the axons of the pyramidal cells in the upper layers of the cortex enter these bundles, it was suggested that each module has its own individual output bundle of axons (Peters and Sethares, 1996).

Another vertical component of this cortex is the horse tails of axons that originate from double-bouquet cells with cell bodies in layer II/III. These horse tails, which can be visualized using antibodies to calbindin, extend as far as layer IVCα. The horse tails are most prominent in layer III, in which they are regularly spaced, and have an average center-to-center spacing of about 23 μm, like that of the clusters and myelinated axon bundles. Indeed, in sections double-labelled with calbindin and MAP2 antibodies, it is evident that each horse tail of axons lies adjacent to an apical dendritic cluster. The relationship between these two vertically oriented entities is being more closely examined, but it is now apparent that not only pyramidal cells, but some nonpyramidal, inhibitory, neurons are regularly arranged and contribute to the vertical neuronal modules within cerebral cortex.

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198.10

Do cortical cells integrate local inputs linearly? Short-range lateral interactions revealed by intrinsic signal and single-unit activity.

L. J. Toth*, D.-S. Kim, M. Sur. Dept. of Brain and Cognitive Sciences, M.I.T. Cambridge, MA 02139.

In cat visual cortex, focal iontophoresis of the $GABA_A$ antagonist bicuculline within a column of known orientation preference shifts the optically imaged orientation preference of a region of cortex (~1.1mm radius) towards that orientation (Kim *et al.*, 1995, *SFN Abst.* 21:771). This effect is dose-dependent (responses become non-specific for orientation at higher doses), $GABA_A$ specific (saclofen, a $GABA_B$ antagonist does not cause this effect, whereas GABA causes the inverse effect), position dependent (moving the pipette tangentially through the cortex alters which orientation becomes overrepresented), and often anisotropic. We therefore believe that the imaged spread of activity reveals a range of local functional connectivity, inclusive of all orientations, from a given cortical point. Because up to 70% of the maximal intrinsic signal can result from subthreshold activity (Toth *et al.*, 1994, *SFN Abst.* 20:836), we investigated whether the change in orientation preference observed in optical maps was also reflected in neuronal spiking. Single-unit recordings between 0.2 and 1.0 mm from the site of bicuculline iontophoresis revealed only small changes (<10°) in a given cell's orientation preference, though such changes were consistently towards the preference at the site of iontophoresis. Other physiological characteristics, such as tuning width, direction selectivity, and spontaneous activity were also affected.

We are faced with the observation that although cells receive strong and functional lateral inputs, those inputs do not significantly alter the orientation preference of the postsynaptic neuron. These results indicate that local information non-linearly modulates cortical responses, rather than being linearly integrated within the cell. Supported by NIH EY07023.

198.11

SPECIFICITY OF CONNECTIONS REVEALED BY LASER SCANNING PHOTOSTIMULATION IN RAT VISUAL CORTEX. A. Sawatari* and E.M. Callaway. Molecular Neurobiology Laboratory, The Salk Institute for Biological Studies, La Jolla, CA 92037.

Do layer specific axonal projections form connections onto specific dendritic elements, or do they synapse indiscriminately onto any dendrite present in the layer? We addressed this question by examining the connectivity of neurons in the rat visual cortex, where both layer 2/3 and layer 5 pyramidal neurons have extensive dendritic arbors in layer 2/3. Axons of spiny stellate cells in layer 4 project to layer 2/3 but the specificity of these afferents is not known. Conceivably, layer 4 axons could form synapses selectively onto the dendritic branches of layer 2/3 cells or layer 5 cells, or make indiscriminate connections to both.

We determined the locations of neurons making functional connections with layer 2/3 and layer 5 cells using laser scanning photostimulation. Living sagittal brain slices from the visual cortex of Long-Evans rats were bathed in ACSF containing "caged" glutamate. Whole-cell, voltage-clamp recordings were made from individual layer 2/3 and layer 5 neurons while brief flashes of light (10 msec) from a UV laser, focused to a small spot by a microscope objective, were delivered to various locations in the slice to uncage the glutamate. The uncaged glutamate induces action potentials in nearby neurons. Monosynaptic excitatory post synaptic currents were recorded by stimulating cells in different layers in this manner. Neurons were also intracellularly labeled with biocytin to determine axonal and dendritic morphology along with laminar localization of the soma.

Our sample to date includes four layer 2/3 pyramidal cells and three layer 5 pyramidal cells. Layer 2/3 cells received input from layer 2/3 and layer 4 with moderate input from layer 5B. One layer 2/3 cell also received strong input from layers 5B and 6, suggesting diversity in layer 2/3 pyramids based on functional connectivity. In contrast, layer 5 cells received considerable input from layers 2/3, 5, and 6, but very little input from layer 4. Thus, our preliminary results suggest that layer 4 neurons connect preferentially to layer 2/3 neurons. These observations suggest that neurons can selectively target specific dendritic elements within a given layer. Further experiments will verify whether these trends are maintained in a larger sample.

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198.12

ASYNCHRONOUS DEVELOPMENT OF RECEPTIVE FIELD PROPERTIES AND CLUSTERED HORIZONTAL CONNECTIONS IN MACAQUE STRIATE CORTEX. J.B. Levitt* and J.S. Lund. Dept of Visual Science, Inst of Ophthalmology, University College London, Bath Street, London EC1V 9EL, UK.

A prominent feature of V1 cortical circuitry whose functional role remains enigmatic is the orderly intra-areal lattice of clustered pyramidal neuron projections spreading parallel to the cortical surface. We examined the anatomical organization of these circuits, together with a number of receptive field properties in infant rhesus monkeys. We sought to determine if there is a parallel relationship between the development of patchy horizontal connections and maturation of response characteristics in V1 neurons. We recorded unit response properties in all cortical layers from the parafoveal representation of V1 in 2 paralyzed, opiate-anesthetized infant macaques (4.5 and 9 weeks old), and compared neuronal responses to adult responses collected at similar eccentricities and under identical conditions. We then made small iontophoretic injections of biocytin or dil to examine horizontal connectivity in these same animals. The lattice system anatomy at 4-5 weeks of age appeared adultlike in all characteristics (terminal patch size, repeat distance, overall connectional field coverage). Infant neurons were as orientation- and direction-selective as adult neurons, although they were somewhat less sensitive and responsive. Although infant V1 minimum response fields were the same size as in the adult, infant V1 largely lacked the neurons summating over distances greater than 5 deg found in the adult. Infant V1 also lacked strongly endstopped units. Furthermore, response modulation from regions surrounding the minimum response field was notably weak or absent in the infants, and did not exhibit the same strong stimulus selectivity of such effects as in the adult. While the lattice connectivity was apparently adultlike in the infants, the spatial characteristics of their V1 neurons were strikingly immature. Either lattice connections do not underly these characteristics, or they have yet to mature their adult synaptic complement. Supported by NIH EY10021 & MRCG9408137.

198.13

VISUAL CALLOSAL FIBERS LINK CORTICAL COLUMNS DOMINATED BY THE SAME EYE IN THE CAT. Jaime F. Olavarria*. Dept. of Psychology, Box 351525, Univ. of Washington, Seattle, WA 98195.

The development of callosal connections in cat areas 17 and 18 is thought to depend upon neuronal correlated activity between the hemispheres. A prevailing hypothesis is that correlated activity is generated from viewing the same object through both eyes. An alternative hypothesis (Olavarria & Li 95; Olavarria 96) proposes that interhemispheric correlated activity may be primarily monocular in origin. Afferents from temporal retina are relayed not only ipsilaterally, but also to the contralateral transition zone (TZ), an area of ipsilateral visual field representation located between areas 17 and 18. Active loci in temporal retina would thus induce synchronous activity in retinotopically corresponding loci in both hemispheres.

The binocular hypothesis predicts that callosal neurons should overlap both right and left eye ocular dominance columns (ODC). In contrast, the monocular hypothesis predicts that callosal neurons should overlap columns dominated by the same temporal retina, namely, ipsilateral ODC in regions outside the TZ, and contralateral ODC in regions within the TZ. These predictions assume that ODC influence callosal development, which is likely since ODC segregate long before callosal development is complete.

To test these predictions, callosal neurons were retrogradely labeled by injections of fluorescent tracers into areas 17, 18 and TZ of normal adult cats. The distribution of labeled cells was then correlated with the pattern of ODC labeled transneuronally by intraocular injections of WGA-HRP.

Tracer injections into areas 17 and 18 outside the TZ labeled callosal cells in the opposite TZ, predominantly over columns dominated by the contralateral eye. On the other hand, injections into the TZ labeled cells outside the TZ in contralateral areas 17 and 18, and these cells were found preferentially over ipsilateral ODC. These data support the hypothesis that correlated activity of monocular origin plays an important role in stabilizing juvenile callosal linkages. Supported by NIH EY09343.

199.1

FUNCTIONAL ANALYSIS OF GANGLION-CELL RECEPTIVE FIELDS IN TURTLE. J.R. Dearworth Jr. and A.M. Granda*. Department of Biological Sciences, University of Delaware, Newark, DE 19716.

Retinal ganglion cells have receptive fields that are defined by the interaction of excitatory and inhibitory components. These components can be manipulated by colored adaptations and stimulations, and by the use of pharmacological agents. The interactions describe receptive-field organizations that are progressively complex: here, a representative symmetrical simple center-only organization, a double-opponent arrangement organized for polarity and for color, and a third arrangement that operates for directional selectivity.

Turtles (*Pseudemys scripta elegans*) were anaesthetized and their optic nerves exposed for extra-cellular recording. Eyes were focussed on a hemispheric surface onto which computer controlled targets of light were projected. Ganglion-cell action potentials were collected in time histograms and displayed in 3-D plots. Drugs were directly injected into the vitreous bodies of left eyes; the time courses of drug effects were tracked.

Colored-light adaptations of particular field regions lessen sensitivity from one region while enhancing sensitivity in another. Drugs that act at glutamate and GABA receptors also upset balances between excitatory and inhibitory regions. The simple center-only receptive-field organization can be described by a double integral of a Gaussian function. The center-surround, double-opponent field can be described by the addition of a Fourier transform to the double Gaussian function. The directional-selective receptive field that is asymmetric can be described by the above-mentioned functions implemented by a cardioid function.

199.2

EXCITATORY INPUT THROUGH GAP JUNCTIONS TO ON-CONE BIPOLAR CELLS IN RAT RETINA. E. Hartveit* Dept. Neurophysiol., Univ. Oslo, Norway.

The glutamatergic photoreceptor input to rod bipolar cells and ON-cone bipolar cells seems to be mediated by an APB-sensitive metabotropic receptor, whereas the input to OFF-cone bipolar cells is mediated by an ionotropic receptor. It is important to determine whether the axon terminals of ON- and OFF-cone bipolar cells overlap in the inner plexiform layer (IPL) or whether they are segregated in separate ON- and OFF-sublaminae. With recent information on rat cone bipolar cell morphology (type CB1-CB9; Euler and Wässle 1995), this problem can be addressed by experiments combining physiological and morphological methods. Whole-cell voltage clamp recordings (int: cesium gluconate, TEA; ext: 2.5-5 mM Co^{2+}) were obtained under visual control (IR-DIC videomicroscopy) from cone bipolar cells in vertical slices of the rat retina. Cells were identified and classified by filling with Lucifer yellow. Responses to glutamate agonists (kainate, AMPA) were obtained by pressure application from a multibarrel pipette. For CB1-CB4, every cell responded with a short-latency (~0.2 s) inward current at V_H= -70 mV, suggesting they were OFF-cone bipolar cells. The current reversed ~0 mV, as expected for non-selective cation channels. In addition, there was evidence for long-latency, indirect GABAergic input to the axon terminals in the IPL (E_{rev}~E_{Cl}; mediated by external Ca^{2+}-independent release; blocked by picrotoxin+3-APMPA). For CB5-CB8, some cells displayed only long-latency GABAergic responses (E_{rev}~E_{Cl}), suggesting they were ON-cone bipolar cells. Surprisingly, others of the same type (CB5-8) displayed short-latency (~0.2 s) responses as well, raising the question whether each type encompasses both ON- and OFF-cone bipolar cells. When the short-latency current responses were studied in isolation, however, E_{rev} was +30 to +40 mV, or could not be reversed at all. This could be explained by assuming that the current was generated in AII amacrine cells, known from morphological investigations to be coupled via gap junctions to axon terminals of some cone bipolar cells. Whole-cell recording of a cone bipolar cell would not give adequate voltage-control of the AII cells. When kainate responses were recorded directly from AII cells, E_{rev} was ~ 0 mV. Current experiments aim at reversibly blocking the gap-junction input.
NFR 101025/310 (NORWEGIAN RESEARCH COUNCIL)

199.3

HOW ON-OFF AMACRINE CELLS WORK. Robert F. Miller* Toby Velte and Nathan Staff. Neuroscience Graduate Program and Department of Physiology, University of Minnesota, Minneapolis, MN 55455.

Electrophysiological studies of On-Off amacrine cells were carried out in the mupuppy retina. Both whole-cell and intracellular electrophysiological recordings were obtained from single amacrine cells in the superfused eyecup preparation; cells were stained intracellularly with either horse radish peroxidase or Neurobiotin. Confocal microscopy was used to evaluate the fine structure of amacrine dendrites and the morphologies of stained amacrine cells were entered into a computer through a commercial tracing program (Eutectic). Modeling studies were carried out on compartmental representations of the cell morphology to simulate the light-evoked synaptic conductance changes and the observed pattern of impulse activity. Simulations of impulse activity were based on 5 non-linear channels, identified in ganglion cells. Successful models of On-Off amacarine cells required impulse generating capabilities in both the dendrites and soma with approximately equal voltage-gated ion channel density. Supported by NIH grant EY03014 to RFM.

199.4

NICOTINIC ACETYLCHOLINE RECEPTORS ARE EXPRESSED BY SOME CHOLINERGIC AMACRINE CELLS IN THE RABBIT RETINA. K. T. Keyser[1*], M.A. MacNeil[1], J. M. Lindstrom[2], F. Wang[2] and R. H. Masland[3] Vision Science Research Center, University of Alabama, Birmingham, AL 35294[1], The Institute of Neurological Sciences, University of Pennsylvania, Philadelphia PA 19104[2], Howard Hughes Medical Institute, Mass. General Hospital, Boston MA 02114[3].

Acetylcholine (ACh) is a transmitter in the retina that affects the response properties of many ganglion cells, including those that display directional selectivity. Three structural (β) and eight ligand binding (α) subunits of neuronal nicotinic acetylcholine receptors (nAChRs) have been purified and antibodies have been raised against many of them. As part of a continuing effort to understand the cholinergic circuitry in the retina, we used a acetylcholine receptor monoclonal antibody (mAb210) and an antiserum against choline acetyltransferase (ChAT), to determine whether or not cholinergic amacrine cells express nAChRs. nAChR immunoreactive cells in the Inner Nuclear Layer (INL) were 7-14µm in diameter and were restricted to the innermost one or two tiers of cells, although occasional cells were found in the outer half of the INL. nAChR containing cells in the Ganglion Cell Layer (GCL) were 7-16µm. ChAT immunoreactivity was found in amacrine cells in the INL and the GCL. In the INL, the 90-95% of the cholinergic cells did not express nAChR immunoreactivity while 5-10% did show colocalization. In the GCL, at least 57% of the ChAT-positive cells, all within the central retina, expressed nAChRs. The percentages represent the lowest estimate of colocalization; a larger proportion of the cholinergic cells may express the nAChR subtype recognized by mAb210 or other nAChR subtypes. Thus, our observations support earlier ultrastructural findings that some cholinergic cells synapse on one another. Supported by EY07845 (K.T.K.), HHMI (R.H.M.) and NS11323 (J.M.L.).

199.5

MORPHOLOGY AND PHYSIOLOGY OF THE AII-AMACRINE CELL NETWORK IN THE MACAQUE MONKEY RETINA. D.M. Dacey.* Dept. of Biological Structure, Univ. of Washington, Seattle WA 98195

In mammalian retina, rod bipolar cells do not contact ganglion cell dendrites but instead direct output to a distinctive amacrine cell type, the AII cell. Synaptic output of the AII-amacrine is mainly to ON cone bipolar cells via gap junctions and to OFF cone bipolar cells via an inhibitory synapse; the AII cells thus provide a pathway by which rod signals can be transmitted to both ON and OFF ganglion cell populations. In primates, the AII cell has been previously identified anatomically (Wässle, et al., J. Comp. Neurol. 361:537 1995); here I report on the light response of the AII cell in the macaque monkey retina and on its gap junctional connections with ON cone bipolar cells. AII cells were identified in an in vitro retinal preparation by selective staining with DAPI (diamidino phenylindole), and targeted for intracellular recording under microscopic control. To photopic luminance-modulated stimuli the AII gave a sustained ON response (~ 5 mV amplitude); the receptive field showed a non-opponent, center-surround organization characterized by additive input from both long (L-) and middle (M-) wavelength sensitive cones. After 30 min dark adaptation, scoptic stimuli evoked a depolarizing response (~ 20 mV amplitude) with characteristic rod spectral and temporal sensitivity. Intracellular injection of Neurobiotin demonstrated the bistratified morphology and coupled network of AII cells. An array of ON cone bipolar cells were also tracer-coupled to the AII network. The spatial densities and morphologies of the coupled cone bipolar cells revealed that both ON-midget bipolar and multiple diffuse cone bipolar types are linked to the AII cells. Thus the AII-cone bipolar gap junctions must be the basis for the strong, cone-mediated center-surround receptive field of the macaque AII cell as well as serving as a channel for rod signals to both midget and non-midget ganglion cell visual pathways. Supported by PHS Grants EY 06678, EY11162 and RR00166.

199.6

ZINC PRESERVES ION-SELECTIVITY AND GATING OF OUTWARDLY RECTIFYING CURRENT IN RETINAL GANGLION CELLS. T. Tabata and A.T. Ishida*. Section of Neurobiology, Physiology & Behavior, University of California, Davis, CA 95616.

Voltage-gated Na^+, K^+, Ca^{2+}, and mixed-cation currents have been identified in retinal ganglion cells of various species. Using whole-cell patch-clamp methods, we have found outwardly rectifying current (I_{out}) in goldfish retinal ganglion cell somata after the above currents were blocked with tetrodotoxin, tetraethylammonium, Co^{2+}, and Cs^+, respectively. In perforated-patch mode, I_{out} activated at membrane potentials more positive than -50 mV, and deactivated at membrane potentials more negative than -60 mV. The amplitude of I_{out} declined with extracellular Cl^- concentration, and was also reduced by furosemide and DIDS. This I_{out} thus appeared to be largely, if not entirely, anionic. However, after repeated depolarizations in ruptured-patch mode, I_{out} showed less selectivity for anions over cations, and its gating kinetics changed irreversibly. Both of these changes could be impeded by including as little as 3-30 nM free Zn^{2+} in the ruptured-patch pipet solution. Under these conditions, with E_{Cl} set either to 0 mV or to -50 mV, the voltage-sensitivity of I_{out} resembled that measured in perforated-patch mode. These results indicate that retinal ganglion cells possess voltage-gated Cl^- current whose relative contribution to I_{out} depends directly or indirectly on intracellular levels of Zn^{2+} or similar divalent cations. Supported by NIH grant EY08120.

199.7

METABOTROPIC GABA RECEPTORS ACT AS DISCRIMINATORS.

J. Zhang, G. Hajduczok* and M. M. Slaughter. Departments of Biophysical Sciences, Physiology, and Ophthalmology. School of Medicine, State University of New York, Buffalo, NY 14214, USA.

The electrogenic properties of the baclofen-sensitive metabotropic GABA receptor were examined in the retinal slice preparation of the salamander, *Ambystoma tigrinum*. Whole cell recordings were obtained from neurons in the ganglion cell layer. Baclofen application activated a conductance that had a reversal potential near that of potassium. This current was not calcium dependent. The conductance was small, but represented a significant fraction of the resting conductance of ganglion cells. Baclofen also reduced a high voltage activated calcium conductance that was sensitive to ω-conotoxin-GVIA, a N-type channel blocker. By down regulating this calcium current, baclofen also produced a reduction of the outward potassium current. The net consequence of these three conductances was that baclofen produced a small inhibitory shunt around the resting membrane potential of the ganglion cell, but a potentiating inward current at more positive potentials. In combination, these conductances would serve to suppress weak depolarizing synaptic currents while enhancing stronger synaptic currents. This was tested by injecting currents of different amplitudes into ganglion cells under current clamp. As predicted, the voltage responses to 15pA currents were suppressed, but the responses to stronger currents (>30pA) were enhanced by baclofen. Therefore, baclofen-sensitive metabotropic GABA receptors function as discriminators, accentuating the differences between the responses to weak and strong synaptic inputs. In the retina, this serves to differentiate the large, phasic synaptic inputs from the smaller, tonic inputs to ganglion cells.

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199.8

PHOTOPIGMENTS IN REGENERATED RETINA

D.A. Cameron*, M.C. Cornwall, and E.F. MacNichol, Jr. Department of Physiology, Boston University Medical School, Boston, MA 02118

Adult fish retinas can regenerate following injury. We have performed a microspectrophotometric analysis of rod and cone photoreceptors in normal and regenerated retinas of adult green sunfish (*Lepomis cyanellus*), in order to determine if the spectral characteristics of regenerated photoreceptors are similar to those of normal photoreceptors. In normal retinas all photopigments are apparently dehydroretinal-based, with all members of double cones containing a long-wavelength pigment (λ_{max} = 620 ± 1 nm, n = 45), all single cones containing a middle-wavelength pigment (λ_{max} = 532 ± 1 nm, n = 24), and all rods containing a different middle-wavelength pigment (λ_{max} = 527 ± 1 nm, n = 21), consistent with an earlier report (Dearry & Barlow, 1987, *J Gen Physiol* 89,745). Photoreceptors derived from patches of regenerated retina six weeks following injury have also been examined. The pigments in the regenerated photoreceptors were not significantly different from normal: double cone members, λ_{max} = 622 ± 2 nm (n = 9); single cones, λ_{max} = 533 ± 1 nm (n = 8); rods, λ_{max} = 526 ± 2 nm (n = 14). There has been no evidence for any short-wavelength or ultraviolet photopigments in either normal or regenerated retina. These results are consistent with the hypothesis that the spectral, and perhaps physiological, characteristics of regenerated photoreceptors are similar to those of photoreceptors in normal retina.

Supported by NIH grants EY-11160 (DAC) and EY-01157 (MCC).

199.9

SCANNING MICROINTERFEROMETRY DEMONSTRATES LONGITUDINAL REFRACTIVE INDEX GRADIENTS IN SUNFISH DOUBLE CONES

M. P. Rowe, N. Engheta, S. S. Easter, Jr., J. M. Corless, and E. N. Pugh, Jr.*

Cone inner segments/outer segments (CIS-COSs) were isolated from the retina of the green sunfish (*Lepomis cyanellus*). CIS-COSs were scanned with a microinterferometer capable of measuring optical path differences (OPD) with a precision of 6 nm. The scanning beam had a diameter of approximately 0.3 μm, and moved in approximately 0.2 μm steps between measurements. Cones were scanned perpendicular to the direction of in vivo light propagation. Such measurements have allowed us to characterize refractive index distributions in single cone CIS-COSs (Rowe, *et al*, JOSA A, *in press*). The refractive indices of double cone CISs cannot be easily calculated from OPD measurements, because unlike single cone CISs they do not have circular cross-sections. Nevertheless, it is possible to make quantitative assessments of the variation in refractive index within a double cone CIS by comparing variations in OPD to variations in double cone size and shape as indicated by transmission electron microscope (TEM) images of tangentially sectioned sunfish retinas. Our analysis indicates that sunfish double cone CISs exhibit longitudinal refractive index gradients qualitatively similar to those measured in single cone CISs and inferred from examination of the electron density of double cones examined via TEM images of radially sectioned sunfish retinas. Double cone COSs can be measured much as those of single cone CIS-COSs (because they do have circular cross-sections), and are found to have refractive indices which are relatively constant (around 1.39) along their longitudinal axes. Our work shows that to accurately characterize waveguiding in real sunfish photoreceptors ,future models will need to incorporate refractive index gradients.

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199.10

CODING VISUAL SCENES WITH ENSEMBLES OF RETINAL NEURONS

C.L. Passaglia*, F.A. Dodge, and R.B. Barlow
Marine Biology Laboratory, Woods Hole, MA. Syracuse University and SUNY Health Science Center, Syracuse, NY 13210.

Many animals use their eyes to seek food, pursue mates, and avoid predators in a visually-rich enviroment. To survive they must process visual information efficiently. Using *Limulus* as a model processing system, we recorded the animal's visual world with a videocamera mounted next to the lateral eye and simultaneously monitored the activity of a single optic nerve fiber with an underwater microsuction electrode. We then computed the eye's response to the underwater scenes by driving a cell-based computer model of the eye with digitized video frames. The model computes the spike rates of all optic nerve fibers and displays them as a sequence of grey-scale images. The pixel array of these "neural images" represents the retinal mosaic of neurons. We verified the model calculations with the response of the recorded single optic nerve fiber.

We found that behaviorally-important underwater objects, i.e. other horseshoe crabs, strongly modulate the computed optic nerve responses in the neural images. Important stimulus characteristics are object size, contrast, and motion as well as natural light fluctuations caused by overhead waves that highlight reflective crab-sized objects. Information about objects appears to be encoded in the coherent activity of a dynamic ensemble of neighboring retinal neurons.

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199.11

THE PRECISION OF RETINAL SPIKE TRAINS.

Michael J. Berry*, David K. Warland, and Markus Meister. MCB Department, Harvard University, Cambridge, MA 02138.

The reproducibility of retinal responses is a fundamental characteristic of the neural code that carries visual information from the eye to the brain. If retinal spike trains are highly deterministic, then a precise visual message can be attached to each spike. We have measured the reproducibility of ganglion cell spike trains, recorded extracellularly from isolated retinae using a multi-electrode array. The stimulus consisted of diffuse random flicker with intensity values chosen randomly every 30 msec from a Gaussian distribution of fixed mean (photopic range) and root-mean-square contrast (35%). The same 20 sec flicker sequence was repeated 100 times.

In both tiger salamander and rabbit retinae, ganglion cells fired action potentials only at sharply defined times. Both the timing and the number of spikes produced during such a firing event were highly reproducible: trial-to-trial timing jitter was as low as 2 msec, and the variance in the spike number up to 30 times smaller than the mean. As a result, the response of ganglion cells is not adequately described by a time-varying firing rate. At lower contrasts (2.3%) the firing events had high timing precision but poor precision of spike number. This suggests that the timing of individual action potentials conveys more information about the visual stimulus than the number of spikes. A calculation of information transmission rates confirms this conclusion.

Supported by a Presidential Faculty Fellowship to M.M.

199.12

MECHANISMS OF CONCERTED FIRING AMONG RETINAL GANGLION CELLS.

I. H. Brivanlou, D. K. Warland,* and M. Meister. MCB Dept., Harvard University, Cambridge, MA 02138.

Parallel observation of spike trains from many retinal ganglion cells has shown that nearby neurons often fire in near synchrony, as evidenced by a strong central peak in their cross-correlograms. We have investigated the circuit mechanisms underlying this concerted firing in the retina of the tiger salamander. During both spontaneous and light-driven activity, different ganglion cell pairs showed 3 types of peaks in the cross-correlogram: broad (100-200 ms width), medium (20-40 ms) and narrow (< 2 ms). When vesicular transmitter release was blocked with Cd^{++}, light responses were abolished. The broad correlations disappeared, but medium and narrow correlations persisted. Occasionally, waves of activity propagated across the retina at high speeds of 20 mm/s.

We propose that nearby ganglion cells share input from a slow neuron via chemical synapses (broad correlations), excitation from fast non-spiking neurons via electrical junctions (medium), and input from spiking neurons via electrical junctions (narrow). Electrical coupling among ganglion cells appears strong enough to sustain propagating bursts under Cd^{++}-block, which may normally be suppressed by a sustained inhibitory input.

Supported by a Markey Fellowship and an NIH grant to MM.

199.13

SPIKE BURSTS IN VISUAL RESPONSES OF RETINAL GANGLION CELLS. S.M. Smirnakis*[,1], D.K. Warland[2], M.J. Berry[2], and M. Meister[2,*,1]Physics Dept. and Medical School, Harvard University; [2]MCB Dept., Harvard University.

How much does the temporal fine structure within a spike train contribute to the neural code? We stimulated isolated tiger salamander retinae with diffuse flicker and recorded ganglion cell spike trains extracellularly with a multi-electrode array. The light intensity was chosen randomly every 30 ms from a Gaussian distribution with photopic mean intensity and 35% root-mean-square contrast. Ganglion cells tended to fire tight bursts with a variable number of spikes. Within a burst, action potentials were separated by a few milliseconds, little more than the cell's refractory period. By contrast, different bursts were separated by several hundred milliseconds. By correlating the stimulus with bursts of different sizes, we found that bursts had qualitatively different visual response properties from those of single spikes. Using a linear decoding algorithm (Bialek *et al.*, *Science* 252, 1854) we estimated and compared the information individual spike trains transmit about the stimulus, before and after identifying the bursts as distinct symbols. If higher visual centers could distinguish between bursts of different sizes rather than treating each spike equally, they could extract 5-50% more information per ganglion cell.

Supported by a Markey Scholarship and a grant from HFSP to MM.

199.14

MULTINEURONAL FIRING PATTERNS AMONG RETINAL GANGLION CELLS CARRY DISTINCT VISUAL MESSAGES. M.J. Schnitzer[1,*] and M. Meister[2]. [1]Depts. of Physics and Molecular Biology, Princeton University, Princeton, NJ 08544, and [2]MCB Dept., Harvard University, Cambridge, MA 02138.

Large neuronal assemblies potentially make use of distributed coding schemes, in which neural messages are conveyed by the simultaneous activity of multiple neurons. In such codes, messages cannot be deciphered by examining the output of single neurons. To determine whether such a coding scheme prevails in the retina, we used a multielectrode array to record the simultaneous activity of 40-60 tiger salamander ganglion cells. Within this population, groups of up to seven cells fired patterns of synchronous spikes with <25 ms delay. This form of concerted activity comprised 30-45% of all spikes recorded in darkness or with a flickering checkerboard stimulus, and over 90% of the spikes from some individual cells. The visual receptive fields of the synchronous firing patterns could not be predicted from those of individual ganglion cells, suggesting that the visual messages conveyed by spike groups carried novel information. It is proposed that retinal ganglion cells use fine structure in their spike trains – on time scales shorter than the ~100 ms photoreceptor integration time – to transmit additional visual messages through the optic nerve bottleneck. Supported by grants to M.M. from ONR and HFSP.

POSTSYNAPTIC MECHANISMS: CHEMICAL EXCITABILITY

200.1

SYNAPTIC INPUTS LIMIT THE PROPAGATION OF SOMATIC ACTION POTENTIALS (APs) INTO THE DENDRITES OF NEOCORTICAL PYRAMIDAL NEURONS IN VIVO. D. Paré*, E. J. Lang and A. Destexhe. Dept. of Physiol., Sch. of Med., Laval Univ., Quebec, Canada G1K 7P4.

To determine if the back-propagation of APs in dendrites occurs *in vivo*, we performed intracellular recordings of layer V pyramidal neurons located within 2 mm of an electrode array that allowed the delivery of electrical stimuli at various depths. Recording pipettes contained K-ac or Cs-ac.

The shape of APs varied depending on whether they were triggered synaptically or by current injection. In comparison to current-evoked spikes, orthodromic APs elicited by intracortical stimuli were reduced in amplitude and duration. The magnitude of this reduction was correlated to the stimulation depth with superficial and deep cortical stimuli producing the smallest and largest decrements, respectively. For instance, in Cs-filled cells, spike amplitudes and duration could be reduced by as much as 30% and 90%, respectively. This phenomenon resulted from the fact that stimuli applied at different depths preferentially activated afferents ending at corresponding dendritic levels as demonstrated by shifts in IPSP reversals and related R_{IN} decreases as a function of the stimulation depth.

These results were replicated in a computational model based on recent data on the distribution of Na^+ channels in the dendrites, soma and axon initial segment of layer V pyramidal cells. Simulated proximal IPSPs were the most effective in preventing the activation of dendritic Na^+ channels by current-evoked spikes. Collectively, these findings suggest that the somatic spike amplitude is critically dependent on the participation of dendritic Na^+ channels and that synaptic inputs impose significant limitations on the retrograde propagation of action potentials in the dendritic tree. Supported by MRC, FRSQ and NINDS.

200.2

THE FUNCTION OF BACKGROUND SYNAPTIC INPUT IN CEREBELLAR PURKINJE CELLS EXPLORED WITH DYNAMIC CURRENT CLAMPING. D. Jaeger* and J.M. Bower. Div. Biology 216-76, Caltech, Pasadena, CA 91125

Dual whole cell recordings of single Purkinje cell somata were obtained in rat slices and artificial synaptic input was injected using dynamic current clamping. The artificial synaptic conductances we employed consisted of mixed excitatory and inhibitory inputs that were obtained from simulations with a realistic Purkinje cell model. Synaptic conductances from all excitatory and inhibitory synapses in the model were summed and stored in a file. The large number of synapses impinging on a single Purkinje cell and the assumed background of random activity in each of these inputs resulted in a considerable level of continuous synaptic conductance. The appropriate current during whole cell recording resulting from these conductance waveforms was calculated with a refresh rate of 10 KHz using a reversal potential of 0 mV for excitation and -70 mV for inhibition. The use of a separate electrode for injecting current eliminated interference between transients in injected current and recorded membrane potential that is typical for single electrode configurations.

In vitro recordings using this type of artificial random background input resulted in a pattern of somatic spiking that was very similar to recordings obtained *in vivo*. Thus, emulating a background of synaptic input transformed the bursty spontaneous activity of Purkinje cells typically seen *in vitro* into an ongoing irregular spike pattern characteristic of *in vivo* recordings. The total injected current was negative during spiking, as predicted from a previous modeling study. This finding indicates that intrinsic Purkinje cell currents are sufficient to keep the cell depolarized and that synaptic input operates as a variable current sink. When the same input pattern was repeated multiple times, we found that a number of spikes was timed accurately within 1 ms for each stimulation, but that these spikes did not result from distinct excitatory inputs. These results suggest a complex input-output function for the cerebellar Purkinje cell. Supported by NIH ns-31378-04, HFSP and the Sloan Foundation

200.3

PATCH CLAMP RECORDINGS OF SYNAPTIC CURRENTS FROM SYMPATHETIC NEURONS DURING NATURAL REFLEX ACTIVITY. [†]V. I. Skok, G. Farrugia, L. G. Ermilov, S. M. Miller and J. H. Szurszewski*. Department of Physiology and Biophysics, Mayo Clinic and Mayo Foundation, Rochester, MN, 55905, USA, and [†]Department of Autonomic Nervous System Physiology, Bogomoletz Institute of Physiology, Kiev 24, Ukraine.

Fast excitatory postsynaptic currents (e.p.s.c.s) elicited by distension of the colonic wall or by electrical stimulation of afferent nerve fibers from the colon were recorded from the neurons of mouse superior mesenteric ganglion (SMG) at 20°C with the whole cell patch clamp recording method. The e.p.s.c.s reversed at -3.5 mV and were abolished by 100 μM hexamethonium in voltage-dependent fashion, indicating that they were mediated by nicotinic acetylcholine receptors (nAChRs). Analysis of the amplitudes and time courses of the e.p.s.c.s evoked by colonic wall distension suggests that at least 25 afferent nerve fibers converged on the same SMG neuron, that the e.p.s.c.s decay phase included two main kinetic components with mean exponentials of 7.8 and 14.5 msec, and, less commonly, longer exponentials ranging up to 70 msec, at -70 mV holding potential. These results provide the first recordings of whole cell currents of single sympathetic ganglion neurons during natural synaptic reflex activity. They indicate that different colonic mechanosensory afferent nerve fibers can activate nAChR channels with markedly different kinetic characteristics. Supported by NIH grant DK17632.

200.4

DOES ACTIVE UPTAKE LIMIT SPILL-OVER OF GLUTAMATE ONTO EXTRASYNAPTIC NMDA RECEPTORS IN THE HIPPOCAMPUS? Fredrik Asztély*, Gül Erdemli & Dimitri M. Kullmann. Dept. Clinical Neurology, Institute of Neurology, Queen Square, London WC1N 3BG, United Kingdom

We have recently proposed that NMDA receptors on a CA1 pyramidal cell can sense glutamate released from terminals which are presynaptic to other cells (Kullmann & Asztély; Erdemli & Kullmann; Soc. Neurosci. Abs. 1996). This could explain why 'pure' NMDA receptor-mediated EPSCs can be elicited with minimal stimulation, and why there is a discrepancy between the amplitude variability of AMPA and NMDA components of population EPSCs: the statistic $1/CV^2$ is larger for the NMDA component, implying a greater quantal content, because NMDA receptors sample more release sites than AMPA receptors. Does active uptake of glutamate limit the extent of activation of extrasynaptic NMDA receptors? Evidence for this includes the observation that the non-competitive uptake blocker dihydrokainate can increase the amplitude of NMDA receptor-mediated EPSCs (Hestrin et al, Neuron 5: 247, 1990). Since the Q_{10} for glutamate uptake is greater than that for diffusion, there should be less spill-over at higher temperatures. We therefore measured $1/CV^2$ for both AMPA and NMDA components in CA1 cells in guinea pig hippocampal slices at different temperatures, using methods described by Kullmann (1994, Neuron 12: 1111). The average ratio [$1/CV^2$ NMDA] / [$1/CV^2$ AMPA] was 2.1 ± 0.1 (S.E.M.) at 20 - 24 °C (n = 42) and 1.4 ± 0.1 at 34 - 36 °C (n = 18). This is compatible with a major role for glutamate uptake in limiting the spatial extent of NMDA receptor activation, and implies that spill-over may be much less prominent at physiological than at room temperatures. To control for other effects of temperature on glutamate release and receptors, we are currently comparing the relative frequency of transmission failures with minimal stimulation in cells held at negative and positive membrane potentials, at the different temperatures. (Supported by the MRC and the Wellcome Trust)

200.5

EVIDENCE FOR AMPA RECEPTOR DESENSITIZATION AT A CENTRAL SYNAPSE. T.S. Otis*, & L.O. Trussell, Dept. of Neurophysiology, University of Wisconsin Medical School, Madison WI 53706.

The AMPA subtype of glutamate receptor desensitizes within milliseconds upon exposure to glutamate (GLU). Even so, no direct evidence demonstrates that desensitization limits receptor availability during physiological synaptic activity. Using whole cell recordings from chick cochlear nucleus neurons (nucleus magnocellularis or NM) in brain slices, we show with 3 independent approaches a rapidly developing, slowly recovering ($\tau > 30$ ms), postsynaptic depression following activation of single presynaptic inputs. 1) Quantal current size (peak mEPSC amplitude) is transiently reduced following evoked EPSCs in $SrCl_2$. 2) GLU released during an EPSC "cross desensitizes" the steady state current activated by an exogenously-applied, non-desensitizing agonist. 3) Currents activated by UV photolysis of caged GLU are depressed following an EPSC. These three methods uncover ~50% postsynaptic depression at 10 ms after the EPSC and a time constant for recovery ranging from 50-100 ms. Furthermore, the resulting synaptic depression is synapse specific, as two neighboring synapses on the same cell body strongly depress themselves, but do not depress one another (intervals from 10-100 ms)

In outside-out patches, the recovery (in the absence of GLU) of the response to a second of a pair of rapidly-applied GLU pulses occurs with τ = 16 ms. We hypothesize that recovery from postsynaptic depression in NM is delayed by the previously described slow removal ($\tau \sim 40$ ms) of tens of micromolar GLU from the calyceal synaptic cleft (*J.Neurosci.* **16**:1634-1644). AMPA receptors cannot recover from desensitization until [GLU] is cleared below μM levels, accomplished in part by GLU transporters. Clearance is slowed with increasing quantal content, thus the magnitude and rate of recovery from synaptic depression will vary according to recent activity. Lastly, these results suggest a potential locus for modulation of short-term depression. Supported by Grants GM16300 (T.S.O.) and NS28901 (L.O.T.).

200.6

TRANSPORTERS BUFFER SYNAPTICALLY RELEASED GLUTAMATE ON A MILLISECOND TIME SCALE. J. S. Diamond* and C. E. Jahr, Vollum Institute, Portland, OR 97201.

Recent studies suggest that synaptically released glutamate remains in the cleft only briefly (τ~1 ms; Clements, et al., 1992); glutamate transporters have been proposed to assist in the rapid clearance of free transmitter by binding glutamate in the first ms following release (Tong and Jahr, 1994). Others suggest that a large fraction of glutamate is cleared via transport >15 ms after release (Mennerick and Zorumski, 1995). We have estimated the effect of uptake blockers on the size and shape of the synaptic glutamate transient in cultured CA1 hippocampal synapses using the rapidly dissociating AMPA receptor competitive antagonist kynurenate (KYN) and a kinetic model of AMPA receptors. Because KYN is displaced from AMPA receptors by glutamate during a synaptic event, the block by KYN is very sensitive to glutamate concentration. The relatively fast kinetics of AMPA receptors and KYN dissociation ($k_{off} \sim 5\ ms^{-1}$) allowed us to estimate the time course of glutamate in the cleft with greater temporal resolution than previous approaches using NMDA receptors (Clements, et al., 1992). A glutamate transient was chosen so that the model replicated the dose-inhibition of the AMPA receptor EPSC by KYN and also the slowing of the mEPSC rise time observed in 100 μM KYN. A good fit to the data was obtained with a glutamate transient that decayed with an exponential time constant of 400 μs from a peak concentration of 1.5 mM. In the continuous presence of 100 μM KYN, blocking glutamate transporters with threo-β-hydroxyaspartic acid (300 μM) further slowed the mEPSC rise time, suggesting that transporters help shape even the earliest components of the glutamate transient and that diffusion and transporters clear glutamate at similar rates. These results are also consistent with the idea that, independent of transporters, removal of glutamate from the cleft is slower than predicted by free diffusion alone (Eccles and Jaeger, 1958; Wahl, et al., 1996).

Supported by the NIH.

200.7

Photostimulation using caged glutamate reveals differential distribution of glutamate receptors on rat neocortical neurons.

H.-U. Dodt*, A. Frick, T. Rüther and W. Zieglgänsberger, Max-Planck-Institute of Psychiatry, Clinical Institute, Clinical Neuropharmacology, 80804 München, FRG.

A combination of infrared videomicroscopy and photostimulation using caged glutamate was used to investigate the distribution of excitatory amino acid receptors on the soma and apical dendrite of neocortical layer V pyramidal neurons in brain slices. Both conventional and laser light sources were used for the localised stimulation of neocortical neurons visualized by infrared videomicroscopy. Responses resulting from L-glutamate released locally by photolysis, were recorded somatically with a patch pipette in the whole-cell current-clamp mode.

Neocortical slices were superfused with medium containing 1 μM TTX and 1 mM caged glutamate. UV-irradiation, provided by a mercury burner, was used to release L-glutamate by photolysis locally (~20 μm spot diameter) at the soma and along the apical dendrite. After the selective glutamate antagonists APV (25 μM, n=10) or CNQX (2.5 μM, n=8) were added to the perfusion medium, stimulation was repeated at the same sites. Blockade of the NMDA receptors by APV was most effective at somatic and proximal dendritic sites. At a distance of 350 μm from the soma the decrease induced by APV was only 38% compared with the soma (=100%). Conversely, CNQX reduced glutamate-evoked responses at this dendritic site by 143%. This signifies a higher contribution of AMPA-mediated currents at remote dendritic regions.

By uncaging L-glutamate with a UV-Laser, we were able to stimulate areas in the 1μm range on the neuronal surface. Glutamate sensitivity was spatially not uniform along the dendrite. In fact, discrete 'hot spots' of glutamate sensitivity could be demonstrated by laser photostimulation. Laser stimulation was also used to activate neighbouring pyramidal neurons. Synaptic connections originating from neurons as remote as 900 μm were found. The data suggest that uncaging of excitatory amino acids by means of laser light allows, besides the detailed analysis of the distribution of neurotransmitter receptors, the characterization of neuronal circuits. Supported by a grant of the BMBF.

200.8

PRE- AND POSTSYNAPTIC FACTORS DETERMINE SYNAPTIC INTEGRATION OF GLUTAMAT-MEDIATED EPSPS IN GABAERGIC INTERNEURONS OF THE HIPPOCAMPUS. J.R.P. Geiger, J. Lübke, M. Frotscher, A. Roth and P. Jonas*, Physiologisches Institut and Anatomisches Institut der Universität Freiburg, D-79104 Freiburg and Max-Planck-Institut für medizinische Forschung, D-69120 Heidelberg, Germany

To investigate excitatory synaptic transmission of glutamatergic principal neurons onto GABAergic interneurons, we performed whole cell patch clamp recordings from pairs of synaptically coupled granule cells (GCs) and basket cells (BCs) in the dentate gyrus of hippocampal slices (at 32 °C). At -70 mV, the GC-BC evoked EPSCs were blocked by 5 μM CNQX, indicating that they were mediated by AMPA-type glutamate receptors. GC-BC EPSCs and EPSPs showed a rapid time-course. The EPSCs had a 20 - 80 % rise time of ≈100 μs and a decay time constant of 563 ± 78 μs (range 200 - 940 μs, n = 8). The EPSPs showed a rise time of ≈500 μs and a decay time constant of 3.3 ± 1.0 ms (n = 7). Hence, EPSCs and EPSPs in the BC were about 5-fold faster than those in hippocampal principal neurons. EPSCs and EPSPs showed a marked paired-pulse depression (PPD); the amplitude of the second EPSC was 44 ± 9 % of that of the first (20 ms interpulse interval). The number of failures in the second response was higher than that in the first, indicating that the PPD was of presynaptic origin. Light microscopical analysis of synaptically coupled pairs of neurons filled with biocytin indicated that 2-5 synaptic contacts on basal dendrites of BCs close to the soma (<100 μm, n = 6) underlie the GC-BC synaptic event. Simulations of EPSPs based on a detailed passive cable model of the BC show that the time course of the EPSP is determined by the kinetics of the postsynaptic conductance change. When the conductance decay time constant was between 250 μs and 1.5 ms, the simulated EPSP decay time constant varied between 3.8 and 7.0 ms. Due to the marked PPD and the very rapid time course of the postsynaptic conductance change, EPSPs caused by single GC activity do not exhibit summation. This suggests that specific pre- and postsynaptic properties of the GC-BC synapse favor coincidence detection of excitatory synaptic events by BCs. Funded by the DFG (SFB 505/C5).

200.9

MUSCARINIC LTP DEFICIENCY IN AGED RATS. J. M. Auerbach, M. C. Bundman* and M. Segal. Neurobiology, The Weizmann Institute 76100 Israel.

We have previously shown two opposing, concentration-dependent effects of the muscarinic agonist carbachol (CCh) on reactivity to afferent stimulation in area CA1 of the hippocampal slice in young adult rats (Auerbach and Segal, J. Physiol. 492:479, 1996). CCh (0.5 μM) induces muscarinic long-term potentiation (LTP_m) whereas ≥ 5.0 μM CCh causes a reversible depression of stratum radiatum EPSP slope. Aged animals are known to be slightly deficient in several cholinergic parameters, as well as in learning and memory. Here we compared slices from aged animals (24-27 months) to those from young animals (2 months), in their ability to express tetanic and muscarinic LTP.

In response to tetanic stimulation (100Hz, 1s), old slices showed post-tetanic potentiation (PTP) indistinguishable from that of young slices. A difference was seen, however, in the maintenance phase of LTP. Forty minutes after the tetanus, EPSP slopes recorded from young slices were stable at 65.4 ± 7.8% above baseline whereas those from aged slices decayed to a level 32 ± 7.1% above baseline. In addition, an age-dependent difference was found in the dose-response of the slices to CCh. While slices from aged animals displayed a depression of EPSP slope in response to high CCh concentrations similar to that of young animals, slices from these animals completely lacked LTP_m.

Furthermore, we found that 0.0001% H_2O_2 blocked LTP_m in young slices, but had no effect either on baseline EPSP slope or on response to high CCh concentrations. We also found that the addition of catalase, which breaks down H_2O_2 to water and oxygen, restored LTP_m in slices from aged animals. These findings indicate that cognitive deficits in aged animals and humans, as well as dementias such as Alzheimer's Disease, may be based on an enzyme imbalance leading to accumulation of H_2O_2 in the brain. The mechanisms associated with the action of H_2O_2 are currently being investigated. Supported by a grant from the Minerva Foundation.

201.1

POSITIVE REGULATION OF CRH-BINDING PROTEIN PROMOTER ACTIVITY BY CRH. A.F. Seasholtz* and D.N. Cortright, Department of Biological Chemistry and Mental Health Research Institute, University of Michigan, Ann Arbor, MI 48109.

Corticotropin-releasing hormone (CRH) is the major hypothalamic releasing factor in the mammalian stress response. A 37 kD CRH-binding protein (CRH-BP) distinct from the CRH receptor has been shown to neutralize the ACTH-releasing activity of CRH *in vitro*. The CRH-BP has been localized to a variety of brain regions and to corticotroph cells in the anterior pituitary, suggesting a role for this binding protein as a modulator of the endocrine and synaptic activities of CRH. In an effort to further elucidate the physiological role of the CRH-BP, we have examined the regulation of CRH-BP gene promoter activity by cAMP and CRH. A 3.5 kb fragment of the rat CRH-BP gene containing 3.4 kb of 5' flanking DNA and 66 bp of the 5' untranslated sequences was fused to the chloramphenicol acetyltransferase (CAT) reporter gene and transiently transfected into COS-7 and α-TSH cells. High basal levels of reporter activity were observed in both cell lines and 24 hour treatment with forskolin/IBMX (10 μM/250 μM) increased activity 11-fold in COS cells and greater than 300-fold in α-TSH cells. Co-transfection of α-TSH cells with the CRH-BP-reporter construct and an expression construct for the CRH receptor showed a time- and concentration-dependent positive regulation of CRH-BP-reporter activity in the presence of CRH. Twelve hours of treatment with 20 nM CRH alone increased reporter activity by 13-fold, while treatment with CRH/IBMX increased activity by greater than 350-fold. These results suggest that CRH-BP gene expression is positively regulated by CRH and cAMP. (NIH DK42730)

201.2

STEROID-INDEPENDENT REGULATION OF GLUCOCORTICOID RECEPTOR FUNCTION. C.M. Pariante*, B.D. Pearce, T.L. Pisell, A.H. Miller. Emory Univ. Sch. of Med., Atlanta, GA 30322.

Data has indicated that glucocorticoid receptor (GR) expression and function can be regulated by a number of factors via steroid hormone-independent mechanisms. Interestingly, these pathways may be involved in the glucocorticoid receptor dysregulation believed to underlie the hyperactivity of the hypothalamic-pituitary-adrenal (HPA) axis found in several neuropsychiatric disorders. For example, in vivo and in vitro treatment with the pro-inflammatory cytokine, IL-1, can induce resistance to the effects of glucocorticoid hormones, while antidepressants have been shown to both upregulate GR expression in the brain and increase glucocorticoid-mediated negative feedback on the HPA axis. Our laboratory is currently examining the in vitro effects of these two modulators of the GR. Mouse fibroblasts (L929 cells) were pre-incubated with IL-1α (1-1000 U/ml) or the antidepressant, desmethylimipramine (DMI), (0.1-10 μM) for 24 hours. Cells were then exposed to the synthetic glucocorticoid hormone, dexamethasone (10 nM), for an additional 90 minutes. Translocation of the GR from the cytoplasm to the nucleus was evaluated using immunofluorescent staining of the GR and a cytosolic radioligand binding assay. In addition, GR-mediated gene transcription was measured by means of L929 cells transfected with a CAT reporter gene behind a glucocorticoid response element. IL-1α (1000 U/ml) was found to significantly attenuate both dexamethasone-induced GR translocation and GR-mediated gene transcription without affecting cytosolic binding. In contrast, DMI (10 μM) was found to facilitate GR translocation from the cytoplasm to the nucleus and to decrease cytosolic receptor binding. We suggest that HPA axis function and dysfunction during disorders involving the neuroendocrine and immune systems may be related to steroid-independent mechanisms of GR regulation. Supported by MH47674, MH00680, CNR (Rome) AI95.00290.04.

201.3

Demonstration of a novel receptor mediating the stress effects of central Neuropeptide Y.

Small, C.J., Meeran, K., Morgan, D.G.A., Heath, M.M., O'Shea, D.*, Gunn, I., Taylor, G.M., Choi, S.J., Smith, D.M., Ghatei, M.A., and Bloom, S.R.
Division of Endocrinology and Metabolic Medicine, Department of Medicine, Royal Postgraduate Medical School, Hammersmith Hospital, Du Cane Road, London W12 ONN, UK.

Hypothalamic neuropeptide Y (NPY) is clearly implicated in the regulation of feeding, the stress response, growth and sexual function. NPY modulates the stress response by stimulation of adrenocorticotrophic hormone (ACTH) secretion via hypothalamic corticotrophin releasing hormone (CRH). Of the four recognised NPY receptors (Y1-Y4), it is not known which, if any mediate the activation of the hypothalamic pituitary adrenal (HPA) axis. We administered Intracerebroventricular (ICV) NPY and analogues of NPY which have a reduced affinity for the Y1 and Y4 receptors (NPY(13-36)), the Y2 receptor ([Pro^{34}]NPY) and the Y3 receptor (PYY) and measured plasma ACTH. All NPY fragments studied caused a significant rise in plasma ACTH compared to saline or a peptide control (random sequence of 31 amino acids) at a 0.72nmol dose ($F(7,42)=3.7$:$p<0.005$) and 7.2nmol dose ($F(7,36)=5.4$:$p<0.001$). In addition the novel NPY fragment [Pro^{34}]NPY(13-36), which binds with low affinity to the Y1 & Y2 receptors, did not stimulate two hour food intake (NPY 2.4nmol 5.6 ± 0.6g, [Pro^{34}]NPY(13-36) 50nmol 0.7 ±0.3g, saline 0.8 ±0.2g) but was as effective as NPY at stimulating ACTH release. These studies demonstrate a novel NPY receptor mediating ACTH secretion and indicate that this receptor is functionally distinct from the previously recognised NPY receptors, and the NPY receptor which mediates feeding.

CJS is a Wellcome prize student. This programme is funded by the MRC.

201.4

REGULATION OF GLUTAMIC ACID DECARBOXYLASE mRNA IN CENTRAL STRESS CIRCUITRY. G.L. Bowers, W.E. Cullinan* and J.P. Herman. Dept of Anatomy and Neurobiology, Univ.of Kentucky Medical Center, Lexington, KY 40536-0084, and Dept. of Basic Health Sciences, Marquette Univ., Milwaukee, WI 53233.

γ-amino butyric acid (GABA) plays a major inhibitory role in neuronal regulation of the hypothalamo-pituitary-adrenocortical (HPA) axis. Glutamic acid decarboxylase (GAD), the precursor enzyme for GABA, exists in two isoforms (GAD 65 and 67). Both are present in areas containing HPA-relevant GABAergic neurons. The present studies examined regulation of GAD 65 and 67 mRNA following exposure to acute restraint or a 14-day chronic variable-stressor paradigm. GAD mRNA was analyzed in HPA regulatory regions projecting to the paraventricular nucleus (PVN), including the bed nucleus of the stria terminalis (BST), preoptic area (POA), and hypothalamus, and in the hippocampal formation. Increased expression of both GAD 65 and 67 mRNA was observed in CA1 and dentate gyrus (DG) and in the anterior dorsal (AD) and anterior medial (AM) regions of the BST sixty minutes following stress. Up-regulation of GAD 67 mRNA also occurred in the arcuate (ARC) and dorsomedial (DMH) nuclei of the hypothalamus, and the medial POA. The 14-day chronic variable-stressor paradigm yielded increased GAD 65 and 67 mRNA expression in the AD and AM regions of the BST and medial POA. GAD 65 mRNA up-regulation was also observed in the anterior hypothalamic nucleus, DMH, and suprachiasmatic nucleus (SCN), while an increase in expression of GAD 67 mRNA also occurred in CA3 and DG. Significant changes were not observed in the caudate nucleus, reticular thalamic nucleus, or frontal cortex, indicating confinement of GAD up-regulation to stress-responsive regions of the CNS. Increased synthesis of GABA in these PVN-projecting regions following exposure to acute and chronic stress is consistent with involvement of these selected cell populations in modulation of HPA activity. The significance of differential up-regulation of GAD isoforms in observed brain regions remains unclear. Supported by MH49698.

201.5

ADRENAL SPLANCHNIC DENERVATION DECREASES PLASMA CORTICOSTERONE RESPONSES TO WATER DEPRIVATION IN RATS W.C. Engeland*, L.M. Rogers, D.A. Fitzgerald and J.T. Walworth Departments of Surgery and of Cell Biology / Neuroanatomy, University of Minnesota, Minneapolis MN 55455, USA

Chronic volume depletion produced by water deprivation (WD) increases sympathetic activity and plasma corticosterone (B). Studies were done to determine whether the plasma B response results from changes in plasma ACTH or in sympathetic input to the adrenal. To determine pituitary-adrenal responses to volume depletion, plasma ACTH and B were measured in rats at 24 or 48h after WD or ad lib water. Both plasma ACTH and B were increased after 24 and 48h of WD. To determine whether adrenal innervation was required for the response, rats were unilaterally adrenalectomized, allowed to recover and underwent thoracic splanchnicectomy (SPLNX) or sham SPLNX (INTACT). After 10 days, rats were WD or hydrated for 48h. Plasma ACTH and B increased after WD in SPLNX and INTACT rats; however, the B response was reduced in SPLNX vs INTACT rats. To determine the possible adrenal site affected by SPLNX, adrenals were examined for differences in steroidogenic enzyme mRNA using in situ hybridization. Adrenals sections were hybridized with ^{35}S-labeled oligonucleotide probes, exposed to film, and autorads were scanned and calibrated to a density scale. In response to WD, expression of cytochrome P450 11ß-hydroxylase, but not 3ß-hydroxysteroid dehydrogenase mRNA was increased; however, no differences were found between SPLNX and INTACT adrenals. These findings show that splanchnic nerves contribute to the plasma B response to WD, but the adrenocortical mechanism affected is unknown. Supported by NSF IBN-9319097.

201.6

CORTICOSTEROID-DOPAMINERGIC INTERACTIONS IN MONKEYS TREATED WITH METYRAPONE. D. Lyons*, Q. Wang, S. Lindley and A. Schatzberg. Department of Psychiatry and Behavioral Sciences, Stanford University School of Medicine, MSLS P111, Stanford, CA 94305-5485.

Corticosteroid-dopaminergic interactions thought to occur during major depressive disorders in humans are examined during social separations in squirrel monkeys treated with metyrapone (an 11β-hydroxylase inhibitor that blocks cortisol biosynthesis). Beginning 1-hr before monkeys were separated from groups and housed alone, eight adult females received either 15 mg/kg metyrapone or the fruit drink vehicle administered at 4-hr intervals for 24 hr in a counterbalanced crossover design. Social separation-induced hypersecretion of cortisol was initially driven by hypersecretion of the adrenocorticotropic hormone ACTH. From 1-25 hr post-separation, however, cortisol remained elevated above pre-separation controls while simultaneous measures of ACTH were reduced. This suggests that pituitary corticotrophes are stimulated by the ACTH-releasing hormone CRH, but this stimulatory effect is suppressed by high levels of cortisol. By removing cortisol feedback with the metyrapone blockade, these overdriven corticotrophs are revealed. In addition to producing a significant decrease (88%) in cortisol and a large increase (380%) in ACTH, metyrapone produced post-separation changes in plasma levels of the dopamine metabolite homovanillic acid (HVA). Although HVA levels were not significantly different from pre-separation controls at 1-hr post-separation, HVA was significantly higher (34%) than controls in metyrapone (but not vehicle) treated monkeys at 25-hr post-separation. These results are consistent with our clinical studies of humans and experimental studies of rats, which suggest that hypothalamic-pituitary-adrenal axis hyperactivity produces delayed changes in peripheral dopamine metabolites and central mesocortical dopamine utilization. This research was supported by NIMH grant MH47573.

201.7

DIFFERENTIAL REGULATION OF HYPOTHALAMIC CORTICOTROPIN-RELEASING HORMONE-mRNA BY CHRONIC AND ACUTE INTERMITTENT STRESS IN INFANT RATS. E.E. Gilles[1,2], C. Guirguis[2], L. Schultz[2], S. R. Snodgrass*[3] and T. Z. Baram[2]. Neurology/USC, Los Angeles, CA[1]; Pediatrics & Anatomy/Neurobiology, UCI, Irvine, CA[2]; Pediatrics, U Mississippi, Jackson, MI[3].

Rationale: The modulation of the hypothalamic-pituitary-adrenal axis by chronic or acute intermittent stress in infancy has not been fully defined. We have created an infant rat model of continuous stress manifested by increased secretion of corticosterone (CORT) and downregulation of pituitary corticotropin-releasing hormone (CRH) receptors. The goal of the current study was to examine CRH-mRNA abundance in the hypothalamic paraventricular nucleus (PVN) in this model, in comparison to a single or intermittent acute cold stress. Methods: Rat pups (n=92) were assigned to 3 treatment groups during postnatal days (PND) 2-9: 1) no handling with limited bedding; 2) no handling; 3) routine bedding-handling. On PND 9, pups from groups 1 and 2 were sacrificed under stress-free conditions. Pups from group 3 were either exposed to maximally tolerated cold stress (Yi and Baram, 1994) 1 or 3 times (AM-PND 9, PM-PND 9 and AM-PND 10) and sacrificed 4 hours after the last cold stress, or were sacrificed under stress-free conditions. CRH-mRNA abundance in the PVN was determined using in situ hybridization and MCID image analysis. Results: CRH-mRNA levels of chronically stressed pups were 57% of nonhandled controls ($p<0.005$). CRH-mRNA levels of the group exposed to a single episode of acute cold stress were 191% of controls ($p<0.01$), while levels of the group exposed to multiple cold episodes were reduced to 49% that of controls ($p<0.01$). Discussion: A single episode of acute cold stress results in increased CRH-mRNA levels while either repeated acute cold stress or severe chronic stress causes a reduction of CRH-mRNA levels. This "exhaustion" of the ability of pups to express CRH-mRNA may limit their ability to respond appropriately to subsequent acute stressors.

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201.8

PERSISTENT EFFECTS OF MATERNAL DEPRIVATION ON HPA ACTIVITY. H.J.J. van Oers[1,2], E.R. de Kloet[2] and S. Levine*[1]. [1] Dept. of Psychology, Univ. of Delaware, Newark, DE 19716-2577, U.S.A.; [2]Div. of Medical Pharmacology, LACDR, P.O. Box 9503, 2300 RA Leiden, The Netherlands.

Maternal deprivation of both male and female neonatal rats for 24 hours enhances the adrenocortical response to stress during the stress hyporesponsive period (SHRP). The current experiments test the hypothesis that such maternally deprived neonatal rats show altered hypothalamic-pituitary-adrenal (HPA) axis regulation not only immediately after deprivation but also at a later time. In addition, we looked at mRNA levels for corticosteroid receptors in the hippocampus.

Litters were deprived for 24 hours on postnatal day (pnd) 3 (DEP3-4), 7 (DEP7-8) or 11 (DEP11-12) and tested for their HPA responsiveness on pnd 20. Basal and stress-induced (saline injection) levels of both ACTH and CORT were determined using radioimmunoassays (RIA). Basal mRNA levels of corticotropin-releasing hormone (CRH), mineralocorticoid receptors (MRs) and glucocorticoid receptors (GRs) were measured in brain sections with in situ hybridization.

Compared with non-deprived (NDEP) rats: (i) DEP3-4 animals showed significantly higher ACTH levels after stress at pnd 20. MR mRNA levels in the CA3 and CA4 region of male hippocampi were elevated, while no changes were seen in the other parameters measured. (ii) DEP7-8 rats showed resembled a difference in hormone levels that did not read significancy. The expression of GR mRNA was decreased in all areas of the male hippocampi and in the dentate gyrus of the females. (iii) DEP11-12 animals had significantly lower ACTH values in response to stress. In this group deprivation resulted in a downregulation of GR mRNA in both sexes and MR mRNA in females. In contrast, all four experimental groups showed comparable values for CORT and CRH mRNA. Taken together, differential persistent effects of maternal deprivation are obtained dependent on the day of separation in the SHRP. Supported by NIMH grant MH 45006 to S. Levine.

201.9

THE EFFECTS OF CHRONIC STRESS AND ANTIDEPRESSANT TREATMENT ON THE CORTICOSTERONE RESPONSE TO RESTRAINT STRESS. C.A. Zimmer, J.F. López, D.M.V. Vázquez* and S.J. Watson. MHRI, University of Michigan, Ann Arbor, MI 48109.

There is evidence from both animal and clinical studies that the final products of the Hypothalamic-Pituitary-Adrenal axis (cortisol in humans, corticosterone in rats) have profound effects on mood and behavior (McEwen 1987; Murphy 1991). We have previously shown that in rats, Chronic Unpredictable Stress (CUS) results in elevated basal corticosterone (CS) levels and antidepressant administration can prevent this increase. In this study, we investigated the acute and long term effects of CUS and antidepressants on the stress response to restraint (RS). Rats were stressed daily for 4 weeks and received antidepressant treatment. Non stressed rats treated similarly served as controls. Six groups resulted: Saline (S), desipramine (D), zimelidine (Z), saline stress (SS), desipramine stress (DS) and zimelidine stress (ZS). Twenty four hours after the last CUS session animals were restrained for 30 minutes and blood samples were taken at 0, 15, 30, 60 and 120 min for CS determination. All animals were then allowed to rest for 4 weeks without stress or antidepressant treatment, at which time they were again exposed to a 30 min restraint. We found that after the first RS, the SS group and animals that received desipramine (D, DS), had lower CS levels at 120 min. After one month of rest, the CS response to RS were higher than on the first RS session for all groups, and all animals that received CUS or antidepressants had lower levels at 60 and 120 min when compared to the S group. We conclude that antidepressant treatment does not prevent the acute CS response to stress but has a long lasting effect, preventing prolonged exposure to elevated CS levels after a stressor. Supported by NARSAD Young Investigator Award (JFL) and NIDA (DMV).

201.10

INVOLVEMENT OF THE LOCUS COERULEUS IN THE NEURONAL INTEGRATION OF STRESS. D.R. Ziegler*, K. Bettenhausen, W.A. Cass, and J.P. Herman, Dept. Anatomy and Neurobiology, University of Kentucky, Lexington, KY 40536.

The locus coeruleus (LC) has been hypothesized to act as a central stress effector pathway. Numerous studies indicate that behavioral and perhaps neuroendocrine changes induced by stress are mediated by the LC. The present studies investigate the possibility that HPA-regulatory and effector roles of the LC occur in the context of acute and chronic stress. First, we hypothesized that lesions of the LC would impair HPA stress responsivity. Second, we predicted that the LC would show stress-induced changes in tyrosine hyrdoxylase (TH) and glucocorticoid receptor (GR) mRNA and, possibly, mRNA encoding the colocalized neuropeptides galanin and neuropeptide Y (NPY). Adult, male Sprague-Dawley rats with 6-OHDA lesions of the LC were subjected to a chronic variable stress regimen which, in sham animals, resulted in increased basal HPA activity (i.e., increased CRH mRNA expression, increased plasma ACTH and CORT levels, adrenal hypertophy). LC lesions resulted in blunted plasma ACTH and corticosterone responses to acute restraint stress. However, recovery from acute stress and basal HPA activity were unaffected by LC lesion. A separate group of (unoperated) rats were also subjected to the chronic variable stress regimen to assess activation of LC gene expression. Relative to control (handled) subjects, the chronic stress group exhibited significantly elevated GR, galanin, and neuropeptide Y (NPY) mRNA expression (34%, 72%, 85%, respectively, $p <= 0.05$). However, control and chronic stress groups did not show significantly different levels of TH mRNA. These results suggest that stress increases both co-transmitter expression and glucocorticoid receptivity in LC neurons. In conclusion, LC function seems to be required for a normal stress response; in addition, LC gene expression appears to be chronically enhanced by stress. How this activation of the LC impacts HPA function remains to be evaluated. Supported by MH 49698.

201.11

LOW FREE CORTISOL RESPONSE TO PSYCHOLOGICAL STRESS IN CHILDREN WITH ATOPIC DISORDERS. A. Buske-Kirschbaum , S. Jobst, K. von Auer, W. Rauh, S. Weis, A. Wustmans, C. Kirschbaum and D. H. Hellhammer*. Center for Psychobiology and Psychosomatic Research, University of Trier, 54286 Trier, Germany.

Atopic disorders are chronically relapsing inflammatory disorders which are often described as the results of an allergic response to various food or inhalant allergens.While most of the research has focused on the pathophysiological role of the immune system, the impact of endocrine signals in the pathology of atopic disorders has received only little attention. However, since the endocrine system is suggested to play an important regulatory role in immune functioning, it might be benficial to study endocrine reactivity in atopic patients.

In a first study, children with AD (n=15) and age and sex matched healthy controls (n=15) were exposed to the "Trier Social Stress Test for Children" (TSST-C) which mainly consists of a free speech and mental arithmetic tasks in front of an audience. Salivary cortisol was measured in ten minutes intervals while heart rate was monitored continuously. Results showed that the TSST-C induced significant increases in cortisol and heart rate. However, AD children showed a significantly blunted cortisol response to the stressor compared to the control group. In order to investigate whether attenuated cortisol response might be a general phenomenon associated with allergic inflammation, a second study was designed investigating patients with AA. Children with allergic asthma (n=20) and healthy controls (n=20) were again exposed to the TSST-C. Salivary cortisol and heart rate were determined as previously described. It was found that again, the TSST-C resulted in significantly elevated cortisol response and heart rate. However, comparable to the previous findings, a significantly decreased cortisol response was found in AA children. These data suggest a hyporesponsive hypothalamus-pituitary-adrenal (HPA) axis in children suffering from atopic disoders.

201.12

COMBINED LOW-DOSE PHYSOSTIGMINE AND AVP CHALLENGE IN MAJOR DEPRESSION. R.T. Rubin*, L.K. Sekula, S. O'Toole, K. Czambel. Neurosciences Research Center, Medical College of Pennsylvania and Hahnemann University, Allegheny Campus, Pittsburgh, PA 15212.

Hyperactivity of the hypothalamo-pituitary-adrenal cortical (HPA) axis occurs in 30-50% of patients with major depression. Central cholinergic neurotransmission stimulates CRH secretion, and increased CNS cholinergic activity relative to noradrenergic activity has been hypothesized as a neurochemical abnormality underlying major depression. This hypothesis has been supported by non-endocrine (sleep, pharmacological) studies. One study has shown that major depressives may have a greater HPA axis sensitivity to cholinergic stimulation than do controls, but at a dose of cholinergic agonist (physostigmine; PHYSO) that produces nausea and vomiting — noxious side-effects that themselves can activate the HPA axis.

We therefore designed a low-dose PHYSO/AVP challenge, uncomplicated by noxious side effects, that reliably stimulates the HPA axis in normal men and women. There are 4 test days, 4-7 days apart: saline/saline, PHYSO/saline, saline/AVP, and PHYSO/AVP. With this challenge we are testing moderate to severe major depressives and individually matched normal controls. Baseline serial blood sampling occurs from 1600 to 1800h; PHYSO or saline is given at 1800h; AVP or saline is given at 1900h; and sampling is continued until 2230h. Plasma is assayed for $ACTH_{1-39}$ and cortisol.

To date, eight female major depressives and eight female controls matched on age, race, and hormonal status have been studied. Average afternoon baseline $ACTH_{1-39}$ across the 4 test days was marginally lower in the patients, whereas baseline cortisol was higher. The patients had a clearly higher $ACTH_{1-39}$ response on the PHYSO-alone day. Patients and controls had similar $ACTH_{1-39}$ responses on the AVP-alone day. On the PHYSO/AVP day, the patients again had a clearly higher $ACTH_{1-39}$ response to PHYSO and a similar response to AVP. Cortisol responses paralleled the ACTH responses.

Our findings thus give further support to the hypothesis of increased CNS cholinergic activity in major depression. (Supported by NIH grants MH28380 and MH47363.)

202.1

RO 61-6270, A NEW HIGHLY SELECTIVE DOPAMINE D4 RECEPTOR ANTAGONIST, INDUCES C-FOS EXPRESSION IN MOUSE CORTEX. D.S. Hartman*, R. Smeyne, M.-T. Zenner, C. Goepfert, E.-J. Schlaeger#, F. Jenck, O. Civelli, T. Godel, and C. Riemer. Pharmaceutical Research, Preclinical Neurosciences, #Biotechnology, Hoffmann-La Roche, Basel, Switzerland.

The dopamine D4 receptor (D4R) has been implicated in affective and emotional disorders including schizophrenia. To identify novel potent and selective D4R antagonists, CHO cell membranes expressing the human D4R (Ashgari et al., 1995, J. Neurochem 65, 1157-1165) were used to screen over fifty thousand compounds. Optimization of one lead structure led to the synthesis of Ro 61-6270, a small molecule with high affinity and high selectivity for the human D4R. In competition binding assays Ro 61-6270 showed an inhibition constant (Ki) of 5 nM at the human D4R , and over 1000-fold selectivity for D4 vs. D1, D2, D3, and D5 receptors as well as 40 additional receptor sites tested including adrenergic, serotonergic, muscarinic, and histamine receptor subtypes. Ro 61-6270 is a competitive D4R antagonist, inhibiting DA-induced GTPγS binding to CHO-D4 cell membranes with an IC50 value of 175 nM. Intraperitoneal administration of Ro 61-6270 (30 mg/kg) to Sprague-Dawley rats resulted in efficient brain penetration measured 30 min after injection.

Transgenic lacZ/c-fos mice treated with Ro 61-6270 (10 mg/kg, i.p.) for 2 hr showed c-fos induction in frontal, cingulate, and piriform cortices, as well as in the paraventricular hypothalamic nucleus and in CA1/CA3 regions of hippocampus. Little or no induction in striatum or substantia nigra was seen. A time course of Ro 61-6270 induced c-fos expression should provide a map of brain areas potentially involved in D4R-mediated events, and will be compared to the pattern of ^{3}H-Ro-61-6270 binding in the brain and periphery.

All funding provided by Hoffmann-La Roche, Basel, Switzerland.

202.2

SPLICING OF AN ATYPICAL ALTERNATIVE INTRON IN DOPAMINE D_3 RECEPTOR PRE-mRNA. C. Schmauss*, B.V. Skryabin, and J. Haesik Yoon. Dept. Psychiatry & Brookdale Center for Molecular Biology, Mt. Sinai Sch. of Med., New York, NY 10029.

Dopamine D_3 receptor encoded pre-mRNA is processed alternatively to yield D_3 receptor mRNA and a truncated mRNA, named D_{3nf}. In human brain both mRNAs are co-expressed and, interestingly, both are translated into protein. *In vitro* splicing of substrate D_3 pre-mRNA in HeLa nuclear extracts yields D_3 mRNA and, via additional removal of a short alternative spliceosomal intron, D_{3nf} mRNA. The analysis of D_{3nf}-specific splicing intermediates by primer extension shows that 3' cleavage of this intron occurs at a noncanonical sequence (GGA:GU) that deviates from the invariant 3' splice site sequence AG:N of the major class of introns. When mutant D_3 pre-mRNA in which the alternative 3' splice site is converted into the consensus sequence of the major-class introns (GAG:G) is spliced *in vitro*, 3' cleavage occurs one nucleotide upstream (GA:GG) of the wildtype cleavage site. Furthermore, D_{3nf}-specific splicing is abolished in a mutant pre-mRNA that carries a single nucleotide mutation within the 3' GGA:G sequence, namely GUA:G. These results indicate that the sequence GA:N is essential for splicing of the D_{3nf}-specific alternative intron. In addition, primer-extension experiments suggest that the branchpoint of this alternative intron is located within sequences that are also atypical for branchpoints of major-class introns. This suggests that the D_{3nf}-specific intron represents a novel intron of the minor class. Interestingly, results from RNase protection experiments show that splicing of this intron is enhanced in brains of patients with chronic psychosis. (Supported by NARSAD and MH45212).

202.3

D_4 DOPAMINE RECEPTOR INVOLVEMENT IN PHOSPHOLIPID METHYLATION AND IT'S IMPLICATIONS FOR SCHIZOPHRENIA R.C. Deth*, P.F. Wick, M.L. Kramer, A. Sharma, D. Liu, C. DuRand, Q.-B. Zhu, M. Nagata, Y. Gou, S. Chari. Dept. Pharmaceutical Sciences, Northeastern Univ., Boston, MA 02115

Phospholipid methylation is thought to regulate the plasma membrane fluidity via a reaction in which S-adenosylmethionine (SAM) serves as a methyl donor for the enzyme phospholipid methyltransferase I. We now provide evidence that the D_4 dopamine receptor can also function as a donor of methyl groups, as a result of the adenosylation of MET313 at the cytoplasmic terminus of helix #6. This adenosylation is carried out by methionine adenosyltransferase, the same enzyme which adenosylates methionine, and requires the active R* state of the receptor. Thus antagonists such as haloperidol and clozapine inhibit adenosylation and phospholipid methylation by shifting receptors to the inactive R state. Adenosylation of the receptor also causes the receptor to exhibit spontaneous activity with regard to the activation of G proteins. A deficiency in methionine metabolism has been well documented in schizophrenia. We therefore compared phospholipid methylation in lymphocytes from patients *vs.*controls and found a greater than two-thirds reduction in schizophrenic individuals. Based upon these results, a unified hypothesis can be developed which combines both the "dopamine hypothesis" and the "single carbon hypothesis", centered upon a reduced level of membrane phospholipid methylation and consequent alterations in membrane fluidity. This reduction results from the combined lower activities of at least three different enzymes which are critical for both the receptor-dependent and receptor-independent pathways of phospholipid methylation.

202.4

LONG TERM STABILITY OF DOPAMINE (D_2) RECEPTOR ACTIVITY MEASURED WITH ^{11}C-RACLOPRIDE AND PET. R. Schloesser, J.D. Brodie, G.J. Wang, N. Volkow, J. Logan, D. Alexoff and S. L. Dewey.* Dept of Psych, NYU Med Center, NY, NY 10016. Dept of Chem, BNL, Upton, NY, 11973-5000

This study evaluated the long term stability of dopamine (D_2) receptor activity in normal human volunteers. Six healthy subjects (range: 24 - 75) were investigated twice with ^{11}C-raclopride under resting conditions with a mean time interval between scans of 14.7 months (range: 10-19). The methods used were similar to those published previously (Volkow et al., J. Nucl. Med. 34, 609-613, 1993). The ratio of the distribution volumes (DVs) of the basal ganglia (DV_{BG}) to cerebellum (DV_{CB}) revealed a mean absolute change of 6.0 % (range: -12.7 to 7.3) between study A and B. Mean change of DV_{BG} was 3.1% (range: -4.2 to 4.3), mean DV_{CB} change was 7.40 % (range: -10.7 to 11.3). The intraindividual differences between both scans in our study were similar to the 24 hour interval test-retest data from Volkow et al. (repeated measures ANOVA with df = 9; F = 0.81; p = 0.389). Age and length of the time between scans did not significantly correlate with the binding parameters of ^{11}C-raclopride. Across subjects, the DV ratio index revealed a mean of 3.11 (range = 2.55 to 3.68, SD 0.44) in study A and of 3.07 (range 2.37 to 3.57, SD 0.45) in study B. These findings support the view that baseline dopamine (D_2) receptor activity, in a given individual, and expressed by the DV ratio as an index of the binding potential, remains stable over many months. Furthermore, these results are consistent with previous findings of a substantial interindividual difference in D_2 receptor binding parameters. Future PET studies directed at measuring changes in dynamic properties (response to pharmacologic challenge) over time are necessary prior to beginning studies designed to investigate the effects of disease state or long term drug therapy on patients suffering from mental illness. MH47277, NS15638, RR00096 from the NIH, the DOE, DA06891 from NIDA, NARSAD and Schl 400/1-1 from the DFG.

202.5

REGIONAL EFFECTS OF A D3-PREFERRING AGONIST ON CEREBRAL BLOOD FLOW. K.J. Black*, T.O. Videen and J.S. Perlmutter. Depts. of Radiology, Psychiatry and Neurology, Washington University School of Medicine, St. Louis, MO 63110.

D3 receptors differ from other D2-like dopamine receptors in anatomic distribution as well as structure, pharmacology and second messenger function. We hypothesized that a D3-preferring agonist would alter neuronal activity in neuronal circuits that express D3 receptors. We performed 6 PET studies in 4 normal baboons. Each study included 3 baseline measurements of quantitative regional cerebral blood flow (rCBF), using PET and the $H_2{}^{15}O$ method, and then 3 rCBF scans after each successively higher intravenous dose of pramipexole (5, 50 and 500 μg/kg, *i.e.* "low," "medium" and "high"). PET scans were aligned with an MRI from each animal using a previously validated automated routine, and then transformed to stereotactic space using proportional measurements. We randomly selected about 1/3 of the scans at each condition to form a hypothesis-generating image in atlas space, and these scans were excluded from further analysis. Four areas of decreased rCBF (relative to global CBF) were identified at both medium and high doses, centered on medial orbitofrontal cortex, medial thalamus, right claustrum and posterior cingulate. An additional decrease (caudate head) and an increase (ventromedial pallidum) were identified at the high dose only. These areas were then tested using the remaining ("replication") scans. Preliminary analysis of the replication scans shows mean changes in rCBF in each of the first 4 regions, at both doses, of -8% to -15%. In the last 2 regions the rCBF change at the high dose was -3% and +4% respectively. Thus the change in each region was in the predicted direction, and even in this small sample uncorrected *p* values (t-test)for the first 4 regions at each dose ranged from 0.0035 to 0.09. We conclude that pramipexole may produce functional changes in ventral basal ganglia-thalamocortical circuits.

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202.6

EVALUATION OF EXTRASTRIATAL DOPAMINE RECEPTORS BY USING ^{18}F-FALLYPRIDE. J. Mukherjee*, Z-Y. Yang, T. Brown, J. Roemer and M. Cooper. Department of Radiology, The University of Chicago, Chicago, Il 60637.

Due to the increasing interest in evaluating the role of extrastriatal dopamine receptors in dopamine-related pathophysiology, we have attempted to identify the value of ^{18}F-fallypride ((*S*)-*N*-[(1-allyl-2-pyrrolidinyl)methyl]-5-(3-[^{18}F]fluoropropyl)-2,3-dimethoxybenzamide), as an extrastriatal dopamine D-2 receptor radiotracer. Fallypride exhibits high affinities for D-2 and D-3 subtypes and low affinity for D-4 (^{3}H-spiperone IC_{50}'s: D-2 = 0.60 ± 0.45 nM (rat striata), D-3 = 0.30 nM (SF9 cell lines, rat recombinant) and D-4 = 240 nM (CHO cell lines, human recombinant). *In vivo* rat biodistribution studies with ^{18}F-fallypride showed predominant binding in the striata followed by binding in thalamus, hypothalamus and frontal cortex. PET experiments with ^{18}F-fallypride in male rhesus monkeys were carried out in a PET VI scanner. In several PET experiments, apart from the specific binding seen in the striatum, specific binding of ^{18}F-fallypride was identified in the thalamus as well. Specific binding in the thalamus was significantly lower compared to that observed in the striata of the monkeys (thalamus/cerebellum = 2, striata/cerebellum = 10).

We have previously shown from PET studies that iv administration of *d*-amphetamine (AMPH) in rhesus monkeys alters binding of ^{18}F-fallypride in the striata. At high doses of AMPH (0.75 mg/kg and up) an immediate effect of increased dissociation rate k_{off} (from $<10^{-5}$ min^{-1} in controls to $>10^{-3}$ min^{-1} in AMPH treatment) on specifically-bound ^{18}F-fallypride occurred resulting in a reduction of specifically-bound ^{18}F-fallypride in the striata. However, effect of AMPH on specifically-bound ^{18}F-fallypride in the thalamus was not evident in several experiments that were carried out at doses ranging between 0.25 mg/kg upto 1.0 mg/kg. Due to its high affinity, fallypride is possibly unable to measure very small changes in dopamine levels in the thalamus, which could account for the absence of a measureable AMPH effect. This result may also be indicative of significantly lower levels of endogenous dopamine in this extrastriatal region.

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202.7

DIFFERENTIAL REGULATION OF D3 DOPAMINE RECEPTORS BY ANTAGONISTS K. D. Burris*, S. M. Fausing, R. L. Bertekap Jr. and P. B. Molinoff. CNS Drug Discovery, Bristol-Myers Squibb Pharmaceutical Research Institute, Wallingford, CT 06492

We have previously reported an increase in the density of D3 dopamine receptors following exposure of transfected HEK-293 cells (HEK-D3 cells) to the agonists, 7-OH-DPAT and quinpirole, and the antagonist haloperidol (Soc. Neurosci Abs. 20: 1066, 1994). These findings have been extended using antagonists from a variety of chemical classes in order to examine the pharmacological specificity of drug-induced increases in the density of D3 receptors. Exposure of HEK-D3 cells for 18 hours to haloperidol, a butyrophenone, resulted in an increase in the density of binding sites measured with the radiolabeled antagonist, [^{125}I]-NCQ-298. The maximum concentration of haloperidol examined, 1 μM, stimulated a 2-fold increase in the density of receptors. Exposure of cells to the (+)- and (-)-enantiomers of butaclamol, a benzocycloheptapyridoisoquinoline, revealed that (+)butaclamol was more potent than (-)butaclamol in stimulating an increase in the density of D3 receptors. This suggests that the increase in the density of receptors was mediated through specific interaction of the drugs with D3 receptors. In contrast to the effects of haloperidol and butaclamol, exposure of HEK-D3 cells to the benzamide sulpiride, at concentrations up to 100 μM, did not result in an increase in the density of D3 receptors. Sulpiride, however, blocked the increase in receptor density stimulated by haloperidol. Consistent with the affinity of D3 receptors for the enantiomers of sulpiride, (-)sulpiride was more potent than (+)sulpiride in blocking the effect of haloperidol. Eticlopride, another benzamide, also blocked haloperidol-stimulated up-regulation of D3 receptors. The mechanism whereby haloperidol and butaclamol interacted with D3 receptors to stimulate an increase in the density of receptors is unknown. The mechanism appears to involve more than a simple occupancy-based decrease in the rate of receptor degradation since sulpiride and eticlopride did not stimulate an increase in the density of receptors, though both antagonists interacted directly with D3 receptors to block the increase stimulated by haloperidol. The observation that haloperidol and butaclamol stimulated, whereas the benzamides blocked, an increase in the density of D3 receptors demonstrate that antagonists vary, perhaps through induction of different receptor conformations, in their ability to modulate D3 receptors.

202.8

STRUCTURAL INSTABILITY ASSOCIATED WITH CONSTITUTIVE ACTIVATION OF A G PROTEIN COUPLED RECEPTOR U. Gether*, E. Sanders-Bush and B.K.Kobilka. Howard Hughes Medical Institute, Stanford University, Stanford, CA 94305.

Discrete mutations in the carboxyterminal part of the third intracelullar loop can cause constitutive activation of G protein coupled receptors (GPCR), but the structural and molecular basis for this agonist-independent activity is unknown. We have expressed the constitutively activated mutant (CAM) of the β_2 adrenergic receptor in SF-9 insect cells and established a purification scheme that allowed the first structural analysis of a constitutively activated GPCR. Most strikingly, it was found that the solubilized, purified CAM was highly unstable as compared to the wildtype protein. Thus, incubation of CAM at 37^{O} C for 1 hour completely abolished binding activity whereas the wildtype retained around 60% binding activity after 1 hour and around 20% after 4 hours. The loss of binding activity could be prevented by incubation with the inverse agonist ICI 118,551. Purified CAM was further analyzed after labeling with the environmentally sensitive, sulfhydryl-reactive fluorophore, IANBD, which we previously have used for monitoringof ligand-specific conformational changes in the wildtype receptor. The data showed that, after pretreatment with ICI 118,551, the agonist isoproterenol produced an approximately two-fold greater decrease in fluorescence intensity from CAM than from the wildtype receptor. This suggests that agonist occupancy can promote a more profound conformational change in the CAM receptor than in the wildtype protein. Summarized, our data support an activation mechanism for GPCR's in which stabilizing conformational constraints keep the unliganded, non-constitutively activated receptor preferentially in an inactive state. In response to agonist binding or selective mutations these conformational constraints are disrupted enabling the receptor to assume an active conformation.

202.9

MAPPING AGONIST-INDUCED STRUCTURAL CHANGES IN A G PROTEIN COUPLED RECEPTOR BY SITE-SELECTIVE FLUORESCENT LABELING WITH A CONFORMATIONALLY SENSITIVE CYSTEINE REAGENT. S. Lin, U. Gether, P. Ghanouni, H.T. Schambye* and B.K.Kobilka. Howard Hughes Medical Institute, Stanford University, Stanford, CA 94305.

To establish a system that would allow site-selective incorporation of biophysical probes in a purified G protein coupled receptor we have systematically mutated cysteines in the β_2 adrenergic receptor and generated a series of mutants with only one, two or three remaining cysteines available for chemical derivatization. The mutant receptors were all expressed in SF-9 insect cells and purified. It was found that the mutants retained the agonist and antagonist binding properties of the wildtype receptor. Furthermore, their coupling to adenylate cyclase were indistinguishable from the wildtype. Previously, we have found that agonist-specific conformational changes in the β_2 adrenergic receptor can be detected following labeling of the purified receptor with the sulfhydryl-reactive fluorophore, IANBD. The fluorescence from IANBD is highly sensitive to the polarity of its molecular environment making IANBD a sensitive molecular reporter of conformational changes. Site-selective labeling of the purified cysteine mutants with IANBD revealed that cysteines in transmembrane segments (TM) III and VI were responsible for the reversible and stereospecific decrease in fluorescence emission observed from the IANBD labeled receptor. This suggest that agonist binding causes a conformational change in the receptor that involves movement of TM III and VI. Site-selective incorporation of biophysical probes in G protein coupled receptors should prove a powerful tool for further characterization of the molecular events and structural changes that occur in the receptors during activation and transmission of signal across the membrane.

202.10

Distribution of α_{1A} Adrenergic Receptor mRNA in the Rat Brain Visualized by *In Situ* Hybridization. A.V. Domyancic and D.A. Morilak* Dept. of Pharmacology, University of Texas Health Science Center at San Antonio, 7703 Floyd Curl Dr., San Antonio, TX, 78284-7764

Norepinephrine has been implicated in a number of physiological, behavioral and cellular modulatory processes in the brain. Many of these modulatory effects have been attributed to activation of post-synaptic α_1 adrenergic receptors. At least three α_1 receptor subtypes have been identified by molecular criteria, designated α_{1A}, α_{1B} and α_{1D}. In order to understand the specific functions of these different subtypes in the brain, it would be useful to specify their differential distributions. The distributions of α_{1B} and α_{1D} receptor mRNA expression in rat brain have been described previously, but the cDNA for the rat α_{1A} receptor has only recently been cloned and characterized. In the present study, we describe the distribution of α_{1A} message expression in the rat brain using a radiolabelled riboprobe derived from the rat α_{1A} receptor cDNA (D. Perez, Cleveland Clinic). High levels of α_{1A} receptor mRNA expression were seen in olfactory bulb, tenia tectae, diagonal band and preoptic area, zona incerta, ventromedial and lateral hypothalamus, lateral mammillary nuclei, ventral dentate gyrus, piriform cortex, medial and cortical amygdala, magnocellular red nuclei, pontine nuclei, lateral vestibular nuclei, brainstem reticular formation and several cranial nerve and spinal motor nuclei. More moderate hybridization signal was seen throughout the neocortex, and in the claustrum, diagonal band nucleus and lateral amygdala. This pattern of expression, when considered in comparison with that previously described for the other α_1 adrenergic receptor subtypes, may shed light on the different roles of the α_1 receptor subtypes in mediating the neuromodulatory effects of norepinephrine in processes such as arousal, neuroendocrine control, sensorimotor regulation and in the stress response. Support provided by research grants from the Whitehall Foundation and the American Heart Association, Texas Affiliate.

202.11

ENHANCED QUINPIROLE-INDUCED ORAL ACTIVITY IN RATS LESIONED WITH 6-HYDROXYDOPAMINE DURING ONTOGENY AND WITH 5,7-DIHYDROXYTRYPTAMINE DURING ADULTHOOD. R.M. Kostrzewa[1]*, R. Brus[2], K.W. Perry[3] and R.W. Fuller[3]. [1]Dept. of Pharmacology, East Tennessee State Univ., Johnson City, TN; [2]Dept. of Pharmacology, Silesian Academy of Medicine, 41-808 Zabrze, Poland; [3]Eli Lilly Research Labs, Indianapolis, IN 46285.

To better understand the modulatory role of serotonin (5-HT) neurons on dopamine (DA) receptors, DA D_1 receptor supersensitivity was produced by lesioning DA neurons with 6-hydroxydopamine (6-OHDA, 134 μg; bilateral icv injection with desipramine pretreatment, 3 days after birth). After observing enhanced oral activity responses to the DA D_1 agonist SKF 38393 and to the 5-HT_2 agonist *m*-chlorophenylpiperazine (*m*-CPP) in these rats in adulthood, 5,7-dihydroxytryptamine (5,7-DHT; 3-30 μg icv) was administered. In rats co-lesioned with 6-OHDA and 5,7-DHT, (a) *m*-CPP-induced oral activity was further enhanced, while (b) previously observed SKF 38393 enhancement of oral activity was absent, and (c) quinpirole-induced oral activity (D_2 agonist effect) became enhanced. In co-lesioned rats neostriatal DA content was reduced >98%, while 5-HT content was elevated vs. the saline control group and was slightly reduced (<15%) or unchanged vs. the 6-OHDA group. When 5,7-DHT (75 μg) alone was administered to neonatal rats, quinpirole responses were likewise enhanced. In the group lesioned neonatally with both 6-OHDA and 5,7-DHT, however, neither SKF 38393 nor quinpirole responses were enhanced. The findings indicate that 5-HT neurons have a major regulatory influence on the sensitivity of both DA D_1 and D_2 receptors. (Supported by NS29505)

202.12

ROLE OF β-ARRESTINS IN THE INTRACELLULAR TRAFFICKING OF G PROTEIN-COUPLED RECEPTORS. S.S.G. Ferguson*, J. Zhang, K. Winkler, W.E. Downey III, L.S. Barak and M.G. Caron. HHMI Labs and Dept. of Cell Biology, Duke University Medical Center, Durham, North Carolina, 27710.

β-arrestins and G protein-coupled receptor kinases (GRKs) are appropriately localized at neuronal synapses to play an important role in the desensitization of G protein-coupled receptors (GPCRs) involved in neurotransmission. However, little is known about the mechanisms contributing to the resensitization of GPCR responsiveness, except that agonist-promoted sequestration might be involved in this process. Recently we found, using a phosphorylation- and sequestration-defective β_2-adrenergic receptor (β_2AR) mutant, that GRK-mediated phosphorylation can facilitate β_2AR sequestration. In the present experiments, we find that β-arrestins, proteins which bind GRK-phosphorylated GPCRs, can promote β_2AR sequestration even in the absence of phosphorylation. In addition, wild-type β_2AR sequestration can be inhibited by a sequestration-specific β-arrestin dominant-negative mutant (V53D). In addition, β-arrestin1-V53D is co-immunoprecipitated in an agonist-dependent manner with the β_2AR following chemical cross-linking, indicating that this mutant is not impaired in its ability to interact with the receptor. Moreover, β_2AR sequestration is dependent upon functional dynamin, a protein required for the formation of clathrin-coated vesicles. However, the internalization of another GPCR, the angiotensin II Type 1A receptor ($AT_{1A}R$), is not dynamin-dependent, indicating that alternate pathways for GPCR internalization exist. In addition, $AT_{1A}R$ internalization is not inhibited by the β-arrestin1-V53D mutant, but in contrast, the magnitude of $AT_{1A}R$ internaliztion is increased by overexpression of wild-type β-arrestin. As expected, this β-arrestin-induced increase in $AT_{1A}R$ internalization is dynamin-dependent. These findings suggest a dual role for β-arrestins in GPCR regulation: β-arrestins are not only involved in GPCR desenstization, they also function as trafficking molecules specifically directing GPCRs for clathrin-mediated endocytosis. Supported by NIH Grant NS19576.

202.13

DOPAMINE D2 RECEPTOR DEFICIENCY REVEALS COMPENSATORY LOCOMOTOR SYSTEMS, M.A. Kelly[1], M. Rubinstein[1], C. N. Lessov[2,3], S. Burkhart-Kasch[2,3], J. R. Bunzow[1]*, C. Saez[1], T. J. Phillips[2,3], D. K. Grandy[1] M. J. Low[1]. [1]Vollum Inst., [2]Dept. Behav. Neurosci., Oregon Health Sci. Univ., [3]VA Med. Cent. Portland OR 97201

A role for the dopamine D2 receptor in locomotion was tested in mice homozygous for a targeted nonfunctional allele (KO). Open field activity of drug naive knockouts showed significantly decreased scores for initiation of movement and total horizontal distance, but normal speed and duration of movements compared to wild-type. KO mice showed no further change in activity when treated with the D2 antagonist haloperidol in marked contrast to significant decreases in initiation of movement and total horizontal distance in wild type and heterozygotes. These data suggest that compensatory mechanisms exist in the mutant mice to maintain locomotor activity at a level intermediate between drug naive and acutely D2 receptor antagonized wild-type mice. KO mice had normal increased locomotion in response to cocaine indicating intact D1 receptor function. The role of monoamines in modulating motor function in the D2 knockout mice was assessed by acute monoamine depletion with a combination of reserpine and AMPT. Akinesia was achieved in all mice and was slightly reversed by the D1-like agonist SKF 38393. The combination of SKF 38393 and the D2-like agonist Quinpirole had no additive effect in KO but increased distance scores in wildtypes. Surprisingly, heterozygotes demonstrated a return to pre-depletion distance scores at lower dosages than wild type siblings. Our studies suggest that the D2 receptor is important for the initiation of spontaneous movement (regulating the set-point) but not required for normal speed and duration of movements. Furthermore, the inability of combined D1 and D2 agonists to restore the basal locomotor activity of acutely monoamine depleted knockout mice implies that the compensatory mechanism(s) in these mice involves non-dopaminergic monoamine systems.

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CEREBELLUM

203.1

AN MRI ATLAS OF THE HUMAN CEREBELLUM IN TALAIRACH SPACE.

J. D. Schmahmann*, J. Doyon, C. Holmes, N. Makris, M. Petrides, D. Kennedy, A.C. Evans. *Department of Neurology, Center for Morphometric Analysis, Massachusetts General Hospital, Boston, MA, 02114; McConnell Brain Imaging Center, Montreal Neurologic Institute, McGill University, Montreal, Quebec, Canada; ICBM Project.*

The aim of thus study was to develop a comprehensive atlas of the human cerebellum within Talairach space. The brain of a healthy young male was imaged using a 1.5 Tesla Philips MRI scanner. Twenty-seven T1 weighted images were obtained within Talairach coordinates (Holmes et al., 1996). Brain slices of 0.5mm thickness were co-registered in sagittal, axial and coronal planes. Using available published data, cerebellar fissures were identified on the midsagittal section and followed to their most lateral extent in the parasagittal plane. The location of the fissures was verified on coronal and axial sections as necessary.

The ability to change between planes of section was a major aid in the localization of the twelve major cerebellar fissures. The precentral, preculminate, intraculminate, primary, superior posterior, horizontal, ansoparamedian, prebiventer (prepyramidal), intrabiventer, secondary, and posterolateral fissures were all reliably marked on the three planes of section. Crus IA and crus IB of the ansoparamedian lobe were readily distinguishable from each other by a previously unnamed fissure which we called the "crus I internal fissure." The horizontal fissure was best seen in more lateral sections, the primary fissure optimally seen in the midsagittal plane. After defining the fissures, it was possible to identify the various cerebellar lobules, sublobules, and subfolia. The deep cerebellar nuclei were also identified.

We have developed a detailed atlas of the human cerebellum within Talairach coordinates using MRI. This will be valuable for the correlation of anatomy, function, and pathology, and for the development of probabalistic maps of the human cerebellum. *(NSERC-GOPIN-012; Milton Fund of Harvard University).*

203.2

THE INVOLVEMENT OF THE HUMAN CEREBELLUM IN NON ASSOCIATIVE- (STARTLE-) AND ASSOCIATIVE (LOWER LIMB FLEXION REFLEX) PROCESSES. ELECTROPHYSIOLOGICAL DATA SUPPORTED BY IMAGING TECHNIQUES.

F.P. Kolb*, D. Timmann+, C. Baier, C. Weiller+, H.C. Diener+. Inst. of Physiol. Univ. of Munich, 80336 Munich, +Dept. of Neurol. Univ. Essen, 45122 Essen, Germany.

Beside the generally accepted participation of the cerebellum in motor control, information about the cerebellum's significant role in associative and non associative motor related processes has been accumulated. The aim of the current study was to compare responses in normal and cerebellar subjects during 1) classically conditioned limb flexion reflexes (associative process) and during 2) the habituation of the startle reflex (non associative process).

Current pulses were used as an unconditioned stimulus (US) and applied to the subjects medial plantar nerve eliciting an unconditioned flexion reflex (UR). Preceding the US by 450ms an auditory stimulus (CS) was applied via headphones. The acoustic startle reflex was evoked with a 95 dB 1 kHz pulse (50ms). The corresponding tibial- and sterno cleido mastoide muscle activity was recorded together with the accompanying cerebellar regional blood flow (rCBF), monitored by PET scan technique. The process of condi-tioning and that of habituation correlated significantly with the rCBF of the medial cerebellum. Cerebellar patients could be hardly conditioned and showed significant differences in temporal UR characteristics compared to control subjects. Our data strongly support the assumption of a medial cerebellar involvement in both, associative and non associative motor related processes. Supported by Wilhelm Sander Foundation (94.090.1) and DFG TI 239/2-1.

203.3

PERCEPTION OF KINESTHETIC CUES REQUIRED FOR ASSESSING THE SHAPE OF TWO-DIMENSIONAL IRREGULAR PROFILES IS IMPAIRED IN CEREBELLAR PATIENTS. Yu. Shimansky[1*], M. Saling[2], D.A. Wunderlich[1], V. Bracha[1], G.E. Stelmach[2], J.R. Bloedel[1]. [1]Neurobiology Div., Barrow Neurological Institute, Phoenix, AZ 85013; [2]Motor Control Laboratory, ASU, Tempe, AZ 85287.

The purpose of this study was to determine the capability of cerebellar patients to acquire and use kinesthetic information in the absence of vision for perceiving the shape of two-dimensional unfamiliar profiles with closed irregular outlines. Patients with cerebellar lesions and age-matched healthy controls were tested on four tasks under two conditions that differed in the way information was acquired regarding the shape of these profiles. In the first condition (kinesthetic acquisition, KA), blindfolded subjects acquired information about the shape of a profile by tracing a grooved template three times. In the second condition (visual acquisition, VA), the subjects examined the profile visually. The *first* task consisted of four series. In each series the subjects were asked to trace a *reference* profile three times and memorize its shape. Then a sequence of five profiles was presented in a pseudo-random order. Two of five were the same as the *reference* profile and three were different. The subjects were required to trace each profile three times and indicate whether its shape was identical to the *reference* one. The number of errors was recorded. In the *second* task, a *reference* profile was presented to the subjects again, and then they were asked to draw it from memory five consecutive times while blindfolded. The *third* task was identical to the second one with the exception that the subjects drew the profile using vision. In the *fourth* task, the subjects were required to recognize the shape of the *reference* profile in a set of pictures. Analysis of errors (failure to recognize the *reference* profile or identification of a non-reference profile as the *reference*) showed that under the KA condition the number of errors was significantly increased in cerebellar patients. Furthermore, unlike the cerebellar patients, the control subjects significantly decreased the number of errors across the four series. No cerebellar patient was able to recognize the shape of the *reference* profile and they were unable to draw its characteristic features. No such deficits were observed in the VA condition. The results suggest that the perception of kinesthetic cues in patients with cerebellar dysfunction is considerably impaired. Consequently, the cerebellum plays an important role in processing this type of information. Supported by NIH grants R01N521958 and P01NS30013 and Flinn Foundation.

M. Saling is a visiting scientist from Inst. Norm. and Pathol. Physiology, Bratislava.

203.4

MOTOR CORTEX ACTIVITY IS ABNORMAL PRIOR TO MOVEMENT IN CEREBELLAR DEGENERATION (CD) J Liepert, E.M. Wassermann*, A. Samii, L.G. Cohen, M. Hallett. Human Motor Control Section, NINDS, NIH, Bethesda, MD 20892

Reaction time (RT) is prolonged in CD and Parkinson's disease (PD). Earlier studies showed that, in PD, motor cortex excitability build-up preceding a movement is abnormal presumably due to reduced thalamic drive. This study tested the hypothesis that patients with CD also have a deficit of pre-movement excitability build-up in motor cortex.

Ten patients with CD and no clinical involvement of basal ganglia or corticospinal tract and 10 age-matched normals performed a reaction time task making rapid wrist flexions in response to a visual go-signal.

To test pre-movement motor cortex excitability, transcranial magnetic stimuli (TMS) below motor threshold were delivered at intervals (0-250 ms) after the go-signal. The number of motor evoked potentials occuring in each 20 ms time bin preceding EMG onset was determined, thus indicating the time-course of excitability prior to EMG onset. In 50% of trials, an additional TMS was given simultaneously with the go-signal to see if pre-activation of motor cortex changed the excitability build-up.

RT in CD was prolonged compared to controls (247 vs 200 ms, p=0.0015). Pre-movement excitability started 100 ms earlier in the patients than in the controls and showed a slower increase. With the additional TMS, the excitability build-up in the patients shortened and approached normal.

We postulate that the prolongation of RT in CD is mainly due to an abnormal excitability build-up in the motor cortex. The most likely cause is impairment of the cerebello-thalamo-cortical loop. Pre-activation of the motor cortex by TMS improves the excitability build-up in CD.

203.5

COMPARISON OF SHORT-LATENCY MAGNETIC EVOKED RESPONSES TO MEDIAN NERVE STIMULATION IN CEREBELLUM WITH INITIAL THALAMIC AND CORTICAL RESPONSES IN NORMAL HUMAN SUBJECTS. C. D. Tesche*, Brain Research Unit, Low Temperature Laboratory, Helsinki University of Technology, FIN-02150 Espoo, Finland.

Synchronized post-synaptic current flow generated by a localized, spatially-ordered neuronal population is a primary source of magnetoencephalographic (MEG) signals. Simultaneous activation of multiple brain areas may be disentangled from MEG data if the topographies recorded by the sensors for each source are sufficiently distinct. In somatosensory afferent pathways, sites of synaptic activity include dorsal column nuclei, thalamus, vermis and paravermal area, and postcentral cortex. Magnetic evoked responses to unilateral median nerve stimulation at the wrist (interstimulus interval 0.5 s, 0.3 ms duration, 15% random omission) were recorded from four adult subjects with a 122-channel MEG array placed over the scalp. Magnetic field patterns were computed for dipolar current flow in the neighborhood of the VPL nucleus, spinocerebellum and primary somatosensory cortex utilizing a realistic model for the conducting volume of the head. Signal-space projection (SSP) was used to identify evoked-response waveforms from the data. Although previously reported thalamic responses at 15–17.5 ms consistently preceded cortical activity by 1–2 ms (range 16–19 ms) (Tesche, in press), responses from the eight recordings were consistent with initial peak activation of cerebellar sources occurring over a wider range (13–19 ms) with no consistent sequential relationship with respect to the initial peak thalamic response. This pattern is consistent with parallel activation of these two structures. There are no previous reports of non-invasive detection of population dynamics in normal human cerebellum. This method provides a valuable new tool for functional studies of this important brain area.

Support: NINDS 3 F33 NS09623-01S1

203.6

MYOCLONIC SEIZURE: NECESSITY OF THE INFERIOR OLIVE, TEMPORAL STRUCTURE, AND ALTERATIONS IN SEROTONIN FUNCTION. J.P. Welsh*, M.E. Menaker, B. Chang and S.A. Aicher. Dept Physiology & Neuroscience, New York Univ Med Center, New York NY 10016 & Dept Neurology, Cornell Univ Med Col, New York, NY 10021.

Genetically Epilepsy Prone Rats (GEPR/3Hsd) were used to determine whether pathophysiological inferior olivary (IO) function was necessary for a myoclonic seizure. Seizures were induced by an ascending frequency ramp auditory stimulus. The seizures of 5 GEPR/3Hsd rats were measured non-intrusively before and after lesion of the IO by i.p. administration of 3-acetylpyridine, harmaline and niacinamide. As previously described (Reigel et al., *Life Sci*, 1986), the seizure consisted of running, clonus, and dorsiflexion of the back. We further found that the clonus, which exhibited a frequency of 2-6 Hz, was always preceded by a 5-10 s period of profoundly exaggerated 9.2 $\pm$ 0.1 Hz tremor. Electromyographic recordings obtained simultaneously from the left and right biceps brachii revealed the tremor to consist of 50 ms bursts of activity that were nearly perfectly synchronized across the midline. The neurotoxin abolished both the tremor and clonus in 4 of 5 rats. By 1 mo post-lesion, 2 rats regained the seizure, but 2 were seizure-free for 3 mos, at which time their brains were removed. A Nissl stain, immunostaining for NSE, and a retrograde tracer revealed a complete loss of IO neurons in the seizure-free rats, but some sparing in the caudal IO in the 2 that regained seizures. In a separate group of 4 GEPR/3Hsd rats, quantitative immunocytochemistry revealed a nearly complete absence of serotonin fibers within the IO. Further experiments revealed a reduced sensitivity of GEPR/3Hsd to the tremorogenic effect of harmaline. The data indicate that the IO, the origin of the cerebellar climbing fibers, may play an important role in the genesis of myoclonus and indicate a site where serotoninergic drugs may have anti-myoclonic actions. (Supported by the Myoclonus Research Foundation & NINDS NS-31224)

203.7

RELATIONSHIP OF CEREBELLAR PURKINJE CELL DISCHARGE TO MOVEMENT PARAMETERS DURING VISUOMOTOR ARM TRACKING. I. SIMPLE SPIKES. T.J. Ebner*, M.T.V. Johnson & J.D. Coltz. Graduate Program in Neuroscience and Depts. of Neurosurgery and Physiology, University of Minnesota, Minneapolis, MN 55455.

In a previous electrophysiological study of cerebellar Purkinje cells in our laboratory (Coltz & Ebner, 1995), we attempted to determine the relationship between simple spike discharge and movement position, velocity, and acceleration in monkeys using two variants of a visuomotor arm tracking task. One task required the animal to track moving targets in two dimensions with a bell-shaped velocity profile, while the other specified a constant velocity. Movements were made at 4-5 peak or constant velocity levels (2-6 cm/s) to endpoints in 8 directions. A multiple linear regression model was used to fit the temporal profile of simple spike discharge to the temporal profiles of position, velocity, and acceleration. Analyses of 86 cells revealed that in both tasks, position, velocity, and acceleration all were important predictors of simple spike rate. On average, however, movement velocity was a better predictor of simple spike rate than position or acceleration (maximal mean R^2 velocity=0.57, R^2 position=0.46, R^2 acceleration=0.36). An additional analysis evaluated the relationship between cell discharge and the kinematic parameters for each direction-velocity combination in the task. There was a tendency for single cells to correlate with position, velocity, and acceleration most strongly at different direction-velocity combinations. That is, for a given cell, a spatial parcellation of parameter encoding was found to exist. These findings demonstrate that Purkinje cell simple spike discharge correlates with kinematic parameters of movement and may do so using a spatial parcellation scheme. Supported by NIH grants NS 18338 and NS 31350.

203.8

RELATIONSHIP OF CEREBELLAR PURKINJE CELL DISCHARGE TO MOVEMENT PARAMETERS DURING VISUOMOTOR ARM TRACKING. II. COMPLEX SPIKES. J.D. Coltz*, M.T.V. Johnson & T.J. Ebner. Graduate Program in Neuroscience and Depts. of Neurosurgery and Physiology, University of Minnesota, Minneapolis, MN 55455.

The relationship between cerebellar Purkinje cell complex spike discharge and limb movements remains poorly understood. In this study, we use a previously described visuomotor arm tracking task, coupled with novel analyses, in an attempt to evaluate this relationship. Purkinje cells were recorded in two monkeys during a 2-dimensional visuomotor task that required tracking of targets moving at 5 constant velocities (2-6 cm/s), reaching from a centrally located start position to endpoints in 8 different directions. The task comprised 2 periods: (1) a cue (nonmovement) period, during which the animal was provided information about the velocity and direction of the upcoming movement, and (2) a track (movement) period, during which the animal moved from start to endpoint. Single-trial analyses of 34 Purkinje cells utilized a multiple logistic regression model in which the complex spike data were related to movement velocity and direction in the cue and track periods. The strength of association between these parameters and cell discharge was quantified by calculating mean partial R^2 values. Significant correlations with both velocity and direction were found; in addition, a temporal ordering of these correlations emerged. The maximal mean R^2 for direction occurred during cue (0.22), dropping to a minimum of 0.06 during track. For velocity, correlations were stronger during track (maximal mean R^2 = 0.23) than cue (0.05). These findings indicate that Purkinje cell complex spike discharge encodes information about both sensory and motor parameters during a visuomotor arm tracking task in primates. Supported by NIH grants NS 18338 and NS 31530.

203.9

SOMATOSENSORY PATHWAYS TO THE CEREBELLUM: SPATIAL ORGANIZATION OF CORTICOPONTINE TERMINAL FIBERS IN THE RAT. K.A Lyngstad[1], J.H. Thompson*[2], J.M. Bower[2] and J.G. Bjaalie[1]. [1]Dept. of Anatomy, University of Oslo, 0317 Oslo, Norway. [2]Division of Biology 216-76, Caltech, Pasadena, CA, 91125.

Information is transferred from the primary somatosensory cortex (SI) via the pontine nuclei (PN) to the cerebellar cortex. SI contains a continuous somatotopic map, whereas the cerebellar hemispheres contain 'fractured' somatotopic maps. We are investigating the somatotopic map at the intermediate level, the PN. Using high density micromapping, we have injected Phaseolus vulgaris - leucoagglutinin (Pha-l) into somatotopically defined regions of SI. The complete distribution of labeled fibers were displayed in computer 3D - reconstructions. With injections covering most parts of SI, labeled fibers were distributed in discontinuous zones located within a large lamella-shaped subspace of the PN, 'the pontine SI-lamella' , with a thickness (from ventral to dorsal) of 300-450 µm. Following injections covering most parts of the SI face representation, labeled fibers occupied a smaller lamella of roughly the same thickness. Within this 'pontine face-lamella', most of the fibers were located in two main zones: one large laterally located and one small medially located zone. These two main zones were connected with thin rims of labeled fibers. A single punctate injection in the SI upper lip (UL) representation gave rise to labeled fibers confined to a considerably thinner lamellar subspace. Labeled fibers within this 'pontine UL-lamella' was completely separated into two zones, corresponding to the lateral and medial zones within the face-lamella. This discontinuous labeling pattern may partly represent the basis for a fractured somatotopy in the further projection to the cerebellum. We are currently exploiting double anterograde tracing with Pha-l and biotinylated dextran amine to further elucidate the principles of the corticopontine somatotopic organization. Supported by USPHS grant MH/DA52145-03 and The Research Council of Norway.

203.10

SOMATOSENSORY INPUT TO THE MONKEY CEREBELLUM: DE-EMPHASIS OF DISTAL EXTREMITIES DEMONSTRATED IN COMPUTERIZED 3-D MAPS. K. Vassbø, G. Nicotra, M. Wiberg§ and J. G. Bjaalie*, Dept. of Anatomy, University of Oslo, 0317 Oslo, Norway. §Dept. of Anatomy, University of Uppsala, 75123 Uppsala, Sweden.

The cerebellum receives somatosensory cortical information via the pontine nuclei. We have investigated the anatomic organisation of corticopontine neurons in the postcentral somatosensory areas 3a, 3b, 1 and 2 of the cynomolgus monkey. Large injections of wheat germ agglutinin - horseradish peroxidase were made in the pontine nuclei. Retrogradely labeled cells were found in cortical layer 5, and their distribution was quantitatively recorded. This distribution was analysed and visualised on a Silicon Graphics workstation running custom software. The software was developed for assembling section-based data into three-dimensional reconstructions for subsequent flattening. 3-D color-coded density graphs were used for visualizing the distribution of labeled cells. We found considerably higher average densities of labeled cells in areas 1, 2 and 3a as compared to area 3b. We also found marked and reproducible density gradients *within* areas. These gradients were compared with previously published somatotopic maps of the somatosensory cortex. Judging from this comparison, the density of corticopontine labeled cells was always higher in parts devoted to trunk and proximal limbs than in parts representing distal limbs. This suggests that impact from distal limbs on cerebellar movement control is quantitatively less important than hitherto assumed. Supported by The Research Council of Norway and The Jahre Foundation.

203.11

CLASSIFICATION OF THE RAT CEREBELLAR BASKET AND STELLATE CELLS: EVIDENCE FOR A CONTINUOUS DISTRIBUTION OF TYPES THROUGH PRINCIPAL COMPONENT ANALYSIS

F. Sultan* and J. Bower Div. of Biology, Caltech, Pasadena, CA 91101

The cerebellar inhibitory interneurons of the molecular layer (i.e., the basket and stellate cells) are probably the second most numerous inhibitory interneurons in the central nervous system. Unfortunately, their classification is still a matter of controversy. In this study we have compared the morphology of these neurons using a multivariate statistical analysis. Thirty six parameters extending over a variety of morphological features (geometrical, topological and metrical) were obtained from 3-dimensional reconstructions of twenty six rat rapid-golgi stained basket and stellate cells. The subsequent principal component (PC) analysis showed that the first PC was strongly correlated with the mean vertical (perpendicular to the pia) location of the axonal fiber swellings (SW), the number of basket collaterals, the vertical location of the soma in the molecular layer, the maximal distance of the dendritic tips from soma, and the dendritic thickness. The second PC was strongly correlated with parameters describing some of the axons' morphology: distal spread of the SW in the horizontal axis (in the parasagittal plane and tangential to the pia), the spread of the SW in the vertical axis and the ratio of the proximal vs. distal portion of the SW distribution. The principal plane showed no clustering of the data points, with respect to either the first PC, the second PC or both. First, this analysis suggests that inhibitory neurons in the molecular layer of the cerebellum represent a continuous distribution of morphological properties which vary dependent on the vertical poistion of the soma in the molecular layer. Thus, cells traditionally classified as either stellate or basket cells appear to be one cell type. Second, it may be possible to link the variations in neuronal morphology to specific features in the development or organization of the cerebellar cortex. For example, the first PC is most likely related to the time of the neurons birthdate. This was first proposed by P. Rakic for the dendritic morphology (J. Comp. Neurol.: 146, 1972). The second PC could be related to activity-dependent plasticity due to synaptic interactions with other neurons (inhibitory interneurons and/ or Purkinje cells), and/ or to differences in the spatial extent of the input to these cells, i.e., the different sizes of the fractured somatotopic patches of the granular layer (Shambes, G.M., Gibson, J.M., and Welker, W. Brain Behav. Evol.: 15, 1978).

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DEGENERATIVE DISEASE: ALZHEIMER'S-BETA-AMYLOID—PROTEIN INTERACTIONS II

204.1

AMYLOID BETA PROTEIN BINDS THE EUKARYOTIC 20S PROTEASOME. L. Gregori*, J. F. Hainfeld[1], M. N. Simon[1], and D. Goldgaber. Department of Psychiatry and Behavioral Science, School of Medicine, State University of New York, Stony Brook, NY 11794. [1]Biology Department, Brookhaven National Laboratory, Upton, NY 11973.

Proteasomes are responsible for the ATP-dependent degradation of ubiquitinated proteins. In Alzheimer's disease (AD) and other age-related neurodegenerative disorders, ubiquitinated proteins are not removed and accumulate into intraneuronal structures. We previously found that amyloid beta protein (Aβ) inhibits ubiquitin/proteasome dependent conjugate degradation. Here we show that Aβ directly binds the 20S eukaryotic proteasome and forms a stable proteasome-Aβ complex which was detected by Western blot using anti-Aβ antibodies. Using a 1.4 nm Nanogold labeled Aβ, we visualized proteasome-Aβ complexes by scanning transmission electron microscopy. Analysis of side and end views of proteasome-Aβ complexes revealed mostly a single gold particle corresponding to one gold labeled Aβ molecule inside the proteolytic structure, along the peptide channel. Direct interaction of Aβ with the inner proteolytic compartment of the proteasome may explain the generation of ubiquitinated lesions observed in AD and other neurodegenerative disorders. In addition, the detection of Nanogold labeled peptide inside the 20S eukaryotic proteasome suggests that the conformational requirements in peptide degradation by eukaryotic proteasomes are distinct from those of prokaryotic proteasomes.

This study was supported by a Zenith award from Alzheimer's Association to D. G.

204.2

INTRACELLULAR DETECTION OF THE C-TERMINAL TAIL CONTAINING APP POLYPEPTIDES IN AD BRAIN TISSUE G. J. Kotwal, , J. Chan[@], M. Tseng[@], L. A. Carr*[±], D. E. Justus, and J. Daly. Departments of Microbiology and Immunology [@]Anatomical Sciences and Neurobiology and [+]Pharmacology, University of Louisville School of Medicine, Louisville KY 40292 .

A major hallmark of Alzheimer's disease (AD) is the presence of extracellular amyloid plaques consisting primarily of beta peptide. The beta peptide is the product of processing of the amyloid precursor protein (APP). During the processing of APP, the cytoplasmic fragment has been suggested to remain within the cell. In order to investigate the fate of this fragment, we generated an antibody specific for a nine amino acid peptide, the sequence of which was derived from the carboxy-terminal putative cytoplasmic tail of APP. Computer analysis of the entire APP gene, searching for regions of greatest antigenicity, surface probability, hydrophilicity, and presence of beta turns, indicated that the cytoplasmic tail region is an immunodominant region of APP. The peptide coupled to keyhole limpet hemocyanin protein (KLH), produced a very high titer antibody (1:1X 10^6). In order to further characterize the antibody, immunohistochemical staining of mouse neuroblastoma cells in cell culture (SN49MA) stably transformed with a full-length APP was done and the intense specific staining only in the SN49MA, but not in the controls was indicative of the specificity of the antibody for APP. EM analysis of rat brains subjected to reperfusion injury confirmed the intracellular localization of the antibody staining. The antibody was also able to specifically detect the accumulation of the stable C-terminal tail containing portion of APP in neurites of the amygdala and hippocampus of human Alzheimer brain tissue, but not in age-matched normal brain tissue. These findings are indicative of a likely role of the C-terminal tail of APP in the pathogenesis of AD. This work was supported by the Humana startup funds and the Graduate Research Council funds awarded to GJK

204.3

HUMAN FE65: IDENTIFICATION AND LOCALISATION OF A PROTEIN INTERACTING WITH THE INTRACELLULAR CARBOXY-TERMINUS OF THE AMYLOID PRECURSOR PROTEIN AND ITS EFFECT UPON PROCESSING OF THE AMYLOID PRECURSOR PROTEIN. D.M. McLoughlin and C.C.J. Miller* Depts. of Neurology and Neuroscience, Institute of Psychiatry, Denmark Hill, London SE5 8AF, UK

The yeast two-hybrid system was used to screen a human brain cDNA library for proteins that interact with the cytoplasmic domain (carboxy-terminal 47 amino acid residues) of APP. Four cDNA sequences coding for such proteins were isolated and identified. Two of these cDNA sequences encoded overlapping regions of the human homologue of a previously described rat neuronal protein, FE65. This has been shown to be developmentally regulated and contains a putative transcription activating domain. We have sequenced the entire cDNA encoding the human version of FE65 and have demonstrated that the amino-terminal approximate 80 amino acids are not required for binding to APP.

In order to study the subcellular localisation of FE65, a myc-epitope tagged version of FE65 was created and expressed in transfected CHO cells. Although previously considered to be a nuclear protein, it appears that human FE65 is localised to the cytoplasm. Wild-type human FE65 was co-transfected with APP 695 in order to investigate it effects upon the processing of APP.

This work was supported by grants from the Wellcome Trust and MRC to C.C.J.M. D.M.M. is a UK Alzheimer's Disease Society Research Fellow.

204.4

NEURONAL PATHOBIOLOGY OF THE CARBOXYL-TERMINUS OF THE AMYLOID PRECURSOR PROTEIN. D.L. McPhie*, K.J. Ivins, M.R. Kozlowski, R.L. Neve. Dept. Genetics, Harvard Medical School, McLean Hospital, Belmont, MA 02178; Geron Corp., Menlo Park, CA 94025.

We have shown previously that the carboxyl-terminal 100-amino acid fragment (C100) of the Alzheimer amyloid precursor protein (APP) is toxic to neurons, and that its expression in the brains of transgenic mice causes Alzheimer disease-like neuropathology and loss of cognitive functions. To begin to clarify the mechanism by which C100 kills neurons, we have sought cDNA clones encoding binding proteins for this fragment of APP. One of these cDNAs codes for a neural-specific P21-activated kinase (N-Pak) that binds both to C100 and to APP. The *n-pak* cDNA represents an ~11-kilobase mRNA with a 1632 base pair open reading frame encoding a 61 kilodalton serine/threonine kinase. RNA blot analyses and *in situ* hybridization histochemistry revealed that *n-pak* mRNA is nervous system-specific and is present at highest levels in regions of the adult brain that are severely affected in Alzheimer disease. Anti-APP immunoreactivity was specifically coprecipitated with N-Pak from neurons, and N-Pak immunoprecipitated from transfected cells demonstrated kinase activity in the presence of the activated p21 proteins rac1 and cdc42. The known involvement of these small G proteins in the machinery controlling axonal outgrowth suggests that disruption of this pathway by C100 or by familial Alzheimer disease APP mutants may play a part in the disassembly of the cytoskeleton that occurs in Alzheimer disease. Supported by AG12954 and Geron Corporation.

204.5

β-AMYLOID PEPTIDE INTERACTS WITH α-TUBULIN (b α 1) IN VIVO.
S. R. Hughes*[1], T. Watanabe[2], J. Cheetham[2], J. Knaeblein[1], J. D. Buxbaum[2], P. Greengard[2], N. G. Riedel[1], and S. R. Sahasrabudhe[1].
[1]Hoechst Marion Roussel Inc., Neuroscience Therapeutic Area, P.O. Box 2500, Somerville, NJ 08876. [2]The Rockefeller University, Laboratory of Molecular and Cellular Neuroscience, New York, NY 10021.

Alzheimer's disease is characterized by the presence of neuritic plaques in the brain containing aggregates of β-amyloid (Aβ) peptide, a 39-43 amino acid peptide derived post-translationally from a larger amyloid precursor protein (APP). We have used the yeast interaction trap or two-hybrid system to clone genes encoding proteins capable of inteacting with Aβ. In this system, a LexA-Aβ fusion protein (bait) occupies LexA binding sites in the upstream DNA region of LacZ and LEU2 reporter genes to inhibit their expression. Expression of an Aβ-interacting protein from a B42 fusion library with the bait allows the activation of the reporter genes by bringing the activator sequence B42 in close proximity to the LexA binding sites. In this study, we report the identification of the clone encoding human α-tubulin (b α1). This protein was capable of interacting with the Aβ peptide, but failed to interact with LexA-bicoid or LexA-C100 fusion proteins, indicating that the observed interaction with α-tubulin was specific. When yeast cells expressing α-tubulin were subjected to immunoprecipitation with anti-LexA antibody, the epitope of which is contained within the bands corresponding to the prey fusion protein, predictive of α-tubulin-B42 complexes, were detected, indicating in vivo interaction between Aβ and α-tubulin. Furthermore, purified preparations of tubulin were shown to bind the Aβ peptide. The presentation will discuss the implications of these findings.

204.6

CHANNEL FORMING ACTIVITY OF BETA AMYLOID PEPTIDES: ROLE OF AMINO ACID RESIDUE IN BETA AMYLOID PEPTIDE 25-35.
Meng-chin Lin*, Tajib Mirzabekov, and Bruce Kagan.
Neuroscience Interdepartmental Program, Brain Research Institute of UCLA Medical School, Los Angeles, CA 90024.

The formation of amyloid plaques has been implicated to play a role in the pathogenesis of Alzheimer's disease (AD). AD-related amyloid beta-peptide (Aβ) and its active fragment Aβ25-35 have been found to be neurotoxic, perhaps by dysregulation of intracellular Ca^{2+} levels. We have reported that Aβ25-35 can form voltage dependent ion channels with multiconductive levels in planar lipid bilayers. The Aβ25-35-containing membranes exhibit slight cation selectivity. The resulting ionic disturbance may lead to neuronal death. In this study, we have examined the channel forming activity of different variants of Aβ25-35. Replacing the C-terminal Met with Cys, Ser, or Asp preserved both neurotoxicity and channel forming ability. Deleting the C-terminal Met caused a loss of both neurotoxicity and channel forming activity. Substitution of Asp for Asn at amino acid reside 27 eliminated Aβ25-35 toxicity, and its channel forming activity. Aβ25-35 with Gln at position 27 exhibited toxicity at high concentration (80μM), and formed ionic channels only at high concentration (80μM) as well. Deletion of two N-terminal amino acid residues (namely, Aβ27-35) diminished toxicity and channel formation. Reversed Aβ25-35 or amidated Aβ25-35 did not have any toxic effect on neurons and could not form channels. Mutations at amino acid residue 35 did not alter properties of channels, whereas variations at amino acid residue 27 changed the voltage dependence. This indicates that residue 27 may play a role as voltage sensor of Aβ25-35 ion channels. The results suggest that channel formation is necessary for neurotoxicity.

(Supported by grants from the Alzheimer's Association and NIH)

204.7

AMYLOID B-PEPTIDE MAY INDUCE EXTRACELLULAR CA^{++} ENTRY PATHWAYS INTO N1E-115 NEUROBLASTOMA CELLS. L. Shajenko* and R. M. Davidson. Neuroscience Graduate Program, University of Connecticut Health Cntr. Medical School, Farmington, CT 06030.

It has been well documented that the mechanism for amyloid β-peptide (AβP) induced neurotoxicity involves, in part, increased extracellular Ca^{++} entry into cells, although, the ionic mechanism(s) to explain AβP induced Ca^{++} influx remains unclear. Based on our earlier findings with N1E-115 neuroblastoma (NB) cells (Davidson et al., 1994), we suggested that AβP may be forming Ca^{++} permeable channels *de novo* and/or causing a change in the activity of endogenous Ca^{++} channels. To test our hypothesis, we have examined the effects of AβP on the NB cell's dihydropyridine-sensitive ("L" type) Ca^{++} channel single channel activity. In addition, we have studied its ability to form new channels using a synthetic, liposomal model of the NB cell membrane.

When cells were incubated with AβP for 4-24 hrs, cell-attached recordings demonstrated a ~30% increase in P_{open} of the L-type Ca^{++} channel ($p<.05$). In addition, a statistically significant increase in P_{open} of the L-type channel was noted when AβP1-40 was added to the bath while recording channel activity in the cell-attached but not the excised-patch configuration. The contol peptides AβP40-1, AβP25-35, human and rat amylin did not produce a statistically significant change in P_{open}. To confirm the electrophysiological finding, the viability tests performed at 48 hrs peptide treatment demonstrated that nimodipine (10nM) can attenuate the toxic effect of AβP1-40 (23μM) on NB cells. Furthermore, AβP1-40 (5μg/ml) incorporated into a synthetic NB membrane bilayer formed nimodipine-insensitive, non voltage-gated, low-conductance channels for both mono- and di-valent cations. The permeability sequence (P_{Ca}^{++} as reference) estimated from the reversal potential of the channel current in asymmetrical solutions, was $P_{Cl}^{-} > P_{K}^{+} > P_{Na}^{+} \geq P_{Ca}^{++}$. Furthermore, Ca^{++} was able to impede monovalent ion conductance through the AβP channels. Physiological levels of liposomal-membrane cholesterol attenuated calcium conductance and reduced the P_{open}. These data demonstrate that AβP1-40 may increase Ca^{++} entry into neuronal cells by "exciting" endogenous Ca^{++} channels and by forming Ca^{++} channels *de novo* on the membrane surface. Understanding the mechanism further may help in designing appropriate pharmacological therapies for Alzheimer's Disease. Supported by NIH/NIDR grant # DE09662.

204.8

CYTOSOLIC Ca^{2+} OSCILLATIONS IN DIFFERENTIATED HUMAN NEURONS: EFFECTS OF β-AMYLOID. **Z. Y. Gao[1*], H. W. Collins[2], F. M. Matschinsky[2], V. M. Y. Lee[1] and B. A. Wolf[1]**. Depts. [1]Pathology & Lab Med., [2]Biochemistry & Biophysics, Univ. Penn. Sch. of Med., Philadelphia, PA 19104

Differentiated NT2N cells are a unique model of human neurons in culture. Measured by fura-2 technique, NT2N cells produced spontaneous Ca^{2+} oscillations, which were absent in undifferentiated NTera2/C1.D1 (NT2) cells. The Ca^{2+} oscillations included Ca^{2+} wave, plateau and spike patterns. Glutamate induced a rapid and sustained cytosolic Ca^{2+} increase while acetylcholine only induced a transient increase. Addition of the glutamate receptor antagonists CNQX (10 μM) + DAP5 (10 μM) or atropine (20 μM) did not block the spontaneous Ca^{2+} oscillations, thus glutamate receptor or muscarinic receptor activation is not required for the oscillations. Omission of extracellular Ca^{2+} completely abolished all types of Ca^{2+} oscillations and decreased the average Ca^{2+} level from 106 ± 14 nM to 59 ± 8 nM. Addition of the Ca^{2+} channel blocker nifedipine (L-type) or GVIA (N-type) significantly suppressed Ca^{2+} oscillations, while MVIIC (P- and Q-types) had no effect. Thapsigargin (endoplasmic reticulum Ca^{2+}-ATPase inhibitor) did not inhibit Ca^{2+} oscillations but induced a transient slight increase in the average cellular Ca^{2+} levels. Four day culture of NT2N cells with 40 μM synthetic ß-amyloid protein (Aß42) abolished Ca^{2+} oscillations. 4 μM Aß42 caused minor inhibition while 0.4 μM had no effect. In conclusion, NT2N cells generate spontaneous Ca^{2+} oscillations by Ca^{2+} influx through L- and N- type channels, which are inhibited by Alzheimer's Aß42.

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204.9

PROGRESS IN UNDERSTANDING THE ACTIVITY-REGULATING AND NEUROPROTECTIVE SIGNAL TRANSDUCTION PATHWAY OF SECRETED APP. M. P. Mattson*, K. Furukawa, B. Sopher, R. J. Mark, and G. M. Martin. Sanders-Brown Center on Aging and Dept. of Anatomy & Neurobiology, Univ. of Kentucky, Lexington, KY 40536. Dept. of Pathology, Univ. of Washington, Seattle, WA 98195.

α-secretase cleavage of the β-amyloid precursor protein (βAPP) liberates a secreted form of APP (sAPPα) that may play important roles in regulating neuronal plasticity and survival. In embryonic rat hippocampal cell cultures sAPPα suppresses neuronal activity by activating K^+ channels (*Nature* 379:74-78 (1996)). We have found that sAPPα also suppresses NMDA currents, but not AMPA or kainate currents. Cyclic GMP appears to mediate sAPPα-induced K^+ activation, suppression of calcium responses to gluta-mate, and protection against excitotoxicity. Structure-activity studies revealed that the C-terminal portion of sAPPα mediates activation of the cGMP pathway, and that a heparin-binding domain therein greatly increases sAPPα activity relative to sAPPβ (see Furukawa et al., this meeting). These data suggest that APP mutations that increase β-secretase cleavage may promote neuronal degeneration in Alzheimer's disease by reducing levels of neuro-protective sAPPα. Most recently, we have initiated attempts to isolate a sAPPα receptor using two approaches. First, using a GST-sAPP444-612 affinity column we have isolated two sAPPα-binding proteins (approximately 80 and 50 kDa) and are in the process of obtaining amino acid sequence information. Second, based upon the possibility that the sAPPα receptor has intrinsic guanylate cyclase activity (*Biochem. J.* 311: 45-47 (1995)) we are determining whether "orphan"or novel receptor guanylate cyclases might be a receptor for sAPPα. (supported by the NIH and the Alzheimer's Association).

204.10

SPECIFIC INTERACTIONS OF ALZHEIMER'S DISEASE MEMBRANE PROTEINS S182 AND STM2 WITH ß-APP ON CELL SURFACES. N. N. Dewji *1 and S. J. Singer 2. University of California, San Diego, Departments of Medicine (1) and Biology (2), 0322, La Jolla, CA 92093.

Mutations in the genes for the ß-amyloid precursor protein (ß-APP), a type 1 single -membrane spanning integral protein, and the more recently identified closely homologous seven-membrane spanning integral proteins S182 and STM2, together account for all early-onset familial Alzheimer's disease. The normal functions of the three proteins are not known nor do we know how their functions are implicated in the disease.

We recently proposed (Science, 271, 159-160, 1996) based on precedents in other systems of an intercellular interaction crucial to development between single membrane spanning and seven membrane spanning integral proteins, that one or more forms of ß-APP and S182 (or STM2) may normally be components of an intercellular signaling system. ß-APP and S182 or STM2 on the surfaces of neighboring cells would bind to one another specifically through their extra-cellular domains protruding from the cell membranes, ß-amyloid (Aß) being a proteolytic by-product of this interaction. To test this proposal full-length cDNAs for S182 and STM2 were cloned by PCR and subcloned into pCDNA3. ß-APP695 cDNA was also subcloned into pCDNA3 and the constructs were used to transiently transfect cultured cells. Polyclonal antibodies raised to peptide sequences of STM2 and S182 demonstrated the presence of the two proteins at the surface of transfected cells. In order to determine if STM2 or S182 on one cell interacts with ß-APP on another, transfected cells expressing STM2 or S182 were mixed with cells expressing ß-APP. Heterotypic cell aggregates were formed, as shown by double labeling with antibodies to ß-APP and STM2 (or S182). Furthermore, this aggregation could be inhibited by excess soluble ß-APP, indicating specificity of the interaction. Our work provides evidence for the direct physical interaction of ß-APP with STM2 and S182, which may be crucial to the generation of Aß and the genesis of Alzheimer's disease.

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205.1

TF NEURO-E: A MOUSE NUCLEAR RECEPTOR INVOLVED IN THE CONTROL OF NEUROGENESIS. A. Maelicke*, U. Bauer, A. Maus, S. Schneider-Hirsch, S. Reinhardt, and M. Rentrop Laboratory of Molecular Neurobiology; Institute of Physiological Chemistry and Pathobiochemistry, Johannes-Gutenberg Univ. Med. Sch., 55099 Mainz, Germany.

We have cloned the full length cDNA of a nuclear receptor, denoted TF neuro E, the transcription of which is induced early after cultured PCC7-Mz1 mouse embryonic carcinoma cells have been exposed to the morphogen retinoic acid (RA). TF-neuro-E expression was observed exclusively in the neuronal derivatives of Mz1 cells but not in glial or fibroblast derivatives. In the developing mouse brain, TF neuro-E was expressed in the early neuroepithelium and in zones of differentiating neurons, In the adult mouse brain, double labeling confirmed that TF neuro-E transcripts only occur in neurons. The coding sequence of TF neuro-E is 1482 bp. Using electrophoretic mobility shift assays, we have identified the direct repeat AGGTCA, without intervening nucleotide (DR0), as the hormone-responsive element of TF neuro-E. Because the E-domain of TF neuro-E does not contain an AF-2 subdomain, the transcription factor may act ligand-independent, either as homodimer, or as heterodimer with RXT. When transfected into PCC7-Mz1 stem cells that have not been exposed to RA, TF neuro-E induced within a day the expression of typical neuronal markers, suggesting that it acts as a determining or stabilizing factor of the neuronal cell lineage.

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205.2

P21 INVOLVEMENT IN NEURONAL DIFFERENTIATION. F. Berger*, P. Taupin, J. Ray, F. Gage. Laboratory of genetics, The Salk Institute, La Jolla. The process of terminal neuronal cell differentiation is intimately coupled to cell cycle arrest in G0. Molecules that inhibit cell cycle have been identified, among them p21 is an universal cell cycle associated kinase inhibitor. The investigation of p21 role in neuronal differentiation was motivated by; (i) its role in G1 restriction point which is essential for post-mitotic cells; (ii) its role in muscle cells differentiation; and (iii) its specific expression in adult neurons. Adult hippocampal neuronal progenitor cell line HC2S2, which contains a tetracycline regulatable v-myc oncogene, was used to investigate the role of p21 in neuronal differentiation. Two days after tetracycline addition, these commited cells stop dividing and terminally differentiate in 5 to 6 days. We also used adult hippocampal progenitors cultured in FGF-2 because of their pluripotential commitment to glial, neuronal and oligodendroglial lineages. HC2S2 cell differentiation is characterized by an early (day 3) p21 cytoplasmic immunodetection followed by a nuclear immunoreactivity of p21 by day 6 at which time the cells are terminally differentiated as demonstrated by tau expression. Adult unmodified progenitors with a neuronal morphology showed similar expression of p21. Immunocytochemistry data was confirmed by immunoblot and immunoprecipitation experiments. A functional study was also performed using antisense oligonucleotides targetting p21 expression. P21 antisense induced cell cycle reentry of differentiated HC2S2 cells. Retinoic acid differentiated progenitors decreased axono-dendritic formations and also reentered cell cycle when treated with antisense to p21. In both cell types sense and random controls did not induce any effect. In conclusion, p21 expression is correlated with neuronal differentiation both in adult progenitors and immortalized HC2S2 progenitor cells. Antisense experiments suggest an important role of p21 in maintenance of post-mitotic neuronal state. Transfection experiments using p21 inducible gene are currently underway to further analyze the inductive role of p21 in neuronal differentiation.

205.3

TRANSCRIPTIONAL ACTIVITY OF MEF2 IS REQUIRED FOR NEURONAL DIFFERENTIATION OF P19 CELLS. Shu-ichi Okamoto, Dimitri Krainc*, Katerina Sherman, and Stuart A. Lipton. Dept. of Neurology, Children's Hospital & Progr. in Neuroscience, Harvard Medical School, Boston, MA 02115.

MEF2A, B, C, and D are transcription factors that belong to the MADS (MCM1-agamous-deficiens-serum response factor) gene family. The expression of MEF2 proteins is highest in brain and skeletal muscle. Recent evidence suggests that MEF2 cooperates with basic helix-loop-helix proteins to induce myogenesis, whereas the function of MEF2 in brain remains unknown. In this work, we used P19 embryonal cells — a line of pluripotent cells that differentiate into the neuronal cell type when treated with retinoic acid (RA). Stable transfection of P19 cells with a dominant-negative form of MEF2 resulted in inhibition of RA-induced neurogenesis. We next examined the effects of ectopic expression of MEF2C on the developmental potential of P19 cells. In stable transfection experiments, most of the MEF2C-expressing cells were transformed into cells with bipolar morphology. The phenotypic characteristics of these cells are being examined in greater detail, but preliminary results suggest that some neuronal and muscle determinants are expressed. These results suggest that MEF2 may play an important role in cell fate determination of mutipotential stem cells. This work was supported by NIH grant P01 HD29587.

205.4

STAGE-DEPENDENT RESTRICTIONS IN THE DEVELOPMENTAL POTENTIAL OF RETINAL PRECURSOR CELLS. T. Belecky-Adams[1], G. Teitelman[1,2] and R. Adler[1]. Wilmer Eye Instit., Johns Hopkins Univ. Sch. Med., Balto., MD 21287[1], SUNY, Brooklyn, NY 11203[2]

Using the "window labeling" technique, that allows cell birth determination with a resolution of hours, we have shown that precursor cells born during embryonic day 5 (WL_5) give rise predominantly to non-photoreceptor neurons when allowed to develop in vivo until embryonic day (ED) 18, or when they are isolated for low density culture after a 3 day exposure to the in vivo microenvironment. The same postmitotic cells, however, give rise predominantly to photoreceptors when isolated for culture 12 hr after terminal mitosis, suggesting that many WL_5 precursors remain plastic after cell birth. An equivalent comparison of the in vivo and in vitro fate of cells born on ED 6 or 7 (WL_6 and WL_7) showed that many WL_6 precursors exhibit similar plasticity after terminal mitosis, whereas most WL_7 precursor cells give rise to non-photoreceptor neurons even when isolated immediately after terminal mitosis. Since there were no detectable differences in cell death between cultures, the data appear to indicate that the developmental potential of retinal precursor cells becomes restricted in a stage-dependent manner. Supported by NIH grant EY04859, NIH fellowship EY06642, and Research to Prevent Blindness Inc.

205.5

SONIC HEDGEHOG INDUCES PHOTORECEPTOR DIFFERENTIATION IN THE MAMMALIAN RETINA. E.M. Levine*, J. Turner, H. Roelink, and T.A. Reh. Department of Biological Structure, University of Washington, Seattle, WA 98195.

Hedgehog is a diffusible protein that is important in many developmental patterning events in Drosophila. Several vertebrate hedgehog proteins have also been identified and one of them, sonic hedgehog (SHH) patterns the dorsal-ventral axis of the neural tube. In the Drosophila eye disc, hedgehog also controls the progression of photoreceptor differentiation in the morphogenetic furrow. We have identified two members of the hedgehog gene family that are expressed in the developing rat retina. In this study we investigated whether SHH is also active in promoting photoreceptor differentiation in the mammalian retina. An N-terminal recombinant SHH (SHH-N) was added to dissociated rat retinal cultures for 2-14 days and the number of retinal cells of various phenotypes was analyzed with immunohistochemistry. We found that the number of rod photoreceptors increased over 10-fold in the SHH-N treated cultures over the sister control cultures. By contrast, the number of retinal ganglion cells and amacrine cells was similar to those in control cultures. Thus, SHH-N specifically induces the generation of rod photoreceptors in rat retinal cultures. We propose that some of the molecular mechanisms for photoreceptor differentiation may be conserved in mammals and Drosophila.

This work was supported by funds from NINDS and NEI.

205.6

INVOLVEMENT OF DELTA-NOTCH SIGNALING IN THE CENTRAL TO PERIPHERAL ASPECTS OF GANGLION CELL DIFFERENTIATION IN THE RETINA. C. Dooley, W.B. Thoreson* and I. Ahmad. Dept. of Cell Biology and Anatomy, and Ophthalmology, University of Nebraska Medical Center, Omaha, NE 68198.

The Notch signaling regulates differentiation of immature cells through cell-cell interactions. We have shown previously that the expression of the Notch-1 receptor and the gene for its putative ligand delta-1 show a central to peripheral gradient during retinal development (Mech Dev. 53:73-85, 1995; ARVOAbst.37: 200 1996). To test the hypothesis that Delta-Notch signaling is involved in the central to peripheral aspect of cell-fate determination in the retina we have studied the effect of antisense oligonucleotide corresponding to Delta-1 transcript on the expression of RA4 antigen, an early marker for retinal ganglion cell (RGC) differentiation. RA4 immunoreactivity shows central to peripheral gradient, being absent from the peripheral retina at stage 22. When the stage 22 retinal explant cultures were treated with 50μM of the Delta-1 antisense oligonucleotide RA4 immunoreactivity was more predominantly detected in the peripheral as compared to the central retina. Also, the number of RA4 positive cells was significantly decreased as compared to the controls treated with sense or missense Delta-1 oligonucleotides. Similar results were obtained with Notch-1 antisense oligonucleotide suggesting that the change in the spatial expression of RA4 was due to the attenuation in Delta-Notch signaling. These observations suggest that the attenuation in Delta-Notch signaling promotes cells in the peripheral retina to express RA4 during RGC differentiation. Supported by EY 10313.

205.7

LOSS OF CHICK ALAR PLATE MARKERS BY ABLATION OF DORSAL RHOMBENCEPHALON IS REVERSED BY BMP-4 *IN VIVO*. H. C. Etchevers*, A.-H. Monsoro-Burq and N. M. Le Douarin. Institut d'Embryologie, 94736 Nogent-sur-Marne, France.

In the light of recently published claims of neural crest respecification in chick after ablation of the rhombencephalic neural folds, we examined effects within the central nervous system. *In situ* hybridization of multiple markers of the dorsal neural tube and examination of dorsally located brainstem nuclei in more mature specimens were performed. Depending on the depth of the ablation, the nested domains of Slug, Msx-2, Wnt-1, Msx-1 and Pax-3 were deleted and not subsequently re-expressed. Substitutions of quail neural folds at the levels of the mesencephalon and rhombomere 7 demonstrated the capacity of the neural crest cells anterior and posterior to the ablation to compensate extensively and form normal crest derivatives for their new positions. On the other hand, embryos up to 11 days show dorsal but not ventral hindbrain disorganization. After implantation of fibroblasts expressing mouse BMP-4, we observed re-expression of Msx-1, Msx-2 and Pax-3 in the previously intermediate to ventral areas of the rhombencephalon, mimicking the *in vitro* or ectopic inductions of these markers by ectoderm seen by other authors. These results emphasize that regeneration in the early embryonic nervous system is more complex than previously implied. HCE is a HHMI Predoctoral Fellow. Supported by the CNRS and the Collège de France.

205.8

THE A-P IDENTITY OF DIENCEPHALIC AND TELENCEPHALIC PRECURSORS IS DETERMINED G. Fishell*, A. Langston, J.E. Johnson#, S.O Huh‡, E. Lai‡ and C. Neyt Developmental Genetics Program, The Skirball Institute and Dept. of Cell Biology, NYU Medical Center, 550 1st Ave, NY, NY, 10016;# UT Southwestern, Cell Biology and Neuroscience, 5323 Harry Hines, Dallas, Tx, 75235-9111; ‡Sloan-Kettering Cancer Center, NY, NY, 10021

Previously we observed that telencephalic precursors from the LGE (lateral ganglionic eminence), the anlagae of the striatum, when transplanted heterotopically to cortex adopt both cortical morphologies and cortical projections. In addition, we observed that these LGE cells integrated in a variety of areas as far posterior as the midbrain. Based on their neuronal morphology it was impossible to infer whether LGE cells which integrate into more posterior divisions of the CNS had maintained a telencephalic phenotype or adopted that of their host site. To resolve this issue, we used two lines of transgenic animals where LacZ expression was directed to either the telencephalon (using a line where LacZ was targeted to the BF1 loci) or ventral diencephalon (expression driven by a MASH1 control elements), as endogenous markers of regional phenotype. Telencephalic cells from each of these lines that integrated into the diencephalon were BF1 positive and MASH1 negative, respectively, suggesting that they maintain a telencephalic phenotype even when integrated into a diencephalic environment. In a set of complementary experiments, we transplanted ventral diencephalic cells into the forebrain. In these cases virtually no cells integrated in the telencephalon but large numbers were found homotopically integrated into the diencephalon. Together these results suggest that neural precursors in forebrain, while still able to change their phenotype within specific forebrain divisions, are not able to modify their A-P position and that cellular integration in itself is not sufficient to infer that the cells phenotype is altered. (supported by NIH grant RO132993-02).

205.9

LASER ABLATION OF NERVE-ASSOCIATED LINEAGE PRECURSORS OF ADULT ABDOMINAL MUSCLES IN *DROSOPHILA*. E. R. Farrell* and H. Keshishian. Biology Dept., Yale University, New Haven, CT 06520.

In *Drosophila*, two nerve-associated cells which express the mesoderm-specific transcription factor Twist serve as the lineage precursors of the lateral muscles of each adult abdominal hemisegment. These cells arise in the embryo and are located on nerve branches to muscle fiber 8. It has been hypothesized that each of these cells is a precursor for a group of 8-10 adult muscle fibers. The Twist expressing precursors proliferate during the second and third larval stages to produce two clusters of approx. 15 cells per hemisegment. During pupation their progeny migrate along the nerves to form the adult muscles. Treatment of larvae with an agent that disrupts cell proliferation has been shown to decrease the number of abdominal muscle fibers in the adult (Broadie and Bate, 1991, *Development* **113**, 103-118). Here we directly examine whether laser ablation of the precursors during early stages of their proliferation has an effect on the number of lateral fibers in the adult. Trypan blue/Twist double labeling following *in vivo* ablation of single precursors confirmed that cell death is confined to the targeted cell. We next ablated cells in second instar larvae, when there are about 2-3 cells per cluster. Following ablation of one cell per cluster, we could find no significant change in the number of lateral fibers in the resulting adults (n = 32 hemisegments in 16 animals). However, multiple cell ablation (two cells per cluster, on average) resulted in a 29-48% reduction in the number of lateral fibers (n = 7 hemisegments in 5 animals). The remaining fibers are distributed equally over the lateral muscle region. In contrast, ventral and dorsal abdominal fibers in these hemisegments were not affected by the ablation. These results confirm the role of the Twist expressing cells in the adult muscle lineage, and indicate that the determinative events occur early in the lineage. Supported by NIH and NSF.

205.10

PURIFICATION AND CHARACTERIZATION OF NEOCORTICAL CALLOSAL PROJECTION NEURONS IN CULTURE. M.W.Arnold, B.R.Leavitt, V.L. Sheen, M.J.Zylka, and J.D. Macklis* Dept. of Neurology & Prog. in Neuroscience, Harvard Medical School; Division of Neuroscience, Children's Hospital, Boston, MA 02115

Reconstruction of complex neocortical circuitry may be possible via the transplantation of defined and controllable neuronal precursors under the guidance of developmentally relevant cellular and molecular control signals. In the neocortex, the effectiveness of potential transplantation therapy may depend critically on whether donor cells can undergo developmentally appropriate migration, differentiation, integration, and connectivity to restore function. Embryonic neurons and multipotent neural precursors transplanted into regions of neocortex undergoing photolytically induced apoptotic neuronal degeneration of pyramidal neurons can respond to reexpressed developmental signal molecules, selectively migrate into these regions, differentiate into pyramidal neurons, accept afferent synapses and re-form specific distant projections, although multipotent precursors do so with relatively low efficiency. The isolation of neuronal precursors with more restricted potential may provide more specific and efficient cellular reagents for reconstruction of neocortical circuitry. The present studies investigate whether relatively immature projection neurons from postnatal cortex can be purified to form such a restricted donor cell population, and begin to characterize their requirements for differentiation and survival. Callosal projection neurons were retrogradely labeled in P0 mice with fluorescent microspheres injected into contralateral projection fields. Labeled cortex was dissociated, and labeled projection neurons were isolated by fluorescence activated cell sorting (FACS) and plated onto poly-L-lysine coated plastic of glass. 85% to 95% pure projection neurons were cultured in conditioned or defined medium, or cocultured with glial cells. Immunocytochemical analysis confirmed that purified projection neurons were glutamatergic, and expressed NSE and mature neuronal markers including NeuN, but not the glial marker GFAP. When cocultured below a glial feeder layer, or in conditioned medium, they acquired mature, complex pyramidal neuron morphology and survived up to 4 weeks in culture. In contrast, purified projection neurons without medium conditioning underwent similar early differentiation, then died between 1 and 2 days *in vitro*. These results demonstrate that callosal projection neurons can be purified, survive, and undergo restricted differentiation retaining relatively homogeneous phenotype. Further characterization of these neurons may allow control over their differentiation and survival, and may suggest strategies to direct the development of less mature precursors toward the reconstruction of neocortical circuitry. Supported by HD28478, Alzheimer's Association IIRG-93-143, MRRC HD18655, Leopold Schepp Fdn., and the Rita Allen Foundation.

205.11

MULTIPOTENT NEURAL PRECURSORS TRANSPLANTED TO REGIONS OF TARGETED NEURONAL APOPTOSIS IN ADULT MOUSE SOMATOSENSORY CORTEX DIFFERENTIATE INTO NEURONS AND RE-FORM CALLOSAL PROJECTIONS. B.R. Leavitt*, M. Canales, E.Y. Snyder and J.D.Macklis Department of Neurology, and Program in Neuroscience, Harvard Medical School, and Division of Neuroscience, MRRC, Children's Hospital, Boston, MA 02115.

In the neocortex, the effectiveness of potential transplantation therapy for diseases involving neuronal loss may depend upon whether donor cells can differentiate appropriately, reestablish precise long distance projections, and reconstruct complex functional circuitry. Previous studies demonstrated that embryonic neurons transplanted into neocortical regions undergoing photolytically targeted apoptotic neuronal degeneration in adult mice respond to reexpressed developmental signal molecules and selectively migrate to these regions, differentiate into pyramidal neurons, accept afferent synaptic input, and re-form specific long distance projections to the original targets of the replaced neurons. 15% of C17-2 multipotent neural precursors transplanted within lamina II/III of these regions also undergo directed neuronal differentiation in response to such signals, but only exhibit glial differentiation in intact or excitotoxically lesioned neocortex. The experiments presented here assess whether transplanted C17-2 cells further differentiate and send long distance axons to contralateral targets. C17-2 cells prelabeled with fluorescent microspheres and/or [^{3}H]thymidine were transplanted into adult mouse primary somatosensory cortex (S1) undergoing apoptotic degeneration of callosal projection neurons (n=28). Three to five months later the retrograde tracers Fluorogold, fluorescent microspheres, or DiI were injected into contralateral S1 cortex or ipsilateral thalamus. Many of the differentiated C17-2 cells in lamina II/III were retrogradely labeled from contralateral S1 cortex, the original target of host neurons undergoing degeneration, and most had a large pyramidal neuron phenotype. They expressed neuronal markers including NSE and NeuN but not the astrocyte marker GFAP. These data demonstrate that C17-2 neural precursors transplanted into adult neocortex undergoing synchronous apoptotic neuronal degeneration are capable of differentiating into pyramidal neurons and extending long distance projections that partially replace prior projections of degenerated callosal neurons. These results suggest that reconstruction of cortical circuitry by transplanted precursors may be possible in the mature neocortex, if appropriate donor cells are provided with the correct local and distant signals within a permissive host environment. Supported by HD28478, Alzheimer's Assoc. IIRG-93-143, MRC 9404FEN-1324, MRRC HD18655, NS33852, The Rita Allen Foundation.

205.12

TIMING AND LOCATION OF NON-RADIAL MIGRATION IN THE CHICK DIENCEPHALON J. A. Golden, J. C. Zitz, and C. L. Cepko*. Dept. of Genetics, Harvard Medical School, Boston, MA 02115

Lineage analysis of the mature chick diencephalon using retroviral vectors has demonstrated that clones are widely dispersed in the medial-lateral, dorsal-ventral, and anterior-posterior planes. To define where and when sibling cells disperse in the chick diencephalon, embryos were infected at stage 10-11 (36 hours incubation) with a retroviral stock encoding a library of molecular tags. Embryos were harvested 4 to 8 days post injection and the brains fixed, serially sectioned and histochemically stained for alkaline phosphatase activity encoded by the retroviral vector. AP+ cells were mapped and clones determined based on the molecular tag in most cases. On embryonic day 4 (E4) all clones were organized in radial columns spanning the neuroepithelium. No migration was seen in the ventricular zone at any age. On E5 most clones remained radial, while a few clones had cells migrating perpendicular to the radial column. Clones dispersed in both the anterior-posterior and dorsal-ventral planes. Migration perpendicular to the radial column began at the limit of the ventricular zone. On E6 and E7 many clones had cells migrating perpendicular and sometimes orthogonal to the radial column that continued to define the clonal origin. By E8, clonally-related cells appeared to be migrating away from the radial column at multiple levels outside the ventricular zone. The proportion of clones showing migration in the anterior-posterior and dorsal-ventral directions was comparable to the percentage found to have dispersed in the mature diencephalon. These data indicate that migration independent of radial glia occurs in the chick diencephalon outside the ventricular zone, similar to non-radial migration in other areas of the central nervous system.

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206.1

CALCIUM CONTROL OF EXOCYTOSIS MEASURED BY FM1-43 FLUORESCENCE IN MAMMALIAN SYNAPTOSOMES.

M.A.M. Prado, M.A. Romano-Silva, P.S.L. Beirão, B. Collier[1]*, M.V. Gomez and C. Guatimosim. Departamentos de Farmacologia, Bioquímica-Imunologia, (ICB-UFMG) Belo Horizonte-MG 31270-901, Brasil and [1]Department of Pharmacology and Therapeutics, McGill University, Montréal H3G 1Y6, Canada.

In the present work, we investigated the exocytosis of synaptic vesicles from rat brain cortical synaptosomes using the styryl dye, FM1-43. The fluorescent labelling of synaptic vesicles from rat brain cortical synaptosomes occurred only if vesicles completed cycles of exo-endocytosis in the presence of dye. The decrease of fluorescence, due to dye release from synaptic vesicles, could be detected when synaptosomes were depolarized by high $[KCl]_o$ (25 or 60 mM) or influx of Na^+ through tetrodotoxin-sensitive channels. The curves for 60 mM KCl were fitted by a double exponential with (τ)s of 10±1 and 126±18, and both components of FM1-43 destaining were dependent on calcium entry into the nerve terminals. The ω-toxins, conotoxin MVIIC and agatoxin IVA, blocked exocytosis induced by 25 mM KCl, but had no effect on the fluorescence drop due to 60 mM KCl depolarization. On the other hand, the ω-conotoxin GVIA, a N-type calcium channel blocker, had only negligible effects on the decrease of fluorescence due to membrane depolarization. The above results suggest that destaining of FM1-43-labelled nerve terminals had several characteristics of the exocytosis of synaptic vesicles and occurred from two kinetically distinct pools. In addition, due to the sensitivity to low concentration of ω-agatoxin IVA, it seems that the P type calcium channel contributed largely to the entry of calcium coupled to exocytosis induced by 25 mM KCl in mammalian nerve endings.

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206.2

FLUCTUATIONS OF EVOKED UNITARY SYNAPTIC RESPONSES IN CORTICAL PYRAMIDAL NEURONS. Shaul Hestrin*. Dept. of Anatomy and Neurobiology, University of Tennessee, Memphis TN 38163.

Important parameters of local cortical circuits are the fluctuations in timing and amplitude of the postsynaptic responses induced by action potentials in a single presynaptic neuron.

Dual whole-cell recording were obtained from layer V pyramidal neurons in the rat visual cortex. The slices were kept at room temperature or at 34-36 °C. Pairs of neighboring pyramidal cells were selected using DIC optics. The average EPSP at different pairs was between 0.2 mV and 3.0 mV and the average EPSC was between 5.9 pA and 48.4 pA. The decay time constants of the EPSPs were between 25 ms and 93 ms whereas those of the EPSCs were between 2.5 ms and 11.3 ms.

In synaptically connected pairs of neurons, the average latencies between the presynaptic action potential and the initial phase of the EPSP(C) in different cell pairs were between 1-6 ms (n=14). However, for any given cell pair the trial to trial latencies fluctuated with standard deviations ranging from 120 µs and 1.0 ms.

Variability of EPSP(C)s amplitudes often reflects previous presynaptic activity. In 13 out of 19 pairs the unitary EPSP(C)s exhibited paired-pulse depression. At intervals of 10 ms to 1 second the response ratio was 0.51 ± 0.2. In most of these pairs (n=11) there was an increase in the rate of action potential failure to produce an EPSP(C). The failure rate increased from 7% in the first EPSP(C) to 29% in second EPSP(C). In two pairs exhibiting depression no failures were detected. These results suggest that paired-pulse depression reflects reduction in transmitter release.

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206.3

QUANTAL VARIANCE REVEALED IN ELECTRON MICROSCOPICALLY IDENTIFIED EXCITATORY SYNAPSES OF THE CAT VISUAL CORTEX.

O. Paulsen*, C. Stricker[1], G. Tamas, T. Szilagyi, P. Somogyi and E. H. Buhl. MRC Anatomical Neuropharmacology Unit, University Department of Pharmacology, Oxford OX1 3TH, U.K., and [1]Division of Neuroscience, John Curtin School of Medical Research, Australian National University, Canberra, ACT 0200, Australia.

In order to extract quantal parameters of excitatory synaptic transmission between layer II/III pyramidal cells and GABAergic interneurons in adult cat visual cortex, intracellular recordings were made with biocytin-filled sharp microelectrodes from pairs of neurons in standard slices. Unitary EPSPs in interneurons (means ranging between 316 and 1224 µV), evoked at 1-2 Hz by action potentials of the pyramidal cell, were subjected to quantal analysis. Using stringent electron microscopic analysis, the numbers of synaptic release sites in each of five connections were determined to be one, two, two, five and seven (Buhl, Tamas, Szilagyi, Paulsen & Somogyi, this meeting).

The amplitude distributions of the EPSPs obtained during stable recording conditions (468-700 events in each cell pair) were fitted with three models based on noise deconvolution as in Stricker *et al.* (1994). The models were: a fully unconstrained mixture model, a quantal model with no quantal variance, and a quantal model with quantal variance. For each of the models, the minimum number of components was determined, for which addition of one more component failed to improve the fit significantly. For all five connections analysed, the first two models gave, excluding the failures, a minimum number of components, which exceeded the number of morphologically determined release sites. The third model, incorporating quantal variance, gave a minimum number of components compatible with the electron microscopically determined number of release sites. For all five EPSPs, a quantal model with quantal variance could be identified, which was compatible with the number of release sites, and which fitted the data as well as models incorporating additional components but no quantal variance. The quantal coefficient of variation (CV) for this best model varied between 0.33 and 0.46. The corresponding quantal sizes ranged between 260 and 657 µV.

We conclude, that there is good agreement between the number of components in the model and the number of identified synaptic release sites. Therefore, a quantal release model can account for the transmission at this junction, but a significant amount of quantal variance has to be incorporated. These data demonstrate the importance of correlated anatomical information in the selection of the most pertinent model of synaptic transmission.

Supported by the British Medical Research Council.

Stricker, Redman & Daley (1994) Biophys. J. 67, 532-547.

206.4

ARE SOME MINIS MULTIQUANTAL? M. Frerking, S. Borges, and M. Wilson*. Section of Neurobiology, Physiology, and Behavior, University of California, Davis, CA 95616.

Amacrine cells from embryonic chick retinae form $GABA_A$ergic autapses in culture. In the presence of external TTX, TEA, and internal Cs^+, small depolarizations elicit Ca^{2+} influx-dependent, miniature IPSCs (minis) at low frequency. The amplitude distribution of these minis is positively skewed, as in other neurons. The origin of skew in the mini distribution is uncertain, but one recent model proposes that large minis represent multiquantal release synchronized by transient Ca^{2+} influx sensed simultaneously at nearby but separate release sites (Edwards, 1995, Physiol. Rev. 75, 759-787).

A prediction of this model is that the skew and large coefficient of variation (CV) of the mini amplitude distribution are both contingent on transient Ca^{2+} influxes. We therefore tested this model by examining minis evoked in the absence of external Ca^{2+} either by internal perfusion of buffered Ca^{2+}-containing solutions or by bath application of the Ca^{2+}-independent secretagogue, α-latrotoxin. Contrary to the predictions of the model, neither the CV nor the skew of the mini amplitude distributions evoked by these methods are resolvably different from the mini distributions evoked by Ca^{2+} influx. We conclude that the mini amplitude distribution does not contain a significant number of Ca^{2+} influx-evoked multiquantal minis. Supported by EY04112 (MW).

206.5

CYCLIC NUCLEOTIDE-GATED CHANNELS IN SYNAPTIC TERMINALS OF RETINAL CONE PHOTORECEPTORS. **A. Savtchenko & R.H. Kramer*** Dept of Molec. & Cell. Pharm., Univ. of Miami Sch. of Med, Miami FL. 33136

Cyclic nucleotde-gated (CNG) channels are crucial for transducing changes in cGMP concentration into electrical signals during light responses in retinal photoreceptors. Here we show that CNG channels are highly concentrated not only in outer segments of rods and cones, but also in presynaptic terminals of cones, suggesting that they play an important functional role in synaptic transmission. These studies were done on enzymatically dissociated cones from lizard (*Anolis carolinensis*). In cones devoid of outer segments, application of pCPT-cGMP, a membrane-permeant CNG channel agonist, activated an inward current of 2-10 pA at -60 mV in 8 of 8 cones with terminals and 0 of 10 cones lacking terminals. Likewise, voltage-gated Ca^{2+} current was detected in all cells containing terminals and none lacking terminals. Hence, aside from the outer segment, the terminals contain nearly all the CNG and voltage-gated Ca^{2+} channels in the cell. Application of cGMP onto excised patches showed that CNG channels were present in 16% of terminal patches, 12% of inner segment patches, and 60% of outer segment patches. However, the CNG channel density in patches that responded to cGMP was much greater when the patch was taken from the outer segment (8-19 channels/patch) or the terminal (20-37 channels/patch) than when the patch was taken from the inner segment (2-5 channels/patch). These observations are consistent with a clustering of CNG channels in terminals, perhaps near sites of transmitter release. Fura-2 Ca^{2+} imaging experiments demonstrate that both membrane depolarization and cGMP analogs trigger Ca^{2+} influx into the terminal. Direct measurements with glutamate receptor "biosensors" are being performed to test if CNG channel activation can indeed trigger transmitter release. Supported by NIH grant NS30685 and AHA grant 9503002.

206.6

DOPAMINERGIC MODULATION OF NUCLEUS ACCUMBENS GABA SYNAPSES. D. Geldwert, S. Rayport*. Depts. Psychiatry, Anatomy & Cell Biology, and Ctr. Neurobiology & Behavior, Columbia Univ.; Dept. Neuroscience, N.Y.S. Psychiatric Institute, NY 10032.

In postnatal nucleus accumbens (nAcc) cultures medium-spiny neurons make inhibitory autapses blocked by the $GABA_A$ antagonist bicuculline (Shi and Rayport, J. Neurosci., 1994), which we confirmed under voltage clamp (n=29). We now report that DA (1 µM, n=29) produces presynaptic inhibition of autaptic IPSCs in 83% and facilitation in 10% of cells. The D1 agonist SKF38393 (1 µM, n=12) and the D2 agonist quinpirole (1 µM, n=14) each mimic DA action, but less strongly; the same is true when the two agonists are applied together (n=4). DA in the presence of the D1 receptor antagonist SCH23390 has a similar incidence and efficacy as quinpirole (n=10); similarly DA in the presence of the D2 antagonist sulpiride (1 µM) resembles SKF38393 (n=10). However, sulpiride and SCH23390 applied together (n=4) do not block DA modulation. Overall, about 85% of cells show both D1- and D2-mediated modulation consistent with extensive colocalization of DA receptors on medium-spiny neuron presynaptic terminals. We also find that 5HT and NE, as well as the $GABA_B$ agonist baclofen (Shi and Rayport, 1994), mediate presynaptic inhibition, adding to the complexity of potential modulatory actions. These autapses model intrinsic GABA synapses in the nucleus accumbens (Shi and Rayport, 1994), and thus are likely to be a major target of DA action, indicating that further examination of these synapses will be important in discerning the signaling role of DA in the mesolimbic system.

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206.7

PRESYNAPTIC α-ADRENOCEPTORS UNDERLIE INDIRECT MODULATION OF EVOKED INHIBITORY EVENTS IN RAT SOMATOSENSORY CORTEX. B.D. Bennett, J.R. Huguenard and D.A. Prince*. Dept. Neurol. & Neurolog. Sci., Stanford Univ. Med. Ctr., Stanford CA 94305

Previous experiments have demonstrated that epinephrine (EPI) reduces the amplitude of monosynaptic, evoked inhibitory postsynaptic currents (eIPSCs) in the majority of neurons studied in P9-12 rat neocortical slices through a presynaptic mechanism. The depression of monosynaptic eIPSCs was always accompanied by a large increase in the frequency of spontaneous IPSCs (sIPSCs) resulting from activation of presynaptic α-adrenoceptors (Bennett et al., Soc. Neurosci. Abst. 21: 431.9). In the present experiments, we tested the hypothesis that the attenuation of eIPSC amplitude might be due to an increase in sIPSCs that would elevate synaptic GABA levels and result in activation of presynaptic $GABA_B$ receptors. Whole-cell voltage-clamp recordings of IPSCs were obtained from layer V pyramidal neurons in slices of rat somatosensory cortex. Monosynaptic eIPSCs were obtained by layer V stimulation during perfusion of slices with ACSF containing 20 μM DNQX and 50 μM APV.

The presence of functional presynaptic $GABA_B$ receptors was confirmed in experiments where a profound reduction of eIPSC amplitude was produced by 5-10 μM baclofen (16±5% of control; n=5) and was antagonized by concurrent exposure to 0.5-1 mM CGP 35348 (91±14% of control; n=4). EPI (10 μM) applied in the presence or absence of 0.5-1 μM CGP 35348 produced an enhancement (121±13% of control; n=9) or depression (73±8% of control; n=15) of eIPSC amplitude, respectively. The blockade of $GABA_B$ receptors did not prevent EPI-induced increases in sIPSC frequency which were observed in all neurons. These data indicate that the depression of eIPSC amplitude observed following EPI application is a consequence of indirect activation of presynaptic $GABA_B$ autoreceptors resulting from α-adrenoceptor-mediated increases in sIPSC frequency. These studies were supported by NIH grant NS 12157 from the NINDS and a Pimley postdoctoral fellowship to BDB.

206.8

MODULATION BY NEUROTRANSMITTERS OF NOREPINEPHRINE SECRETION FROM SYMPATHETIC GANGLION NEURONS DETECTED BY AMPEROMETRY. D.-S. Koh and B. Hille*. Dept. Physiol. & Biophys., Univ. Washington, Seattle, WA 98195.

Patch clamp studies of acutely isolated superior cervical ganglion (SCG) cells revealed inhibition of N-type Ca^{2+} currents in the cell soma by many neuromodulators (TINS, 17:531). To test if the same neuromodulators can reduce release of norepinephrine (NE) at varicosities and terminals of SCG cells, we measured NE secretion using carbon-fiber amperometry at presumed sites of terminals in cultures of SCG neurons stimulated electrically. The pharmacological properties of NE release had many parallels to those of N-type Ca^{2+} channels: Release was blocked by Cd^{2+} or ω-conotoxin GVIA. It was reduced 50 and 62% by 10 μM NE or 2 μM UK14,304, an α_2-adrenergic agonist. It was reduced 63% by 10 μM oxotremorine-M, a muscarinic agonist, which was antagonized by M_2 and M_4 receptor antagonists methoctramine and tropicamide. After overnight incubation with pertussis toxin (PTX), the inhibition by UK14,304 and oxotremorine-M was much reduced. Several other neuromodulators known to inhibit Ca^{2+} channels in these cells, including adenosine, prostaglandin E_2, somatostatin, and secretin, also depressed secretion 34-44%. In cultures treated with ω-conotoxin, it was possible to evoked secretion dependent on L-type Ca^{2+} channels with long KCl depolarizations. This secretion was not sensitive to UK14,304.

In conclusion, the neuromodulators that act on SCG somata also act on their secretory terminals. The depression of NE release at terminals parallels depression of N-type Ca^{2+} currents in the soma.

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206.9

SELECTIVE ENHANCEMENT OF GLUTAMATE RELEASE BY INTERACTION OF PEROXYNITRITE AND A VOLTAGE-GATED CALCIUM CHANNEL SIGNAL. M.J. Friedlander* and C. Gancayco. Neurobiology Research Center, University of Alabama at Birmingham, Birmingham, AL 35294.

We evaluated the mechanism by which N-methyl-D-aspartate receptor (NMDAR) activation and nitric oxide (NO) production selectively modulate L-glutamate release in the cerebral cortex. Cortical synaptosomes were prepared from ether-anesthetized adult guinea-pigs (Montague et al., Science, 263:973, 1994). Release of endogenous glutamate was evaluated by a luminometric assay. The thirty-fold enhancement of glutamate release evoked by a single pulse of 100 μM NMDA application was completely blocked by pre-treatment of the synaptosomes with either the nitric oxide synthase (NOS) inhibitor, L-nitro-arginine, (1 μM LNA, n=64) or by application of the N-type voltage gated calcium channel (VGCC) blocker, ω-conotoxin (0.5 μM CTX, n=14). The application of exogenous NO (1-20 μM NO gas) directly enhanced glutamate release by tenfold (n=20) but this action also was completely blocked by CTX (n=8). Since NO readily reacts with superoxide anion (O_2^-) to form peroxynitrite ($ONOO^-$), we evaluated whether this reaction plays a role in modulation of cortical glutamate release. Application of superoxide dismutase (5,000 units SOD) completely blocked the enhanced glutamate release evoked either by endogenous NO production subsequent to NMDAR activation (n=12) or by exogenous NO application (n=8). Moreover, direct application of $ONOO^-$ (1-20 μM) was sufficient to elicit enhanced glutamate release (n=10), unless VGCCs were blocked (100nM CTX, n=6). SOD did not interfere with the ability of $ONOO^-$ to enhance glutamate release in the synaptosomes where VGCCs were not blocked (n=6). We conclude that NMDAR activation leads to NOS activation, NO production, and reaction with O_2^- to form $ONOO^-$. The $ONOO^-$ signal interacts either directly with VGCCs or with a calcium signal in active presynaptic terminals, selectively boosting glutamate release at active synapses in local volumes of cerebral cortex. Supported by NIH Grant EY-05116.

206.10

PHOSPHORYLATION MODULATION OF THE INTERACTION BETWEEN N-TYPE CALCIUM CHANNELS AND SYNAPTIC CORE COMPLEXES. C.T. Yokoyama, Z-H. Sheng, and W.A. Catterall*. Department of Pharmacology and Graduate Program in Neurobiology and Behavior, University of Washington, Seattle, WA 98195.

N-type calcium channels contain a synaptic protein interaction (synprint) site in the cytoplasmic loop between homologous domains II and III which interacts with the synaptic core complex proteins syntaxin and SNAP-25 in a biphasic, calcium-dependent manner (Sheng et.al. (1996) *Nature* **379**:451-454). We report here the identification of second-messenger-activated protein kinases which phosphorylate the synprint and alter interactions with syntaxin and SNAP-25. Of a panel of protein kinases tested, only protein kinase C (PKC) and calcium/calmodulin-dependent protein kinase type II (Cam KII) phosphorylated a his-6-synprint fusion protein with a stoichiometry of at least 1.0. Two-dimensional tryptic phosphopeptide mapping correlated with the stoichiometry measurements and indicated that each kinase phosphorylated a distinct pattern of 2 or more sites within the synprint. Phosphorylation of the his-6-synprint with PKC or Cam KII strongly inhibited the ability of the synprint peptides to interact in a binding assay with immobilized GST-syntaxin or GST-SNAP-25, even at concentrations of free calcium which stimulate maximal binding. Binding assays with his-6 fusion proteins of smaller regions of the synprint indicate that disruption of binding is mediated by multiple phosphorylation sites. Furthermore, PKC and CaM KII phosphorylation of the his-6-synprint fusion protein inhibited interactions with native rat brain synaptic core complexes containing syntaxin and SNAP-25. These results suggest a candidate presynaptic mechanism whereby PKC and CaM KII phosphorylation of the synprint serves as a biochemical switch for interactions between N-type calcium channels and synaptic core complexes. Supported by NIH grants NS22625 and GM07108-21.

206.11

BIPHASIC CHANGES IN THE PHOSPHORYLATION STATE OF IDENTIFIED PRE- AND POSTSYNAPTIC PKC SUBSTRATES DURING LTD

P.N.E. De Graan*, G.M.J. Ramakers, I.H. Heinen, and W.H. Gispen Rudolf Magnus Institute for Neurosciences, P.O. Box 80040, 3508 TA Utrecht, The Netherlands

LTP and LTD are two forms of activity-dependent long-lasting changes in synaptic efficacy. Activation of protein kinases is important in the proces of LTP, whereas protein phosphatases have been implicated in LTD. B-50 (a.k.a. F1, GAP-43, neuromodulin) is a well characterized presynaptic PKC substrate. More recently RC3 (a.k.a. neurogranin, BICKS) has been identified as a postsynaptic, neuron-specific substrate for PKC. These two proteins share an 18 amino acid sequence, containing the unique PKC phosphorylation site and a calmodulin binding domain. Previously, we have shown that the phosphorylation of both B50 and RC3 is increased during narrow and partially overlapping time windows after the induction of LTP. In this study we investigated the effect of LTD induction on B-50 and RC3 phosphorylation. Hippocampal slices were prepared from young rats (60-80 g), and were labelled with $^{32}P_i$. After stable baseline recordings of dendritic field EPSPs (fEPSPs) in the CA1 field of the hippocampus, LTD was induced by a 15 min, 1 Hz stimulation at test intensity of the afferent fibres. This stimulation paradigm induced a 50 % reduction in the slope of the fEPSP, which could be blocked by the NMDA receptor antagonist AP5 (50 μM). Control slices in the same chamber only received test stimulation. The in situ B-50 and RC3 phosphorylation state was determined in individual slices using quantitative immunoprecipitation and phosphorimaging at different time points after LTD induction. One hour after LTD induction we find a NMDA receptor-dependent increase in B-50 phosphorylation, but no change in RC3 phosphorylation. At shorter times after LTD induction, B-50 and RC3 phosphorylation was below control levels. Thus our data show biphasic changes in the in situ phosphorylation state of pre- and postsynaptic PKC substrates, and indicate that phosphatases and kinases may be activated in distinct time windows following LTD induction. (This work was supported by an ENP grant of the ESF)

206.12

SYNAPTIC TRANSMISSION AND BEHAVIORAL ABNORMALITIES IN MUTANT MICE LACKING mGluR4. A. Baskys*, R. Gerlai, R. Pekhletski, J. Roder and D. R. Hampson. Dept. of Physiology and Fac. of Pharmacy, Univ. of Toronto, Mount Sinai Hosp., Toronto, Ont. M5S 1A8, Canada.

To understand the role of type 4 metabotropic glutamate receptor (mGluR4) in synaptic transmission we studied field EPSPs (fEPSPs) evoked in dentate gyrus neurons in in vitro slices from mutant mGluR4-deficient (M) (n=27) and wildtype (W) mice (n=23). Paired pulse (interval=50 ms) stimulation of the lateral perforant pathway (LPP) revealed no significant difference in paired pulse facilitation or fEPSP/fibre volley ratio between the groups. In W mice, fEPSPs remained stable at the control stimulation rate of 0.1 Hz but decreased significantly following a five minute period of continuous stimulation at 0.5 Hz. Responses returned to control following a 10 min. pause in stimulation. Prolonged (10 min.) stimulation at 0.5 Hz produced only short-term depression (STD) and did not lead to long-term depression of the fEPSP slope. $GABA_A$ receptor/channel antagonist picrotoxin (50 μM) or NMDA receptor antagonist D-APV (50 μM) did not affect STD. There was no significant STD in slices from mGluR4-deficient mice. A putative agonist for mGluR4, L-AP4 (25 and 50 μM), significantly ($p<0.05$) suppressed fEPSPs in M (n=9) and W (n=8) slices with no significant difference between the groups. Depression of fEPSPs by metabotropic agonist 1S, 3R-ACPD (25 μM) was not different in W (n=5) and M (n=6) slices. In the Morris water maze paradigm both groups showed a similar decrease in escape latency over the course of 14 training sessions. Significantly shorter escape latencies were recorded in sessions 2 and 3 in M mice upon removal of the platform to a different quadrant but no significant difference was found in sessions 1, 4, 5 and 6. These results indicate that mGluR4 may be responsible for STD in LLP but is not responsible for L-AP4-induced depression. Improved learning of the new platform location by mGluR4-deficient mice may be related to diminished STD or to other processes influenced by this receptor. Supported by MRC.

206.13

ENDOGENOUS ADENOSINE ON MEMBRANE PROPERTIES OF CA1 NEURONS DURING NORMOXIA AND ANOXIA. P.J. Zhu* and K. Krnjević, Anaesthesia Research and Physiology Depts., McGill Univ., Montréal, Québec H3G 1Y6, Canada

Ongoing effects of endogenous adenosine on CA1 neurons in hippocampal slices from Sprague-Dawley rats were studied by extra/intracellular and whole-cell recordings. The slices were submerged and kept at 33°C. During normoxia, an A1 antagonist (8-SPT), or adenosine deaminase potentiated both spontaneous (n=7) and evoked EPSPs (n=10), but not monosynaptic IPSP (n=6); and caused a minimal depolarization (by 1 mV) -- probably due to the enhanced synaptic activity -- but no significant change in input conductance (n=9). Hypoxia (4-5 min) induced an outward current and increase in input conductance. 8-SPT (10 μM) reversibly attenuated this effect (n=6). In the presence of Ba (1 mM), hypoxia produced no significant change in input conductance and the outward current was replaced by a small inward current. These data indicate that ongoing adenosine release tonically inhibits excitatory synaptic transmission; has no direct effect on inhibitory synaptic transmission; and contributes to hypoxic hyperpolarization.

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TRANSPORTERS II

207.1

LOCALIZATION OF mRNA FOR VESICULAR MONOAMINE TRANSPORTERS, VMAT1 andVMAT2, IN THE DEVELOPING RAT EMBRYO.

Stefan R. Hansson*, Eva Mezey@ and Beth I. Hoffman.
Lab of Cell Biology, NIMH and @ Clinical Neurosciences Branch, NINDS, Bethesda , MD 20892

Monoaminergic neurons (dopamine, norepinephrine, epinephrine, serotonin, histamine) are physiologically functional prior to synapse formation. Catecholamines have been shown to induce morphogenesis such as closure of the neural folds, and to induce changes in the developing cortex. Following their synthesis, monoamines are packaged into synaptic vesicles or secretory granules by vesicular monoamine transporters (VMATs). The cDNA cloning of rat VMATs has revealed the existence of two highly homologous but distinct gene products. In the adult rat, VMAT1 is thought to be limited to the adrenal medulla. We have used in situ hybridization histochemistry with RNA probes to delineate gene expression of the two VMATs during rat development. VMAT2 was detected earlier and was more abundant than VMAT1 mRNA. In the central nervous system (CNS), VMAT 2 mRNA was first detected in cells of the neural tube at embryonic day 11 (E11). By E13, VMAT2 mRNA was localized to monoamine cell body-containing regions in the brainstem and hypothalamus. In addition regions which receive monoaminergic innervation contain VMAT2 mRNA: rhinencephalon (E13), the pallidal neuroepithelium of the basal ganglia (E13), striatal part of the basal ganglia (E14), septum (E15), thalamus (E16), tegmentum (E17). VMAT1 mRNA was detected in pallidal neuroepithelium, striatal subventricular zone, cortex and hippocampus (E18) and the external germinal layer of the cerebellum (E18). The presence of VMAT1 mRNA in the subventricular zone (E18) and VMAT1 mRNA in the neural tube (E11) suggests a role for monoamines in differentiation. From early gestation, VMAT1 and VMAT2 mRNA have an overlapping distribution in sympathetic and autonomic ganglia, heart and adrenal medulla. In the gut VMAT1 mRNA was localized to the inner nerve plexus while VMAT2 mRNA was present in the outer enteric plexus. The presence of mRNA for VMATs implies possible regulated release, or a need for sequestration of monoamines before synapses are formed. mRNA for VMATs in non-monoaminergic cells of the limbic system as well as the basal ganglia suggest an unexpected role for these transporters.

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207.2

SYNAPTIC VESICULAR MONOAMINE TRANSPORTER GENE KNOCKOUTS. N. Takahashi@*, J. Sora@, R. Revay@, D.M. Donovan@, H.F. Liu@, L.L. Miner@, G.R. Uhl@ @Molec. Neurobiol., NIDA-IRP, NIH; #Dept. Neurol. & Neurosci., JHUSM, Balto., MD 21224

The synaptic vesicular monoamine transporter (VMAT2) accumulates brain monoamines into synaptic vesicules. Amphetamine and its derivatives disturb dopamine storage in vesicules, resulting in dopamine release into cytoplasm where it could contribute to amphetamine toxicity. Overexpression of VMAT2 can suppress the toxicity of the Parkinsonism inducing toxin MPP+ *in vitro*. These observations imply that VMAT2 could protect dopamine neurons from toxicities of dopamine and MPP+. To study these effects, we have isolated genomic clones from a murine 129SvJ genomic library that contain the all exons of the VMAT2 gene and 5' flanking sequences. A VMAT2 gene targeting vector deleted the first three exons encoding the first two VMAT2 transmembrane domains and the large lumenal loop, replacing them with a pgk neomycin resistance gene. Eight AB1 embryonic stem call lines harboring the desired homologous recombination were identified by Southern blot analysis, and several several animals produced by blastocyst injection of two of these cell lines. The molecular, pharmacological and behavioral features of heterozygous and homozygous VMAT2 knockout phenotypes will advance our understanding of VMAT2 function.

207.3

OVEREXPRESSION OF PUTATIVE VESICULAR ACETYLCHOLINE TRANSPORTER INCREASES QUANTAL TRANSMITTER PACKAGING IN DEVELOPING XENOPUS NEURONS: H-j. Song[1*], G-l. Ming[1], E. Fon[2], B. Bellocchio[2], R. H. Edwards[2], and M-m. Poo[1]. [1]Dept. Biol., UCSD, La Jolla, CA. 92093, [2]Depts. Neurol. and Physiol., UCSF, San Francisco, CA. 94143.

Putative vesicular acetylcholine transporter (VAChT) genes from several species have been cloned and both the mRNA and protein are localized in cholinergic neurons. However, the function of the putative transporter has not been clearly demonstrated. In this study, we tested the function of VAChT by overexpressing the protein in developing neurons when quantal transmitter secretion is not yet mature. Rat VAChT cDNA was injected into one of the early blastomeres of *Xenopus* embryos, together with a fluorescence marker FITC-dextran. Expression of VAChT was elevated in all cells derived from the injected blastomere, including spinal neurons. At the neuromuscular synapses made by these neurons, the spontaneous miniature endplate currents (MEPCs) showed a significant increase in the median amplitude (458.8±67.8 pA, SEM, n=30), as compared to that at control synapses (192.7±32.2 pA, SEM, n=26). Analysis of the amplitude distribution of MEPCs indicates a significant shift to higher amplitudes for neurons overexpressing VAChT. The increase in the quantal size is also accompanied by an increase in the MEPC frequency: 34.2±10.4 min^{-1} (SEM, n=30) and 9.5±3.5 min^{-1} (SEM, n=26), for the overexpressing and control neurons, respectively. These results supports the putative role of this protein in promoting ACh packaging into secretory vesicles. (Supported by NIH grant NS31923)

207.4

TARGETED DISRUPTION OF THE MURINE SEROTONIN TRANSPORTER GENE. D. Bengel[1,2], D. L. Murphy[1*], D. Feltner[3], M. Seemann[2], A. Heils[2], A. Grinberg[3], H. Westphal[3], and K.-P. Lesch[2]. [1] Lab. of Clinical Science, NIMH, NIH, Bethesda, MD 20892. [2] Dept. of Psychiatry, Univ. of Würzburg, 97080 Würzburg, Germany. [3]Lab. of Mammalian Genes & Development, NICHD, NIH, Bethesda, MD 20892.

The brain serotonin transporter (5-HTT) plays a central role in the modulation of serotonergic neurotransmission. It has been associated with mood disorders, anxiety and aggression and is the primary target for widely used serotonin uptake inhibiting antidepressant drugs such as fluoxetine. Interest in the mechanism of disease- and therapy-induced modification of 5-HTT function and its impact on early brain development and event-related synaptic plasticity is widespread and intensifying. To analyze the function of the 5-HTT, we have generated transgenic mice lacking the 5-HTT by means of homologous recombination in ES cells. The second exon was deleted, and homologous recombination was confirmed by Southern analysis in two embryonic stem cell clones. Four male chimeric mice, upon mating with wild-type mice, gave rise to germline transmission of the mutant allele. Homozygous mice were obtained by intercrossing of heterozygotes. Despite evidence that excess serotonin during embryonic development, including that produced by fluoxetine, may lead to severe craniofacial and cardiac malformations, no obvious developmental defects have been observed in mutant mice. Feeding behavior as well as weight gain are normal in the 5-HTT deficient mice. Further characterization of the molecular, pharmacological, and behavioral features of the homozygous mutant phenotype is underway.

207.5

ACUTE REGULATION OF THE HUMAN NOREPINEPHRINE TRANSPORTER IN NATIVE AND HETEROLOGOUSLY TRANSFECTED CELL LINES. S. Apparsundaram*, and R.D. Blakely, Department of Pharmacology and Center for Molecular Neuroscience, Vanderbilt University Medical Center, Nashville, TN 37232-6600.

Active transport of norepinephrine (NE) into presynaptic nerve terminals via NE transporter (NET) is the primary means of modulating synaptic NE levels after release. Although many reports have demonstrated that NET activity can be both up- and down-regulated by hormones and neurotransmitters, the cellular mechanisms involved in acute regulation of NET activity remain to be defined. Activators of PKC (ß-PMA and ß-PDBu) produced a rapid and concentration-dependent inhibition of NE transport in human noradrenergic SK-N-SH, hNET stably transfected HEK 293 and transiently transfected COS cells. The inactive α-isomers of these agents were ineffective. Kinetic analysis indicated that PKC activators produced a 40% decrease in Vmax with little or no change in Km of NE transport. Acute staurosporine treatment did not alter basal hNET activity but abolished ß-PMA-induced inhibition of NE transport in hNET transfected cells. Since hNET contains multiple consensus Ser/Thr residues for phosphorylation in its putative cytoplasmic NH_2 and COOH domains, *in vitro* phosphorylation experiments were carried out using glutathione-S-transferase (GST) fusion proteins containing either NH_2 or COOH tails of hNET. Purified PKC, PKA and PKG phosphorylate both NH_2 and COOH tails with little or no phosphorylation of GST component. Cell-surface biotinylation and *in vivo* metabolic labeling are being used to determine whether PKC-mediated changes in NE transport involve surface redistribution, protein phosphorylation or both. (Supported by NINDS Grant NS 33373)

207.6

CHIMERIC HUMAN AND *DROSOPHILA* SEROTONIN TRANSPORTERS TO PROBE REGIONS INVOLVED IN LIGAND BINDING AND ION DEPENDENCE. E.L.Barker*, M.A.Gray, E.A. Camp, and R.D.Blakely, Dept. of Pharmacology and Ctr. for Mol. Neuroscience., Vanderbilt Univ. Sch. of Med., Nashville, TN 37232

Pharmacological studies using the cloned human (hSERT) and *Drosophila* (dSERT) serotonin transporter cDNAs have revealed differential sensitivities to substrates and antagonists across species. The tricyclic antidepressants and citalopram were found to be less potent at the dSERT as compared to the human homologue, whereas the antagonist mazindol was significantly more potent at the *Drosophila* transporter. Although cocaine showed no difference in potency across species, the cocaine analog RTI-55 was much less potent at dSERT than hSERT suggesting that cocaine analogs recruit additional sites of interaction that are not present in dSERT. Chimeras between human and *Drosophila* SERTs have localized the region of TMD2 (amino acids 118-136) as playing a major role in mazindol and citalopram recognition. This region does not appear to influence potency for other ligands exhibiting differences between hSERT and dSERT. Using site-directed mutagenesis, the eight amino acids that differ between hSERT and dSERT in the TMD2 region will be explored as potential candidates mediating the species-selectivity of mazindol and citalopram. In addition to identifying ligand binding domains, cross-species chimeras can also track domains responsible for other functional differences observed for the two SERT homologues. For example, serotonin transport by hSERT exhibits absolute dependence upon Na^+ and Cl^-, whereas dSERT transport activity is also dependent upon Na^+, but only facilitated by Cl^- with approximately 20% of uptake activity remaining in the absence of Cl^-. Functional studies using the wild-type and chimeric SERTs also have implicated the TMD2 region as being involved in Na^+ sensitivity, whereas the region distal to TMD2 appears important for the absolute dependence of hSERT on Cl^-. Together these studies suggest new opportunities for identification of critical domains and residues involved in SERT function. (This work supported by NIH grants DA07390 and DA05679)

207.7

ACTIVATION OF PROTEIN KINASE C DOWNREGULATES SEROTONIN TRANSPORTERS VIA ALTERED CELL SURFACE DISTRIBUTION. S. Ramamoorthy*[1], Y. Qian[2], A. Galli[1], Louis J. DeFelice[1] and R.D. Blakely[1]. [1]Dept. Pharmacol. and Ctr. for Mol. Neurosci., Vanderbilt Univ., Nashville, TN 37232; [2]Program in Neurosci., Emory Univ., Atlanta, GA 30322.

The plasma membrane serotonin (5HT) transporter (SERT) has been extensively investigated over the past decade as a mechanism for the clearance of synaptic and plasma 5HT, as a target of cocaine and antidepressants, and as a biological marker of mental illness. Multiple reports demonstrate a capacity of SERTs to be acutely regulated by second messenger-linked systems. Previously, we have shown that short term treatment of PMA causes a dose-dependent, stereospecific, and staurosporine-sensitive inhibition of 5HT uptake in HEK-293 cells that stably express human SERT (293-hSERT cells). The effect of PMA on 5HT uptake is mainly through a decrease of 5HT transport Vmax, with little change in Km. PMA treatment of 293-hSERT cells under voltage clamp also reveals a loss of SERT-mediated currents. In the present study, we labelled cell surface proteins using a nonpermeablizing biotinylating agent, harvested the biotinylated proteins on steptavidin agarose beads and detected cell surface SERT protein by immunobloting with a SERT-specific polyclonal antibody. In a paradigm of PMA treatment that causes a significant decrease in 5HT uptake, we observed in parallel a similar decrease of cell surface SERT protein. Total amount of SERT in the cell is not changed by PMA treatment. Consistent with the decrease of cell surface SERT, the nonbiotinylated SERT protein is also elevated in PMA treated cells. The effect of PMA on both 5HT uptake and cell surface SERT density is blocked by cotreatment with staurosporine. In conclusion, activation of PKC causes a decrease in 5HT uptake via decreasing cell surface SERT density, presumably by altered plasmalemmal translocation of SERT protein. This work is supported by DA07390.

207.8

COCAINE REGULATION OF SEROTONIN TRANSPORTER mRNA AND BINDING SITES IN HUMAN BRAIN

KY Little*, ZS DelProposto, PR McFinton, DP McLaughlin, CS Livermore, GW Dalack, SJ Watson

Department of Psychiatry, University of Michigan, Ann Arbor, MI

Chronic cocaine exposure in human users appears to increase dopamine transporter binding sites in post mortem brain, as measured with [^{3}H]WIN 35428. Although cocaine also binds to and inhibits the serotonin transporter (SERT), there is less information available about the effects of chronic cocaine on the SERT in humans or animals. In this series of experiments, we assessed brainstem SERT mRNA levels employing in situ hybridization, and midbrain, hippocampal, and striatal binding sites utilizing [^{125}I]RTI-55 and quantitative autoradiography. Brain specimens from 32 cocaine users and age-, sex-, and PMI-matched controls were dissected at autopsy, as authorized by the Medical Examiner. SERT mRNA was labeled using a 920 bp riboprobe and high stringency washes. mRNA OD's were quantified in dorsal raphe, median raphe, and B9 cell groups. [^{125}I]RTI-55 binding was quantified at .035, .35, and 3.5 nM (.1 Kd, Kd, and 10xKd) in the presence of 10 nM RTI-121, with specificity defined with citalopram(100 nM). mRNA levels were not different between groups, but binding was significantly increased in caudate(19%), putamen(22%), and accumbens(26%). In contrast, SERT binding was significantly decreased in the midbrain over the substantia nigra, but not linear nucleus or dorsal raphe. An acute conformational change (increasing binding) may have been superimposed on chronic cell loss, especially prominent in substantia nigra where both projecting and terminal neuronal loss would magnify the chronic effect. **Supported by NIH award DA09491.**

207.9

NITROGEN-BASED DRUGS ARE NOT ESSENTIAL FOR BLOCKADE OF DOPAMINE TRANSPORT OR BINDING

Bertha K. Madras*[1], Zdenek B.Pristupa[2], Hyman B. Niznik[2], Anna Y. Liang[3], Peter C. Meltzer[3]. Harvard Medical School[1], Southborough, MA 01772, Clarke Institute of Psychiatry[2], Toronto, Canada, Organix[3] Inc., Woburn, MA.

Drugs such as cocaine, methylphenidate and antidepressants that block monoamine transport are invariably nitrogen-based. The nitrogen is presumed to be necessary for anchoring the drug to the same aspartic acid residue on the transporter protein that bonds the nitrogen of a monoamine neurotransmitter. We now report a novel series of drugs which possess no nitrogen and bind to the dopamine, serotonin and norepinephrine transporters with relatively high affinity. A lead compound bound to the human dopamine transporter in putamen (IC_{50}: 5.01 ± 1.74 nM), in cos-7 cells transiently expressing the human dopamine transporter cDNA (IC_{50}: 2.1 ± 0.75 nM) and blocked [^{3}H]dopamine transport in the cells (IC_{50}: 7.2 ± 4.24 nM). The compounds did not fully [^{3}H]WIN 35,428 binding to monkey and human dopamine transporter. These innovative drugs offer unique tools for modeling drug-transporter complexes and highlight the potential for developing a new generation of non-nitrogen drugs targeted to monoamine transporters and receptors. DA06303, DA09462, RR00168, DA 4-8309.

207.10

PHORBOL ESTER INDUCED PHOSPHORYLATION OF DOPAMINE TRANSPORTERS IN RAT STRIATUM. R.A. Vaughan*[1], R.A. Huff[1], G.R. Uhl[1], & M.J. Kuhar[2]. [1]Molec. Neurobiol. Br., NIDA IRP, Balto., MD 21224, Depts of Neurology & Neuroscience, JHUSM, & [2]Neurosci. Div., Yerkes Regional Primate Ctr., Emory Univ., Atlanta, GA 30322

The primary amino acid sequence of dopamine transporters reveals consensus phosphorylation sites for several protein kinases including PKA, PKC, and CaM kinase. Phorbol esters regulate dopamine uptake in synaptosomes and in heterologous expression systems, and direct phosphorylation of DAT has been found in a stably expressing cell line (Huff, et al., Society for Neuroscience Abstract, 1996). We report here the demonstration of DAT phosphorylation in brain. Rat striatal synaptosomes were incubated with [^{32}P]-orthophosphate, and radiolabelled of DAT detected by immunoprecipitation, SDS-PAGE, and autoradiography. A phosphorylated protein that co-migrated on gels with [^{125}I]IDEEP-photoaffinity labeled DAT was extracted using immune but not preimmune serum. Immunoprecipitation was blocked by the addition of the immunizing but not irrelevant peptides, and the protein was not isolated from cerebellum, a brain region devoid of DAT. The level of DAT phosphorylation increased in a dose- dependent manner by the addition of up to 10 µM okadaic acid. DAT was phosphorylated at a basal level in the absence of kinase activators, but treatment of synaptosomes with PMA increased DAT phosphorylation 3-4 fold. This effect was dose-dependent with an IC_{50} of about 300 nM, while the inactive phorbol ester 4αPDD at 10 µM was without effect. Increased phosphorylation was observed within minutes, and was maximum by 20 min. These results provide the first demonstration of neurotransmitter transporter phosphorylation in brain, and raise the possibility that rapid phosphorylation-induced functional regulation of DAT could significantly alter synaptic dopamine dynamics. Supported by the National Institute on Drug Abuse.

207.11

ENHANCEMENT OF DOPAMINE TRANSPORTER PHOSPHORYLATION BY PHORBOL ESTERS: PARALLELS WITH PHORBOL-INDUCED DECREASES IN TRANSPORT. R.A. Huff[1], R.A. Vaughan[1], M.J. Kuhar[2], & G.R. Uhl[1,3]. [1]Molec. Neurobiol. Branch, NIDA IRP, Balto., MD 21224; [2]Neurosci. Div., Yerkes Regional Primate Ctr., Emory Univ., Atlanta, GA 30322; [3]Depts. Neurol. & Neurosci., JHUSM, Balto., MD 21224.

Dopaminergic neurotransmission is terminated by presynaptic reuptake of dopamine by the dopamine transporter (DAT). The primary sequence of the dopamine transporter contains multiple potential phosphorylation sites, suggesting that the function of the transporter may be regulated by phosphorylation. We have examined DAT phosphorylation *in vivo* by labeling LLC-PK$_1$ cells expressing DAT with [^{32}P]orthophosphate followed by immunoprecipitation and electrophoresis of DAT. We have identified DAT as a phosphoprotein that migrates with the same relative mobility as [^{125}I]DEEP-labeled DAT, is immunoprecipitated in the presence of irrelevant peptide but not antigenic peptide, and is absent from nontransfected cells. Activation of protein kinase C with the active phorbol ester PMA enhances modest basal levels of phosphorylation by 3 fold and this enhancement is blocked by the protein kinase inhibitor staurosporine. The inactive phorbol ester 4α-PDD does not produce this effect. The enhancement of DAT phosphorylation by PMA is concentration- and time-dependent, showing increases as early as 4 min after PMA treatment. The concentration and time dependency of the effect of PMA, as well as its staurosporine-sensitivity, parallel previously documented PMA decreases in dopamine transport V_{max}. These parallels suggest that phosphorylation of DAT could contribute to rapid down-regulation of transporter function, and thus provide a novel mechanism whereby modulation of protein kinase C activity in dopaminergic neuronal processes could dynamically alter extraneuronal dopamine levels.

207.12

Activation of Purified Inositol Hexakisphosphate Kinase by Protein Kinases. S. M. Voglmaier*, C. -F. Huang, and S. H. Snyder. Johns Hopkins University School of Medicine, Department of Neuroscience, Baltimore, MD 21205.

Two inositol phosphates which possess pyrophosphate bonds, diphosphoinositol pentakisphosphate (PP-IP5) and bisdiphosphoinositol tetrakisphosphate (bis-PP-IP4), were recently identified. We have purified an inositol hexakisphosphate (IP6) kinase from rat brain supernatants. The purified protein with a molecular weight of 54 kD has high affinity (K_m=0.7 μM) and specificity for IP6 as substrate. During the purification process, phosphatase inhibitors were required to maintain the IP6 kinase activity. This indicates that phosphorylation of IP6 kinase may be an important regulatory mechanism *in vivo*. We have found that (1) calcium is a necessary cofactor for IP6 kinase, (2) upon protein kinase C (PKC) phosphorylation, IP6 kinase activity increases about 500%, (3) in the absence of diacylglycerol and phosphatidylserine, the IP6 kinase activity cannot be upregulated by PKC, and (4) in the presence of PKC inhibitors, the activation effect is abolished.

PAIN MODULATION

208.1

INTRADENTAL RECEPTORS ARE NOT SENSITIZED DURING PULPAL INFLAMMATION IN THE CAT. D. Andrew and B. Matthews*. Department of Physiology, University of Bristol, Bristol BS8 1TD, U.K.

We have investigated the effects of inflammation of dental pulp on the properties of intradental receptors in 8 young adult cats. At an initial operation under general anaesthesia, a cavity was cut on the buccal side of each lower canine tooth. To induce pulpitis, dental caries from a human tooth was sealed into the cavity with dental amalgam (Mjör and Tronstad, *Oral Surg.* 34, 102-108, 1972). After 1 week, the animals were anaesthetized with sodium pentobarbitone and recordings were made from single fibres dissected from the inferior alveolar nerve (IAN) during the application to dentine at the tip of the tooth of a range of stimuli that would cause pain in man (mechanical, thermal, osmotic, hydrostatic pressure changes and drying). Pulpal blood flow was recorded with a laser Doppler flow meter during electrical stimulation of the IAN at different intensities. At the end of the experiment the animals were perfused with fixatives and sections of decalcified dentine and pulp from all 4 canines were examined in the light microscope. Some sections were immuno-labelled with antibodies to either substance P (SP) or calcitonin gene-related peptide (CGRP).

Compared with normal teeth studied previously, resting pulpal blood flow was up to 200% greater but it increased less on IAN stimulation. The responses of the 79 single units studied were similar to those of controls, and the same proportion failed to respond to dentine stimulation. There was histological evidence of pulpal inflammation in all the treated teeth but SP and CGRP expression was similar to controls. The cats showed no evidence of pain during the week following insertion of the caries. These results suggest that, in man, the pain of pulpitis may be due to central sensitization in nociceptive pathways.

Supported by The Wellcome Trust.

208.2

Novel interaction of α2-adrenergic with μ-opioid and A1-adenosine agonists in peripheral antinociception in the rat

K.O. Aley, P.G. Green* and J.D. Levine
University of California San Francisco, CA 94143-0452

We evaluated for interactions between the peripheral antinociceptive effect of clonidine, an α2-adrenergic agonist, DAMGO (μ-opioid agonist), and CPA (A1-adenosine agonist) using the Randall-Selitto paw withdrawal test. A rapid (within four hours) development of tolerance and cross-tolerance was observed if any of these agents were administered repeatedly (hourly). Furthermore, the α2-adrenergic antagonist yohimbine, produced withdrawal hyperalgesia in paws made tolerant to clonidine, DAMGO or CPA.

In evaluating for cross receptor-specific block of antinociception, naloxone (μ-opioid antagonist) and PACPX (A1-adenosine antagonist) were found to block clonidine antinociception. Yohimbine (α2-adrenergic antagonist) blocked both DAMGO and CPA antinociception. In studies of cross receptor-specific block of withdrawal, naloxone-induced withdrawal hyperalgesia in DAMGO-tolerant paws and PACPX-induced withdrawal hyperalgesia in CPA-tolerant paws was reversed by co-injection of clonidine with naloxone and PACPX, respectively.

Since all three classes of peripherally-acting antinociceptive agents block PGE_2 hyperalgesia, and all three receptors interact in their peripheral antinociceptive effects we suggest that all three receptors are located on the same subpopulation of primary afferent nociceptors. Interactions thought to occur at the level of the second messenger, mediating the development of tolerance, were found to be completely promiscuous. In addition, a novel interaction may also occur at the level of the receptors, centered on an interaction of the α2C-adrenergic with the μ-opioid and A1-adenosine receptors.

Funded by NIH Grant DE08973

208.3

A PERIPHERAL FACTOR OF COLD ALLODYNIA AND HYPERALGESIA. HYPOTHESIS AND MODELING. L. de Medinaceli*, J-C. Hurpeau, H. Begorre. IEBM, 54511 Vandoeuvre, & CRAN-INPL, 54516, Vandoeuvre, France.

Allodynia and hyperalgesia are relatively frequent sequelae of peripheral nerve injury. These symptoms are worsened by cold but their mechanism is not well understood. We suggest that incomplete recovery of diameter of regenerated fibers is one of the factors involved in cold intolerance after nerve damage.

Conduction velocity is correlated to fiber diameter, and is slowed down by cold. In normal subjects, cold does not desynchronize the volleys of sensory impulses sufficiently to change the intelligibility of the peripheral "messages." Sensory perception remains accurate although it acquires a characteristic numbness. On the other hand, posttraumatic reduction in fiber diameters causes a permanent distortion of the messages. We considered that when the distortion is severe, the resulting messages may be perceived by the centers as containing nociceptive components. We further hypothetized that, even in cases of moderate permanent distortion, cold acts by increasing the posttraumatic abnormalities of impulse synchronization. In winter, decompensation is observed when a threshold of desynchronization is reached.

We devised a model of peripheral nerve messages, in an attempt to represent the distortion brought about by cold and crush damage. Although our model was quite simple, a great number of parameters could vary. The results of statistical analyses indicated that peripheral desynchronization might explain, at least in part, the painful sensations experienced in winter by many patients after peripheral nerve injury.

208.4

ANTI-NGF PREVENTS THERMAL ALLODYNIA IN CHRONIC CENTRAL PAIN FOLLOWING SPINAL CORD INJURY. M.D. Christensen*, A. Everhart and C.E. Hulsebosch. Dept. Anat. & Neurosci. and Marine Biomed. Inst., Univ. Texas Med. Br., Galveston, TX 77555-1069.

Spinal cord injury frequently results in dysesthesias. We report that the development of chronic central pain following spinal cord hemisection is prevented by continuous infusion of antibodies to NGF (ANTI-NGF). The spinal cords of male Sprague-Dawley rats were hemisected at T13 and PE 10 tubing was inserted intrathecally to the T12 level. Alzet pumps (2 ml capacity, 10 μl/hr, 14 days) containing vehicle (pH 7.2 buffered saline) (N=5), ANTI-NGF (40 μg/hr, N=5) or NGF (35 ng/hr, N=5) were connected to the tubing and inserted subcutaneously. The animals were tested both preoperatively and postoperatively for mechanical and thermal thresholds for paw withdrawal and for supraspinal responses. We report the results of the behavioral tests which support the development of mechanical allodynia but the prevention of thermal allodynia in the ANTI-NGF treated group compared to vehicle controls after 14 days. In contrast, the NGF group had heightened mechanical and thermal allodynia and were terminated at 7 days for humane reasons. CGRP peptide is involved in pain transmission, located in A delta and C fibers and upregulated by NGF. Data will be presented to demonstrate that downregulation of CGRP in ANTI-NGF treated rats and inhibition of A delta and C-fiber intraspinal sprouting are two mechanisms that can account for the inhibition of thermal allodynia. (Supported by NS 11255 and the Kent Waldrep National Paralysis Foundation.)

208.5

EFFECTS OF SUBACUTE INTRAVENOUS SNX-111 INFUSION ON MECHANICAL ALLODYNIA IN A RAT MODEL OF PAINFUL PERIPHERAL NEUROPATHY. Y.-X. Wang, M. Pettus, T. Singh*, S. S. Bowersox and R. R. Luther. Neurex Corporation, Menlo Park, CA 94025.

SNX-111, the synthetic version of the naturally occurring ω-conopeptide MVIIA, is a selective antagonist of neuronal N-type voltage sensitive calcium channels (VSCC). This compound has been shown to block mechanical allodynia in a rat model of painful peripheral neuropathy when it is administered spinally (Chaplan *et al.* J. Pharmacol. Exp. Ther. 269: 1117, 1994) and is now under clinical evaluation for the treatment of chronic pain. The current study evaluated the effect of subacute (6 days), intravenous SNX-111 infusion on mechanical allodynia thresholds in rats with a painful peripheral neuropathy induced by tight ligation of the L_5/L_6 spinal nerves at sites distal to the dorsal root ganglia.

Twenty-three rats were divided into four treatment groups: vehicle (0.9% saline, 1 µl/hr, n=6), SNX-111 (3 µg/kg/hr, n=4), SNX-111 (10 µg/kg/hr, n=6) and SNX-111 (100 µg/kg/hr, n=7). After confirming the presence of mechanical allodynia, test and control articles were administered by continuous, constant-rate intravenous infusions for 6 days using osmotic minipumps. Hindlimb withdrawal responses to tactile stimuli (mechanical allodynia) were measured daily before, during and after test/control article infusion. Intravenous infusion of vehicle did not significantly alter mechanical allodynia thresholds during or after treatment. SNX-111 delivered at doses of 3 or 10 µg/kg/hr was ineffective; however, SNX-111 at 100 µg/kg/hr significantly attenuated mechanical allodynia. Analgesia was present 1-2 days after initiating infusion and was maintained for the duration of the treatment period. The anti-allodynic effect of SNX-111 gradually declined after discontinuation of infusion, with mechanical allodynia thresholds returning to pretreatment levels within 3 days of terminating treatment.

These results show that subacute intravenous infusion of the selective N-type VSCC blocker SNX-111 causes a dose-dependent and reversible blockade of mechanical allodynia in rats with an experimentally-induced painful peripheral neuropathy.

208.6

EXPERIMENTAL PAINFUL MONONEUROPATHY: INHIBITORS OF TNF-α-PRODUCTION INDUCE A DECREASE IN HYPERALGESIA AND AN INCREASE OF SPINAL MET-ENKEPHALIN C. Sommer* and M. Marziniak, Neurologische Universitätsklinik Würzburg, Germany

The aim of the present study was to investigate the effects of TNF-inhibiting drugs in experimental painful mononeuropathy. Thalidomide (50 mg/kg) and pentoxifyllin (100 mg/kg), both known to inhibit macrophage production of TNF-α, were administered to rats with a unilateral sciatic nerve chronic constriction injury (CCI). Vehicle treated rats developed heat hyperalgesia and tactile allodynia from postoperative day 3. Both behaviors were consistently present until the end of the experiment on day 14. Pentoxifyllin-treated rats were protected from maximal heat hyperalgesia throughout the course of the experiment, and from tactile allodynia during the first postoperative week. Thalidomide treated rats had decreased heat hyperalgesia on postoperative days 3 and 5 and decreased allodynia during the first postoperative week. Spinal cord tissue harvested on postoperative day 14 was immunostained for neuropeptides. Preliminary results show a moderate increase in met-enkephalin-immunoreactivity in CCI-animals as compared to controls and a significant increase in thalidomide- and pentoxifyllin-treated rats when compared to either controls or to vehicle treated CCI-rats. We conclude that drug-induced attenuation of hyperalgesia and allodynia is paralleled by increased activity of the endogenous pain-modulating system in this model. Funding: DFG So 328/2-1

208.7

CLONIDINE PREVENTS NEUROTOXIC SIDE EFFECTS WHILE ENHANCING THE NEUROPATHIC PAIN RELIEVING ACTION OF MK-801 J.W. Olney*, S. Eye, D.F. Wozniak and V. Jevtovic-Todorovic. Depts of Anesthesiology and Psychiatry, Washington Univ Med School, St. Louis, MO, 63110.

Recent studies show that NMDA glutamate receptor antagonists can alleviate neuropathic pain. However, NMDA antagonists have the liability of causing psychotomimetic reactions in adult humans and adverse effects ranging from memory and locomotor impairment to injury of cerebrocortical neurons in adult rats. Alpha-2-adrenergic agonists prevent the cerebrocortical neurotoxic side effects of NMDA antagonists. For example, clonidine at 0.05 mg/kg sc prevents the neurotoxic side effects of the powerful NMDA antagonist, MK-801(0.5 mg/kg sc). In the present study we used sciatic nerve ligation and subsequent thermal paw stimulation to elicit a thermal hyperalgesic response (common component of neuropathic pain syndrome) in adult female rats, and studied the ability of clonidine to abolish this response by itself or to modify the analgesic action of MK-801. MK-801 abolished the thermal hyperalgesic response at a dose ≥ 0.05 mg/kg sc but not at 0.025 mg/kg sc. The hyperalgesic response was not abolished by clonidine at 0.05 mg/kg sc, but was by combined treatment with MK-801 (0.025 mg/kg sc) and clonidine (0.05 mg/kg sc). The dose of MK-801 required to induce cerebrocortical neuronal injury is approximately 0.2 mg/kg sc. Therefore, MK-801 by itself abolished the hyperalgesic response at a dose lower than that required to produce neurotoxic side effects and when combined with clonidine, which protects against these side effects, the analgesic action of MK-801 is augmented, thereby allowing MK-801 to achieve pain relief at a 50% reduction in dose which further widens its margin of safety. Supported by AG 11355, DA 05072 and RSA MH38894 (JWO).

208.8

ROLE OF NITRIC OXIDE (NO) IN CUTANEOUS VASODILATION INDUCED BY SPINAL CORD STIMULATION IN THE RAT. K.W. Barron*, J.E. Croom, M.J. Chandler, M.C. Koss, and R.D. Foreman. Depts. of Physiology and Pharmacology, Univ of Oklahoma Health Sciences Center, OKC, OK 73190

Spinal cord stimulation (SCS) increases blood flow to the extremities and may have a limb saving effect in addition to treatment of refractory chronic pain in patients with peripheral vascular disease. The purpose of this study was to examine the importance of NO in cutaneous vasodilation due to SCS. Male rats (300-500 gm) were anesthetized with pentobarbital (60 mg/kg i.p.). A unipolar ball electrode was placed on the left side of the exposed spinal cord at approximately L1-L2. Blood flow responses were concurrently recorded from both hindpaw footpads with laser doppler flowmeters (Vasomedic, 403A). Blood flow responses were assessed during 1 min of SCS (0.6 mA at 50 Hz, 0.2 msec pulse duration). NO synthase was inhibited with i.v. N^G-Nitro L-arginine methyl ester (L-NAME). L-NAME markedly attenuated the cutaneous vasodilation due to SCS in two separate groups of rats receiving either a 2 mg/kg (n=6) or 10 mg/kg (n=7) dose. The NO synthase inhibitor, N^G-mono-methyl-L-arginine (L-NMMA, 10 mg/kg, i.v., n=6), also attenuated the SCS response. The ganglionic receptor antagonist, hexamethonium (10 mg/kg, i.v., n=7), did not affect cutaneous vasodilation due to SCS. After hexamethonium, L-NAME administration (10 mg/kg) no longer attenuated the SCS response. Our results demonstrate that NO but not autonomic ganglionic function plays a significant role in the SCS-induced increase in cutaneous hindpaw blood flow in the rat. (Support: NIH Grant HL22732 and the Presbyterian Health Foundation)

208.9

CUTANEOUS VASODILATION DUE TO SPINAL CORD STIMULATION (SCS) IS DORSAL ROOT DEPENDENT AND MEDIATED BY CALCITONIN GENE-RELATED PEPTIDE (CGRP). J.E. Croom*, R.D. Foreman, M.J. Chandler and K.W. Barron. Dept. of Physiology, Univ. of Oklahoma Health Sciences Center, Oklahoma City, OK 73190

SCS is used clinically to provide pain relief from peripheral vascular disease and has the benefit of increasing cutaneous blood flow to the affected extremities. The purpose of this study was to examine the role of dorsal roots, CGRP, and substance P (SP) in the cutaneous vasodilation induced by SCS. Male rats (280-400 gm) were anesthetized with pentobarbital (60 mg/kg i.p.). A unipolar ball electrode was placed unilaterally on the spinal cord at the L1-L2 spinal segment. Blood flow was recorded in each hindpaw foot pad with laser Doppler flowmeters. Blood flow responses were assessed during 1 min of SCS (either 0.2 mA (subdural) or 0.6 mA (epidural) at 50 Hz, 0.2 msec pulse duration). Dorsal rhizotomy of L3-L5 (n=5) abolished the cutaneous vasodilation to subdural SCS whereas removal of T10-T12 (n=5) and T13-L2 dorsal roots (n=5) did not attenuate the SCS-induced vasodilation. The CGRP antagonist, CGRP(8-37) (2.6 mg/kg i.v., n=7), abolished the epidural SCS-induced vasodilation whereas the substance P receptor antagonist, CP-96,345 (1 mg/kg i.v., n=6), had no effect. In summary, L3-L5 dorsal roots and CGRP are essential for the SCS-induced vasodilation. We propose that SCS antidromically activates afferent fibers in the dorsal roots that causes peripheral release of CGRP producing cutaneous vasodilation. (Supported by NIH Grant HL22732, AHA-Oklahoma Affiliate, and the Presbyterian Health Foundation)

208.10

SPINAL NEURONAL RESPONSES TO INNOCUOUS STIMULATION "WIND UP" IN THE PRESENCE OF I.T. STRYCHNINE. George L. Wilcox*[1], Kelley F. Kitto[1] and Christopher Loomis[2]. [1]Dept. of Pharmacology and Graduate Program in Neuroscience, University of Minnesota, Minneapolis MN 55455 and [2]School of Pharmacy, Memorial University, St. Johns, Newfoundland, Canada A1B 3V6.

Normally, A-fiber input neither signals nociception nor elicits wind-up (the temporal summation of slow synaptic potentials evoked by repetitive noxious stimulation). However, inappropriate nociception, termed allodynia, accompanies 1) neuropathic states and 2) the acute blockade of spinal glycine receptors with intrathecal (i.t.) strychnine. Thus, light tactile stimulation provokes nocifensive behaviours in conscious rats, and cardiovascular/withdrawal responses in anesthetized rats, comparable to those evoked by noxious thermal, mechanical or chemical stimulation without strychnine. To test the hypothesis that wind-up accompanies these abnormal behavioral and autonomic responses, we characterized the responses of spinal cord neurons to repeated brushing in the peripheral receptive field, before and after the iontophoretic application of strychnine. Extracellular single neuron recordings were made in urethane-anaesthetized (1.2 g/kg initial dose, i.p.) male Sprague-Dawley rats (400-500 g). Seven-barreled iontophoretic glass micropipettes were used to record single-unit action potentials through the low impedance (1-3 MW) carbon fiber center barrel and drugs delivered from the surrounding outer barrels: strychnine HCl 10 mM in 190 mM NaCl (pH 4.0), 100 mM N-methyl-D-aspartate Na (NMDA) in 100 mM NaCl (pH 8.0), 10 mM (R,S)-a-amino-3-hydroxy-5-methylisoxazole-4-propionic acid HBr (AMPA) in 190 mM NaCl (pH 8.0). Recordings were made from dorsal horn neurons (L3-L5) responding to noxious and/or innocuous (brush) and noxious (pinch, squeeze) stimuli applied to the ipsilateral hind paw receptive fields. Most low threshold neurons displayed wind-up to repeated A-fiber activation by natural innocuous stimulation. Fewer than half of the wide dynamic range (multireceptive) neurons showed increasing responses to repeated activation. We interpret these results to indicate that the prohibition of wind-up in response to non-nocious stimulation is dependent on glycine. When glycine transmission is blocked, these compensatory changes fail to occur, producing wind-up in the absence of nociceptive primary afferent fiber recruitment. (Supported by NIDA grant R01-DA-04274 to GLW and MRC of Canada to CL.)

208.11

ELECTROPHYSIOLOGICAL EVIDENCE FOR CANNABINOID- MODULATION OF SPINAL NOCICEPTIVE PROCESSING. A.G. Hohmann*, K. Tsou and J.M. Walker. Schrier Research Laboratory, Department of Psychology, Brown University, Providence, RI 02912.

Electrophysiological methods were used to study the role of cannabinoid receptors in spinal nociceptive processing and to determine whether these effects are mediated by direct or indirect actions in the spinal dorsal horn. Extracellular recordings were obtained from the lumbar dorsal horn of urethane anesthetised rats. Noxious thermal stimulation was applied with a Peltier device to regions of the ipsilateral hindpaw corresponding to the receptive field of isolated WDR neurons. Both the classical cannabinoid CP55,940 (125 μg/kg, i.v.) and the non–classical cannabinoid WIN 55,212–2 (125 μg/kg, i.v.) suppressed noxious heat–evoked activity. The suppression of noxious heat–evoked activity was blocked by pretreatment with SR141716A (1 mg/kg, i.v.), a competitive antagonist for the central cannabinoid receptor. Intravenous administration of either vehicle or the receptor–inactive enantiomer WIN55,212–3 (125 μg/kg) failed to alter noxious heat–evoked activity. A subsequent experiment was conducted to determine the site of action for these effects. Intraventricular administration of WIN55,212–2 suppressed noxious heat–evoked activity in lumbar dorsal horn neurons of intact animals. By contrast, intraventricular administration of the enantiomer of the active compound did not differ from treatment with vehicle. These results are consist with previous work suggesting that cannabinoid–induced suppression of noxious heat–evoked activity is attenuated following spinal transection. The results of these experiments, taken together, suggest that cannabinoids selectively modulate the activity of nociceptive neurons via actions at central cannabinoid receptors. These data also provide converging lines of evidence for a role of descending antinociceptive mechanisms in cannabinoid–modulation of spinal nociceptive processing.

Supported by a grant from NINDS to JMW and KT (R01–NS33247) and a predoctoral fellowship from NIDA to AGH (DA05725).

208.12

CP 55,940 PRODUCES ANTINOCICEPTION THROUGH A CANNABINOID RECEPTOR MECHANISM IN THE BRAIN. A.H. Lichtman*, K.R. Dimen, L.A. Showalter, and B.R. Martin. P.O. Box 980613, Dept. Pharmacol. & Toxicol., Medical College of Virginia-Virginia Commonwealth University, Richmond, VA 23298.

The observation that the potent cannabinoid agonist CP 55,940 injected into the periaqueductal gray (PAG) of the rat elicits an antinociceptive response that is pertussis toxin sensitive and exhibits enantioselectivity suggests a cannabinoid receptor mechanism of action. In the present study, the competitive cannabinoid antagonist SR 141716A was employed to test whether cannabinoid-induced antinociception is mediated through a specific receptor mechanism. Male Sprague Dawley rats, implanted with cannulae aimed at either the lateral ventricle or the ventrolateral PAG, were given microinjections of SR 141716A or DMSO vehicle 10 min before CP 55,940 or DMSO and then assessed in the tail-flick test to radiant heat. As previously reported, CP-55,940 produced antinociception when administered into both the ventricle (ED_{100}=50 μg) and the PAG (ED_{100}=20 μg). Intracerebroventricular administration of SR 141716A at a dose of 100 μg shifted the ED_{50} value of CP 55,940 from 22 to 46 μg and 300 μg of the antagonist completely blocked the antinociceptive effects of CP 55,940. In contrast, SR 141716A failed to attenuate the antinociceptive effects of morphine (10 or 20 μg). In the PAG, SR 141716A (30 μg) antagonized the antinociceptive effects of CP 55,940. These results provide strong evidence that cannabinoids produce antinociception through a receptor mechanism of action. Supported by NIDA grants DA-08387 and DA-03672.

NEUROTROPHIC FACTORS: BIOLOGIC EFFECTS

209.1

GDNF KNOCKOUT MICE DIED WITHIN 24 HOURS AFTER BITH, DISPLAY RENAL AGENESIS, ABSENCE OF ENTERIC NEURONS, REDUCTION IN MOTORNEURON NUMBERS AND NORMAL COMPLEMENT OF MIDBRAIN DOPAMINERGIC NEURONS. I. Silos-Santiago[1]*, M. Sánchez[2], J. Frisén[1], B. He[1], M. Barbacid[1]. [1]Dept. of Molecular Biology, Bristol-Myers Squibb Pharmaceutical Research Institute, Princeton, NJ 08543; [2]Centro de Biología Molecular, C.S.I.C., Madrid, Spain.

The glial cell line-derived neurotrophic factor (GDNF), promotes the survival of midbrain dopaminergic neurons in vivo and in vitro. Furthermore, protects dopaminergic, noradrenergic and motor neurons from degeneration paradigmes. And similarly to other growth factors of the neurotrophin family GDNF is also a potent survival factor for embryonic motorneurons, and for sensory and autonomic neurons. These results have suggested that GDNF may be a candidate for the treatment of degenerative diseases. To analyze the role of GDNF during the development and maintenance of the nervous system, we have generated a GDNF null mice. These knockout mice, die within the first 24 hours of life due to renal agenesis. Within the nervous system, there is absence of enteric neurons and a reduction in the number of trigeminal and spinal motorneurons. However, we did not find changes in the number of TH positive neurons in the midbrain. These data indicate that GDNF is a survival factor for enteric neurons and for certain subpopulation of motorneurons, and also suggest that GDNF is not a survival factor for dopaminergic neurons, at least during embryonic development.

209.2

PHARMACOLOGICAL STUDIES OF GLIAL CELL LINE-DERIVED NEUROTROPHIC FACTOR (GDNF) ON ADULT MOTONEURONS IN VIVO. *Q. Yan*, C.R. Matheson, O.T. Lopez, J.L. Urich, A.A. Welcher, and F. Collins.* Department of Neuroscience, Amgen Inc., Thousand Oaks, CA 91320.

Motoneurons require neurotrophic factors for their survival and maintenance of function during development and in adulthood. Recent evidence indicates that GDNF is a potent trophic factor for neonatal as well as lesioned adult motoneurons. In this study, we have examined if adult motoneurons can retrogradely transport exogenous GDNF and whether normal un-lesioned adult motoneurons are responsive to GDNF. We found that adult spinal motoneurons readily retrogradely transported ^{125}I-GDNF in a receptor-mediated fashion after injection into the sciatic nerve. Systemic administration of GDNF resulted in a dose-dependent up-regulation of CGRP mRNA level in both normal and axotomized facial motoneurons. GDNF treatment did not affect the choline acetyltransferase immunoreactivity (ChAT IR) in the normal motoneurons but did attenuate the axotomy-induced decrease of ChAT IR. Using CGRP and ChAT expression levels as endpoints, we compared the effect of varying the frequency or routes of administration of GDNF treatment. We found that a subcutaneous (sc) daily bolus was better than higher but less frequent dose, and sc infusion was better than sc bolus or iv bolus. We also found that intracerebral ventricle infusion, as an approximation of intrathecal delivery of GDNF, was the most effective way to influence adult facial motoneurons.

This study is supported by Amgen Inc.

209.3

DEMONSTRATION OF NEUROTROPHIN 3 AS A TARGET-DERIVED NEUROTROPHIC FACTOR FOR RAT SYMPATHETIC NEURONS X.-F. Zhou*, Z.-W. Qi and R. A. Rush. Department of Physiology and Centre for Neuroscience, Flinders University of South Australia, Australia.

In a previous study using an antiserum specific for neurotrophin 3 (NT3)*in vivo*, we have demonstrated that endogenous NT3 is required for the survival of most sympathetic neurons in postnatal rats (Zhou and Rush, J. Neurosci. 15: 6521-30, 1995). The mechanisms underlying this NT3 action on sympathetic neurons, however, is not known. Whether NT3 is a target-derived factor like NGF or acts in an autocrine fashion is not determined. In the present study in rats, using immunohistochemical and reverse transcription-polymerase chain reaction techniques, we have demonstrated that NT3-mRNA is abundantly expressed in many sympathetic effector tissues including mesenteric arteries, heart, salivary gland and kidney, but it is hardly detectable in adult superior cervical ganglia (SCG). The expression of NT3-mRNA in these tissues is developmentally regulated and increases with age in atrium, salivary gland and mesenteric artery. The levels of NT3-mRNA in effector tissues are highest in densely innervated tissues such as the mesenteric arteries and lowest within the SCG. Almost all sympathetic neurons are immunoreactive for both the NT3 high affinity receptor TrkC and the NGF receptor TrkA. Sympathetic neurons accummulate NT3-immunoreactivity (ir) which is abolished by administration of NT3 antibodies *in vivo* or axotomy of postganglionic neurons. Furthermore, NT3-ir accumulates on the distal but not the proximal side of a lesion on the internal carotid nerve as early as 3 hours post-constriction, indicating endogenous NT3 is retrogradely transported by sympathetic neurons. These studies provide evidence indicating that NT3 acts in parallel with NGF as a target-derived neurotrophic factor for sympathetic neurons both during development and in adult animals.

209.4

OVEREXPRESSION OF NT-3 IN MUSCLE STIMULATES THE FORMATION OF SUPERNUMERARY MUSCLE SPINDLES D.E. Wright*, L. Zhou, J. Harding, and W.D. Snider. CSNSI, Washington Univ., St. Louis, MO, 63110.

Neurotrophin-3 (NT-3) is required for the survival of primary sensory neurons mediating the sense of proprioception. Remarkably, muscle spindles never develop in NT-3 deficient animals, demonstrating that these sensory end-organs are also directly or indirectly regulated by NT-3. To further examine this issue, we examined neurotrophin receptor expression in developing myotubes and generated transgenic mice that overexpress NT-3 in muscle. We show that trkC, the signaling receptor for NT-3 is not expressed by myotubes or developing spindles, suggesting no direct effect of NT-3 on spindle formation. To overexpress NT-3 in muscle, fertilized mouse embryos were microinjected with a construct containing an NT-3 cDNA under the control of the muscle specific promoter myogenin (Myo/NT-3 mice). In Myo/NT-3 mice, increased levels of NT-3 mRNA expression are apparent in developing muscle by embryonic day (E) 12. The elevated NT-3 expression persists for at least two weeks postnatally and is detectable in both extrafusal and intrafusal muscle fibers. To assess the consequence of increased levels of NT-3 on spindle formation, the number of spindles were counted in two hindlimb muscles. Myo/NT-3 mice have three times the normal number of spindles in hindlimb muscles. The supernumerary spindles have the standard complement of intrafusal fibers and are positioned adjacent to nerves in the hilum region of the muscle. Our results that spindle numbers are altered in response to altered NT-3 levels is consistent with findings in NT-3 heterozygote null mutant mice (+/-) which have 50% of spindles in the soleus muscle (Ernfors et al., 1994). To determine whether the effects of NT-3 on spindle number result from proprioceptive neuron survival and/or axonal branching, the number of proprioceptive neurons were examined. Myo/NT-3 mice have a 65% increase in the number of DRG neurons that express parvalbumin mRNA, a marker for proprioceptive neurons. Conversely, NT-3 (+/-) mice have a 50% loss of parvalbumin (+) DRG neurons. Together, these results reveal that levels of NT-3 in muscle regulate the development and number of muscle spindles. We provide evidence that this regulation is indirect and due in part to effects on proprioceptive neuron survival. Supported by NIH.

209.5

DELETION OF THE p75^{NTFR} GENE ATTENUATES SEPTAL CHOLINERGIC CELL LOSS IN MICE HETEROZYGOUS FOR A DELETION OF THE NGF GENE. H. Sauer, M.C. Nishimura, H.S. Phillips* Dept. of Neuroscience, Genentech, Inc., 390 Point San Bruno Blvd., South San Francisco CA 94080.

Mice heterozygous for a targeted disruption of the *NGF* gene display atrophy and a partial loss of medial septal cholinergic neurons, and a functional impairment in spatial learning tasks. This is consistent with reduced brain tissue levels of NGF and NGF mRNA (Chen *et al.*, Soc. Neurosci. Abstr. 19, 1683). The present study was undertaken in order to investigate the role of the p75 low affinity neurotrophin receptor (p75) in mediating septal cholinergic cell loss in mice displaying a partial NGF depletion.

By cross-breeding mice heterozygous for a deletion of the *p75*-gene with mice heterozygous for the disruption of the *NGF* gene, the following genotypes were obtained: (*p75+/+; NGF+/+*), (*p75+/+; NGF+/-*), (*p75-/-; NGF+/+*), (*p75-/-; NGF+/-*). Mice were perfused at 3 months of age and serial sections through the septum were processed for choline acetyl transferase (ChAT) immunocytochemistry.

In *p75* wild-type mice we confirmed that the *NGF+/-* genotype results in a partial decrease in the total number of ChAT-positive septal neurons (866±36; n=14) when compared to *NGF+/+* mice (1058±112; n=7; $p<0.05$). Disruption of the *p75* gene led to a total attenuation of septal ChAT-positive cell loss in *NGF+/-* mice (1193±38; n=13; $p<0.01$ for *p75-/-NGF+/-* vs. *p75+/+NGF+/-*). In addition, these mice displayed no significant cell loss compared to their *NGF+/+* littermates (1240±73; n=7). These findings suggest that the p75 receptor plays an important role in mediating septal cholinergic cell loss after partial NGF depletion.

209.6

NEURON-SPECIFIC REGULATION OF EXPRESSION OF THE NGF GENE IN THE BRAIN. M. Cartwright[1,2], P. Isackson[3], and G. Heinrich*[2]. [1] Dept. of Pharmacology and [2] Evans Dept. of Clinical and Research Medicine, Boston Univ. Med. Center Hospital, Boston MA., [3] Mayo Clinic, Jacksonville, FL.

The gene encoding nerve growth factor (NGF) is expressed by neurons in the cortex, thalamic nuclei and the hippocampus. In many neurons, NGF mRNA levels are increased by seizure-induced stimulation of neuronal activity. The increase occurs in two distinct patterns. In cortical neurons the induction is slow, with a peak at 12 h. In contrast, in the dentate granule cells the induction is rapid, peaks at 6 h, returns to base line by 12 h, and peaks again at 24 h (Lauterborn et al., Exp. Neuro. 125, p22, 1994).

To identify the regions of the NGF gene that mediate the transcriptional contribution to this induction, we expressed two previously identified promoters. The NGF sequences extended from -5 kb to +8 in exon 1B and were linked to a human growth hormone gene reporter. Northern blot hybridization analyses demonstrated that the transgene was expressed in the brain. In situ hybridization studies revealed that transgene mRNA was present in neurons in the cortex, thalamus and the pyramidal cells of Ammon's horn. Interestingly, the transgene mRNA could not be detected in the granule cells of the dentate gyrus even following kainate induced seizure activity. One explanation for the failure to express in these cells could be that the transgene is lacking critical cis regulatory elements. Alternatively, expression in the dentate granule cells may be mediated by another promoter. The unique rapid and biphasic induction of NGF mRNA in the dentate granule cells is also consistent with transcription initiation from a novel promoter. We have identified NGF transcripts whose 5' ends are consistent with this possibility. Related transcripts were previously considered splicing intermediates of the NGF gene primary transcript (Selby et al., Mol. Cell. Biol. 7:9, p3057, 1987). We detected the presence of these transcripts in mouse hippocampus, cortex, and SMG. This suggests that the regulation of NGF gene expression involves multiple promoters. Supported by NIH RO1 AG10565 (GH).

209.7

AMYLOID PRECURSOR PROTEIN POTENTIATES THE NEUROTROPHIC ACTIVITY OF NGF THROUGH THE INSULIN SIGNALING PATHWAY, WC Wallace*, CA Akar, HK Kole, JM Egan, and B. Wolozin. GRC/NIA, Baltimore, MD and NIMH, Bethesda, MD.

Cortical amyloid precursor protein (APP) is induced and secreted in response to subcortical lesions of cholinergic innervation. To understand the physiological role of induced APP, we have characterized its neurotrophic activity on PC12 cells. APP (50-1000 pM) induced outgrowth of neurites. At lower concentrations, (10-50 pM), APP also synergistically potentiated the neurotrophic effects of NGF either by simultaneous addition of the two factors or by a priming pretreatment with APP. This neurotrophic activity was reduced by tyrosine kinase inhibitors. Insulin receptor substrate-1 (IRS-1) and mitogen activated kinases (ERKs 1 and 2) were identified among the APP-stimulated tyrosine phosphorylated proteins. In contrast, neither phospholipase-γ1 nor *trk*A exhibited such phosphorylations. In addition, a role for phosphotidylinositol 3-kinase was implicated by wortmannin inhibition. The potentiation of NGF activity was reflected in a corresponding synergistic elevation in the tyrosine phosphorylation of IRS-1. The pattern of tyrosine phosphorylations indicates a signal transduction pathway that is distinct from the NGF/*trk*A and suggests that APP potentiates the responsiveness of neurons to neurotrophins via the insulin signaling pathway. This potentiation may facilitate neuronal recovery following injury. (Supported by IRP, NIA.)

209.8

BRAIN DERIVED NEUROTROPHIC FACTOR (BDNF) REGULATES STRIATAL DARPP-32 AND ARPP-21 *IN VIVO* AND *IN VITRO*. S.Ivkovic, O.Polonskaia, and M.E.Ehrlich*. Psychiatry and Cell Biology, NYU Med.Ctr., NY,10016.

DARPP-32 and ARPP-21 are dopamine and cyclic AMP-regulated phosphoproteins and are coordinately expressed in most of the striatal dopaminoceptive medium size spiny neurons (MSNs). Dopamine regulates the phosphorylation state of DARPP-32 and ARPP-21, but does not regulate their transcription. DARPP-32/ARPP-21 are first detectable in post-migrational neurons, and are phenotypic markers of MSN maturation. BDNF has trophic effects on striatal neurons *in vitro* (Mizuno et al., 1994; Ventimiglia et al., 1995), and a null mutation in the BDNF gene produces a decrease in MSN calbindin expression (Jones et al., 1994). We demonstrate that immunocytochemical levels of DARPP-32 and ARPP-21 are markedly decreased in postnatal day 0 (PO) and P10 BDNF-/- animals (provided by Drs. L. Reichardt and I. Farinas). In dissociated, densely plated (1 X 10^6 cells/ml) cultures derived from embryonic day 13 (E13) and E15 mice, addition of BDNF (50-100 ng/ml) to serum-free media increases the number of DARPP-32-positive cells 5 and 10-fold respectively after one day *in vitro* (1DIV). This effect is apparent at 12 hours, and is abolished by actinomycin D (2 *ug*/ml). ARPP-21 is not detectable until 5-7 DIV, at which time the number of immunopositive cells is increased greater than 10-fold as a consequence of the addition of BDNF. These data suggest that BDNF regulates DARPP-32 and ARPP-21 transcription. We are also analyzing proliferative and survival effects of BDNF on DARPP-32/ARPP-21-positive MSNs. (NIMH R29-47028 and K02-00945)

209.9

TRKB FUNCTION IN THE DEVELOPING XENOPUS RETINA, Z.Z. Liu and F.F. Eide*. Department of Neurology, University of Chicago, Chicago, IL 60637.

Over-expression of a trkB ATP binding mutant receptor in early blastomere-injected *Xenopus* revealed a novel role for this receptor in early retinal development. Severely-affected mutants showed near-complete loss of the laminar organization of the retina, with arrested development of neural and non-neural components of retina. Co-injection of wildtype trkB receptors rescued eye abnormalities. *In situ* hybridization demonstrated the precise regulation of trkB mRNA in germinal retinal neuroepithelium, differentiating pigment epithelium, and developing photoreceptors. BDNF stimulation of dissociated optic vesicles confirmed roles in early retinal differentiation.

F.F.E is supported by grants from the National Institute on Aging (K11AG00568) and Chicago Brain Research Foundation.

209.10

NEUROTROPHINS PRODUCED IN THE THALAMUS MAY BE NECESSARY FOR THALAMIC GROWTH AND SURVIVAL BEFORE THALAMOCORTICAL AXONS ARE ESTABLISHED. R.B.Lotto* and D.J.Price, Dept. of Physiol., Univ. Med. Sch., Teviot Place, Edinburgh EH8 9AG, U.K.

The onset of naturally-occurring cell death in the thalamus coincides with the arrival of thalamocortical axons to the developing cortex, which is around embryonic day 16 (E16) in mice. We showed that, before E16, dissociated thalamic neurons survive and grow in high density, serum free, primary cultures. Immunocytochemistry revealed that they express all the neurotrophins (NT) and their high affinity tyrosine kinase receptors. We added K252a, a selective inhibitor of NT receptors (in the nM range), to these high density cultures. This reduced thalamic growth and survival. In low density cultures, most E16 thalamic neurons die, but their growth and survival increase with the addition of either thalamic conditioned medium or any of the NTs. These data provide the first evidence that a battery of NTs have direct trophic effects on thalamic neurons, suggest that they act in an autocrine fashion early in development, and support the notion of trophic redundancy in the CNS.

Supported by the MRC and The Wellcome Trust

209.11

DEPOLARIZATION PROMOTES SPIRAL GANGLION NEURON SURVIVAL VIA MULTIPLE MECHANISMS INCLUDING AN AUTOCRINE NEUROTROPHIC RESPONSE. M.R. Hansen, and S.H. Green*. Depts. of Biological Sciences & Otolaryngology, University of Iowa, Iowa City, IA 52242.

Previous studies have shown that BDNF, NT-3, and permeant cAMP analogs promote spiral ganglion neuron (SGN) survival *in vitro* in an additive manner; depolarization promotes survival with even greater efficacy. *In situ* hybridization and immunohistochemistry show up-regulation of BDNF and NT-3 synthesis within depolarized neurons *in vitro*. Addition of TrkB-IgG fusion protein abolishes the trophic effect of BDNF and partially inhibits the trophic effect of NT-3; TrkC-IgG has no effect with BDNF but abolishes the trophic effect of NT-3. The Trk-IgG fusion proteins, individually or in combination, inhibit the trophic effect of depolarization by only ≈30%. The MEK inhibitor PD098059 abolishes trophic support by neurotrophins but inhibits the trophic effect of depolarization by only ≈30%. A combination of PD098059 and Trk-IgG proteins inhibits survival only slightly more than do Trk-IgG proteins or PD098059 alone. The trophic effect of permeant cAMP analogs is unaffected by the MEK inhibitor and by Trk-IgG proteins. These data indicate that neurotrophins promote survival via the Ras-ERK pathway and that depolarization promotes survival, in part, by inducing an *autocrine* neurotophic pathway and, in part, by a distinct pathway not involving ERK activation. Trophic signaling by cAMP defines another pathway. This latter pathway also appears to be recruited by depolarization. Rp-cAMPS, a specific PKA inhibitor, abolishes cAMP-dependent survival but has no effect with neurotrophins. Rp-cAMPS inhibits depolarization-dependent survival by ≈30%; this inhibition is additive with that of Trk-IgG proteins. Supported by AOS and NIH training grant DC00040.

209.12

PARACRINE INTERACTIONS BETWEEN NEURONS AND GLIAL CELLS MEDIATED BY BDNF. C. Kalcheim*, D. Shelton# and M. Pruginin. Dept. of Anat. and Cell Biol.-Hebrew Univ. of Jerusalem-Hadassah Med. School-Jerusalem 91120-P.O.B. 12272. Israel and #Dept. of Neurosciences, Genentech, Inc. 460 Point San Bruno Boulevard,South San Francisco, CA 94080. USA

Avian sensory neurons synthesize primarily BDNF, to a lesser extent NT-3, but no detectable NGF, raising the possibility that specific neurotrophins mediate local activities of paracrine or autocrine type in adition to well known target-derived effects. We have tested the hypothesis that neuron-derived BDNF mediates a neuronal effect on glial differentiation.

Coculturing neurons with developing glial cells of quail DRG stimulates within a day a 2.5-fold increase in the proportion of nonneuronal cells that express the constitutive glial marker SMP when compared to glial cultures devoid of neurons. This effect was found to be mediated by BDNF because incubation of mixed neuron-glia cultures with a specific trkB immunoadhesin that sequesters BDNF, but not with trkA or trkC immunoadhesins, abolishes this stimulation. Furthermore, the effect of the neurons can be mimiqued by treatment of glial cultures with soluble BDNF that stimulates the conversion of SMP-negative glial cells into cells that expresses the SMP phenotype with no effect on their survival or proliferation. That BDNF is the endogenous neuron-derived factor affecting glial development in culture was confirmed by the observation that BDNF immunoreactive protein is expressed by cultured sensory neurons, but not by the ganglionic satellite cells. Preliminary evidence suggests that the p75 receptor is likely to mediate the BDNF-dependent upregulation of SMP, because specific neutralization of p75 abolishes neuronal stimulation of SMP expression. These results show for the first time a local, paracrine role for BDNF in mediating the action of ganglionic neurons on differentiation of adjacent satellite cells.

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LONG-TERM POTENTIATION: PHYSIOLOGY I

210.1

HIGH LEVELS OF CALCINEURIN INHIBITED ADENYLYL CYCLASE TYPE 9 (AC9) IN HIPPOCAMPAL PROJECTION NEURONS. F. A. Antoni*, M. Palkovits, R. Rosie, S. M. Smith, J. Simpson, G. Fink, J. M. Paterson. MRC Brain Metabolism Unit, Edinburgh, U.K., Lab Cell Biology, NIMH, NIH, Bethesda, MD.

Long-term potentiation in the hippocampus involves cAMP signaling with Ca^{2+}/calmodulin activated adenylyl cyclases (types 1 and 8) playing a key role. Adenylyl cyclases inhibited by Ca^{2+} (AC5 and AC6) have not been demonstrated in hippocampal neurons, although AC5 is prominent in rat striatum. Hippocampal long-term depression involves calcineurin, a Ca^{2+}/calmodulin activated protein phosphatase, that also controls the activity of AC9. In the present study riboprobes and specific antisera raised against the C- and N-terminal portions of AC9 were used to examine the distribution of this cyclase in rodent brain. Northern analysis indicated the presence of a single species of 9kb RNA in mouse striatum and whole brain extracts. Western blots reveal the presence of a single 160K immunoreactive band in hippocampus and hypothalamus, radioimmunoassay suggests that the concentration of adenylyl cyclase in membranes from whole hippocampus is c.190 ng/mg protein. *In situ* hybridization showed a very intensive signal in all hippocampal structures including the anterior hippocampus, induseum griseum, pyramidal layers of CA1, CA2, CA3, the dentate gyrus as well as the subiculum. No clear indication of RNA signal in hippocampal interneurons was found. Hippocampal pyramidal neurons contain high levels of adrenal corticosteroid receptors and AC9 is important for corticosteroid feedback regulation of adenohypophysial corticotrope cells. In this respect it is of note that in addition to hippocampus, areas thought to be important for corticosteroid feedback action in the brain such as the hypothalamic paraventricular nucleus, the prefrontal cortex, the cingulate cortex, the amygdala and the anterodorsal thalamic nucleus all contained relatively high levels of AC9 mRNA, although none of these regions matched the hippocampus in intensity. It is proposed that AC9 in hippocampal pyramidal neurons may contribute to long-term changes in neurotransmission and is in all probability co-localized in the same pyramidal neurons that produce AC1 and AC9. Taken together with the functional analysis of pituitary corticotropes, the overall distribution of AC9 in the rodent brain supports the hypothesis that it has an important role in corticosteroid modulation of neurotransmission.

210.2

POSTSYNAPTIC Ca^{2+}-DEPENDENT PROTEIN KINASE AND PHOSPHATASE ACTIVITIES DYNAMICALLY CONTROLLING SYNAPTIC STRENGTH THROUGH INTRACELLULAR Ca^{2+} SIGNALING. JIN-HUI WANG* & PAUL T. KELLY, *Department of Neurobiology and Anatomy, University of Texas Medical School at Houston, Texas 77030.*

The role of protein phosphorylation and dephosphorylation in regulating synaptic transmission and synaptic plasticity was examined by postsynaptic injections of modulators of CaM-KII and PKC or calcineurin (CaN) in area CA1 of adult rat hippocampal slices. Postsynaptic injections of pseudosubstrate inhibitors of PKC plus CaMKII [PKC(19-31)/CaMKII(281-302)] did not affect basal synaptic transmission in naive pathways, but significantly attenuated the maintenance of LTP in pre-tetanized pathways (254±37% vs. 133±22%). Postsynaptic injections of a specific CaN inhibitor (FK-506) induced significant synaptic potentiation in pre-tetanized (239±20% vs. 174±17%) and naive pathways (196±15% vs. baseline values 100%). These results indicate that postsynaptic PKC and CaM-KII activities are essential for LTP maintenance, but CaN activity limits synaptic strength at a stable level during basal and potentiated synaptic transmission (i.e., the dynamic balance among their activities that sets synaptic strength is dominated by CaN). To elucidate the postsynaptic mechanisms responsible for synaptic potentiation induced by CaN inhibitors, a variety of agents including BAPTA, a calmodulin-binding peptide, PKC(19-31)/CaMKII(281-302), or heparin/dantrolene (inhibitors of Ca^{2+} release from intracellular stores) were co-injected with FK-506. Each agent significantly attenuated FK-506-induced synaptic potentiation. Our results indicate that the down-regulation of synaptic strength by postsynaptic CaN activity is mediated by decreasing intracellular Ca^{2+} signaling and the activity of downstream components which contribute to the balance among protein kinases and phosphatases.

210.3

THE ROLE OF CALCIUM IN HOMOSYNAPTIC LTD AND LTP IN HIPPOCAMPAL CA1 PYRAMIDAL NEURONS.
B. R. Christie*, and D. Johnston Division of Neuroscience, Baylor College of Medicine, Houston, TX 77030

Hippocampal neurons exhibit various forms of synaptic plasticity, including long-term potentiation (LTP) and long-term depression (LTD). The induction of LTD depends, among other things, upon a rise in postsynaptic $[Ca^{2+}]_i$. To investigate the changes in $[Ca^{2+}]_i$ during LTD, we have combined fura-2 imaging with whole-cell recordings of visually identified CA1 neurons.

Hippocampal slices (400 μm) were obtained from 2–3 week old rats using standard procedures and maintained at 32°C in a submerged chamber. To measure fluorescence changes in intracellular Ca^{2+}, fura-2 (160 μM) was included in the pipette solution, and relative changes in $\Delta F/F$ were measured using a cooled CCD camera in sequential frame transfer mode (25 ms frame interval). Whole-cell recordings were made from CA1 neurons located within 50 μm of the slice surface. Following a 10–20 min baseline period, cells were synaptically activated at 1–50 Hz for a total of 900 pulses. In all cases, EPSPs subthreshold for action potential generation were paired with somatically generated action potentials and changes in fluoresence were monitored. LTD, which lasted at least 30 min, occurred in neurons that received 1–20 Hz stimuli, while LTP was produced in cells administered 30–50 Hz stimuli. The transition from LTD to LTP was accompanied by increased $[Ca^{2+}]_i$ in both the soma and dendritic regions of recorded cells. Both the LTD and the LTP were NMDA-dependent, but LTD induction could also be reduced or prevented with L-, R-, and T-subtype voltage-gated Ca^{2+} channel antagonists. These data suggest that the Ca^{2+} entry caused by back-propagating action potentials is involved in the induction and maintenance of both hippocampal LTD and LTP (MH44754, MH48432, NS11535, NSERC103103).

210.4

THE ROLE OF DENDRITIC ACTION POTENTIALS IN THE MODULATION OF SYNAPTIC EFFICACY
J. Magee* and D. Johnston Division of Neuroscience, Baylor College of Med., Houston, TX 77030

Neuronal dendrites are the primary site for the coordination and storage of synaptic information. Dendritic voltage-gated Na^+ channels allow axonally-generated action potentials to propagate back into the dendrites and activate voltage-gated Ca^{2+} channels. We have used whole-cell patch-clamp techniques in conjunction with high-speed fluorescence imaging to determine the role of back-propagating action potentials in the induction of long-term changes in synaptic efficacy in CA1 pyramidal neurons. A subthreshold, theta-like, synaptic stimulation was delivered to dendrites > 200 μm from soma. When subthreshold trains of EPSPs were delivered, only slight (<3% $\Delta F/F$) increases in dendritic $[Ca^{2+}]$ and no significant changes in synaptic efficacy were observed. When subthreshold EPSP trains were paired with 5–15 action potentials, dendritic $[Ca^{2+}]$ was significantly elevated (>10% $\Delta F/F$) and a long lasting increase in synaptic efficacy observed. The increase in EPSP amplitude (~250%) peaked ~20 mins after theta stimulation and was observed for > 1 hr. Trains of action potentials themselves did not induce any long-term changes in EPSP amplitude. To confirm that *dendritic* action potentials were required for synaptic plasticity, axonal spikes were blocked from propagating into the dendrites by focal application of 10 μM TTX to the proximal dendritic arbor. Large amplitude Ca^{2+} transients and action potentials could only be recorded in segments of the neuron that are proximal to the TTX block. Small spikes that produce no Ca^{2+} transients were recorded from the dendritic regions located distal to the block. Pairing of subthreshold EPSP trains and non-backpropagating spikes did not result in any significant change in EPSP amplitude, whereas, subsequent pairing of EPSPs with back-propagating spikes did result in a long lasting increase. These data provide evidence that spike-dependent elevation in dendritic $[Ca^{2+}]$ provides a feedback signal to the synapses that an output of the neuron has occured.(NS11535,MH48432,MH44754)

210.5

CREB AS A STIMULUS DURATION-DEPENDENT SWITCH FOR SYNAPSE-TO-NUCLEUS SIGNALING.
H. Bito*, K. Deisseroth, and R. W. Tsien. Dept. of Mol. Cell. Physiology, Stanford University, Stanford, CA 94305-5426.

Using cultured hippocampal neurons, we studied the requirement for synaptic activity in triggering CRE-mediated gene expression. We also investigated in parallel the pattern of nuclear CREB Ser-133 phosphorylation and dephosphorylation at the single cell level. A prolonged (180s), but not a short (18s), train of synaptic stimulation at 5Hz was found to efficiently induce a significant increase in the immunoreactivity of c-Fos and SS-14, two genes with prototypical CRE promoters. Both stimuli, short or long, were equally effective in inducing nuclear CREB phosphorylation, but prolonged stimulation favored slower recovery from phosphorylation. Detailed and quantitative analyses revealed that a CaM kinase kinase/CaMKIV cascade and CaN/PP1 regulated the phosphorylation and dephosphorylation of CREB in a stimulus duration-dependent manner. CRE-regulated gene expression was always associated with the persistence of nuclear pCREB, rather than the induction of pCREB itself. Further experiments suggest that CaN plays an essential role in selecting the signals capable of eliciting CRE-mediated gene expression and in shaping the stimulus duration-dependence of this switch. Supported by NIMH Silvio Conte Research Center, Mathers Foundation (RWT), and fellowships from HFSP (HB) and MSTP (KD).

210.6

MECHANISMS UNDERLYING THE UNIQUE ROLE OF THE L-TYPE CA^{2+} CHANNEL IN SYNAPSE-TO-NUCLEUS SIGNALING.
K. Deisseroth*, H. Bito, and R.W. Tsien. Department of Molecular and Cellular Physiology, Stanford University Medical School, Stanford, CA 94305-5426.

While many types of voltage-activated Ca^{2+} channel can be found on the dendrites of mammalian central neurons, we and others have found that L-type Ca^{2+} channels play a prominent role in signaling leading from the surface membrane to the nucleus. Here we investigate the nature and mechanisms of the involvement of this channel in synaptically-evoked nuclear CREB phosphorylation. L-type blockade with 10 μM nimodipine strongly inhibited synaptically-induced CREB phosphorylation while N-type Ca^{2+} channel inhibition with 1 μM ω-conotoxin GVIA had no effect, in the presence or absence of additional NMDA receptor blockade with 25 μM D-AP5. The comparatively slow activation kinetics of the L-type channel appear to allow the CREB pathway to be selectively responsive to synaptic events. While neither action potentials nor brief (1 ms) voltage-clamp depolarizations could trigger CREB phosphorylation, both synaptic events and slightly longer (5-10 ms) depolarizations succeeded. The brief stimuli failed because insufficient Ca^{2+} influx occured through the slowly-activating L-type channels; selectively increasing L-type flux with 5 μM S-(-) Bay K 8644 enabled action potentials to give rise to CREB phosphorylation. On the other hand, the comparatively slow *in*activation kinetics of the L-type channel may be important in allowing continued Ca^{2+} influx and CREB phosphorylation over the long stimulation timescale necessary to give rise to CRE-mediated gene expression. Thus, multiple intrinsic characteristics of the L-type channel appear to make it better-suited than the N-type channel to mediate synapse-to-nucleus signalling through CREB. Supported by NIMH Silvio Conte Research Center (RWT) and fellowships from the MSTP (KD) and HFSP (HB).

210.7

DIFFERENT CA^{2+} SIGNAL AMPLITUDES ARE REQUIRED FOR THE INDUCTION OF LTP AND LTD IN PYRAMIDAL CELLS OF THE RAT VISUAL CORTEX.
C. Hansel*, A. Artola[1] and W. Singer. Max-Planck-Institute for Brain Research, Deutschordenstr. 46, 60496 Frankfurt, FRG. [1]Present address: Dept. of Neurobiology, ETH-Hoenggerberg, CH-8093 Zürich.

Previously, we have shown that in layer II/III pyramidal cells of the rat visual cortex the induction of LTP is associated with higher rises of dendritic $[Ca^{2+}]_i$ than the induction of LTD (Hansel et al., *Soc. Neurosci. Abstr.* 21: 711.3, 1995). This finding was based on Ca^{2+} measurements using the fluorescent Ca^{2+} indicator fura-2. As the Ca^{2+} buffering capacity of the dye might influence the induction of LTP or LTD we performed the adjustment of LTP and LTD inducing tetanization protocols and the measurements of associated Ca^{2+} signals in different groups of cells to avoid false correlations. Using Ca^{2+} chelators we now directly tested the functional significance of the observed difference in the Ca^{2+} signal amplitude and its potency to determine the direction of synaptic gain change. Neocortical slices (200-250 μm) were obtained from rats aged 5-7 weeks. Sharp microelectrodes were used to measure responses to stimulation in either layer IV (conditioned pathway) or lateral layer II (control pathway). As previously reported, LTP was induced in 6 out of 7 cells by pairing of five 50 Hz stimulus trains in layer IV with a postsynaptic 20 mV-depolarization ($[Mg^{2+}]_o$= 1mM). The application of the same protocol induced LTD in 3 out of 5 cells when the cells were filled with fura-2 and induced LTD in 4 out of 5 cells when the cells were filled with the non-fluorescent Ca^{2+} chelator BAPTA (chelator concentration in the electrode tip: 4mM). In none of the cells LTP was induced. In combination with the previously reported observation that the induction of LTP is associated with stronger Ca^{2+} elevations than the induction of LTD these results provide compelling evidence for the hypothesis that the amplitude of the postsynaptic $[Ca^{2+}]_i$ rise is a key factor for the determination of the polarity of synaptic gain changes. *This study was supported by the Max-Planck-Society.*

210.8

KINETICS OF NMDA RECEPTOR ACTIVATION BY CAGED GLUTAMATE IN THE HIPPOCAMPAL SLICE: CONSTRAINING FUNCTIONAL SYNAPTIC GEOMETRY.
Z. F. Mainen* and R. Malinow. Cold Spring Harbor Lab., Cold Spg. Hbr., NY 11724.

The spatiotemporal concentration profile of synaptically released glutamate is central to the physiology of excitatory synapses but is impossible to measure directly. Of particular interest is the possibility that low concentrations of glutamate, sufficient to activate the NMDA receptor but not the lower-affinity AMPA receptor, may "spillover" from one release site to neighboring synapses. To study the non-equilibrium kinetics of glutamate receptor activation at intact synapses, we have used rapid photolysis of caged glutamate during whole-cell recordings from pyramidal neurons in the CA1 region of the hippocampal slice.

Varying the intensity of brief full-field UV light pulses, we find that the decay of the NMDA response (measured at +40 mV in the presence of NBQX and BMI) is sensitive to the amount of glutamate uncaged, with the fastest responses (at the weakest light intensities) being significantly shorter than synaptic responses. Several observations suggest that the decay time reflects primarily a sensitivity of NMDA receptor kinetics to transmitter concentration rather than prolonged activation by slowly-cleared glutamate. The NMDA decay measured at -40 to -60 mV is insensitive to the amount of uncaged glutamate. In addition, the response time course is not affected by the amount of uncaging in the presence of high concentrations of the open channel blocker MK-801, a condition in which the response is expected to approximate the first latency distribution of receptor openings.

These caged glutamate experiments suggest that the decay time of synaptic NMDA responses may be used to quantify the concentration of glutamate by which they were activated. In support, we find that synaptic NMDA responses following a brief tetanus (3–5 pulses, 100 Hz) are slowed compared to single stimuli. However, we find that "silent synapse" responses (responses having NMDA but not AMPA components) have NMDA time courses identical to the NMDA time course for mixed AMPA–NMDA synapses. Furthermore, concentrations of uncaged glutamate large enough to mimic the decay of synaptic NMDA responses are sufficient to activate AMPA receptors. These observations are difficult to reconcile with most scenarios for spillover activation of NMDA receptors.

Supported by NIH grants to Z.F.M. and R.M.

210.9

EFFECT OF LONG-TERM POTENTIATION INDUCING STIMULI ON EXPRESSION OF CALCIUM CALMODULIN DEPENDENT KINASE II ISOFORMS, ALPHA AND BETA, AND PROTEIN PHOSPHATASE INHIBITOR 1.
L. W. Chiang, K. Deisseroth, R. W. Tsien, and H. Schulman*. Stanford University School of Medicine, Stanford, CA 94305.

Long-term potentiation (LTP), a stable increase in synaptic strength that can last for days, is sensitive to inhibitors of protein synthesis and RNA transcription. Activity-dependent transcriptional regulation of protein phosphatase inhibitor 1 (INH-1) and calcium/calmodulin-dependent kinase II (CaMKII), signal transduction molecules that modulate postsynaptic responses to presynaptic stimuli, could contribute to long-lasting changes in synaptic transmission. Previously, single-cell PCR experiments demonstrated marked heterogeneity in the relative expression pattern of INH-1, α- and β-CaMKII isoforms among single CA1 pyramidal neurons in adult rat hippocampal slices. In order to test whether relative transcript levels could have been affected by high frequency stimulation, we measured mRNA levels at various times following LTP-inducing stimuli (3 trains of 18 s at 50 Hz) in cultures of hippocampal neurons. We observed an induction of immediate early genes (*c-fos* and NGFIa) by one hour after stimulation that rapidly decays to baseline, and an increase in α-CaMKII at late times (24 hours). Novel findings were apparent increases in total β-CaMKII transcripts at late times, as well as activity-dependent differential splicing from embryonic (β_e and β'_e) to adult isoforms (β and β'). We speculate that differential expression of β-CaMKII could contribute to maintenance of LTP by increasing the affinity of the CaMKII holoenzyme for activation by calcium. This work was funded by an NIMH Silvio Conte Center for Neuroscience Research Grant.

210.10

CaMKII ACTION ON MICROTUBULES PROMOTES CALCIUM-EVOKED DENDRITIC EXOCYTOSIS.
M. Maletic-Savatic*, T.Koothan and R. Malinow, Cold Spring Harbor Laboratories, Cold Spring Harbor, NY 11724.

We have recently demonstrated that regulated dendritic exocytosis is a Ca^{++}- and CaMKII-dependent process which might be involved in long-term potentiation. Here, we wish to test the hypothesis that CaMKII promotes Ca^{++}-evoked dendritic exocytosis (CEDE) by action on microtubules. Toward this goal, we have used the quantitative time-lapse imaging of the fluorescent membrane probe FM1-43 to monitor the exocytosis of labeled compartments in cultured hippocampal neurons. Overnight exposure of cultured neurons to 1.5μM FM1-43 preferentially labels dendritic compartments. Exocytosis of these compartments, observed as a robust loss of FM1-43 fluorescence, was induced by a Ca^{++} ionophore, A23187. To test the role of microtubules in CEDE, we exposed mature cultured neurons to 2.5 μg/mL nocodazole for 30 min at 37^0C. A23187-induced exocytosis was completely blocked with nocodazole. To test the association of CaMKII and microtubules in CEDE, we introduced a constitutively active CaMKII into mature cultured neurons via a recombinant vaccinia virus (VV). Spontaneous exocytosis was monitored after overnight culture infection with 10 MOIs of CaMKIIVV. Cultured neurons infected with a b-galactosidaseVV and uninfected cultures served as controls. Spontaneous exocytosis in CaMKIIVV-infected neurons was significantly greater than exocytosis observed in control neurons. This exocytosis was blocked by 2.5 μg/mL nocodazole, added to culture medium 30 min before monitoring spontaneous exocytosis. These results indicate that CaMKII activity mediates CEDE via a microtubule-dependent action.

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210.11

PAIRING-INDUCED LTP IN HIPPOCAMPAL SLICE CULTURES IS NOT STRICTLY INPUT-SPECIFIC. F. Engert and T. Bonhoeffer*. Max-Planck-Institut für Psychiatrie, 82152 München-Martinsried, FRG.

Several groups have shown that pairing-induced long-term potentiation (LTP) is not specific on the presynaptic fibers: it spreads to neighboring cells which have not participated in the induction procedure (Bonhoeffer et al., '89; Schuman and Madison, '94).

We have now tried to test whether LTP in the Schaffer collateral/CA1 pathway in rat hippocampus is specific on the postsynaptic side, that is whether only the synapses on the stimulated fibers are enhanced or whether other, nearby synapses between other presynaptic elements and the same postsynaptic dendrite are also affected. So far this has mostly been examined by stimulating two separate pathways onto the same postsynaptic cell. However, the spatial resolution of this approach is limited and does not permit rigorous assessment of the specificity of LTP in the tens of micrometer range.

We attempted to achieve our goal as follows: as a first step we blocked voltage activated calcium channels in the entire culture by replacing the standard extracellular solution with a solution containing cadmium and low calcium (0.8 mM) abolishing all normal synaptic transmission. We then re-established synaptic transmission only in a small spot of ~30 µm diameter by using a local superfusion system which applied normal extracellular solution focally to the dendritic tree of an intracellularly recorded postsynaptic neuron. Using this "superfusion spot" like a narrow light beam to search for single groups of synapses we could individually activate small groups of synapses no further apart than 30-40 µm. We then applied the pairing protocol to a first group of synapses (test) while in the neighboring group (control) synaptic transmission was blocked by the cadmium in the extracellular solution.

Moving the superfusion spot - after the pairing procedure - to the control site we observed, to our surprise, that synaptic enhancement at the test-site had also led to substantial enhancement at the control-site. This spread of enhancement occurred only when test and control site were less than 50 µm apart. No increase was observed when the control spot was located more than 100 µm away from the test spot.

These data indicate that there is no strict input-specificity of LTP, at least in hippocampal slice cultures. Rather LTP "spreads" over a relatively short distance to neighboring synapses on the same dendrite even if these synapses were silent during the induction procedure.

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210.12

ACTIVITY-DEPENDENT ENHANCEMENT AND SUPPRESSION OF LONG-TERM HETEROSYNAPTIC FACILITATION MEDIATES PATHWAY-SPECIFIC PLASTICITY AT CONVERGING INPUTS ON A COMMON TARGET. S. Schacher*, F. Wu and Z.-Y. Sun. Cntr. Neurobiol. & Behav., Columbia Univ. Coll. of P & S, NYSPI, New York, NY 10032.

Activity-dependent modulation of synaptic function with temporal pairing of stimuli is a cellular mechanism for associative conditioning. Although such a mechanism might explain cellular and behavioral changes associated with a single pairing, it is not sufficient to account for pathway-specific plasticity with multiple pairings. To explore the mechanisms associated with cell-specific plasticity at convergent pathways, we examined activity-dependent modulation of 5-HT facilitation of sensorimotor connections of *Aplysia* re-established in cultures that had 2 sensory neurons (SNs) and 1 target L7. Whereas 1X tetanus (20 Hz for 2 sec) or 1X 5-HT evoked only a short-lasting change, a single pairing of the stimuli evoked a significant long-term change only in the SN given the paired stimuli. This change is mediated primarily by a presynaptic mechanism. Repeated (4X) tetanus at 25 min intervals failed to evoke any long-term change, while 4X 5-HT alone evoked a long-term change in both SN connections. By contrast, 4X pairing of tetanus plus 5-HT evoked both a significant increase in the efficacy of the SN connection given the paired stimuli and a significant reduction of the facilitation normally evoked by repeated 5-HT in the other SN connection. These changes involved both an enhancement and a suppression of facilitation; 30 min after the last treatment the efficacy of both unpaired and paired SN connections were enhanced. Moreover, the activity-dependent changes in long-term facilitation appear to be mediated by activity in the motor cell. Both hyperpolarization of L7 and incubation with APV blocked the enhancement and suppression without interfering with long-term facilitation evoked by 5-HT alone. The results suggest that pathway-specific modulation, a feature of long-term synaptic plasticity in many systems, is intrinsic to the neural circuitry that is modified, and requires activity-dependent changes in the postsynaptic target.

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DEVELOPMENTAL GENETICS I

211.1

SHARP-1,-2: NOVEL HELIX-LOOP-HELIX PROTEINS WITH RAPID INDUCTION BY NGF IN PC12 CELLS

M.J. Rossner, J. Doerr, P. Gass, M. Schwab, and K.-A. Nave*
Center for Molecular Biology (ZMBH), University of Heidelberg, D-69120 Heidelberg, Germany.

We have amplified the cDNA of a new basic helix-loop-helix protein from the adult rat brain which shows a notable sequence homology to Drosophila neurogenic factors. The search for a full-length cDNA clone of SHARP-1 (enhancer-of-split and hairy related protein-1) yielded a second, closely related gene product, SHARP-2. Both proteins are distinct from mammalian HES proteins and may define a new bHLH subfamily. Both mammalian genes are regulated throughout development and show a highly region-specific expression in the adult central nervous system. By *in situ* hybridization, the 4.5 kb SHARP-1 mRNA is most prominent in the CA1 region of the hippocampus, the dentate gyrus, and the internal granule cell layer of the cerebellum. Neuronal expression increases throughout brain development and is highest in the adult CNS. SHARP-1 and -2 are weakly expressed in exponentially growing PC12 cells but both gene transcripts are rapidly induced by the addition of NGF. In Drosophila, *hairy* and *E(spl)* proteins are transcriptional repressors of neuronal differentiation, and we investigate a similar role of SHARP proteins in mammalian development. Protein kinase raf mediates some of the actions of NGF in PC12 cells, and preliminary data suggest that in cotransfected PC12 cells, SHARP-1 counteracts c-raf effects in the NGF signal transduction pathway.

(Supported by a DFG grant to K.A.N. and a graduate training grant of the DFG to M.J.R.).

211.2

KNOCKOUT OF GPI-LINKED CELL RECOGNITION MOLECULE T-CADHERIN IN MOUSE

M.Garlatti, B.Ferreiro and B. Ranscht*. *The* Burnham Institute, La Jolla, CA 92037, USA.

T-cadherin, a GPI-linked cell adhesion/ recognition molecule of the cadherin gene family identified in chick, delineates avoided regions in the motor axon pathway and is repulsive to motor axons (Fredette and Ranscht, J.Neurosci.14:7331-7346 and submitted). To study T-cadherin function in the mouse, we are generating null mutations for T-cadherin by homologous recombination. Using PCR primers, we have isolated mouse T-cadherin cDNA that predicts a protein with 75 % amino acid identity to chick T-cadherin. Expression of the cDNA in COS7 cells results in cell surface expression of a GPI-anchored protein that is immunoreactive with anti-chick T-cadherin antibodies. The gene targeting vector was constructed from mouse genomic DNA by insertion of a neomycin casette into the exon encoding the amino terminal portion of the first extracellular domain EC1. Herpes simplex thymidine kinase was used as a negative selection marker. The gene targeting vector was electroporated in J1 embryonic stem cells. G418-resistent clones were selected and tested for the mutated allele by Southern blotting. Two targeted ES cell clones were injected into mouse blastocysts and reimplanted in pseudo-pregnant foster mothers. Chimeric founder males mated with Black Swiss females carry the mutation into the germline. Matings for mice homozygous for the T-cadherin null mutation are underway. The mice will be analyzed for developmental defects, including misrouting of motor axons projections. A preliminary assessment of the phenotype will be presented.

211.3

INDUCIBLE AND CELL TYPE-RESTRICTED GENE KNOCKOUT IN THE CENTRAL NERVOUS SYSTEM OF MICE. M. T. Hasan, H. Bujard, E. Mercer, D. Anderson, J. Morgan, D. L. King and S. Tonegawa* Center for Learning and Memory, Massachusetts Institute of Technology, Cambridge, MA 02139.

We have produced transgenic mice in which cre gene expression in the brain can be induced by oral ingestion of a tetracycline derivative, doxycycline. Our system utilizes the reverse tetracycline transactivator (rtTA) under the control of the brain-specific promoter, L7, and the cre gene under the control of a tetracycline-based minimal promoter. Patterns of cre expression in brain tissues can easily be visualized by utilizing a lacZ reporter system. To accomplish this, these cre-expressing mice were mated to mice carrying copies of lacZ gene under the control of a chicken β-actin promoter. Between the promoter and the lacZ elements was inserted translational STOP sequences flanked by a pair of loxP sites. Progeny mice which carry rtTA, cre and lacZ transgenes were treated for five days with doxycycline in the drinking water. The occurence of efficient cre/loxP-mediated recombination events in the neocortex and hippocampus was indicated by the presence of blue cells after staining of brain sections with X-gal. In the absence of doxycycline treatment, no blue cells were detectable. These results indicate that cre/loxP-dependent recombination events can occur in postmitotic neuronal cells, and that genes of interest can be knockout in an inducible manner in neuronal cells.

Howard Hughes Medical Institute (S.T.)
National Institute of Health grant RO1 NS32925 (S.T.)

211.4

DEVELOPMENTAL RESCUE OF DROSOPHILA CEPHALIC DEFECTS BY THE HUMAN OTX GENES.

T. Nagao[1], S. Leuzinger[2], D. Acampora[3], A. Simeone[3], H. Reichert[2] and K. Furukubo-Tokunaga[1,2*]. 1) Inst. Biol. Sci., Univ. Tsukuba, Tsukuba 305, Japan. 2) Zoologishes Institut der Universität Basel, CH-4051 Basel, Switzerland. 3) Internat. Inst. Genet. & Biophys. CNR, I-80125 Napoli, Italy.

Recent studies suggest that the fundamental genetic programs for the development of the cephalic structures including the brain might be highly conserved. Seminal to these processes are the evolutionally conserved homeobox genes, otd/Otx and ems/Emx genes, which are expressed in segmental patterns in the developing brains in both flies and mice. In order to examine functional conservation of the otd and the Otx genes, we have introduced the human Otx homologs into flies, and examined their developmental control activity in a fly otd mutant, *ocelliless*, which fail to develop ocelli and associated vertex structures as well as the protocerebral bridge in the brain. By inducing expression of the transgene via a heat shock promoter, we find that both the human Otx1 and Otx2 genes are as potent as the fly otd gene in rescue of the epidermal defects in the mutant. We discuss our results in the light of the origin of cephalogenesis and brain formation in evolution (supported by the Swiss NSF and the Univ. Tsukuba).

211.5

Targeted inactivation of the *hoxd-10* gene affects the development of hindlimb innervation. E. M. Carpenter*, J. M. Goddard, A. P. Davis, and M. R. Capecchi. Dept. of Human Genetics, University of Utah, Salt Lake City, UT 84112

We have disrupted the mouse *hoxd-10* gene by inserting the neomycin resistance gene into the homeobox using homologous recombination in embryo stem cells. Mice carrying this mutation are viable and survive to weaning and adulthood. Mutant mice show gait defects primarily affecting their hindlimbs. Severely affected mice hyperextend their hindlimbs and drag the dorsal surface of their hindfeet along the ground. The hindlimbs appear stiff, but not spastic. Mutant mice are also unable to use their hindfeet to grip an object positioned along their ventral midline, though control mice can grasp similar objects with ease. Gross morphology of mutant hindlimbs appears normal. There is no evidence of muscle wasting or large bone deformation. Sections of the hindlimb indicate that the major muscle groups of the hindlimb are present, although some disorganization of the muscles near the distal end of the femur is evident. Analysis of skeletons of mutant mice reveals changes in the organization of the knee, particularly in the placement of the patella. We have also examined the nervous systems of these mice. Carbocyanine dye tracing has shown that mutant mice have a slightly smaller complement of lumbar motor neurons and a smaller number of spinal nerves providing hindlimb innervation. Histological sections show that those motor neurons that are present appear normal in size and position. Immunohistochemical labeling of the hindlimbs in embryonic mice using antibodies directed against neurofilament protein indicates that the developing peroneal nerve is absent in some mutants. Anti-neurofilament labeling and carbocyanine dye tracing also indicate that there is decreased arborization of the developing fibularis nerve. These observations document changes in the skeleton, musculature, and nervous system of *hoxd-10* mutant mice and may provide an explanation for the observed gait defects. Supported by the Howard Hughes Medical Institute and the National Institutes of Health.

211.6

TRANSCRIPTIONAL REGULATION OF GAP-43 PROMOTER BY BASIC HELIX-LOOP-HELIX PROTEINS. M. N. Uittenbogard, D. R. Peavy, T. Neuman and A. Chiaramello*. Department of Biochemistry and Molecular Biology, Colorado State University, Fort Collins, CO 80523.

The GAP-43 gene is expressed at high level in the growth cones of neurons during axonal growth and synapse formation. The *GAP-43* promoter region contains seven E-boxes (E1 to E7) that are organized in two clusters, a distal cluster (E3 to E7) and a proximal cluster (E1 and E2). Deletion analysis and site-directed mutagenesis of the *GAP-43* promoter region showed that only the most proximal E1 E-box significantly modulates the *GAP-43* promoter activity. This E-box is conserved between the rat and human *GAP-43* promoter sequences, in terms of flanking sequence, core sequence, and position. We found that endogenous E-box binding proteins present in neuroblastoma N18 cells recognize the E1 E-box and activate *GAP-43* promoter. The transcriptional activity of the *GAP-43* promoter was repressed not only by the negative regulator Id2 protein, but also by two non-tissue specific class A bHLH proteins, E12 and ME1a. *In vitro* analyses showed that both ME1a and E12 bind to the E1 E-box as homodimers. By Northern analyses, we established an inverse correlation between the level of *E12* and *ME1a* mRNA and *GAP-43* mRNA in various neuronal cell lines as well as in ME1a-overexpressing PC12 cells. Therefore, we have identified a *cis*-acting element, the E1 E-box, located in the *GAP-43* promoter region which either modulates positively or negatively the expression of *GAP-43* gene depending on which E-box-binding proteins occupy this site.

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211.7

B-50/GAP-43, A MARKER OF NEURAL DEVELOPMENT IN *XENOPUS LAEVIS*. L. H. Schrama[1*], G. Lepperdinger[4], A. Moritz[1], N. van den Engel[1], A. Marquart[1], A. B. Oestreicher[2], B. J. L. Eggen, W. J. Hage[3], K. Richter[4], and O. H. J. Destrée[1] [1]Lab. Physiol. Chem., [2]Med. Pharmacol., RMI for Neurosciences, Utrecht University, Universiteitsweg 100, 3584 CG Utrecht, [3]Hubrecht Laboratory, NIOB, Uppsalalaan 8, 3584 CT Utrecht, The Netherlands. [4]Inst. für Genetik und Allgemeine Biologie, Universität Salzburg, Hellbrunnerstraße 34, A-5020 Salzburg, Austria.

To study the regulation and function of the growth-associated protein B-50 in *Xenopus laevis*, B-50 cDNAs were isolated and characterized. The deduced amino acid sequence reveals potential functional domains of *Xenopus* B-50 that may be involved in G-protein interaction, membrane-binding, calmodulin-binding and protein kinase C phosphorylation. The expression of B-50 at the RNA and protein level during development was investigated using the *Xenopus* cDNA and the monoclonal B-50 antibody NM2. The antibody NM2 recognizes the gene product on western blot and in whole-mount immunocytochemistry of *Xenopus* embryos. Moreover, visualization of the developmentally regulated appearance of B-50 immunoreactivity shows that this mode of detection may be used to monitor axonogenesis under various experimental conditions. In adult *Xenopus*, B-50 mRNA was shown to be expressed at high levels in brain, spinal cord and eye using northern blotting. The earliest expression was at developmental stage 13 with poly(A) RNA. By whole-mount immunofluorescence, applying the CSLM, the protein is first detected in embryos from stage 20, where it is expressed in the developing trigeminal ganglion. Also later in development the expression of the B-50 gene is restricted to the nervous system in *Xenopus*.

XB-50/GAP-43 is a good marker to study the development of the nervous system in *Xenopus laevis*. The endogenous expression of B-50 correlates largely with the previously observed neural-preferred expression in vivo of a rat B-50 promoter-reporter construct in *Xenopus*. This indicates that elements that govern B-50 expression in the nervous system are conserved during evolution.

Supported by NWO grant 903-42-001.

211.8

LEAD INDUCES GAP-43 mRNA AND ODC ACTIVITY IN THE NEOCORTEX. A. Hilliard, N.H. Zawia, J.G. Townsel* and R. Sharan. Dept. of Pharmacology, Meharry Med. College, Nashville, TN 37208.

Studies in our laboratory found a similar ontogenetic pattern of expression for both ornithine decarboxylase (ODC) activity and growth associated protein 43 (GAP-43) mRNA expression in various brain regions. GAP-43 is a marker of neuronal differentiation and axonal elaboration and the polyamines are produced during periods of peak synaptogenesis. In an attempt to examine a possible link between GAP-43 and the ODC/polyamine system, we challenged animals by exposure to the neurotoxicant lead (Pb), a known modulator of both systems. We found that both ODC activity and GAP-43 mRNA were induced in the neocortices of Pb-exposed female rat pups. However, induction of ODC activity (PND 3) preceded that of GAP-43 mRNA (PND 5). This indicates that the ODC/polyamine system may be related to the expression of GAP-43 mRNA. Further studies in our lab are aimed at investigating common signal transduction mechanisms that may mediate the induction of both proteins. This work was supported by NSF grant number HRD-9550699.

211.9

SELECTIVE MODULATION OF Sp1 DNA-BINDING BY LEAD. N.H. Zawia*, T. Crumpton & R. Sharan. Dept. of Pharmacology, Meharry Medical College, Nashville, TN 37208.

Novel studies in our lab have demonstrated that exposure to lead (Pb) *in vitro* alters the DNA binding of zinc finger proteins (ZFP). These transcription factors play key roles during growth/differentiation. Using the gel shift mobility assay, HeLa cells nuclear extracts, the consensus oligonucleotide for Sp1, and similar concentrations of cadmium (Cd) and calcium (Ca), we examined if such perturbations were selective for Pb. We found that low concentrations of Pb (<1.0 mM) produced a dramatic enhancement of Sp1 DNA binding, while higher concentrations of Pb (>5 mM) abolished the DNA binding activity of Sp1. While both high and low concentrations of Ca had no effect on Sp1 DNA-binding, we found that low concentration of Cd inhibited such binding and high concentrations formed unresolved complexes on the gel. These studies suggest that Pb alters Sp1 DNA-binding in a specific dose-dependent manner and thus may partially impact the expression of target genes through such an action. This work was supported by grants from NIH (5 G12RR0303208) and NSF (HRD-9550699).

211.10

RECEPTOR TYROSINE PHOSPHATASES EXPRESSED BY DEVELOPING NEURONS IN THE LEECH. T.R. Gershon*, M. Nitabach, M. Baker, P. Wu and E. R. Macagno. Columbia Univ. Dept. of Biological Sciences, New York, NY, 10027.

In order to better understand how neurons develop different arbors, we searched for cell surface receptors expressed on different subsets of neurons during embryogenesis. Using RT-PCR we cloned fragments of leech homologues of receptor tyrosine phosphatases (rPTPs) and screened them by whole mount in situ hybridization. We found 2 leech homologues of the rPTP leukocyte antigen related protein (LAR) that are expressed by different small sets of neurons. We designated these genes HmLAR1 and HmLAR2, obtained the full coding sequences, expressed portions of them in E. coli, and raised antisera to the exogenously expressed protein. We are also in the process of expressing the extracellular domains in insect cells using baculovirus in order to obtain bio-active molecules. Here we present the coding sequences, expression patterns, and protein localization patterns of HmLAR1 and HmLAR2. HmLAR1 notably has a shorter extracellular domain than HmLAR2, and is more closely homologous to the rat protein PTP NE-3 than to LAR. HmLAR2, in contrast appears to by the true LAR homologue. We have identified the normal characterisitcs of the HmLAR expressing neurons and we plan to block HmLAR function through a variety of techniques in order to change their course of development.

Funded by NSF grant #IBN-94-09996.

211.11

ALTERING GENE EXPRESSION IN IDENTIFIED EMBRYONIC NEURONS BY INJECTING mRNAs INTO SINGLE CELLS. L. R. Wolszon*, G. Aisemberg, V. Wong and E. R. Macagno. Columbia Univ. Dept. of Biological Sciences, New York, NY, 10027.

The use of invertebrate preparations, such as the medicinal leech, are highly advantageous in developmental studies of neuronal outgrowth, morphology and plasticity. However, the inaccesibilty of traditional genetic approaches in this system has made it difficult to determine the functions of developmentally relevant genes. We present here a technique that allows us to alter specific gene expression in identified embryonic neurons, using pressure-injection of mRNAs into individual central neurons of live leech embryos.

The skin is opened over a segmental ganglion of an immobilized E8-E13 *H. medicinalis* embryo. A fine-tipped microelectrode, pulled from silanized and baked thin-walled glass, is filled with 0.5 ul of the mRNA solution (plus a tracer dye) and beveled. The electrode tip is micromanipulated into the target neuron and 2-3 250-ms pulses of 5-10 psi are applied to the back. The embryo is then allowed to develop for 2-3 more days, and is processed for antibody staining of the relevant protein.

Funded by NIH grant #NS-34545.

211.12

DEVELOPMENTAL REGULATION OF GENE EXPRESSION IN THE MURINE CNS. S. Tontsch, O. Zach, G. Lepperdinger, G. Webersinke, H.Bauer, K. Richter° and H.C. Bauer*. Inst. f. Molekularbiol. Austr. Acad. Scs. A-5020 Salzburg and °: Inst. f. Genetik, Univ. Salzburg, A-5020 Salzburg, Austria.

Migration and differentiation of neurons in the developing cerebral cortex starts at an advanced gestational stage in the mouse. In order to identifiy genes which show stage-specific expression during neurogenesis, we subtracted first strand cDNA from E 12 brains with mRNA from brains derived from newborn animals. By subsequent differential screening two full-length cDNA sequences (A13 and C2) were identified, both corresponding to genes which we have found to be highly expressed between E 10 and E17 but not in adult and early embryonic animals. Whole mount in-situ hybridization has revealed that expression of A 13 and C2 is most prominent in the hindbrain and in the spinal cord. These newly identified cDNA sequences have no homology to already known sequences found in the databases. In a second experiment we have cloned and sequenced the mouse homologue of the C. elegans *unc 33* gene, which was previously suggestd to be involved in neurite outgrowth. Again, expression of unc 33 was highest during later stages of development and was located in the eyes, the rhombencephalon and in the spinal cord.

DEVELOPMENTAL GENETICS II

212.1

EVIDENCE FOR A DELAY IN THE FORMATION OF THE EXTERNAL GRANULE CELL LAYER IN MEANDER TAIL MUTANT EMBRYOS. K.M. Hamre* and D. Goldowitz. Dept. of Anat. & Neurobiol., Univ. of Tenn. Coll. of Med., Memphis, TN 38163

In homozygous meander tail mutant mice (gene symbol: *mea*), cerebellar granule cells are virtually absent from the anterior lobe due to the gene acting intrinsically in granule cell precursors (Hamre and Goldowitz, 1995). Results of the analysis of chimeric cerebella suggest the hypothesis that this loss is due to a delay in the formation of the external granule cell layer (EGL). We are testing this hypothesis by examining the morphology of the EGL in homozygous *mea* compared to wild-type (C57BLKS/J) embryos at embryonic days (E) E12.5-14.5. In wild-type (+/+) embryos, a clear EGL is present by E13.5. In E13.5 *mea* embryos, the EGL is either not present or, if present, is smaller and more disorganized. In addition, the cells beneath the EGL (largely Purkinje cells) appear more disorganized. A smaller EGL was also observed at E14.5. To determine whether this difference could be due to alterations in the cell cycle of granule cell precursors, the mitotic labeling was examined in the EGL and germinal trigone of embryos at E12.5-E14.5. Dams carrying homozygous *mea* or wild-type embryos were given an injection of 5-bromo-2'-deoxyuridine (BrdU), allowed to survive for 1 hour and processed for the demonstration of BrdU. The number of labeled and unlabeled cells were computed to determine the labeling index (percentage of cells labeled). At E13.5 and E14.5, the labeling indices of cells in the germinal trigone (the source of EGL neuroblasts) are equivalent in +/+ and *mea* cerebella. Further, in both groups, the labeling index within the germinal trigone decreases as the age of the embryo increases. In the EGL, the labeling indices are also similar between +/+ and *mea* cerebella. However, in *mea* cerebella that appear to lack an EGL there are, as expected, no BrdU-positive cells. These results suggest that there is a delay in the development of cells in the EGL which is not due to alterations in mitotic activity. To test this hypothesis, we are studying the expression of molecules that mark EGL development in *mea* cerebella. Support: NS23475.

212.2

LEVELS OF NMDA-R1 , GAD_{67}, AND GABA-T mRNAs IN PURKINJE NEURONS ARE IDENTICAL IN WILD-TYPE, *pcd* MUTANTS, AND *Hu-Bcl-2* TRANSGENIC MICE.

H. Zanjani*, N.J.K. Tillakaratne, J.C. Martinou, N. Delhaye-Bouchaud, J. Mariani, and A.J. Tobin. UCLA, Los Angeles, CA, USA; Institut des Neurosciences, UPMC and CNRS, Paris, France; and Glaxo Imb., Geneva, Switzerland

We have used non-radioactive *in situ* hybridization to study the cerebellar expression patterns of three neuronal mRNAs -- NMDAR1 , GAD_{67}, and GABA-T -- in wild-type mice, *pcd* (Purkinje cell degeneration) mice, and transgenic mice that express the human *Bcl-2* gene. In *pcd* mice, Purkinje cells are destined to die as a result of a defect intrinsic to the dying cell, with apoptotic death occuring between P20 and P35. At P18, all Purkinje neurons showed indistinguishable patterns of expression of the three mRNAs examined. We found no evidence for a subset of Purkinje neurons in which the pattern of gene expression could predict a distinctive susceptibility to *pcd*-induced cell death. The cerebella of *Bcl-2* transgenic mice contain 40% more Purkinje neurons than wild-type controls. Again, our *in situ* hybridization studies revealed no heterogeneity in the expression of these three neuronal mRNAs, suggesting that the neurons "rescued" by Bcl-2 are identical to all other Purkinje neurons. Thus, we found no evidence for a subset of Purkinje neurons that might have a distinctive susceptibility to apoptosis. (Supported by FRM, INSERM and NINDS)

212.3

ANALYSIS OF RETINAL PHENOTYPE IN BF-1 KNOCKOUT MICE. S. O. Huh*, V. Hatini, R. C. Marcus°, C. A. Mason°, and E. Lai Cell Biology and Genetics Program, Memorial Sloan-Kettering Cancer Center, New York., NY 10021. °: Dept. of Pathology, Anatomy and Cell Biology, Columbia University, New York, NY10032

BF-1 winged-helix transcription factor is expressed in the anterior hemiretina and the telencephalon. The BF-1(-/-) embryos develop with reduced telencephalic vesicles, rudimentary basal telencephalon and distorted retinal morphology. Here, we investigated the molecular basis and ontogeny of eye abnormalities in BF-1(-/-) embryos. Morphological analysis at E13.5-16.5 reveals that retinae in homozygotes becomes enlarged and the whole optic cup expresses Pax-6. The optic stalk normally constricts and decreases in diameter and is eventually replaced by retinal ganglion cell axons. In the BF-1 mutant, however, the optic stalk at E10.5 does not constrict and remain open and the retinae are in broad continuation with third ventricle. The timing of retinal cell differentiation is similar between mutant and wild type, suggesting that the enlarged retinal cells did not arise from unchecked proliferation of the retinal precursor cells. We previously hypothesized that BF-1 might specify positional identity of RGC and regulate axonal targeting at the optic chiasm. DiI-labelled nasal RGCs successfully crosses at the chiasm suggesting that BF-1 is not required for proper targeting. At present, we can not distinguish whether BF-1 functions in the retinal field in an autonomous mode or whether BF-1 is required to signal the retinal field non-autonomously. We are currently attempting to distinguish between these two possibilities (supported by NIH grant, EY11124).

212.4

MAPPING QUANTITATIVE TRAIT LOCI THAT CONTROL RETINAL GANGLION CELL NUMBER USING F2 INTERCROSS PROGENY R.C. Strom*, D. Goldowitz, and R.W. Williams. University of Tennessee, Memphis, Center for Neuroscience, Memphis, TN 38163.

Variation in retinal ganglion cell number is distributed approximately bimodally among 17 standard inbred mouse strains and also among 26 BXD recombinant inbred strains. This bimodal distribution indicates that one or two genes have large effects on retinal ganglion cell number. Using BXD recombinant inbred strains we have mapped a gene (*Rcnc1*) with a major effect on retinal ganglion cell number to chromosome 11, near *Tstap91a* at ~58 cM. To confirm the precise location of *Rcnc1* on Chr 11 and to locate new loci controlling ganglion cell number, we have produced 105 F2 intercross progeny. These progeny were produced by crossing BALB/cJ and CAST/Ei strains. Axons of retinal ganglion cells were counted in optic nerve cross-sections using electron microscopy. The retinal ganglion cell strain averages for BALB/cJ and CAST/Ei are 63,393 and 45,047, respectively. The strain average for 11 BALB/cJ x CAST/Ei F1's is 59,454 ± 3,445(SD). The phenotypic distribution of the F2s is unimodal, and the average is 57,208 ± 7,644 (SD). Using Wright's equation the number of genes affecting variation in the F2s was estimated to be one. To identify loci affecting ganglion cell number a whole genome-scan was performed by typing microsatellite markers at intervals of ~30 cM throughout the genome. Microsatellites were typed by PCR. To expedite marker analysis, PCR was performed using pooled DNA from the ten highest and lowest cases. Linkage analysis of the pooled DNA indicated linkage with markers located on Chr 11. Individual F2s used in the pools were then genotyped using Chr 11 markers. Linkage probability was calculated using Map Manager QT. Linkage was found between a target gene controlling retinal ganglion cell number and markers on Chr 11 near ~50 cM, (nominal P <0.00001). This region at 50 cM is just proximal to the region identified previously in our analysis of BXD recombinant inbred strains. Supported by RO1 NS 35485.

212.5

MAPPING QUANTITATIVE TRAIT LOCI THAT CONTROL NORMAL VARIATION IN BRAIN WEIGHT IN THE MOUSE

R. W. Williams*, R. C. Strom, and D. Goldowitz. University of Tennessee, Center for Neuroscience, Memphis, TN 38163

Genes that control natural variation in brain weight are likely to play important roles in the production, survival, and growth of neurons and glial cells. Isolating these polymorphic genes has been difficult because complex traits such as brain weight are influenced by many loci. Here we show that a small number of quantitative trait loci (QTLs) have sufficiently large effects on brain weight to be mapped using recombinant inbred strains (RI) and F2 intercrosses. Fixed brains were bisected behind the cerebellum and weighed (n ~850 adults). Inbred strain averages range from 374 ± 8 mg in CAST/Ei to 516 ± 11 mg in BALB/cJ. Differences between sexes are trivial (<10 mg). The coefficient of variation in isogenic strains averages 4.0% (~60 strains), whereas variation in outbred groups averages 6.5%. Heritability (h^2) is ~0.65. A comparison of variance between intercross progeny (CAST/Ei x BALB/cJ F2) and isogenic groups indicates that no less than three QTLs control normal variation in brain weight. At least one QTL has a major effect, influencing weight by more than 40 mg. Single gene effects of this size can be mapped using fewer than 200 F2 progeny. The weak correlation between body and brain weight in F2 crosses ($r^2 = 0.14$, n = 223) suggests that these QTLs do not have notable effects on body size. BXD and BXH recombinant inbred strains (n = 38) and F2 progeny were used to map major effect loci. The strongest candidate regions are on chromosome 8 near *Cpe*, Chr 9 near *Xmv15*, and Chr 11 near the centromere (nominal $P < 0.0006$ in these regions). Supported by RO1 NS 35485 and EY-9586.

212.6

States, Trajectories and Attractors: Modeling the Complexity of Developmental Genetic Networks using Boolean Networks

R. Somogyi*, M. Askenazi[1], A. Wuensche[1], B. Sawhill[1], S. Fuhrman, S. Kauffman.[1] LNP, NINDS, NIH, Bethesda, MD; [1]Santa Fe Institute, Santa Fe, NM.

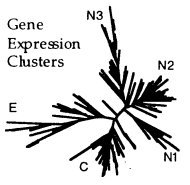
Understanding the computational principles of genetic networks may become necessary for solving complex problems like brain development. The unfolding of the developmental program is manifested in the interdependent activation and inactivation of networks of genes. The complexity of multiple molecular interactions is captured in Boolean networks by allowing any set of genes within the system to serve as an input to any chosen gene. The *state* of a gene (on/off) is computed from the *state* (on/off pattern) of the input elements through a combinatorial Boolean rule. Such networks can be used to calculate state sequences in time, or *trajectories*. Because a Boolean network has a limited number of states, each trajectory inexorably leads to a single repeating state (point *attractor*) or a cycle of states (dynamic *attractor*). Trajectories can be likened to the transient gene expression patterns of development, and attractors to the resulting differentiated cell types. The higher level properties of these systems allow the identification of ordered, chaotic and complex systems' domains, the complex regimen representing an optimum of diversified yet stable behavior. In random Boolean nets, the complex domain is favored through judicious choice of canalyzing rules, i.e. rules in which an input element state overrides the input states of other elements in the rule. Profound predictions of this order may be tested on experimental systems today. A comprehensive synthesis of complex network models with experimental data may be achieved through *reverse engineering* of genetic network architectures from experimentally measurable trajectories, e.g. gene expression transients during CNS development or responses of cells to targeted perturbations. This approach is being successfully applied to model binary trajectories; using the GeneTool algorithm, a minimum number of trajectories generated by a Boolean net allow the extraction of the underlying network wiring and rules.

212.7

From Genes to Dynamic States: Mapping Spinal Cord Development with the Gene Expression Matrix Reveals Functional Gene Clusters

X. Wen, S. Fuhrman*, S. Smith, M. Askenazi[1], J. L. Barker, R. Somogyi. LNP, NINDS, NIH, Bethesda, MD; [1]Santa Fe Institute, Santa Fe, NM.

Development can be described as an unfolding genetic program, controlling cell proliferation and differentiation. The primary step in this process is gene activation and inactivation, generating mRNA expression patterns. Simplifying the complexity of these processes will require a detailed characterization of these states. We examined mRNA samples from rat cervical spinal cord from embryonic day 11 (E11) to adult, focusing on genes which mediate intercellular communication, the initiators of cell differentiation: peptide and receptor genes for the neurotrophins, insulin-like factors and heparin-binding growth factor family members, as well as neurotransmitter receptors and metabolizing enzymes. To relate these to major phases of CNS development, we examined the expression of early and late markers. Using ratiometric RTPCR and PAGE product analysis, we analyzed >100 genes, and found distinct expression patterns which were used to create gene expression clusters with PHYLIP software (see graph). Genes highly expressed solely during early embryonic development (E) generally were limited to peptide factors, their receptors, and several markers. Neurotransmitter signaling genes generally followed a progressive activation between E11 and E15 and either gradually increased until adult (N3), or reached maximal expression late in development and declined to medium (N2) or minimally detectable (N1) levels in the adult. Housekeeping genes remained largely constant (C). The patterns suggest that these genes may affect each other's expression. This conjecture should be confirmed by comparative analysis of mutual information levels, allowing us to distinguish potentially interacting genes.



212.8

PHYSICAL MAPPING OF X-LINKED LISSENCEPHALY AND SBH (XLIS): A CNS NEURONAL MIGRATION GENE.

ME Ross[1]*, AK Srivastava[2], GJ Gleeson[3], KM Allen[3], CA Walsh[3], JH Young[1], WB Dobyns[1]. Neurology Dept, Univ of Minnesota, MPLS, MN[1]; Greenwood Genetic Ctr, Greenwood SC[2]; & Neurology Dept, Beth Israel Hosp, HMS, Boston, MA[3].

The failure of neuronal migration can produce cortical malformations with associated cognitive and motor impairment and epilepsy. Two striking human malformations are subcortical band heterotopia (SBH or double cortex) and lissencephaly (LIS). The coincidence of LIS in males and SBH in females from several families suggests that a single gene mutation on X may be involved in both disorders. We have mapped the gene responsible for this condition to chromosome Xq22.3-q23 by linkage analysis in 4 multiplex families and physical mapping of an X-autosomal translocation in a girl with LIS. Linkage analysis determined a critical region of approximately 30 cM between Xq21.3 and Xq24, flanked by markers DXS1002 and DXS1001 with a maximum 2 point LOD score of 2.4 (K Allen, et al., Soc. Neurosci. Abst. '96). This region contains the Xq breakpoint of a girl (XLI-01) with classical LIS and a de novo X;2 translocation with breakpoints in Xq22.3-q23 and 2p25.1. Multiple STS markers from the critical region on X were used to physically map a somatic cell hybrid, which retains the der(2). Markers DXS87, DXS1105 and COL4A5 were placed centromeric and DXS1072 telomeric to the breakpoint. This was confirmed by fluorescence in situ hybridization (FISH) using genomic probes from the region to examine metaphase chromosomes from XLI-01. These studies reduced the critical region to 1-2 Mb. We conclude that both X-linked lissencephaly and SBH are caused by malfunction of a single gene (XLIS) located in Xq22.3-q23 and that the less severe, SBH phenotype in females is due to random X-inactivation (Lyonization). We propose that mutational heterogeneity contributes to the observed variation in the severity of the XLIS phenotypes. Identification of the XLIS gene will be important for understanding the molecular basis of cortical neuronal migration. (MMF & NIH)

212.9

LINKAGE ANALYSIS OF "DOUBLE CORTEX"/ X-LINKED LISSENCEPHALY, A HUMAN MUTATION CAUSING ABNORMAL NEURONAL MIGRATION

K.M. Allen[1]*, J.G. Gleeson[1,2], M.E. Ross[3], I. Scheffer[4], S. Berkovic[4], M. Berg[5], J. Motte[6], W.B. Dobyns[3] and C. Walsh[1]. Beth Israel Hosp., Neurology Dept., Boston, MA[1], Children's Hosp., Boston, MA[2], Univ. of Minnesota Med. School, MN[3], Austin Hosp., Heidelberg, AU[4], Univ. of Rochester Med. Ctr., Rochester, NY[5], American Memorial Hosp., Reims, FR[6].

Analysis of cerebral cortical development is facilitated by studying genetic disorders with abnormal neuronal migration. Two striking examples in humans are "double cortex" and lissencephaly. Double cortex, also known as subcortical band heterotopia, shows an abnormal band of neurons in the white matter underlying an apparently normal cortex, while lissencephaly shows an abnormally thick cortex with decreased or absent surface convolutions. Affected patients have varying degrees of epilepsy and mental retardation. Inheritance of double cortex in daughters and lissencephaly in sons of double cortex females suggested X-linked dominant inheritance (Pinard et al., JNNP 1994;57:914). We analyzed four families with double cortex and/or X-linked lissencephaly with approximately 40 X-linked STS markers and established linkage to Xq21.3-Xq24 with a maximum two-point LOD score of 2.4 (θ=0). Recombination events with the proximal marker DXS1002 and distal marker DXS1001 define the candidate region. This represents a physical distance of about 30 cM but this region is further refined by a girl with classical lissencephaly and a balanced X-autosomal translocation at Xq22.3-Xq23 (see M.E.Ross et al., this meeting). These results suggest that there is a single gene defect on the X chromosome responsible for the abnormal neuronal migration; the difference in phenotype between affected males and females may be due to X-inactivation of the normal gene in the carrier females. Human inherited malformations may provide a means of elucidating genetic mechanisms of normal migration and cortical development. (Supported by HSFP, NIH and Klingenstein Fdn.)

212.10

GCM FAMILY: A NOVEL FAMILY OF POTENTIAL TRANSCRIPTIONAL REGULATORS EXPRESSED IN THE DEVELOPING NERVOUS SYSTEM.

T.Hosoya[1], H. Baba[1], Y. Akiyama[2], A. M. Poole[2], K. Ikenaka*[3] and Y. Hotta[1,2]. [1]Molec. Genet. Lab., [2]Grad. Sch. of Sci., Univ. of Tokyo, Tokyo 113, [3]National Inst. of Physiology, Okazaki 444.

The *glial cells missing* gene (dGCM1) was identified as a switch gene that controls glial vs. neuronal developmental decision in *Drosophila*. It encodes a novel nuclear protein with no known motif characterized to date. Our search for *gcm* homologues resulted in the isolation of several homologues from human and mouse mRNA, and one from *Drosophila*. These homologues have many common amino acids in the N-terminal 150a.a. region. We call this conserved amino acid sequence "gcm box". We showed that the gcm box is a novel DNA binding motif that specifically binds to an octamer sequence. This gcm binding sequence is repeated in the 5'-region of a Drosophila gene whose expression is dependent on dGCM1, strongly suggesting that gcm proteins bind the upstream regions of their target genes, thereby controling the gene expression. Thus the group of gcm homologues, or "gcm family", is most likely a novel family of transcriptional regulators.

RT-PCR experiments detected expression of the mammalian homologues in the embryonic brain. We performed *in situ* hybridization of a mouse homologue (mGCMb) to find strong expression both in the central and in the peripheral nervous system. The signal profile in the nervous system suggests that mGCMb expression begins when cells become postmitotic and start differentiation. The expression is confined in the developing stages, and can hardly be detected in the adult brain. These results raise the possibility that gcm family proteins may regulate cellular differentiation processes in the mammalian nervous system.

This work was supported by grants-in-aid from Ministry of Education, Science and Culture, and from Science and Technology Agency of Japan.

212.11

NEURONATIN-α IN PC12 CELLS: DOWNREGULATION BY NERVE GROWTH FACTOR. Rajiv Joseph*, Wayne Tsang, Dexian Dou, Kevin Nelson and Klaus Edvardsen. Department of Neurology, K-11, Laboratory of Molecular Neuroscience, Henry Ford Hospital, Detroit, MI.

Neuronatin, first cloned from neonatal rat brain (*BBRC 1994; 201:1227-1234*), is expressed during mammalian neurogenesis (*Brain Res 1995; 690:92-98*). There are two alternatively spliced forms, α and β. The α-form contains all three exons, whereas, the β-form contains only exons 1 and 3. The 81 bp middle exon, encoding 27 amino acids, has been spliced out in *neuronatin-β*. More recently we have sequenced the human *neuronatin* gene (*Genomics, in press*), and assigned it to chromosome 20q11.2-12 (*Brain Res, in press*). In an attempt to understand the function of this gene, its expression in PC12 cells, an established model of neuronal growth and differentiation, was investigated. Of the two spliced forms, only the α-form was expressed in PC12 cells. Although, *neuronatin-α* mRNA was abundant in undifferentiated PC12 cells, neuronal differentiation induced by NGF resulted in marked downregulation, and removal of NGF was associated with a return of *neuronatin-α* mRNA levels to baseline. These effects were not seen with TGFβ1, EGF, TPA and dexamethasone. Although, bFGF also reduced *neuronatin-α* mRNA levels, the effect was less pronounced than with NGF. The NGF-induced decrease in *neuronatin-α* mRNA levels occurred even when protein and RNA syntheses were blocked. The finding that only the α-form is expressed in proliferating PC12 cells, may implicate the middle exon of *neuronatin* in the mechanisms of cell growth and proliferation. Supported by grants from the NIH (NS-01521) and American Heart Association-National (92-132) (to R.J.).

212.12

CHANGES WITH THE DEVELOPMENT OF NOMIFENSINE-INDUCED c-fos mRNA EXPRESSION IN THE RAT BRAIN. M. Murata[1,3], A. Oshima[1], A. Kashiwa, [1] T. Nishikawa[1] K. Emori[2]* and M. Kurachi[2]. [1]Dept. of Mental Disorder Research, National Institute of Neuroscience, NCNP, Kodaira, Tokyo 187, Japan; [2]Dept. of Neuropsychiatry, Toyama Med. & Pharmaceut. Univ., Toyama 930-01, Japan; [3]Tanino Gozan Hospital, Toyama 930-01, Japan.

Using Northern blot analysis, we examined the postnatal development of c-fos mRNA expression in the rat brain induced by systemic administration of nomifensine (NOM). Subcutaneous injection of NOM (40mg/kg) induced after one hour a high level of c-fos mRNA in the neocortex, striatum and cerebellum, but not in the hippocampus and paleocortex as compared with the saline injected control animals. In neocortex and cerebellum, induction of the c-fos mRNA increased gradually from postnatal day 8 to 49 and dramatically increased from day 23 to 49, especially in the cerebellum. In the striatum, c-fos mRNA induction increased dramatically from day 49. Although c-fos mRNA expression increased gradually every period, the induction was smallest in the hippocampus and no significant difference was observed in the paleocortex. The present results suggest that the maturation of certain neuronal circuits might be needed to develop behavioral sensitization to NOM.

212.13

ONTOGENY OF TYPE 4 PHOSPHODIESTERASE IN RAT BRAIN. K. Zhang, S. Farooqui, N. Leidenheimer* and J. M. O'Donnell. LSU Medical Center, Shreveport, LA 71130. Type 4 Phosphodiesterase (PDE4) is a family of PDEs that have a high affinity for cAMP and are sensitive to a class of selective inhibitors that includes rolipram. Experiments were carried out to determine the development of PDE4 expression and activity. This was accomplished by measuring the kinetics of rolipram-sensitive PDE activity and through the use of immunoblot using an antibody developed against PDE4 (K116). On postnatal day 1 (PND1), the PDE4 activity was about half of that of the adults, and remained so until PND10. On PND15, the PDE4 activity reached adult value and remained so thereafter. This change in PDE4 activity was due to an increase in the Vmax and not a change in Km. Immunoblot of the adult cerebral cortical proteins with K116 showed 4 protein bands at 115, 105, 100 and 93 KD respectively. The expression of 115 and 93 KD protein was low at birth and reached adult value on PND10. The expression of 105 KD protein was much higher at birth when compared with that of the adult and remained so until PND21. An extra band at 103 KD was found in the cerebrocortex from the neonates. This 103 KD protein diasappeared on PND21. Other brain regions such as brainstem and cerebellum were also studied.

GENESIS OF NEURONS AND GLIA: EGF AND FGF EFFECTS

213.1

ISOLATION OF PROGENITOR CELLS FROM THE OLFACTORY EPITHELIUM WHICH GIVE RISE TO NEURONS, ASTROCYTES, AND OLIGODENDROCYTES. A. Gloster* and F.D. Miller. Montreal Neurological Institute, McGill, Quebec, Canada H3A 2B4.

We have isolated proliferating progenitor cells from the olfactory epithelium of neonatal (P1-9) mice. Two days after isolation in F12/DMEM media supplemented with EGF and B27, most cells were dead or dying. However, a small number of large phase bright cells were present, most of which were attached to the flask bottom, and which over the next 2-6 days began dividing to produce a cluster of cells. On day 4 there were approximately 500 of such cell clusters per pup used in the original isolation. Over the next few days, most of these cell clusters began to lift off from the flask surface to form floating balls of cells. These cells were immunoreactive for nestin, a marker of neuronal stem and progenitor cells. They were induced to differentiate into cells with astrocytic, oligodendritic, and neuronal phenotypes. Six days after the original isolation the cell clusters were transferred to polylysine coated dishes with F12/DMEM media containing B27 and 2% fetal bovine serum. The balls of cells plated down, and within a day began to migrate out of the cell clusters and morphologically differentiate. One month after plating the cells were processed for indirect immunohistochemistry. Some cells were still immunoreactive for nestin, while others were immunoreactive for glial fibrillary acidic protein, galactocerebrosidase, neuron specific β3 β-tublin or neurofilament. Recently we have isolated proliferating cells from adult mouse and rat olfactory epithelium and vomernasal organs which are also nestin positive, and are presently characterizing their phenotype after their differentiation *in vitro*. These results demonstrate the isolation of progenitor cells from the olfactory epithelium, and suggests that these cells are multipotential, and not restricted to becoming olfactory neurons, their normal *in vivo* fate. Supported by Canadian Neuroscience Network.

213.2

GLIAL GROWTH FACTOR ENHANCES SUPPORTING CELL PROLIFERATION IN RODENT VESTIBULAR EPITHELIA CULTURED IN ISOLATION. R. Gu[1], M. Marchionni[2], and J.T. Corwin[1]*. [1]Depts of Otolaryngology-HNS & Neuroscience, Univ. of VA, Charlottesville, VA 22908, [2]Cambridge NeuroScience, Cambridge, MA 02139.

We have investigated the potential of Glial Growth Factor (rhGGF2) to enhance cell proliferation and self-repair in damaged mammalian vestibular sensory epithelia. Sensory epithelia from the utricles of 66 adult female rats were isolated by a one-hour treatment with thermolysin at 500 μg/ml. All edges were trimmed away to remove any non-sensory epithelial cells, then each epithelium was bisected transverse to the striola. Each piece initially was cultured in DMEM/F-12 with 10% FBS for 8 days. Then, experimentals were cultured in that medium supplemented with one of the following factors: rhGGF2 at 12.5 ng/ml, 25 ng/ml, 50 ng/ml, or 100 ng/ml, or TGF-α at 50 ng/ml, or FGF2 at 25 ng/ml for 5 days before fixation. BrdU (3 μg/ml) was added for the last 2 days. Each condition was tested on 20-37 pieces of epithelium. The average number for BrdU-labeled nuclei per epithelium was 1.4±0.4 in controls cultured without the test factors. In epithelia cultured in rhGGF2 the number of BrdU-labeled nuclei was 10.2*±2.5 at 12.5 ng/ml; 19.2*±4.3 at 25 ng/ml; 31.3*±5.5 at 50 ng/ml; and 29.8*±6.3 at 100 ng/ml. Epithelia cultured in TGF-α contained 4.9*±1.3 labeled nuclei. Those in FGF2 contained 0.8±0.3. Additional epithelia were used to assess the effect of FBS concentration on the response to rhGGF2. These were cultured in DMEM/F-12 supplemented with 10% FBS for the first 8 days, then in DMEM/F-12 supplemented with rhGGF2 at 100 ng/ml with different concentrations of FBS (0%, 2.5%, 5% or 10%) for 5 days. BrdU was added for the last two days. BrdU-labeled nuclei averaged 7.7±2.5 in epithelia cultured with 0% FBS; 48.0*±9.1 in 2.5% FBS: 39.4*±5.5 in 5% FBS: and 29.8*±6.3 in 10% FBS. In medium that contained 10% FBS the most effective concentration of rhGGF2 was 50 ng/ml . In the presence of 100 ng/ml rhGGF2 the optimal level of FBS was 2.5%. The results show that rhGGF2 significantly enhances supporting cell proliferation in sensory epithelia isolated from rat utricles. *$p<0.05$ (Supported by NIDCD grant RO1-00200 and the LOVHF.)

213.3

LOCAL REGULATION OF ASTROGLIAL PROLIFERATION THROUGH SPECIFIC CELL-CELL INTERACTIONS. Yuji Nakatsuji* and Robert H. Miller. Department of Neurosci. Case Western Reserve Univ. School of Medicine, Cleveland OH.44106

During development and maturation of the vertebrate CNS, proliferation and survival of distinct populations of neuron and glial precursors is closely regulated. The mechanisms that regulate the proliferation of astroglial precursors are not clearly understood. Using purified populations of neural cells in vitro, we have investigated the growth factor requirements for proliferation and the effects of glial/glial and neuron/glial interactions on the proliferation of type-1 astrocytes and their precursors. We show that type-1 astrocytes and its precursors proliferate in response to serum factors, epidermal growth factor and fibroblast growth factor (FGF). Serum driven proliferation of type-1 astrocytes was specifically inhibited by density dependent contact inhibition but unaffected by soluble factors from high density astrocyte cultures. The inhibition of cell proliferation was cells type specific. Co-culture of type-1 astrocytes with smooth muscle cells had no effect on their proliferation. Similarly, the proliferation of O-2A progenitor cells was not inhibited by type-1 astrocytes. In co-cultures of type-1 astrocytes and neurons, local interactions between neurons and astrocytes altered the morphology of the glial cells and promoted their proliferation. Since during development, the majority of astrocyte proliferation occurs after the majority of neurons are born. It may be that the primary inhibitor of astroglial proliferation in the mature CNS is mediated through contact between adjacent astrocytes. Such inhibition is locally lost in pathological conditions such as CNS injury leading to astrocyte proliferation.(Supported by NIH grant 25597)

213.4

ONTOGENY OF CYSTEINE STRING PROTEINS IN PRENATAL RAT BRAIN AND STEM CELL CULTURES.

M.L. Cordeiro[1], S.D. Zurcher[1], A. Mastrogiacomo[1], C. Gundersen[1*]; and H.I. Kornblum [1,2] Depts. of Molecular and Medical Pharmacology[1] and Pediatrics[2], UCLA School of Medicine, Los Angeles, CA 90024.

Cysteine string proteins (CSPs) are intrinsic components of synaptic vesicle membranes. These relatively low mass proteins appear to be involved in the interaction of docked synaptic vesicles with presynaptic calcium ion channels. Recently, CSP immunoreactivity was shown to be abundant in synapse rich areas of the adult rat brain. However, little information is available concerning the role of CSPs in developing brain. We therefore have examined the ontogeny of CSP mRNA in embryonic rat brain (ages E11-P0) using *in situ* hybridization with ^{35}S-labeled CSP cRNA. By E11, CSP mRNA was relatively homogeneously distributed throughout the CNS. This pattern persisted through E15. By E18, hybridization could be seen predominantly in gray matter and germinal zones, with little expression in white matter. This pattern became even more prominent by the day of birth. Particularly high levels of hybridization were present in the developing cortical plate and hippocampus at these later stages of embryonic development. Because CSP expression was observed in germinal epithelia, we investigated CSP expression in neural stem cells generated from embryonic rat (E17-E19) basal ganglia or cerebral cortex in the presence of TGFα. Western blot identified CSPs in these stem cell cultures. These results suggest that CSPs may have an important role in early development of the CNS.

(Supported by the UCLA Dept. of Molecular and Medical Pharmacology, Dana Foundation, and NIH).

213.5

NEURONAL INDUCTION OF EMBRYONIC STEM CELLS IN SERUM-FREE MEDIUM. M.F.A. Finley*, S. Devata, and J.E. Huettner. Department of Cell Biology & Physiology, Washington University, St. Louis, MO 63110

Following aggregation and treatment with retinoic acid (RA), embryonic stem (ES) cells differentiate into neurons and glia. Previous work has shown that a variety of cell phenotypes are produced, including both excitatory (glutamatergic), and inhibitory (glycinergic and GABAergic) neurons. In order to learn more about the control of neuronal development, we have begun to study the effect of a variety of growth and differentiation factors on the mature phenotype of ES-derived neurons.

As a prerequisite for this work we have developed appropriate conditions for the efficient induction of neurons in serum-free, defined medium. Initial tests compared Opti-MEM and Neurobasal Medium + B27 supplements (NB + B27) to our standard induction medium: DMEM with nucleosides and 20% calf serum. ES cells survived poorly in Opti-MEM and failed to differentiate. In contrast, ES cells aggregated for 4 days without RA and 4 days with RA (4- / 4+) in NB + B27 yielded a similar proportion of neurons as in complete DMEM (about 40%). Compared to complete DMEM, however, there were roughly half as many cells in total at the end of the induction period in NB + B27.

In the absence of RA, ES aggregates maintained for 8 days in complete DMEM failed to produce neurons; instead, they gave rise to a significant proportion of contractile cells. In contrast, aggregates maintained for 8 days in NB + B27 without RA yielded a small percentage of neurons but no contractile elements. For both 4- / 4- and 4- / 4+ protocols in NB + B27, the addition of 5 ng/ml bFGF and / or TGFα had little effect on either the proportion or total number of neurons obtained.

NIH NS30888; M.F.A.F. holds a Predoctoral Fellowship from the NSF

213.6

CULTURED SPHERES OF CNS PROGENITOR CELLS FROM THE E13 RAT STRIATUM EXPRESS MEMBERS OF THE JAK-STAT FAMILIES.

C. De Fraja[1], L. Conti[1], A. Vescovi [2], M.Mora[2*], S. Govoni[3] and E. Cattaneo[1]

[1]Inst. of Pharmacol. Sciences, Univ. of Milano, Via Balzaretti 9, Milano; [2]Besta National Neurological Institute, MI; [3]Inst. of Pharmacology, Univ. of Pavia, Pavia-IT

Stem/progenitor cells from the CNS of different mammalian species have been isolated by different laboratories, expanded for several periods in culture and their differentiation potential analyzed. We started from very early rat striatal material (E13) which had been mechanically dissociated and plated at different densities onto untreated culture dishes. Cells were exposed to EGF or bFGF, alone or in combination, in the absence of a coating substrate. Rat cells detached from the plate within 1-3 days from plating and floated in suspension. Round aggregates of cells (spheres) were soon becoming visible. By 6-7 DIV their size had highly increased. After this period single spheres were dissociated and cells plated at very low density in growth factor enriched medium. Single cells could then be identified which were capable of generating secondary clones in vitro. Several of these subclones were grown separately and expanded for over two months. It has been shown that similarly derived spheres contain cells that can differentiate into both neurons and glia. By growing these cells we obtained enough material to perform biochemical analyses. We were interested in whether these proliferating CNS progenitor cells expressed members of the JAK-STAT families of proteins, necessary to transduce from the non tyrosine kinase receptors. Western blot analyses and immunoprecipitation studies were performed on lysates obtained from these expanded CNS progenitor cells. Our data show that JAK2 was abundant in these cells. Furthermore, specific expression of some of the Stat molecules has been identified. Selective recruitment of members of the JAK-STAT by specific stimuli may elicit important biological functions in CNS cells. (Funded by Natl.Res.Coun.Italy)

213.7

NON-VIRALLY MEDIATED GENE TRANSFER INTO HUMAN CENTRAL NERVOUS SYSTEM STEM CELLS. A.L.Vescovi*, L.Conti§, P.Frölichsthal, A.Gritti, S.Govoni°, and E.Cattaneo§. National Neurological Institute "C.Besta", via Celoria 11, § Inst. of Pharmacological Sciences, Univ. of Milan, Via Balzaretti 9, 20133 Milan, and ° Institute of Pharmacology, Univ. of Pavia, Italy.

The introduction of foreign genes into cells of the central nervous system (CNS) is a method of fundamental relevance for studying the events occurring in the brain under physiological or pathological conditions. Thus far, the use of such a technique to study the basic events underlying the proliferation, fate commitment and differentiation of human CNS precursor cells has been hampered by the lack of both a suitable source of human multipotential CNS cells and of a reliable method to deliver genes into these specific cell type. We report here the successful delivery of foreign genes into multipotential human CNS stem cells. By means of lipofectamine-based transfection the bacterial β-galactosidase or the temperature sensitive allele of the SV40 Large T antigen gene were delivered into foetal (10.5 weeks post-conception) forebrain stem cells that had been grown and expanded for over one year *in vitro*. A transfection efficiency of 7.4% was achieved and expression of the foreign genes was detected in both undifferentiated cells and differentiated neuronal and glial cells. Thus, our data show the possibility of targeting exogenous genes to undifferentiated human CNS stem cells and to their progeny, and define an efficient transfer approach alternative to viral-based methodologies. Supported by the Alzheimer's Association/The Hearst Corporation Pilot Research Grant 94-057 to E.C. and by the Ministry of Health, Italy (ICS49.2/RS93.42) to A.L.V.

213.8

DIFFERENTIATION OF EGF-RESPONSIVE STEM CELL PROGENY; A TIME COURSE ANALYSIS UNDER VARIOUS CULTURE CONDITIONS.

A.Gritti, M.Ferrario, L. Cova, R.Galli, E.Parati* and A.L.Vescovi. Natl. Neurol. Inst. "C.Besta", Via Celoria 11, 20133 Milan, Italy.

The establishment of a culture technique enabling the continuous and expansive growth of multipotential CNS stem cells, in the presence of EGF, has raised the intriguing possibility of preparing mixed neuronal/ glial cultures avoiding the repeated use of primary brain tissue. Unfortunately, cultures from EGF-generated cells that are induced to differentiate by removal of EGF contain only a small percentage of neuronal cells, as compared to those obtained using traditional techniques. In order to clarify this phenomenon, we studied the time course of expression of antigenic markers specific for neuronal, astroglial or oligodendroglial cells, in cultures of EGF-generated cells differentiating under various conditions. We found that the percentage of neuronal cells increased at day 2, 4 and 6 after EGF removal, reaching a maximum of 10-12% at this later time point. The number of neurons then dropped over time, reaching a minimum (3-5%) at 12 days, regardless of the presence or absence of serum. Conversely, astrocytes were barely detectable at day 2 but their number increased overtime reaching a maximum at 12 days. The presence of 2% serum strongly affected the percentage of astrocytes in the culture. Serum-containing cultures embodied 15-25% more astrocytes (reaching a maximum of 60-70% at 12 days) than serum-free cultures. Our data indicate that culture conditions can strongly affect the differentiation of stem cell progeny and show that a great deal of death occurs in these cultures during early stages of neuronal differentiation. Further investigations are currently underway to confirm preliminary results showing that FGF-2 may antagonise neuronal death in this system. Supported by The Ministry of Health, Italy (ICS49.2/RS93.42).

213.9

INSULIN-LIKE GROWTH FACTOR-I (IGF-I) IS A POTENT DIFFERENTIATION FACTOR FOR NEURONAL PRECURSORS GENERATED BY EGF-RESPONSIVE CNS STEM CELLS

Yvan Arsenijevic and Samuel Weiss*. Neuroscience Research Group, University of Calgary Faculty of Medicine, Calgary, AB, Canada.

Little is known regarding the action of IGF-I on neuronal precursors of the CNS. We examined the actions of IGF-I, IGF-II and insulin on neuronal precursors generated by embryonic EGF-responsive stem cells. When neuronal precursors were plated in the presence of 1% serum, but in the absence of any insulin family member, very few neurons were detected after seven days in vitro (DIV). IGF-I induced a dose-dependent (EC_{50}, 5.5±1.8 nM) 10-40 fold increase in neuron number. IGF-II and insulin were equieffective, but 7- and 50-fold less potent, respectively. The presence of IGF-I receptor immunoreactivity and IGF-I actions in low density platings suggest a direct action on neuronal cells. Only 10% of the neurons observed in the presence of IGF-I incorporated bromodeoxyuridine, suggesting that its actions are largely not mitogenic in nature. In addition, both short term and delayed administration of IGF-I closely mimicked its actions when present during the entire culture period, suggesting that IGF-I promotes differentiation and not survival. Further support for this conclusion was a dose-dependent increase in neurite number by IGF-I, which did, however, require the continued presence of the growth factor. These results suggest that IGF-I is a potent differentiation factor for CNS stem cell-generated neuronal precursors.

Supported by the Medical Research Council of Canada and a Swiss Foundation for Medicine and Biology Fellowship to Y.A.

213.10

EPIDERMAL GROWTH FACTOR-RESPONSIVE NEURAL STEM CELLS FROM VARIOUS REGIONS OF MURINE EMBRYONIC CNS: DIFFERENCES IN POTENTIAL FOR NEURONAL DIFFERENTIATION.

T.J. O'Connor*, A.L. Vescovi, P. Hettiaratchi, and B.A. Reynolds. NeuroSpheres Ltd., Calgary, Alberta, Canada T2N 4N1

We have previously isolated epidermal growth factor (EGF)-responsive multipotential neural stem cells from embryonic E14 mouse striatum (Reynolds et al, 1992; J. Neurosci. 12:4565-74), and the subependymal layer of adult mouse striatum (Reynolds and Weiss, 1992; Science 255:1707-10). In this study, we investigated the distribution of EGF-responsive stem cells throughout the developing (E14) mouse CNS. EGF-responsive cells could be isolated from murine embryonic cortex, thalamus, ventral mesencephalon, and spinal cord, as well as striatum. Cultures from all regions could be passaged repeatedly to generate large numbers of progeny, which retained the capacity to differentiate into neurons, oligodendrocytes, and astrocytes. Intriguingly, significant differences in the capacity to produce neuronal progeny were found among cultures from the various regions, with stem cells from more rostral regions generating more neuronal progeny. These findings may suggest the presence of distinct populations of EGF-responsive precursor cells in the embryonic CNS.

213.11

PACAP LIGAND AND RECEPTOR EXPRESSION IN EMBRYONIC CEREBRAL CORTEX

N. Lu* and E. DiCicco-Bloom. Dept. of Neurosci. & Cell Biol. UMDNJ/Robert Wood Johnson Medical School, Piscataway, NJ 08854

While the transition from proliferation to differentiation in cerebral cortex precursors is well-characterized *in vivo*, regulatory signals remain undefined. Previously, we found that the 38 amino acid peptide, PACAP, promoted this ontogenetic switch in cultured E13.5 cortical precursors, inducing mitotic arrest while enhancing differentiation. Further, precursor expression of PACAP and receptor as well as peptide blocking studies indicated a functional autocrine circuit *in vitro*. To define function *in vivo*, we have examined PACAP ligand and receptor expression in developing brain.

To evaluate mRNA expression *in vivo*, 30µg of total RNA from E13.5 cortex was used for Northern analysis. Two bands at 2.8kb and 3.7 kb were detected with a cDNA fragment hybridizing to the full PACAP coding region. In addition, a 7.5kb band for type I receptor mRNA was detected, consistent with previous studies in adult brain. Further, since PACAP type I receptor exhibits 5 splice variants, RT-PCR was employed to define region-specificity: embryonic cortex exhibited the "short" variant, while embryonic sympathetic ganglia expressed the "hop" insert variant. Thus, both ligand and receptor mRNAs are expressed during cortical neurogenesis.

To define protein expression and localization, we performed immunohistochemistry on E13.5 brain sections. PACAP expression was most abundant in cortex and dorsal hippocampus, and was localized to the cytoplasm of ventricular zone precursors. To define relationships of peptide and receptor to proliferation, acutely dissociated cells were plated for 3 hrs, and double-labeled with PACAP/receptor and mitotic marker, BRDU. PACAP and receptor were expressed by >70% of cortical precursors. Further, both mitotic and non-mitotic precursors expressed peptide and receptor, consistent with PACAP inhibition of proliferation and promotion of differentiation. In sum, the expression of PACAP and receptor mRNA and protein in embryonic cortex suggests that the peptide plays a critical autocrine/paracrine role in neurogenetic regulation. (Supported by RO1 NS32401)

213.12

SELECTIVE TRANSFER OF BASIC FIBROBLAST GROWTH FACTOR (bFGF) ACROSS THE MATURE BLOOD-BRAIN BARRIER.

J.P. Wagner*, J.B. Black, & E. DiCicco-Bloom. Dep't of Neurosci. & Cell Biol., UMDNJ/Robert Wood Johnson Med. Sch., Piscataway, NJ.

Since most polypeptide growth factors do not cross the blood-brain barrier (BBB), their therapeutic utility in neurodegenerative disease has been limited by the development of appropriate delivery systems. Previously, we reported that a small dose of bFGF (5 ng/g body weight) injected subcutaneously (s.c.) into adult rats stimulated mitosis in multiple brain regions, suggesting that bFGF can exert effects across the mature BBB. The current study investigates bFGF transfer and defines molecular selectivity.

To verify entry of bFGF into the brain, 28 day old rats (n=3) were given 5 µCi of ^{125}I-bFGF s.c. After four hours, cerebrospinal fluid (CSF) and blood were collected, and levels of ^{125}I radioactivity were quantitated. Approximately 0.06% of the total injected dose was measured in the CSF, while CSF/blood ratio of the factor was 3.5%, values similar to those obtained from other compounds which readily cross the BBB, such as morphine. Furthermore, ^{125}I -bFGF was localized within the brain parenchyma *per se*, as determined by a capillary depletion assay, suggesting that peripherally administered factor permeates the BBB, gaining access to both the CSF and brain.

To define the molecular selectivity of this transport mechanism, we first assessed the mitogenic capacity of multiple members of the FGF family on cerebellar granule neurons in culture. Six FGF's were equally effective in stimulating mitosis *in vitro* (aFGF, bFGF, and FGF-4-7); we next defined the activity of these six factors *in vivo*. Although all six family members stimulated cerebellar mitosis after s.c. injection, mitogenic activities varied markedly; bFGF and FGF-7 were most effective. However, in the hippocampus, another potentially responsive region, only bFGF stimulated mitosis after s.c. injection. This region-selective activity of peripherally administered FGF's, possibly due to regional differences in uptake and/or FGF-receptor expression, is currently being evaluated to characterize this potentially useful therapeutic strategy. (Support: NICHD23315; Trophix Pharm.)

213.13

EXPRESSION OF NOTCH AND JAGGED IN TGFα-GENERATED NEUROSPHERES

S.D. Zurcher[1]*, C.J. Shawber[2], G. Weinmaster[2], and H.I. Kornblum[1,3], Depts.of Molecular and Medical Pharmacology[1], Biological Chemistry[2] and Pediatrics[3] UCLA, LA, CA 90095

Transforming growth factor alpha (TGFα) binds and activates the EGF receptor to induce proliferation of neural stem cells to form spheres. These "neurospheres" are composed of cells that can give rise to both neurons and glia. Cell-cell interactions may regulate cell type differentiation of these stem cells. However, the molecular mechanisms that underlie these decisions are not well defined. In *Drosophila*, cell-cell interactions between the receptor *Notch* and its ligand *Delta* have been shown to regulate cell fate choices. In vertebrates, a family of *Notch* receptors, comprised of 4 *Notch* genes, and a family of potential *Notch* ligands (*Jagged*, *Serrate-2* and *ratDelta1*) have also been isolated. To investigate a role for *Notch* and its ligands in the proliferation and differentiation cells within the neurospheres, we have examined the expression of *Notch1*, *Notch2*, *Jagged* and *ratDelta1*. Neurospheres were generated from rat (E17-19) embryonic rat striatum and were stimulated to divide by the addition of TGFα for 2 weeks before processing. Cells from these neurospheres have the property of self-renewal and that they generate neurons and glia. By Nothern blot analysis, the neurospheres express *Notch1*, *Notch2*, *Jagged* and *ratDelta1* mRNA. Western blot analysis confirmed the expression of *Jagged* protein in the cells from neurospheres. Cells cultured from dissociated neurospheres revealed that both neurons and Nestin positive undifferentiated cells express *Notch1* and *Jagged* by immunocytochemistry. The expression of *Notch* and its ligands in cells within the neurospheres, as well as those cells derived from the neurospheres, is consistent with the proposed role for these genes in the development of the mammalian brain. (Supported by the Dana Foundation, NIH/NINDS)

213.14

BASIC FIBROBLAST GROWTH FACTOR AFFECTS THE PROLIFERATION AND DIFFERENTIATION OF RAT CORTICAL PROGENITORS IN VITRO, BUT DOES NOT ALTER THEIR CELL CYCLE.

I.F.R.Cavanagh, M.C.Mione* and J.G.Parnavelas, Dept. Anatomy & Dev. Biol., University College London, London WC1E 6BT, UK.

We have examined the effect of basic fibroblast growth factor (bFGF) on cortical progenitor cells in vitro, using cells prepared from E16 rat embryos. Even after 48 h, bFGF had doubled the rate of proliferation of these cells, as measured using BrdU incorporation. Analysis of cell cycle parameters using the labelled mitosis method revealed that this increase was not due to any alteration of the lengths of either the cell cycle or any of its phases. This growth factor was also found to prolong the mitotic ability of these cells. Retrovirally-labelled clones grew significantly larger in the presence of bFGF, and BrdU labelling further showed that nearly all the cells in these clones were still cycling after 4 DIV. In defined medium, most of the clonally-related cells ceased dividing after 2 DIV, equivalent to only four cell cycles. An additional effect of bFGF on cortical progenitor cells in culture was to stimulate previously quiescent cells to reenter the mitotic cycle. All of those attempting to do so in defined medium instead underwent apoptosis. The extent of neuronal and glial differentiation was studied after 5 DIV. In the presence of bFGF, the percentage of cells immunoreactive for MAP-2 was less than half that of control cultures. Conversely, the percentage of cells immunoreactive for nestin (a marker of progenitor cells) was as high in the presence of bFGF as at the start of the experiment. These experiments show that bFGF, a potent mitogen for cortical progenitor cells, has no effects on the parameters of the cell cycle but extends the proliferative period of these cells, promotes their survival, and delays their differentiation into neurons.
Supported by the Wellcome Trust.

213.15

SYNERGYSTIC EFFECTS OF EGF AND FGF-2 ON THE EXPANSION OF RAT CNS PRECURSOR CELLS FROM PRIMARY EMBRYONIC TISSUE. M.G. Terborg, A.E. Rosser, P. Tyers, S.B. Dunnett* and C.N. Svendsen
MRC Cambridge Centre for Brain Repair, University of Cambridge, UK

Both EGF and FGF have been shown to promote the division of CNS precursors from either embryonic or adult rat CNS tissues. Although it has been suggested that EGF may drive a multipotent stem cell while FGF may effect a bi-potent neuronal/glial precursor, the contribution of each to the division of specific cell types remains poorly understood. In this study precursor cells were isolated from the striatal eminencies of E15 rat embryo's and cultured in EGF, FGF-2, or a combination of EGF and FGF-2 for a period of 5 weeks at an initial plating density of 200,000 cells per ml in 20 mls of medium, and subsequent densities of 100,000 cells per ml at each passage (every 7d). No substrate and no serum was used and the cells generally grew as free floating or attached spheres. The number of viable cells plated and the number of viable cells at the end of each week were assessed and an "expansion ratio" generated (number of cells at end divided by number of cells at start) where 1 indicates no change, <1 a decrease in cell number and >1 an expansion in cell number. FGF-2 alone did not result in any expansion of striatal precursors between any of the passages with expansion ratios of approximately 0.5, 0.7, 0.1, 0.0 and 0.0 at 1, 2, 3, 4 and 5 weeks respectively. EGF resulted in expansion ratios of approximately 2.5, 5.6, 3.6, 0.7 and 0.0 at each week suggesting strong initial growth followed by a sharp reduction and cell senescence. Combined application of EGF and FGF-2 resulted in expansion ratios of 5.2, 5.1, 1.7, 0.8 and 0.1 at each week showing a strong synergy over the first week in vitro, a possible reduction in expansion between 2 and 3 weeks and a lack of effect on the late senescence of the rat precursors. These results suggest a strong interaction between EGF and FGF-2 on striatal precursors after plating from primary tissue, but that continued application of both factors may decrease cell expansion thereafter. Supported by the Wellcome Trust and MRC.

213.16

b-FGF AS CELL CYCLE MODULATOR *IN VITRO* IN THE NEOCORTICAL PROLIFERATIVE EPITHELIUM OF EMBRYONIC MICE. T. Goto[1,2*], T.Takahashi[1,2], P.G.Bhide[1], S.Miyama[1,2] and V.S.Caviness, Jr[1].
1.Department of Neurology, Massachusetts General Hospital, Harvard Medical School, Boston, MA 02124, 2.Department of Pediatrics, Keio University School of Medicine, Tokyo 160.

b-FGF is an established mitogen for the neocortical pseudostratified ventricular epithelium (PVE)(*Ghosh and Greenberg, Neuron 15:89-103, 1995*). Here we examine the effect of b-FGF upon progression of cells of the PVE into S phase in explants of the cerebral wall *in vitro*. Explants of the full width of the cerebral wall at the 13th embryonic day (E13) were "punched out" by glass pipette trocar, immediately implanted in collagen gel, cultured at 37°C for 5 hr in DMEM/F12 media only (control) and in DMEM/F12 containing 100 ng/mL of b-FGF and then for an additional 6 hrs after the addition of 10 μM of the S phase marker, BUdR. Cells marked with BUdR were stained immunohistochemically and the labeling indices (LI) within coronal sectors of the PVE, subdivided in its radial dimension into 10 μm bins, were determined in the medial cortical (MCZ) and lateral cortical (LCZ) zones, corresponding to distances of approximately 300 μm and 1000 μm, respectively from the caudato-pallial angle of the VZ. The overall LI for the full thickness of the PVE after 6 hr exposure to BUdR is 0.6 in the MCZ and 0.4 in the LCZ of both control and b-FGF-exposed PVE. However, in the LCZ, the LI in the outer half of the PVE (S-phase zone, a zone where the PVE cells are undergoing S phase) are higher in the b-FGF-exposed PVE than control. These findings are consistent with an action of b-FGF to accelerate the entry of G1 cells into the next S-phase or, alternatively, to reduce the fraction of cells which leaves the cycle after division. Such an effect of b-FGF is not observed in the MCZ where the duration of G1 is only 60 % of that in the LCZ (Miyama et al., unpublished).
Supported by NIH grant NS12005.

213.17

A DIFFERENCE IN GROWTH RATES BETWEEN RAT AND MOUSE EPIDERMAL GROWTH FACTOR RESPONSIVE CNS PRECURSOR CELLS. C.N. Svendsen*, A.E. Rosser, M. Terborg, P. Tyres and T. Rykin MRC Cambridge Centre for Brain Repair, University of Cambridge, UK

There are numerous reports where populations of precursor cells have been isolated *in vitro* from both the embryonic and adult rodent CNS which can then be induced to divide using epidermal growth factor (EGF) as a mitogen. In addition, EGF responsive precursor cells within the adult subventricular zone have also been shown to divide, both *in vivo* and *in vitro*, suggesting that similar cells may exist in specific brain regions throughout life. However, many of these studies have focused on the mouse rather than the rat. More specifically, continued expansion of EGF responsive precursors *in vitro* over a period of months has only been described using mouse tissues. We have performed a direct comparison of the growth characteristics of EGF responsive precursors from either the rat or mouse embryonic striatum and shown that while the mouse cells could be continually expanded as spheres of cells for over 50 days, under identical culture conditions rat cells could only be expanded for between 21 and 30 days, after which the cells reduced in number and died. The centre of the rat spheres were full of dying cells while dividing cells could be seen towards the outside based on exclusion of trypan blue, confocal imaging of rhodamine uptake up and electron microscopy. Various additions to the medium including B27, n-acetyl acetate or LIF did not prevent this senescence of the rat cells. Our data suggest that mouse CNS precursor cells have inherent properties which allow them to divide for extended periods of time as free floating spheres of cells in stark contrast to rat cells which have only limited proliferative potential under the conditions described here. Supported by the Wellcome Trust and MRC.

GENESIS OF NEURONS AND GLIA: MECHANISMS AND KINETICS

214.1

DIFFERENTIAL GENE EXPRESSION IN THE DEVELOPING TELENCEPHALON OF MUTANTS LACKING THE WINGED HELIX TRANSCRIPTION FACTOR BF-1. C.A. Baptista*, G. Balas and E. Lai, Cell Biology and Genetics Program, Division of Endocrinology, Cornell University Graduate School of Medical Sciences, Memorial Sloan-Kettering Cancer Center. New York, NY 10021.

We previously reported that the transcription factor Brain Factor-1 (BF-1) is required for the normal development of the cerebral hemispheres after embryonic day 10 (E10; Xuan et al., 1995). In animals homozygous for the BF-1 deletion the ventral telencephalic neuroepithelial cells stop proliferating after E10 causing the complete absence of the basal telencephalon. In contrast, dorsal telencephalic neuroepithelial cells continue to proliferate, however, they originate smaller dorsal structures because of a premature exit from the cell cycle and differentiation into neurons. We postulated that BF-1 normally carries out its function by upregulating genes that promote cell cycle progression and/or downregulating genes that inhibit progression through the cell cycle thus, keeping telencephalic neuroepithelial cells actively proliferating. In order to find BF-1 target genes we initiated a subtractive hybridization procedure. Briefly, directional cDNA libraries were constructed in phagemid vectors from E10 wild type and mutant animals just before the mutant phenotype is apparent. Single stranded DNA and biotinylated RNA, each made from one of the cDNA libraries, were hybridized and the mixture incubated with streptavidin to remove the commom sequences. The single stranded DNA in the unbound fraction (enriched for wild type or mutant specific DNA sequences) was collected and used to construct subtracted libraries. We will report the results of the ongoing analysis of differentially expressed clones. These genes will further clarify the molecular mechanisms which control the development of the cerebral hemispheres.
Supported by NIH grant RO1 HD29584.

214.2

THE ROLE OF THE RETINOBLASTOMA PROTEIN IN NEURONAL COMMITMENT AND TERMINAL DIFFERENTIATION R.S.Slack, H.El Bizri, J.Wong, D.J.Belliveau, A.Gloster, R.Varma, A. Speelman, F.D.Miller*, Montreal Neurological Institute, McGill University, Montreal, P.Q. H2A 2B4

We questioned whether the retinoblastoma protein (pRb) is required to make a commitment decision, or do neurons commit to their fate, express neuronal markers, and undergo apoptosis due to failure to undergo terminal mitosis. To distinguish these possibilities we have used pRb nullizygous mice (Jacks et al., 1992) and established a colony expressing the Tα1 α-tubulin promoter driving a nuclear β-galactosidase transgene. The reporter gene is induced at the point of commitment to a neuronal fate, coincident, or in many cases prior to, terminal mitosis (Gloster et al., 1994). β-galactosidase expression was analyzed throughout the nervous system of these mice at timepoints ranging from E10.5 to E14.5. We detected abnormal neuronal development throughout the nervous system, including the cortex, the retina and the olfactory epithelium, regions which were previously reported to be spared. Our observations support the hypothesis that neuronal commitment occurs prior to terminal mitosis in all regions examined. However, once migration has commenced and cells fail to exit from the cell cycle, apoptosis takes place. This is best exemplified in the developing olfactory epithelium in which the loss of LacZ-positive cells coincides with the appearance of TUNEL-positive cells. Consistent with this interpretation is the fact that nestin staining in Rb -/- mice is similar to controls. Cortical progenitors cells in which the pRb family has been ablated with specific deletion mutants of the adenovirus E1A oncoprotein undergo apoptosis concomitant with terminal mitosis. These studies indicate that pRb is not essential for the mainenance of progenitor cells but is essential for terminal mitosis and differentiation of cortical neurons. Supported by the Neuroscience Network and the Medical Research Council of Canada.

214.3

ALTERED CELL PROLIFERATION IN THE SPINAL CORD OF THE MOUSE NEURAL TUBE MUTANT PAX3 SPLOTCH-DELAYED. C.R. Keller-Peck* and R.J. Mullen. Program in Neuroscience, Dept. of Neurobiology & Anatomy, University of Utah School of Medicine, Salt Lake City, UT 84132.

Homozygous Splotch-delayed mouse embryos develop lumbosacral myeloschisis (an open neural tube) as well as abnormalities in neural crest migration. A single base pair substitution in the *Pax3* gene produces this phenotype. The Pax3 protein, expressed in the dorsal neural tube and dermomyotome beginning at embryonic day 8 (E8), is likely regulating extracellular matrix and cell adhesion molecules. We have utilized this mutant to investigate the role normal neural tube closure plays in neuronal proliferation. The thymidine analog BrdU was injected intraperitoneally into pregnant +/$Pax3^{Spd}$ females between E10 and E15. Embryos were removed 24-48 hours after injection, embedded, serial sectioned transversely through the entire lumbosacral region, and stained immunohistochemically. PCR primers were devised to genotype embryos so that heterozygous embryos could be differentiated from their wild-type littermates. Cell counts were made by focusing through the section and counting heavily labeled nuclei. At E10, the number of BrdU positive cells is decreased relative to controls. In contrast, there are significantly more cells born in affected embryos between E11 and E13. Our results indicate that more total cells are born in the spinal cord of spina bifida embryos ($Pax3^{Spd}/Pax3^{Spd}$) than are born in the spinal cord of normal (+/+) littermates. Closure of the neural tube may have an antimitotic activity on proliferating cells of the nervous system. (Supported by NIH Grant HD28845)

214.4

THE ROLE OF NEUROBLAST PROLIFERATION IN THE ABNORMAL DEVELOPMENT OF THE MOUSE TRISOMY 16 CEREBRAL CORTEX. **T.F. Haydar*, B.K. Krueger, and P.J. Yarowsky#. Depts. of Physiology, and #Pharmacology, University of Maryland School of Medicine, Baltimore, MD 21201.**

Neuroblast proliferation subserves two distinct roles during corticogenesis. Initially, dividing neuroblasts in the ventricular zone give rise to daughter cells which re-enter the cell cycle, causing an exponential tangential expansion of the proliferative epithelium. This tangential cortical expansion is followed by radial growth of the pallial layers, beginning around embryonic day 11 in the mouse, as daughter cells become post-mitotic and migrate outward, away from the pool of dividing cells. Previously, we described abnormalities in corticogenesis and neuronal differentiation in the trisomy 16 (Ts16) mouse cortex, a model of Down syndrome {*Soc. Neurosci. Abstr.* 21:30 (1995)}. We found that radial expansion of the cortex was abnormal in Ts16. Here we show that two separate defects are present during morphogenesis of the Ts16 cortex. First, gross brain measurements indicate that the tangential expansion of the Ts16 cortex is persistently retarded compared to normal littermate brains. Second, we confirmed our earlier finding that beginning at E14, Ts16 cortical growth is characterized by a thinner cortical plate and intermediate zone although the ventricular zones appear normal. We now report that this aberrant radial growth is just a delay, as the Ts16 cortex and cortical layers attain normal thickness shortly before birth. We hypothesized that decreased proliferation, of Ts16 neuoroblasts could adversely affect the progression of the tangential and radial cortical expansion. To explore this possibility, we used bromodeoxyuridine and ^{3}H-thymidine to characterize the cell cycle of the cortical progenitor population. We report that cell cycle kinetics in the Ts16 cortex are slower than controls. This may partly explain the observed growth retardation of the cortex. **NIH T32GM08181, AG10686, and SRIS, Univ. Maryland.**

214.5

PROX1 PROTEIN EXPRESSION IN MOUSE CNS DEVELOPMENT H. Dou and C. Q. Doe*. HHMI, Dept. of Cell and Structural Biology, Univ. of Illinois, Urbana, IL 61801.

Cellular diversity within the Drosophila and vertebrate CNS is generated, in part, by the asymmetric division of neural precursor cells. In Drosophila, the prospero (pros) gene encodes a homeodomain transcription factor that is asymmetrically segregated into the daughter cell during mitosis of the neural precursor. Prox1, a murine homolog of pros, is transcribed in the CNS and many other tissues during development. Here we examine the protein distribution of Prox1, the mouse pros homolog, focusing on the PROX1 distribution in dividing ventricular zone (VZ) precursors.

A rabbit polyclonal antibody was made against a C-terminal 30 amino acid peptide shared by all known vertebrate pros homologs (frog, chick, mouse, human; S. Tomarev, pers. comm.; C. Kintner, pers. comm.). Affinity purified PROX1 antibody specifically detects a protein of 75 Kd in adult brain Western blots, although other bands are detected in embryonic extracts. Histochemical staining of tissue sections from embryos (E10, E12, E14) and adult brain shows nuclear localization of PROX1. Confocal immunofluorescent imaging of mitotic VZ precursors shows that VZ precursors with both vertical or horizontal divisions have cytoplasmic PROX1 protein distribution; no evidence for asymmetric cortical localization is observed. PROX1 is detected in an increasing number of spinal cord and brain VZ cells from E10 to E14, and in a subset of differentiated neurons persisting into the adult. PROX1 is detected in both lens and retinal cells in the eye. In the adult, the highest level of PROX1 is in cerebellar Purkinje cells, both in the nucleus and in dendritic processes. Our data suggests that PROX1 is a transcription factor involved in the early CNS development as well as in the function of mature neurons in the adult.

Supported by NIH 27056 and HHMI. C.Q. Doe is an Assistant Investigator of HHMI.

214.6

TEMPORAL AND SPATIAL EXPRESSION OF $P21^{Cip1/WAF1}$ IN THE DEVELOPING MURINE NEOCORTICAL CEREBRAL WALL. I.Delalle* and V.S. Caviness, Jr. Neurology, Massachusetts General Hospital and Harvard Medical School, Charlestown, MA 02129.

The length of the cell cycle (Tc) in the murine neocortical pseudostratified ventricular epithelium (PVE) increases from ~10 to ~20 hrs during the neurogenetic interval E11-E17. The increase in Tc is due solely to an increase in the length of the G1 phase (Tg1). In the E12-E15 interval Tg1 is distributed as a rostrolateral to caudomedial gradient with values of Tg1 laterally greater than those medially. We have previously determined that the lateral to medial gradient in Tg1 between E12 and E15 is associated with down regulation in the rate of expression of cyclin E, a regulatory subunit of cyclin-dependent kinase 2 (Cdk2) and necessary for the G1 to S phase transition. Here we explore, by means of *in situ* hybridization with S^{35}-labeled riboprobe, the lateral to medial expression at E12 and E15 of $p21^{Cip1/WAF1}$, a cell-cycle inhibitor, that binds and inhibits Cdk2. At both E15 and E12, p21 mRNA is present in both the proliferative and nonproliferative populations of the cerebral wall with the density of expression variably 30% to 2 times greater in strata dominated by nonproliferative cells than in the PVE. The average density of p21 expression in the PVE, in contrast to that determined earlier for cyclin E, is indistinguishable at E12 and E15, whether in the medial or lateral cerebral wall. The presence of p21 mRNA in the PVE suggests that this inhibitor plays a role in cell cycle regulation of this proliferative population. Because average expression of p21 mRNA in the PVE is invariant across widely ranging values of Tg1, it is probable that the level of p21 activity is regulated posttranscriptionally as appears to be the case for the cell cycle inhibitor $p27^{Kip1}$. (by NINDS grant NS12005).

214.7

REGIONAL DIFFERENCES IN COMPOSITION OF RETROVIRALLY LABELED CLONES DURING EARLY DEVELOPMENT OF THE MOUSE NEOCORTEX. R.S. Nowakowski*, L. Cai, and N.L. Hayes. Department of Neuroscience and Cell Biology, UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ 08854.

A lateral-to-medial gradient in maturation of neocortex during development has been well-documented (Smart, 1985). To investigate the relationship between regional maturation and differences in proliferative behavior in the pseudostratified ventricular epithelium (PVE), we injected a replication-incompetent retrovirus into the lateral ventricles of fetal mouse brains on embryonic day 11 (E11); animals were sacrificed 3 days post-injection at E14. AP-labeled clones were evenly distributed across the entire surface of the developing neocortex indicating that there were no regional differences in the likelihood of infection of the retrovirus. However, the clone composition defined by the inclusion of proliferative cells and post-mitotic cells varied regionally. Clones containing only postmitotic cells (Q-clones) were concentrated in the ventrolateral region, whereas clones containing only proliferating cells (P-clones) and clones containing both proliferating and postmitotic cells (PQ-clones) were concentrated in a band extending from rostral-to-caudal across the dorsal region of the neocortex. The differences in clone compostion were correlated with the maturity of the cortex as assessed by the thickness of the cortical plate and corresponded to the known lateral-to-medial gradient in maturation. This correlation indicates that clone composition within the developing cortex is determined by the progression in the proportion of PVE cells leaving the cell cycle (Q) vs those re-entering S (P). In addition, since PQ-clones tend to be largest clones, the state of maturation of the PVE in different regions will affect the sizes of retrovirally labeled clones found in different regions of cortex.

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214.8

CLONE SIZE DURING NEOCORTICAL DEVELOPMENT: AGREEMENT OF EXPERIMENT AND PREDICTION. L. Cai*, N.L. Hayes and R.S. Nowakowski. Dept. of Neuroscience and Cell Biology, UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ 08854.

Neuronogenesis in the developing cortex of the mouse occurs in a pseudostratified ventricular epithelium (PVE) over a 6 day period beginning at embryonic day 11 (E11) and ending on early E17. During this period: there are 11 cell cycles that lengthen from about 8 hr to about 20 hr, the proportion of cells that leave the cell cycle to become postmitotic or quiescent (Q) increases monotonically from 0 to 1, and the complementary proportion of cells that continue to proliferate (P) decreases from 1 to 0. To investigate the effects of these dynamic changes in the proliferative behavior on clone size, we injected a replication-incompetent retrovirus encoding human placental alkaline phosphatase (AP) into the lateral ventricle of E11 fetal mouse brains. After survival for 2, 3 and 4 days, AP-labeled clones were evenly distributed across the neocortex. The number of cells in each clone was counted, and the distribution of clone sizes was compared with the distribution of clone sizes calculated from cell cycle lengths, Q and P [as determined using double labeling with bromodeoxyuridine and tritiated thymidine (Takahashi, et al., 1995)] for the corresponding developmental period. The important finding is that the distribution of clone sizes from the retroviral experiments was in substantial agreement with the calculated distribution. This agreement of experimentally determined clone sizes with calculated histograms made from Q and P measurements means that the average behavior of the population (i.e., the S-phase label data) can be used to predict the range of behaviors of the individual members of the population (i.e., the retroviral clone size data). Thus, during early development of the neocortex the major determinant of clone size in retrovirally labeled lineages is the developmental progression of Q and P. (Supported by NIH (NS33443) and NASA (NAG 2-950).)

214.9

THE RATE OF CELL OUTPUT AND THE PATTERN OF CELL MIGRATION IN THE EMBRYONIC MOUSE CORPUS STRIATUM. P. G. Bhide* and A. N. Sheth, Neurology Department, Massachusetts General Hospital and Harvard Medical School, Charlestown, MA 02129.

The rate of cellular output is a major regulator of cytogenesis because it is vulnerable to the action of a variety of cell-intrinsic and extrinsic factors. We examined this parameter in the mouse striatum on embryonic day 11 (E11; E0=day of conception), the earliest stage of striatal cytogenesis. Tritiated thymidine and bromodeoxyuridine were used in combination to distinguish between mitotically active and postmitotic progeny. About 30% of the daughter cells "born" on E11 become postmitotic; the remaining re-enter the proliferative pool. The majority (~94%) of the postmitotic cells rapidly exits the proliferative zone in a tight cluster leaving in its trail a slowly migrating minority. As the cells enter the postmitotic zone on E12, they apparently freely intermingle leaving no indication of their previous segregation. Thus, cellular migratory cues in the proliferative zone may differ from those in the postmitotic zone. The daughter cells which re-enter the cell cycle on E11 also partition into 2 spatially distinct cohorts within the proliferative zone: some remain near the ventricular border as a part of the pseudostratified ventricular epithelium while the others migrate away to "seed" a secondary proliferative population. The relatively high rate of cellular output and the partitioning of the proliferative cells into 2 groups by E11 are the earliest signs of the emergence of a cytokinetically distinct striatal proliferative domain within the seemingly homogeneous telencephalic neuroepithelium and this may presage the eventual formation of striatal and neocortical compartments of the forebrain. *Supported by NINDS grant #NS-R29-32657.*

214.10

SEX DIFFERENCES IN THE CELL CYCLE PARAMETERS OF THE PROLIFERATIVE VENTRICULAR EPITHELIUM (PVE) IN THE DEVELOPING RAT PREOPTIC AREA. V.K. Chetverukhin*[1], E.V. Chernigovskaya[1], O.A. Danilova[1] and R.S. Nowakowski[2]. [1]Sechenov Institute of Russian Academy of Sciences, 194223 St. Petersburg, Russia, [2]Dept. of Neuroscience and Cell Biology, UMDNJ-RWJMS, Piscataway, NJ 08854, USA.

To elucidate developmental mechanisms of the sexual differentiation in the mammalian brain we have investigated possible sex differences in the cytokinetic behavior within the proliferative ventricular epithelium (PVE) of the developing rat preoptic area (POA). Wistar rat embryos ranging in age from embryonic day 13 (E13) through E16 were exposed to a sequential double S-phase labeling first with 3H-thymidine followed 2 hours later with bromodeoxyuridine (BUDR) and sacrificed 0.5 hours after BUDR administration. The sex of each embryo was determined by counting Barr bodies, i.e., the inactivated X-chromosome (Barr & Bertram, 1949, Nature, 163:676-677) in postmitotic neurons of the brains and spinal ganglia. Labeling indices (ratios of the number of nuclei labeled with one or both markers to all nuclei) were used to calculate the duration of the cell cycle (Tc). Within the PVE, we have found sex differences in Tc that vary with the developmental stage. No differences in Tc were found in POA at E13 (Tc=11 hrs). In contrast, at E14 and E15, Tc was much shorter in males (Tc=13 hrs) than in females (Tc=16 hrs). However, these sex differences are reversed at E16, by which time Tc has become longer in males (Tc=23 hrs) than in females (Tc=17 hrs). Thus, our study has provided the first evidence for the existence of sex differences in the proliferative behavior of cells of the embryonic rat POA. We suggest these sex differences in cell cycle regulation may contribute to, and, indeed presage, the formation of the sexual dimorphism in the adult mammalian POA. (Supported by ISF Grants NVK000 and NVK300 to VKC and by the James S. McDonnell Foundation to RSN.)

214.11

DYNAMIC MODELISATION OF CORTICAL NEURON PRODUCTION. B. Moraillon, F. Polleux, C. Dehay, H. Kennedy* , INSERM U371, 18 Ave du Doyen Lépine, 69675 BRON (France).

The cerebral cortex is parcelled in distinct areas that are characterised by their cytoarchitectonics which can be defined on the basis of the number of neurons forming each layer.Previously we have shown that individual cortical areas are characterised by differences in the proliferative rates of their progenitor cells (Dehay et al., 1993, Polleux et al., 1995, 1996). We have designed a computational model in order to test whether the known variations in cell-cycle kinetics of cortical precursors influences the numbers of postmitotic neurons in individual areas. We have simulated neuron production in which the following parameters can be modulated independently and in different combinations (i) the number of precursors, (ii) the cell-cycle duration, (iii) the modes of division (proportions of proliferative divisions -where daughter cells remain in the cell cycle, proportions differentiative divisions -where daughter cells quit the cell cycle and proportions of mixed divisions -where one daughter cell remains in the cell cycle and the other quits the cycle), (iv) cell death. Covariation of these parameters shows that changes in modes of division and duration of cell cycle can have a complex effect on neuron production.

The model reveals how the observed regional variations in the cell cycle kinetics can lead to the observed differences in neuron number of individual layers in the cortical areas. These results show that modulation of the cell cycle almost certainly plays a significant role in establishing the cytoarchitectonics of the cortex

Bibliograhy: Dehay et al., Nature (1993) 366:464-466; Polleux et al., Neurosci Abst(1995) 21(2):1513; Polleux et al. (1996) Submitted.

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214.12

BIRTHDATING EXPERIMENTS IN EMBRYONIC MONKEY REVEALS DISTINCT PATTERNS OF CELL PROLIFERATION AND MIGRATION IN PRESUMPTIVE VISUAL AREAS 17 AND 18. H. Kennedy, C. Dehay*, G. Ziegel, I. Smart°, P. Giroud and M. Berland. INSERM U371, 18 Ave du Doyen Lépine, 69675 BRON (France). °Dundee University Dundee, Scotland.

Direct examination of the proliferative activity of the monkey cortical precursors shows that modulation of the cell-cycle kinetics parameters is responsible for the histogenesis of distinct cortical areas in the primate (Dehay et al.,Nature, 1993) and that cell-cycle duration was shorter in area 17 precursors than in area 18 precursors (Kennedy et al.,1995) during the generation of supragranular layers. Here we show that after long survival times, labelling patterns in the adult cortex reflect differences in the areal kinetics. Using long survival times, tritiated thymidine (H3-Thy) pulse injection at E65 results in heavily labelled cells being located in layer 6 in area 17 and in layer 5 in area 18 of the mature cortex. Injection at E78 resulted in heavily labelled neurons being located in layers 2/3 in both areas. Computation of the Labelling Index in the mature cortex indicates differences in the relative duration of S phase with respect to the total cell-cycle duration between area 17 and area 18 precursors during the generation of supragranular layers whereas no difference in the cell cycle kinetics can be detected in the two sets of precursors generation of layer 5. The transition from low values of LI in area 18 to higher values in area 17 is abrupt and coincides with the cytoarchitectural boundary.

The germinal zones giving rise to areas 17 and 18 exhibit conspicuous morphological specialisations which are related to neuron production. Pulse injections of H3-THY made at regular intervals between E61 and E94 and combined with short survival times allows the identification of the germinal zones and reveal that the major phase of neuron output in area 17 is provided by the subventricular layer that expands enormously between E61 and E71 while the ventricular layer is regressing. Pulse injection of H3-Thy at E64 followed by a 7 day survival time shows that migration of neuroblasts from the germinal zone to the cortical plate is considerably faster in presumptive area 17 than in presumptive area 18. This increased rate of migration accomodates the higher rates of proliferation characteristics of A17 ventricular zone. This means that complex mechanisms are regulating the migration rate of neuroblasts originating from distinct regions of the ventricular zone and that there is a precise orchestration of the timing of early events of areal histogenesis.

Bibliograhy: Dehay C. et al., Nature(1993); Kennedy et al., Neurosci. Abst.(1995); Supported by EEC grant SCI*CTIO622, HFSP grant RG-55/94B and MRE grant 92C0678.

214.13

PROLIFERATIVE COMPARTMENTS IN THE POSTNATAL SUBVENTRICULAR ZONE. J.R.L. Menezes and R. Lent*. Depto. de Anatomia, Inst. de Ciências Biomédicas, UFRJ, Rio de Janeiro, 21941-590, Brazil.

The postnatal subventricular zone (SVZ) is highly proliferative and a site of neurogenesis. It surrounds the lateral ventricle and extends toward the olfactory bulb (OB). We have studied the BrdU incorporation after short survival periods, to determine the number and distribution of the S-phase cells within the postnatal SVZ. BrdU injections (IP) were given to P2 and P4 mice. Animals were allowed to survive for 15, 30, 60 and 120 min., then killed by ether inhalation and perfused with warm HistoChoice. Brains were embedded in paraffin and cut parasagittally at 5-6 µm, then processed immunohistochemically for BrdU and counterstained. The cell distribution was analyzed with the aid of a microscope coupled to a computer with a tracing software. There was a very high number of BrdU+ cells within the SVZ, usually arranged in clusters and distributed in a decreasing gradient towards the OB. In addition, BrdU+ cells were concentrated along the periphery while the core was less densely populated. Moreover, nuclei with heavily labeled uniform chromatin were more numerous along the periphery of the SVZ, while nuclei with non-uniform heterochromatin labeling were predominant within the core. This result suggests a restricted spatial distribution of early and late S-phase cells within the postnatal SVZ, probably reflecting distinct compartments for proliferation and migration within this zone. *Financial support:* CNPq, FINEP.

214.14

MITOTIC NEUROBLASTS ENGAGE IN AXONAL OUTGROWTH AND PATHFINDING *IN VIVO* E. Wolf, I. B. Black, and E. DiCicco-Bloom*, Dept. Neurosci. & Cell Biol., UMDNJ/Robert Wood Johnson Medical School, Piscataway, NJ 08854

Neuroblast proliferation (neurogenesis) is believed to precede axodendritic outgrowth during neuronal development. However, we previously showed that 1) mitotic sympathetic neuroblasts and cerebellar granule cells in culture elaborate neurites before mitosis, 2) the morphology of these "paramitotic" neurites is heritable, and 3) some mitotic sympathoblasts *in vivo* project axons toward targets before dividing. These findings suggested that mitotic neuronal precursors, in addition to postmitotic neurons, initiate pathfinding.

To begin characterizing the role of paramitotic axonal outgrowth in superior cervical ganglion (SCG) development, we examined ganglia from embryonic ages E15.5 and E16.5. Mitotic neuroblasts projecting axons out of the ganglion were identified by combined retrograde tracing of the internal carotid nerve (ICN) and bromodeoxyuridine (BrdU) labeling of S-phase neuroblasts. We found that a substantial fraction of the mitotic population has axons in the ICN during early neurogenesis *in vivo* (12% at E15.5 and 7% at E16.5).

To assess the incidence and topography of this phenomenon, both the ICN and the external carotid nerve (ECN) of E16.5 ganglia were retrograde-traced. With this procedure, the fraction of S-phase, axon-bearing cells nearly doubled (reaching 13%), and these cells were not regionally restricted within the ganglion. Thus, axon elaboration and pathfinding by mitotic neuroblasts is common and occurs thoughout the developing ganglion *in vivo*.

These results raise the possibility that neurogenesis and neuronal connectivity are simultaneously orchestrated through direct interaction of proliferative populations with distant targets. As cerebellar granule cells exhibit paramitotic neurites in culture, this principle may apply more generally to nervous system formation. Supported by NIH HD 23315 and Trophix Pharm.

215.1

ISOLATION AND CHARACTERIZATION OF tsA58-INFECTED RAT OLFACTORY EPITHELIAL CELLS *IN VITRO*. A. N. Polzin* and D. D. Hunter
Depts. of Neurosci. and of Anatomy and Cell Biol., Tufts Univ., Boston, Mass.

The neurons of the olfactory epithelium continue to turn over and differentiate throughout the life span of an animal; however, the molecular mechanisms underlying these phenomena are not entirely clear. Attempts to elucidate the regulation of proliferation and differentiation of the olfactory epithelium have been hampered by technical limitations both *in vivo* and *in vitro*. For example, primary cell culture systems have been characterized with limited success in part due to the difficulty of isolating pure populations of each cell type present in the olfactory epithelium. Consequently, we have begun to develop cell lines derived from the rat olfactory epithelium that would allow for more accessible biochemical manipulation. This may be potentially useful to search for molecules that are involved in the development of the olfactory epithelium, as well as to identify factors that are either intrinsically or environmentally determined.

To construct cell lines from the olfactory epithelium, we infected the epithelium, dissociated from newborn rats, with a replication-incompetent retrovirus carrying an oncogene. We used a temperature-sensitive mutant of SV40 large T antigen that should promote the proliferation of infected cells at 33°C, but lose its effect at 39°C, thereby allowing the infected cells to differentiate. Following selection in geneticin, eighteen colonies were isolated; five of these (AP7, AP8, AP9, AP11 & AP12) have continued to grow well and adhere tenaciously to the substrate. As expected, when grown at 39°C, cells showed morphological characteristics of further differentiated state, including longer processes. As an initial step to analyze the biochemical characteristics of the cell lines, we looked for the presence of two neuronal markers, NCAM and MAP5, by immunocytochemistry. At 33°C, the cells express little NCAM and no MAP5; in contrast, at 39°C, the cells express both NCAM and MAP5, suggesting that they are neuronal.

To further characterize the cells, we are currently determining the expression of other cell type-specific markers. If the lines are biochemically established to be derived from the olfactory receptor neuron (ORN) lineage, we are interested in testing environmental factors that may control the proliferation and differentiation of progenitor cells and ORNs. The establishment of cell lines of the globose basal cell/ORN lineage may provide insight into mechanisms by which the olfactory epithelium regenerates and factors controlling this process.

Support: NIH; Grant #NS29785

215.2

TASTE BUD DEVELOPMENT IS AUTONOMOUS IN ANTERIOR ENDODERM ISOLATED FROM AMPHIBIAN GASTRULAE. L.A. Barlow* and R.G. Northcutt. Dept. of Neurosciences, UC San Diego, La Jolla, CA 92093.

The predominant model of embryonic taste bud development has held that ingrowing nerve fibers induce the differentiation of taste buds late in embryonic development. However, several recent findings indicate that the differentiation of embryonic taste buds is independent of innervation. Using salamander embryos, we have now also eliminated the potential inductive role of cephalic neural crest. Taste buds will develop *in vitro* in tissue devoid of neural crest (Barlow and Northcutt, 1995). We have further investigated the ability of isolated cephalic endoderm to generate taste buds under culture conditions by pushing our experimental paradigm back in developmental time. We explanted the anterior ventral endoderm of early salamander embryos (stage 13) and placed the tissue in simple culture medium (60% L15 with Gentamycin and Fungibact) for 10 -12 days, until intact control embryos had taste buds. The majority of explants survived and developed taste buds which were recognized using several immunocytochemical markers. Based on these results we have formulated a new model of taste bud genesis. We propose that the initial event in taste bud patterning occurs at gastrulation and involves the specification of anterior (head) endoderm. Subsequently, local cell-cell interactions within this cephalic endoderm trigger differentiation of scattered taste cell progenitors which ultimately produce taste buds. We are currently testing the components of this model.

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215.3

FGF AND ACTIVIN SIGNALLING PATHWAYS IN THE DETERMINATION OF RETINAL CELL FATES. Kathryn B. Moore and Sally A. Moody[1]* Department of Anatomy and Cell Biology and Neuroscience Program[1], The George Washington University, Washington, DC 20037.

In vertebrates, retinal progenitors begin as multipotent cells which undergo a systematic restriction in cell fate, yet the signalling pathways that determine these fates have not been well defined. In *Xenopus*, both cell position and competence determine whether embryonic progenitors will enter the retinal lineage (Huang and Moody, 1993, *J. Neurosci* 13, 3193-3210). To identify the molecular basis of these events, we have begun to examine which potential signals (position) and signal transduction machinery (competence) are necessary and/or sufficient. Our first step has been to test specific signals involved in the induction of dorsal mesoderm, the first known signalling event in *Xenopus* development. mRNAs encoding functional and dominant-negative forms of FGF receptors mixed with mRNA for Green Fluorescent Protein as a lineage tracer, were targeted to retinal precursor cells (D1.1.1) in the 32-cell embryo. When the FGF pathway was eliminated in D1.1.1 cells by overexpression of the dominant-negative FGF receptor, these blastomeres continued to contribute to the retina. However, a subset of D1.1.1 descendants was blocked from assuming dorsal mesoderm and ventral brain fates. Wild type FGF receptors were ectopically expressed in V2.1.1, a blastomere that does not normally contribute to retina, and is not competent to produce retinal lineages when transplanted to the D1.1.1 "retinogenic" region of the embryo. Regardless of whether the blastomeres were left in situ or transplanted to the D1.1.1 position, descendants of V2.1.1 only populated the gut and lateral mesoderm, as are their normal fates (Moody, 1987 *Develop Biol*, 122 300-319). Together, these results demonstrate that an intact FGF signalling pathway is not sufficient to specify a blastomere contribution to retinal lineages. Results from the genetic manipulation of functional and dominant-negative activin receptors also will be presented. Supported by NIH grants EY10096 (SAM) and EY06649 (KBM).

215.4

THE PROGENITOR CELLS IN THE *XENOPUS LAEVIS* NEURULA ORIGINATE DIFFERENT RETINAL CELL LINEAGES. Sen Huang*, Ida Chow and Sally A. Moody, Department of Anatomy and Cell Biology and Neuroscience Program, George Washington University and Department of Biology, American University, Washington D.C.

We previously demonstrated that as early as the 32-cell stage *Xenopus laevis* blastomeres are biased to produce different amacrine neurotransmitter phenotypes in the retina. The present study aims in determining if the neuroectodermal progenitor cells in the neural plate and neural fold are restricted in their production of different retinal cell types. A fluorescent lineage tracer, Texas Red-Dextran Amine, was iontophoretically injected into the stage 14 to 20 embryos' neuroectodermal cells. At stage 44/45 tadpole retinas were serially sectioned (14 μm) and immunostained to show the GABAergic amacrine cells in the lineage labeled clones. On average, stage 14 progenitors produced clones with 11 cells, stage 16 progenitors produced clones with 7 cells, and stage 20 progenitor clones had 6 cells. Normally, half of the retinal cells are interneurons, about a quarter are photoreceptors, another quarter ganglion cells and some glial cells. However, in the labeled clones 80% of the retinal cells were interneurons, 14% photoreceptors, 4% ganglion cells and 2% glial cells. Two types of clones were observed: 1) radial clones (61%) span all layers of the retina and contain almost all retinal cell types; and 2) tangential clones (39%) spread within a single retinal layer and contain only restricted cell types, mostly horizontal and bipolar cells. Among the lineage labeled amacrine cells, GABA-positive and GABA-negative cells could be found in the same clone. These results suggest that the neuroectodermal retinal progenitors in the neurula are not homogeneous; some of them are determined to produce restricted retinal cell types. (Supported by NIH grant EY10096)

215.5

OTX2 EXPRESSION IS RESTRICTED TO OUTER LAYERS OF THE EMBRYONIC MAMMALIAN RETINA. K. C. Wikler*, F. M. Vaccarino and D. L. Stull. Section of Neurobiology, Yale Sch. of Medicine, New Haven, CT, 06510.

The homeobox gene *orthodenticle* (*otd*) plays a critical role in photoreceptor cell development in the Drosophila eye (Vandendries et al., Dev. Biol., 173, 243- 255). Specifically, *otd* participates in the relative positioning of photoreceptor subtypes within each ommatidium. We are examining the expression of Otx2, the vertebrate homologue of *otd*, in the embryonic mouse and monkey retina to determine whether it serves a similar function in the development of the mammalian photoreceptor mosaic.

Mouse retinae from embryonic (E) day E10, E11, E12, E13, E14, E18, and E20, and macaque monkey retinae from E68 and E84 were sectioned and either immunoreacted with a polyclonal antibody to Otx2 protein or probed for Otx2 message using digoxigenin-UTP labeled human Otx2 mRNA. At E11, the onset of cone genesis in central retina, Otx2- positive cells were identified in this region near the proliferative neuroepithelium. By E18, when cone genesis is complete, Otx2 expression extends into the retinal margins, but remains restricted to the outer layers of the retina. This consistent laminar distribution of Otx2 mRNA and protein is distinct from the uniform expression patterns of other homeobox genes in the embryonic murine retina (e.g. Dlx-1). This pattern of Otx2 expression was observed in the fetal monkey retina as well; at E68 and E84, Otx2 immunoreactivity is restricted to the outer layers of the retina and appears to label cone photoreceptors.

The restricted expression pattern of Otx2 is strikingly similar during the development of the mouse and monkey retina, suggesting that its role in generation and differentiation of photoreceptors may be conserved across species. Supported by EY09917 (KCW).

215.6

EARLY DIVERGENCE OF POSTMITOTIC CELLS INTO MAJOR GANGLION CELL CLASSES IN THE EMBRYONIC MONKEY RETINA. P. Rakic* and K. C. Wikler. Sect. of Neurobio., Yale Sch. Medic., New Haven, CT.

M and P ganglion cells project to magno- and parvocellular laminae of the lateral geniculate nucleus (LGN) respectively, forming separate and parallel pathways within the primate visual system. M and P ganglion cells can be identified in the adult macaque by antibodies that recognize specific POU domain proteins (Brn-3a, Brn-3b) (Xiang et al., 1995, J. Neurosci., 15, 4762). In the present study we mapped the distribution of Brn-3 proteins in the fetal monkey retina to determine the time and mode of emergence of M and P ganglion cells in relation to the development of their connections within the retina and with the LGN.

Retinae from embryonic (E) day E68, E82, E93, E120 and adult macaque monkeys were serially sectioned and immunoreacted with either the Brn-3a antiserum, which labels all M cells lightly and a subset of P cells intensely, or the Brn-3b antiserum, which labels all P cells intensely. Adjacent sections were labeled alternatively with either the Brn-3a or b antiserum. Analysis of these sections indicated that, as early as E68, these antisera identify separate sets of cells in the ganglion cell layer at more mature, central regions of the retina. Strikingly, these antisera label separate sets of migrating cells near the proliferative neuroepithelium in less mature, peripheral regions of the same retina. These data indicate that Brn3 antibodies identify different subsets of cells prior to their arrival in the ganglion cell layer.

Although the final proportion of M and P cells may be sculpted through differential cell death after retinogeniculate connections have been established, our results suggest that M and P ganglion cells diverge into their respective subtypes coincident with their final mitotic division, prior to the formation of any synaptic contacts within the retina or with the LGN. Supported by EY09917 (KCW) and EY02593 (PR).

215.7

ASYMMETRIC EXPRESSION OF RETINOID RECEPTORS DIVIDES THE EMBRYONIC MOUSE RETINA INTO DORSAL AND VENTRAL COMPARTMENTS. D. L. Stull* and K. C. Wikler Sect. of Neurobio., Yale Sch. of Med., New Haven, CT.

We are interested in cellular and molecular mechanisms that regionalize the embryonic mouse retina and segregate middle (MWS) and short (SWS) cones into their respective dorsal and ventral fields. We demonstrated a regional heterogeneity in the response of neuroblasts to endogenous retinoids using indicator mice that possess a retinoid dependent *lacZ* reporter transgene (Stull and Wikler, Soc. Neuro. Ab. 21:1529). Transgene activation is restricted to dorsal retina prior to cone genesis and ventral retina coincident with cone genesis. It remains unclear whether this regional response to retinoic acid (RA) is due to local differences in the synthesis of RA and/or the expression pattern of retinoid receptors.

If local availability of RA regulates transgene activation, then augmenting RA levels should alter the pattern of *lacZ* expression. To test this hypothesis, pregnant indicator mice were given either 50 mg/kg all-trans RA in oil or oil alone 14 hours prior to sacrifice at embryonic (E) days E9.5, E10, E11, and E13. Embryos were sectioned, immunoreacted for β -galactosidase (β-gal), and counterstained with bis-Benzimide. Exogenous RA did not alter the pattern of transgene activity: β -gal positive neuroblasts were restricted to dorsal retina between E9.5 and E11 and ventral retina by E13. To determine if differences in the expression patterns of retinoid receptors underlie dorsal/ventral differences in transgene activation, we mapped the distribution of retinoid (RXR) and RA receptor (RAR) mRNAs across the neuroepithelium. RXRα, RXRβ, and RXRγ are ubiquitously expressed in the retina at E11 and E12; RXRγ becomes restricted to the ganglion cell layer and the proliferative zone by E14. In striking contrast to these regionally uniform expression patterns, RARβ expression is restricted to dorsal retina at E11 and E12 and extends into ventral retina by E13. These results suggest that the asymmetric response of the neuroepithelium is determined by regional differences in the expression of retinoic acid receptors, and not by local differences in RA synthesis. Supported by EY09917 (KCW).

215.8

POSTINFLAMMATORY MUSCLE NEOGENESIS, A.B. Drakontides,* M.J. Danon, and S. Levine, Depts. of Cell Biology and Anatomy, Neurology, Pathology, New York Medical College, Valhalla, NY 10595, and N.S. Kline Institute, Orangeburg, NY 10962.

Recent studies (Levine & Saltzman, Exp. Mol. Pathol. 60:60, 1994) have demonstrated formation of ectopic skeletal muscle cells at the peritoneal surface of rat diaphragm following a single injection of chemical irritant. Current studies present further observations of this inflammatory-induced myogenesis. Rats (150-300g) were killed 1 to 180 days (ds) after a single IP injection of sodium dodecyl sulfate, 100 mg/kg. Samples of diaphragm were processed for histochemical and EM studies. The peritoneum overlying intrinsic muscle was thickened and subdivided into an outer inflammatory and inner dense connective tissue (ct) layer. At 4 ds, between these two layers, large myoblast-like cells with central nuclei and bundles of myofibrils were seen. Myotubes and mature muscle cells were evident at 7 and 14 ds. Myogenic elements were always oriented perpendicular to intrinsic muscle and separated from it by a layer of dense ct. Ectopic muscle cells were still present after 180 ds; at this time smooth muscle cells were also evident. The source of skeletal muscle cells is unknown. Muscle satellite cells are excluded, as dense ct would create a barrier for migration. Chemical insult and subsequent sequelae may induce resident fibroblasts, mesothelial cells or stem cells to differentiate into muscle.

215.9

***IN VITRO* DIFFERENTIATION OF EMBRYONIC STEM CELLS INTO MOTONEURON-LIKE CELLS**

S. Alonso, Y. Renoncourt, P. Filippi, B. Pettmann*, D. Salaun, V. Arce and P. Carroll. INSERM U382, IBDM, Case 907 Luminy, 13288 Marseille Cedex 09, France.

Our aim is to better understand the mechanisms that control early motoneuron differentiation. *In vitro* studies of motoneuron differentiation from progenitor cells have been hampered by the lack of established cell lines but recently it has been shown that pluripotent embryonic stem cells can differentiate into various cell types including neurons. We show that retinoic acid (RA) alone is able to induce ES cells from cell line D3 to differentiate along a neuronal pathway characterized by the expression of CD24, GAP43 and NF200. Neurotrophin-3 (NT-3) has previously been shown to induce motoneuron differentiation from avian neural tube progenitor cells (Averbuch-Heller et al., 1994). NT-3 alone is unable to induce ES cells from D3 to differentiate into neuron-like cells. Although RA induces neuronal differentiation, it cannot induce the expression of the LIM homeoprotein Islet1/2 characteristic of motoneurons. We show that NT-3 in combination with RA induces the appearance of a sub-population of neuron-like cells which express the neurofilament subunit NF200 and Islet. We also tested bFGF in presence of RA with or without NT-3 and found no Islet-positive neurons. Efficient production of motoneurons from ES cells would considerably facilitate the understanding of motoneuron differentiation.

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215.10

LINEAGE ANALYSIS IN THE EARLY CHICK THORACIC SPINAL CORD

E. B. Cornbrooks* and C. J. Forehand Department of Anatomy and Neurobiology, University of Vermont College of Medicine, Burlington, VT 05405

In the embryonic neural tube, morphologically similar neuroepithelial precursor cells give rise to a variety of cell types, including motor, sensory and glial cells. In an effort to understand the factors influencing development of the different cell phenotypes, we have begun a lineage analysis of precursor cells in the chick thoracic neural tube. Due to our particular interest in sympathetic preganglionic neurons, we have labeled single progenitor cells in the ventral neural tube at stages encompassing the birth of preganglionic and motor neurons. A fluorescent tracer, Texas red labeled dextran amine, was iontophoretically injected into single neuroepithelial cells in the ventral thoracic neural tube of stage 14-22 chicken embryos. Successful injection restricted to a single neuroepithelial cell was confirmed by visual inspection. Embryos were fixed 2-3 days after injection and the thoracic spinal cord was serially sectioned. Clonal analysis revealed labeled cells in the thoracic region grouped closely to the site of injection; a labeled cell in the ventricular zone, presumed to be the progenitor, could be clearly identified in most embryos at these early time points. Clone size ranged from 12-28 cells, dispersed laterally to the progenitor cell. Typically, cells adjacent to the ventricular zone were morphologically undifferentiated, while more laterally positioned cells exhibited numerous processes. In some clones, labeled cells in a ventrolateral position in the spinal cord had rich arbors and an axon extending out the ventral root, clearly identifying these cells as motor neurons. Supported by the VT Affiliate of the AHA (EBC) and NIH R01 NS30062 (CJF).

215.11

ENGRAILED-1 IS EXPRESSED IN A SUBSET OF SPINAL INTERNEURONS DURING MOUSE DEVELOPMENT

Michael P. Matise* and Alexandra L. Joyner, Skirball Institute, New York University Medical Center, New York, NY 10016.

The homeobox-containing gene Engrailed-1 (*En-1*) is expressed in the developing nervous system in neuroepithelial cells spanning the mesencephalic-metencephalic junction, as well as in a bilateral stripe of cells in the ventral hindbrain and spinal cord. Targeted deletion of *En-1* in mice results in the loss of midbrain and cerebellar structures derived from *En-1* expressing regions, suggesting that *En-1* is required for the survival of these precursor cells. In contrast, the spinal cord and hindbrain appear to develop normally. In the spinal cord, *En-1* is initially expressed in cells immediately dorsal to motoneurons, at 9.5-10 days post coitum (dpc). Later, *En-1* cells are located dorsally and laterally, with scattered cells within the motor column in limb regions, but throughout the ventrolateral cord in non-limb regions. As the ventral ventricular zone contracts, *En-1* expression persists at its margins. All *En-1* cells express class III β-tubulin and are thus postmitotic neurons. In addition, *En-1* neurons comprise a subpopulation of *Lim-1/2* expressing cells. The earliest *En-1* expression at ~10 days marks the ventral limit of *Lim* expression dorsal to developing motoneurons. Later, *Lim* expression expands ventrally predominantly into the medial portion of the lateral motor column (LMC), but also some expression is seen in more lateral LMC regions. These latter *Lim* + cells also express *En-1*. No *En-1* interneurons express Islet-1/2 proteins.

To study the fate of *En-1* cells mice were generated in which the reporter gene *lacZ* has been targeted into the first exon of *En-1*, resulting in a loss-of-function of *En-1* while simultaneously providing a marker for *En-1* expressing cells (Hanks et al., 1995). In heterozygote embryos, the pattern of expression of β-galactosidase (β-gal) is similar to *En-1* as assessed by antibody or in-situ analysis. In homozygotes, β-gal activity in the spinal cord is initiated and maintained in a manner virtually indistinguishable from heterozygous littermates, at all stages examined (10-17.5 dpc). This result indicates that *En-1* is not required for the survival of *En-1* expressing cells. The presence of *En-1* interneurons in the knockout will allow us to begin to analyze the effects of the loss of *En-1* on the development of these cells and the spinal cord. *M.M. is supported by an NRSA postdoctoral training grant.*

215.12

NEUROEPITHELIAL STEM CELLS; ISOLATION, CHARACTERIZATION AND CLONAL ANALYSIS. M. S. Rao*, A. Kalyani, and K. Hopson, Department of Neurobiology and Anatomy, University of Utah School of Medicine, Salt Lake City, UT 84132.

To study rat spinal cord development we have established cultures of E10.5 neuroepithelial cells (NEP cells) from the caudal neural tube. NEP cells require both FGF (fibroblast growth factor) and CEE (chick embryo extract) to proliferate and to maintain an undifferentiated phenotype. Epidermal growth factor (EGF) does not support E10.5 NEP cell survival and NEP cells do not form neurospheres in EGF supplemented medium. NEP cells express nestin and lack all lineage specific markers for neuronal and glial sublineages. Cultured and passaged NEP cells do however, retain their pleuripotent character and can differentiate into neurons and oligodendrocytes when plated on laminin in the absence of CEE. In addition, NEP cells differentiate into astrocytes when supplemented with fetal bovine serum. Thus NEP cells can generate all major CNS cell types in culture. NEP cells grown at clonal density generate colonies that vary in size from single cells to several thousand cells. Approximately 40% of the clones are multipotent and contain A2B5 (an oligodendrocyte marker), B-111 tubulin (a neuronal marker) and GFAP (an astrocyte marker) immunoreactive cells. NEP cells also differentiate into ChAT and p75 immunoreactive cells, likely to be motoneurons, in both mass and clonal cultures. Double labeling of clonal cultures shows that ChAT immunoreactive neurons always arise in mixed cultures with other differentiated cells. Thus, motoneurons differentiate from a pleuripotent NEP progenitor cell. The relationship of the NEP stem cell with other CNS derived stem cells is discussed. *Supported by the Muscular Dystrophy Association and a University of Utah faculty award.*

215.13

NEUROEPITHELIAL CELLS FROM EMBRYONIC SPINAL CORD CAN GIVE RISE TO GLIAL PRECURSORS. Margot Mayer-Proschel* and Mahendra. S. Rao. *Department of Oncological Science, University of Utah/Huntsman Cancer Inst., Biopolymers. Res. Buildg. 570, Tel 585-7170, Salt Lake City, Utah 84132

In embryonic rat spinal cord the ventricular zone of neuroepithelial cells differentiates into neurons, astrocytes and oligodendrocytes. We have observed that cultured E10.5 neuroepithelial cells (NEP), which do not express lineage markers for oligodendrocytes, astrocytes or their precursors can be induced to differentiate into A2B5 immunoreactive cells in culture. A2B5 is an antigenic marker which is expressed on Oligodendrocyte-Type-2 astrocyte progenitor cells (O-2A cells) which have been characterized extensively in the postnatal optic nerve. Clonal analysis of the induced A2B5 expressing NEP cells shows that the immunoreactive population arise from multipotent precursor cells. Double-labeling of the A2B5 positive cells in mixed cultures demonstrates that a subset of the cells can give rise to oligodendrocytes (determined by GalC and O4 immunoreactivity) and astrocytes (identified as GFAP expressing cells), depending on the culture conditions. Selecting the A2B5$^+$ population by appropriate techniques and culturing them in oligodendrocyte and astrocyte promoting medium showed that this population of cells can generate both oligodendro-cytes and astrocytes in the absence of the A2B5$^-$ cell population. Single cell cloning experiments showed that individual cells were capable of generating both astrocytes and oligodendrocytes suggesting that this differentiation does not require cell-cell contact or signals from other cells. Thus, the A2B5 immunoreactive population derived from NEP cells may represent cells similar to the bipotential O-2A progenitor cell. The NEP culture system therefore provides an unique opportunity to study the cellular and molecular mechanisms that regulate commitment of a multipotent presursor cell into a cell with a more limited developmental potential. This system will also allow us to determine the complex relationship between glial cell precursors isolated from various region of the brain during different stages of glial cell development.

Supported by the Muscular Dystrophy association, a University of Utah faculty award and the Huntsman Cancer Institute at the University of Utah.

215.14

EN-1 AND EN-2 CONTROL MIDBRAIN DEVELOPMENT IN A GENE DOSE-DEPENDENT MANNER H.H. Simon* W. Wurst# and D.D.M. O'Leary, Molecular Neurobiology Laboratory, The Salk Institute, La Jolla, CA #GSF-Research Center, Oberschleissheim, Germany.

The embryonic expression domains of *En-1* and *En-2* in the murine brain overlap to a large extent. They are expressed in the dorsal aspect of the most rostral hindbrain and throughout the neuroepithelium in the caudal midbrain. These expression domains coincide with the embryonic regions which give rise to the cerebellum, the inferior and superior colliculi, and more ventrally located mesencephalic nuclei. Homozygous mice for the *En-2* mutation are viable and fertile, those deficient for *En-1* die at birth and the entire cerebellum, inferior colliculus and the caudal superior colliculus are absent. The phenotype of the *En-1/En-2* double mutant reflects the early ontogenetic expression of the two genes. In addition to the deficiencies of the *En-1* mutants, the superior colliculus is completely ablated and deeper mesencephalic nuclei are also effected; the noradrenergic locus coeruleus, the mesencephalic dopaminergic cells groups and the serotonergic dorsal raphe nucleus are all absent in these mice.

Here we describe midbrain development within the context of the four engrailed alleles. The phenotype of the P0 mutant mouse heterozygous for *En-1* and homozygous for *En-2* (*En-1* -/+;*En-2* -/-) is undistinguishable from the wildtype. In contrast En-1 -/-;En-2 -/+ mutant mice, their phenotype is stronger than the single mutant (*En–1-/-*) but less severe than the double mutant (En–1–/–;En-2-/-). Dorsally, the superior colliculus is further truncated in comparison to the *En-1* mutant but not ablated as observed in the double mutant. Ventrally, noradrenergic, dopaminergic and serotonergic cell clusters are all reduced in size but still present. The reduction in size of these chemically defined neuronal cell clusters in the *En-1-/-;En–2* -/+ mutant mice is already apparent by the lower number of these neurons in E12 embryos, at which age they can be first defined. The phenotype of the *En–1–/–;En-2* -/+ mutant suggests that the engrailed genes control the midbrain formation in a gene dose-dependent manner. Supported by HFSPO LT-526/94 and NS31558

215.15

EXPRESSION OF *Dfd* HOMOLOGUES IN IDENTIFIABLE NEURONS OF LEECH. V.Y. Wong*, H.-J. Wang, and E. Macagno, Dept. Biol. Sci., Columbia Univ., New York, NY 10027

To further explore the roles of *Antennapedia*-class homeobox genes in the determination of neuronal cell identity, we have isolated two leech *Dfd* homologues from an embryonic cDNA library and have begun to characterize their expression. The deduced amino acid sequences of *Lox6* and *Lox20* suggest that both are homologues of the *Drosophila* gene *Deformed*.

Thus far, we have only analyzed expression patterns of *Lox6*. Northern blot hybridization indicates that it is expressed in juvenile leeches. *In situ* hybridization shows that expression begins in the CNS at about embryonic day 7 (E7), in the subesophageal ganglion as well as in midbody ganglia 1 (MG1) through 21. At E8, the expression is observed in all ganglia. Interestingly, by E12, only a few pairs of neurons continued to express *Lox6*, with the strongest expression detected in MG1.

The unique expression pattern of *Lox6* in the CNS suggests that this gene may have important roles in determining the identities of specific neurons. We are currently trying to identify these cells by *in situ* hybridization coupled with dye-filling techniques. (Supported by NIH grant HD20954).

215.16

OLIGOSPHERES AS A MODEL TO STUDYI MIGRATION *IN VIVO* AND *IN VITRO*. Avellana- Adalid V., Nait-Oumesmar B., Lachapelle F., Aigrot M.S., Baron-Van Evercooren*A.

Cell migration is an essential phenomena in plasticity of the developing and lesioned Central Nervous System (CNS). After CNS demyelination, oligodendrocyte progenitors (OP) but also Schwann cells (SC) contribute to the repair of the lesion. However, their contribution to this phenomena is often limited suggesting ineffective migration in the adult CNS environment.

Migration will depend on interactions that cells establish with the sourrounding substrates and the soluble factors present around the lesion. We have designed a strategy to produce homotypic aggregates of purified primary rat OP which we have called "oligospheres" and which can be expanded for over one year in culture. When seeded onto an adhesive substrate they serve as focal reservoirs of OP. Within minutes, OPs emerge synchronously and radially from the sphere onto the substrate (e.g. poly-ornithine). They migrate as bipolar cells aligned against each other and with a speed of 50-60μm/h. Seeded onto adult spinal cord sections and organotypic cerebellum slices in vitro, oligospheres derived OPs migrate over white matter and the ependymal wall but not over grey matter. Grafted at a distance from a LPC induced lesion of the mouse spinal cord, oligospheres derived OPs followed the same routes of migration before reaching the lesion. Similar data were obtained with the CG4 rat OP cell line (induced to form oligospheres).Oligospheres are, in contrast to dissociated or clumped cell suspensions, easy to handle focal sources (restricted localisation) of OP. Thus oligospheres are a powerfull tool to study the mechanismes which are involved in the migration of OP.

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215.17

HPC-7, A NOVEL STAGE SPECIFIC OLIGODENDROCYTE MARKER D.Baas, R. Hofstein, M.S. Ghandour* and C.J. Barnstable Dept. of Ophthalmology and Visual Sciences, Yale School of Medicine, New Haven, CT 06520. *Centre de Neurochimie, Strasbourg, France

The identification of cell-type specific molecules expressed at different developmental stages can help to elucidate the regulatory mechanisms governing the survival, differentiation and development of cell in the central nervous system (CNS). Oligodendrocytes (OL) form the myelin sheath and are essential for propagation of nerve impulses. In culture, OL arise from bipotential (O-2A) progenitor cells in chemically defined medium. These O-2A cells give rise to type-2 astrocytes in presence of serum.

Mouse monoclonal antibody HPC-7 was secreted from hybridomas produced by fusion of P3-NSI/1-Ag4-1 plasmacytoma cells with spleen cells from BALB/c mice immunized with rat hippocampal membranes. Immunofluorescence on adult rat brain sections showed selective labelling of white matter and other myelinated fibers. Staining of rat secondary cultures of OL, O-2A and type-2 astrocytes (> 90 % pure in each case) showed selective labelling of OL. To determine at what stage in the OL lineage HPC-7 is expressed, cultures of O-2A progenitors and their progeny were examined. O-2A progenitor cells (A2B5 positive) and type-2 astrocytes (GFAP and A2B5 positive) were HPC-7 negative but OL (galactocerebroside positive) cells were HPC-7 positive so this antigen is only expressed in differentiated OL. In order to caracterize more fully at which stage of differentiation HPC-7 appears, we performed double labelling with other antibodies including vimentin, Jones, OL-1, O1 and MBP.

Supported by grants from NIH and the Kemper fund.

215.18

ORIGIN OF OLIGODENDROCYTES EXPRESSING FERRITIN IN THE RAT BRAIN , P. Cheepsunthorn*, S. Levison and J. Connor, Dept. Of Neuroscience & Anatomy, M.S. Hershey Med. Ctr. Penn State College of Medicine, Hershey PA 17033

Ferritin is the intracellular iron storage protein and is expressed in oligodendrocytes. In the white matter, ferritin positive oligodendrocytes occur in discrete patches of cells and not all oligodendrocytes contain ferritin. This observation that subsets of oligodendrocytes express ferritin suggests either that ferritin (and iron) containing oligodendrocytes are programmed (genetically determined) to migrate to specific areas within the white matter or they are influenced by their position (epigenetic factors) to express ferritin. In this study, the subventricular zone of rats at 2 days of age was injected with retrovirus carrying the E. coli beta-galactosidase gene. At 28 days of age the rats were sacrificed and processed for beta-galactosidase and ferritin double-label immunofluorescence. A subpopulation of the SVZ derived oligodendrocytes that migrated to the white matter expressed ferritin. These cells had the morphological appearance of immature oligodendrocytes. These results demonstrate that 1) ferritin positive oligodendrocytes orginate from the SVZ and 2) ferritin is expressed in oligodendrocytes before these cells begin to myelinate. Studies are underway to characterize oligodendroglial clones to establish whether ferritin expression is related to a cells' lineage or whether it is induced by microenvironmental cues.

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215.19

HEAVY CHAIN FERRITIN BINDS DNA K. Manges* and J. Connor. Dept. of Neuroscience and Anatomy, Pennsylvania State University, M. S. Hershey Medical Center, Hershey, Pa 17033

Ferritin has long been known to be the major iron storage protein. As part of ferritin's regulatory repertoire, the molecule prevents the formation of hydroxyl free radicals by scavenging free ferrous iron and converting the iron to Fe^{3+} where it is then stored within the core of the molecule. The ferritin molecule (450 kDa) consists of 24 subunits in varying ratios of Heavy (H) and Light (L) subunits. The subunits are genetically and functionally distinct. The L subunit primarily aids in the formation of the iron core within the molecule. The H subunit is also able to form an iron core, however, the H subunit contains the essential ferroxidase center which is responsible to the rapid uptake of the ferrous iron and subsequent conversion to the ferric form. Unique also to the H chain is an increase in the subunit seen during development, differentiation, proliferation, and stress. This paper presents evidence that H ferritin may also function as a DNA binding protein. Immunohistochemical studies in four week porcine old brains revealed that H chain ferritin stained selective neuronal nuclei while the L chain stained only the cytoplasm. Slot blot analysis of isolated nuclear extract from whole porcine brain indicated that the H subunit was present at a concentration of 4.73 ng ferritin/ ug protein. Incubation of FITC-rH ferritin with isolated nuclei pre-incubated with wheat germ agglutinin did not block entry of H ferritin into the nucleus suggesting the presence of a ferritin receptor on the nuclear membrane. Heart ferritin, which is H subunit rich, bound isolated genomic DNA from brain in a saturable and reversible manner and the recombinant H ferritin bound a specific DNA sequence on gel shift assays. L chain ferritin did not bind DNA. Poly dIdC:dIdC (1000 ng) was used in each gel shift assay to ensure that binding was specific. These data indicate that H ferritin can and does enter the nucleus and binds DNA. Future studies are directed at determining whether H ferritin is acting as a transcription/repressor factor or if H ferritin is protecting DNA from iron-induced damaged. Supported by NS22671 (NIH).

CELL DIFFERENTIATION AND MIGRATION IV

216.1

PRIMARY NEURON GROWTH ON STABLY-MODIFIED GLASS SURFACES. W. K. Scholz* and B. M. Sullivan. Nalge Nunc International, 2000 N. Aurora Rd, Naperville, IL 60563.

Growth substrates are known to affect the adhesion, morphology and differentiation of neurons in culture. For many applications, glass is the preferred substrate, but the glass surface must be adequately modified to support the growth of neurons. This is usually accomplished by the application of polylysine, laminin or fibronectin. We have examined other, more stable surface modifications to soda-lime glass slides to compare their abilities to support the growth of primary neurons. We modified the surface of glass microscope slides by exposing the glass to a corona discharge, by using a newly developed coating procedure, or by coating with diaminopropylsilane as described by Klienfeld, et al. (1988; J. Neurosci. 8, 4098). Surface energies of the modified glass were measured using specific testing solutions graduated from 30-56 dynes/cm (3DT) and the concentration of primary amines on the surface of the glass was measured using an o-phthalaldehyde-based procedure (Pierce). After surface modification, the slides were assembled into Lab-Tek® Chamber Slide™ culture vessels and plated with primary chick brain cells. After 1 day in culture, the culture medium was changed to the defined neuronal medium N2.1 and after four days, the primary neurons were treated with a mitotic inhibitor. Primary neurons did not adhere to any uncoated glass surfaces. Modification of the glass surface by corona discharge, which significantly increased the surface energy, did not improve neuron attachment. In contrast, the diaminopropylsilane coating enhanced neuron attachment and process outgrowth, even though it decreased the surface energy. A newly developed coating procedure improved the attachment and differentiation of neurons and inhibited the proliferation of non-neuronal cells from the brain. Unlike many cell types that prefer highly charged substrates for growth, neurons require specific substrates for differentiation and survival.

216.2

POTENTIAL LAMININ HETEROTRIMERS THAT MAY GUIDE RETINAL DEVELOPMENT. D.D. Hunter*[1], Y. Xu[1], R.T. Libby[2] and W.J. Brunken[2]. [1]Dept. Neurosci., Tufts Univ., Boston; [2]Biology Dept., Boston College, Chestnut Hill, Mass.

Many environmental signals guide the development of the vertebrate nervous system; such signals include components of the extracellular matrix (ECM). We have previously shown that one ECM component, the laminin β2 chain (formerly s-laminin), is expressed in adult and developing retina, and that laminin β2 is likely to be involved in the differentiation of rod photoreceptors and rod bipolar cells. We have also previously shown that the classical laminin, laminin-1 (a complex of the α1, β1, and γ1 chains), is not expressed in the retina, suggesting that laminin β2 is not complexed with the α1 or γ1 chains in this tissue. As it is likely that all laminins exist as heterotrimers consisting of an α, a β, and a γ chain, we have now asked whether other α and γ chains are present in the neural retina, in order to gain insight into the potential chain composition of retinal laminins containing the β2 chain.

Immunological reagents do not exist for many of the laminin chains in rodents; therefore, we used non-radioacitve *in situ* hybridization to detect RNA encoding the α1,2,3,4; β1,2,3; and γ1,2 chains in sections of rat retinae. In the adult neural retina, laminin β2 RNA is present in Müller cells (see accompanying poster); of the other chains, RNA was detected most strongly for the α4 and β3 chains, and less strongly for α3. There was no detectable signal for the known γ chains, nor for α1, α2, or β1. In preliminary immunohistochemistry on human tissue (for which there are more immunological reagents), we detected reactivity for the α3 chain, as well as for laminin-5 (α3/β3/γ2). Together, our immunohistochemical and RNA expression data suggest that the laminins of the neural retina consist of α3, α4, β2, β3, and an unknown γ. As new reagents become available, we will confirm the expression of laminin chain proteins in the rodent retina. In addition, we are in the process of attempting to identify additional γ chains that may be present in heterotrimeric neural laminins. Support: Foundation Fighting Blindness (WJB); NS29785 (DDH).

216.3

IDENTIFICATION OF THE CELLULAR SOURCE OF β2 LAMININS IN ADULT AND DEVELOPING VERTEBRATE RETINAE. R.T. Libby*[1], R.L. Cyr[1], Y. Xu[2], D.D. Hunter[2] and W.J. Brunken[1], [1]Dept. of Biology, Boston College, Chestnut Hill, MA 02167; [2]Dept. of Neuroscience, Tufts Univ. Sch. of Med., Boston, MA. 02111.

Molecules of the extracellular matrix, including laminins, are important in directing some aspects of neural development; specifically, β2 laminin (β2L) has been implicated in the phenotypic differentiation of rod photoreceptors and rod bipolar cells. Developmentally, the spatial and temporal pattern of β2L expression is consistent with a role in rod morphogenesis; however, the cellular source of retinal β2L in the vertebrate retina is unknown. Here we employ several techniques to identify this source. Western blot analysis of proteins isolated from matrix and cytosolic fractions of skate retinae, using two antisera directed against the β2 chain, demonstrate that one antiserum, GP1, recognizes matrix epitopes whereas the other, R49, recognizes cytosolic epitopes. Therefore, R49 may be useful in identifying the source of β2L. In adult skate retinae, as shown previously, GP1 immunoreactivity (IR) was present in the interphotoreceptor matrix. On the other hand, R49-IR spanned the neural retina from the outer to inner limiting membranes and was co-localized with vimentin, a marker for Müller cells (MCs). In the rat retina, β2 chain RNA was detected in what appears to be MCs by *in situ* hybridization. Together, these data show MCs are likely to be a source of β2L in the adult retina. However, during development the β2 chain is present prior to the genesis of MCs, suggesting there is an additional source of β2L. R49-IR in embryonic skate retinae and RNA localization in embryonic rat retinae suggest the neuroepithelial cells are one likely source of this β2L. In addition, R49-IR in skate suggests that the retinal pigmented epithelium may be another source of β2L.
Supported by: Boston College Research Expense Grant and Foundation for Fighting Blindness (WJB) and NS29785 (DDH).

216.4

MOLECULAR CUES THAT INFLUENCE SEGMENTAL PATTERNING OF TRUNK NEURAL CREST MIGRATION C.E. Krull*, A. Collazo, R. Lansford, S.E. Fraser, M. Bronner-Fraser. Division of Biology, California Inst of Technology, Pasadena, Ca 91125

In the avian embryo, trunk neural crest cells migrate in a striking segmental manner through the somites, entering rostral but not caudal sclerotome. Previous studies have shown that cues intrinsic to the somites are responsible for the patterning of neural crest. Attractive/permissive cues present rostrally, inhibitory cues located caudally or a combination of the two influences could establish this remarkable cellular pattern. Recently, we demonstrated that blocking PNA-binding molecules permits neural crest entry into caudal somite. Time-lapse analysis revealed a difference in the cell migratory behavior in rostral versus caudal somite, suggesting that an important cue located rostrally may be necessary to coordinate efficient migration. To this end, we are examining whether other differentially expressed cell-surface and extracellular matrix molecules influence the migratory process. For example, previous studies have suggested that β1 integrin antibodies affect neural crest migration at cranial levels. It is not clear, however, whether β1 integrin plays a role in patterning trunk neural crest. Antibodies against β1 integrin were added to trunk explants and the subsequent effects on neural crest migration were examined using time-lapse videomicroscopy. Neural crest cells migrate in rostral somitic regions in the antibody-treated explants but at rates significantly faster than cells in control explants. The explant paradigm allows cell migration to be followed over time in the presence of various blocking reagents, helping to unravel the mechanisms that guide this critical developmental process. (Funding support: NRSA/NIH #09459 to CEK; NIMH Silvio Conte Center to AC, RL, SEF; MDA#19796 to MBF)

216.5

PLATELET-DERIVED GROWTH FACTOR-INDUCED CHEMOTAXIS OF NEUROEPTHELIAL PROGENITOR CELLS FROM THE EMBRYONIC RAT CORTEX. K. Forsberg-Nilsson 1)3)*, T. N. Behar 2), J.L. Barker 2) and R.D.G. McKay 1), 1) LMB and 2)LNP, NINDS, NIH, Bethesda, MD 20892. 3) Present address Dept of Pathology, University of Uppsala, 75185 Uppsala, Sweden.

The ability of differentiating cells of the neuronal and glial lineages to migrate within the developing central nervous system (CNS) depends on extrinsic guidance signals, some of which are growth factors. We have investigated the chemotactic response to platelet-derived growth factor (PDGF) of progenitor cells from E14-E15 rat cortex. Multipotential cells from the developing CNS can be maintained and expanded in vitro under serum-free conditions in the presence of bFGF. Northern blot analysis of PDGF receptor expression revealed both α-and ß-receptors after culture in bFGF whereas on acutely dissociated cells from the E15 cortex, only the α-receptor could be detected. Both PDGF-AA and PDGF-BB readily induced migration of these cells as measured in a microchemotaxis assay. Complete blocking of the migratory response was achieved by incubation with PDGF isoform-specific antibodies. Immunocytochemical analysis of the migrated cells show more than 90% to be nestin positive. A low proportion possessed markers for oligodendrocyte precursors (O4) or neurons (NFM) but no cells were detected with a glial marker (GFAP). These findings together with reports that PDGF immunoreactivity can be detected in the ventricular zone at this embryonic age suggests a role for PDGF in stem cell migration in the developing cortex.

This study was supported by a grant from the Swedish Cancer Society (K.F.)

216.6

DEFECTS IN THE ROSTRAL MIGRATORY STREAM OF NCAM/mCD24 DOUBLE MUTANT MICE. G. Chazal*, H. Moreau, G. Monti, H. Cremer, and G. Rougon. LGPD, Luminy, 13288 Marseille, France.

PSA-NCAM was shown to be expressed in the adult brain in areas undergoing secondary neurogenesis. Recently, we showed that mCD24, a GPI-anchored molecule transiently expressed during development, is colocalized with PSA-NCAM on newly-generated neuroblasts in adult mice. These cells originate in the ventricular zone of the lateral ventricle and migrate along a well-defined pathway, the rostral migratory stream (RMS), to reach their final destination in the olfactory bulb (Calaora et al., Neurosci., in Press). The participation of these two molecules in the differentiation and migration of neuroblasts was investigated in NCAM (Cremer et al., Nature,1994) and mCD24 (Wenger et al., Transgenic Res., 1995) mutant knock-out mice. RMS morphology and cellular composition was analyzed by immunohistochemistry and EM. Perturbations in the organization of the chain migration of neuronal precursors were apparent in the single-mutant mice and amplified in the double ones. They consisted essentially in : a) an enlargement all along the tract of the migratory pathway from the subependymal layer of the lateral ventricle to the olfactory bulb, b) an increase of BrDU labelled migrating cells and c) and increase of GFAP positive cells which may indicate that the absence of both molecules influences neuro-glial interactions and possibly generated a reactive gliosis. Several hypothesis concerning how the lack of expression of these cell surface molecules may influence neuroblasts chain migration will be discussed.

216.7

EXTENSIVE NETWORK OF CHAINS FOR TANGENTIAL MIGRATION OF NEURONAL PRECURSORS. **F.Doetsch, J.M. Garcia-Verdugo, D. Vicario*, A.Alvarez-Buylla. The Rockefeller University, New York, NY 10021.**

Proliferating neuronal precursors persist in the subventricular zone of adult mammals. In the postnatal and adult mouse, cells from the most anterior portion of the subventricular zone (SVZ) migrate tangentially along the rostral migratory stream to reach the olfactory bulb where they differentiate into granule and periglomerular neurons. These neuronal precursors migrate in chains, revealed by anti-PSA-NCAM staining, without guidance from radial glia or axons (Lois et al. Science 271:978-981, 1996). The fate of SVZ cells which continue to divide more caudally in the walls of the lateral ventricle is, however, not well understood; it has been suggested that they die or only give rise to glia. Here we report that the SVZ throughout the lateral walls of the lateral ventricle of adult mouse brain is organized as a network of longitudinally oriented PSA-NCAM positive chains. In order to test the fate of the cells in this network, we transplanted SVZ carrying a neuron-specific reporter gene at different A/P levels of caudal SVZ of non-transgenic hosts. These grafts reveal that SVZ cells migrate to the olfactory bulb even from caudal-most SVZ and differentiate into neurons. Microinjection of DiI to label a restricted cohort of endogenous SVZ cells at different rostro-caudal levels also shows this tangential migration of neuronal precursors. Pre-embedding immunocytochemistry and ultra-thin electron microscopy of the SVZ in the lateral wall of the lateral ventricle indicate that the PSA-NCAM-positive chains are composed of migrating neuronal precursors and are ensheathed by GFAP-positive cells. Contrary to previous studies, these findings reveal that the SVZ of the adult mammalian brain is dynamically organized as a network of chains for tangential migration of neuronal precursors. **(NIH Grant HD32116)**

216.8

MIGRATION OF SUBVENTRICULAR ZONE CELLS AFTER OLFACTORY BULB REMOVAL IN ADULT MOUSE BRAIN. **B. Kirschenbaum* and A. Alvarez-Buylla. The Rockefeller University, New York, NY, 10021.**

Interneurons of the adult mouse olfactory bulb are continually generated. The precursors of these new neurons originate in the subventricular zone (SVZ) of the lateral ventricle and migrate along the rostral migratory stream (RMS) to the olfactory bulb where they differentiate. What determines the migration of these precursors towards the olfactory bulb is not known. One possible hypothesis is that the olfactory bulb secretes a diffusible factor that attracts the migrating precursors. In order to test this hypothesis, we have studied the migration of cells along the RMS after unilateral olfactory bulb removal. The right olfactory bulb was removed by suction at the level of the olfactory peduncle. Six weeks later the overall shape and dimensions of the RMS were similar between the operated and unoperated side. The RMS was, however, different close to the site of excision, where an accumulation of cells formed. To confirm that rostral migration of cells from the SVZ of the lateral ventricle occurs in the absence of the bulb, we did the following. Two weeks subsequent to unilateral olfactory bulbectomy, BrdU was microinjected bilaterally into the SVZ, a treatment that labels a restricted cohort of dividing SVZ cells close to the injection site, at the level of the anterior lateral ventricle. One week after BrdU microinjection, BrdU labeled cells had reached the olfactory bulb in the unoperated side. In the bulbectomized side, BrdU labeled cells migrated rostrally to the anterior part of the RMS and amassed at the site of the stump. These results indicate that the removal of the olfactory bulb did not prevent the rostral migration of precursor cells from the SVZ towards the bulb. We suggest that the directional guidance of precursor cells within the RMS is intrinsic to the RMS and the migrating cells therein.

Supported by NIH Grant DC03046 and HD32116

216.9

CHAIN MIGRATION OF NEURONAL PRECURSORS *IN-VITRO*. H. Wichterle and A. Alvarez-Buylla*. The Rockefeller University, New York, NY 10021.

Neuronal precursors in adult mice migrate from their site of birth in the subventricular zone (SVZ) of lateral ventricle along a restricted tangential pathway, the rostral migratory stream (RMS), into the olfactory bulb. A previous study (Lois, García-Verdugo and Alvarez-Buylla, Science 271:978-981, 1996) showed that these cells do not use glial or axonal processes for guidance and it was suggested that the neuronal precursors migrate along each other organized as chains. To test this possibility, we developed a system to study this novel form of migration *in-vitro*. SVZ dissected from the anterior horn of lateral ventricle of 5 day old mice was explanted into a 3D substrate (Matrigel™) and cultivated in serum free medium. Long chains of cells grew from these explants 5-40 hrs after explantation. The morphology of cells in these chains (size, growth cone, leading and trailing process) corresponded to the morphology of neuronal precursors found in RMS. Immunocytochemistry demonstrated that all cells in the chains are positive for Tuj1 (early neuronal marker) and PSA-NCAM (embryonic form of NCAM) and negative for Vimentin (early marker for radial glia), GFAP (marker for astrocytes) and MAP2 (marker for differentiated neurons). Time-lapse videomicrography was performed on these chains during the time period between 20 to 50 hrs in culture, when the length of the chains was maximal (100-400µm long and 10-30µm wide). This analysis revealed bipolar cells migrating along each other in both directions with speeds varying from 50 to 150µm/hr. Periods of migration of individual cells were interspersed with stationary periods, during which some cells retracted their leading process and changed direction of movement. Occasionally cells extended their leading process between two parallel chains and moved from one chain to the other. Cell bodies of these "jumping" cells were usually stationary until the highly motile growth cone established connection with cells in the parallel chain. This interaction resulted in pulling and/or pushing the cell body through the leading process. Our study reveals the dynamic properties of SVZ migrating cells and demonstrates that these neuronal precursors migrate bidirectionally crawling along each other without need for processes from other cell types. *(NIH Grant HD32116)*

216.10

A SEPTUM-DERIVED CHEMOREPULSIVE FACTOR FOR THE MIGRATING OLFACTORY INTERNEURON PRECURSORS. H. Hu and U. Rutishauser*. Department of Genetics and Neurosciences, Case Western Reserve University School of Medicine, Cleveland, OH 44106

During mammalian brain development immature neurons often migrate considerable distances to reach their final location. A dramatic example is the rostral migration of olfactory interneuron precursors from the vicinity of the septum to the olfactory bulb in the anterior forebrain, via a specific subventricular pathway. Heterotopic transplantation experiments have been carried out to establish that this migration is unidirectional and that guidance cues operate over a considerable distance. The mechanisms that provide guidance cues for this translocation have not been identified. We have carried out cocultures of the explants from the subventricular zone and the surrounding tissue regions. The results showed that the cells migrated away from caudal part of the septum but not influenced by olfactory bulb, cerebral cortex and caudate putaman. The present studies provide the first evidence that a diffusible chemorepulsive factor, in this case secreted by caudal septum but not by other tissue regions surrounding the pathway, may be involved. This activity is functionally distinct from that produced by factors that influence vertebrate axon outgrowth, such as netrins 1 and 2 and collapsin-1/semaphorin-III. The presence of this activity in the floor plate/ventral spinal cord as well as the septum suggests that this mechanism may influence other forms of cell migration during development. (Supported by NIH grants HD18369 and EY06107)

216.11

ESTROGEN REGULATES NgCAM-DEPENDENT CALCIUM-SIGNALING DURING MIGRATION OF NEW NEURONS INTO THE ADULT SONGBIRD BRAIN. S. Williams,[1]* J. Barry,[1] V. Lemmon,[2] M. Nedergaard[3], S. A. Goldman[1] Depts. of [1]Neurology, Cornell U. Med. Col. NYC, 10021, [2]Neuroscience, Case-Western Res. Sch. Med., Cleveland, OH, [3]Cell Biol., NY Med. Col., Valhalla, NY.

The adult avian forebrain continues to generate neurons from subependymal zone (SZ) precursors, whose neuronal progeny then migrate into the neostriatum. In explant cultures of the adult SZ, neuronal migration is disrupted by antibodies against the Ig-adhesion molecule NgCAM, and new neurons respond to NgCAM protein with increments in cytosolic calcium (Neuron 13:567, 1994.). The calcium response to NgCAM is developmentally restricted, to bipolar migrants during the period from 6-9 days in vitro (Molec. Cell. Neurosci. 7:29, 1996). This corresponds to the post-mitotic age at which new neurons leave the adult SZ, to traverse a layer of estrogen-receptive "gatekeeper" neurons, whose activity modulates the survival of the new migrants (J. Neurobiol. 27:470, 1995). Since the passage of new neurons through the estrogen-receptive layer coincides with their acquisition of NgCAM-dependent calcium signaling, we asked whether the development of the CAM-driven calcium response depended upon estrogen. Among cultures of adult female zebra finch SZ raised in gonadal steroid-depleted media, those supplemented with estradiol developed calcium increments of 82 ± 21% (n = 110 cells) to NgCAM. In contrast, neither their unsupplemented nor testosterone-supplemented controls exhibited such calcium responses to NgCAM (13 ± 8%, n=222, and 13 ± 7%, n=148, respectively) ($p < 0.001$ by ANOVA). The groups exhibited equivalent calcium responses to depolarization, and appeared equally viable to 9 DIV. However, SZ-derived neurons not exposed to estradiol died soon thereafter, significantly more rapidly than their counterparts raised in estrogen. These findings suggest that estrogen, or estrogen-induced paracrine agents, can regulate the coupling of a stably expressed adhesion molecule to calcium-dependent signaling pathways during neuronal migration.

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216.12

DEVELOPMENTAL EXPRESSION AND POSSIBLE ROLE OF FASCICLIN II IN THE GUIDANCE OF MIGRATORY ENTERIC NEURONS. P.F. Copenhaver*, J. Wright, M. Snyder, and S. Combes. Cell Biology L215, Oregon Health Sciences U., Portland, OR 97201.

During the formation of the enteric nervous system (ENS) in the moth, *Manduca sexta*, a population of ~300 neurons (the EP cells) migrate onto a set of pre-formed pathways on the visceral musculature. In particular, a subset of these cells must contact one of eight longitudinal muscle bands that form on the midgut to differentiate normally. In an investigation of the molecular mechanisms underlying this interaction, we have identified the cell adhesion receptor fasciclin II as a likely guidance cue that supports EP cell migration. Antisera against the moth form of fasciclin II (MFas II; a putative homophilic receptor in the immunoglobulin superfamily) stained both the EP cells and the muscle bands during migration, while blocking antibodies against MFas II inhibited EP cell migration in embryonic culture. Using microsequence data from the MFas II protein, we cloned cDNAs encoding two isoforms of MFas II, a transmembrane form and a GPI-linked form. *In situ* hybridization histochemistry with probes derived from these clones confirmed that one or both forms of this receptor is expressed by the EP cells throughout development, but they only transiently appear in the muscle bands during the migratory period. Removal of GPI-linked receptors from the gut surface with PI-specific phospholipase C caused a substantial loss of MFas II protein from the ENS and inhibited EP cell migration. We are currently using clone-specific probes to determine which isoform of MFas II is expressed by each of these cell types, and we have begun to investigate the intracellular signaling mechanisms by which MFas II may affect neuronal migratory behavior. Supported by NIH # NS35369.

216.13

CALCIUM-DEPENDENT REGULATION OF NEURONAL MIGRATION BY THE HETEROTRIMERIC G PROTEIN Go_α. Angela M. Horgan* & P.F. Copenhaver. Dept. Cell & Developmental Biology, L215, Oregon Health Sciences U., Portland, OR. 97201.

During formation of the enteric nervous system (ENS) of the moth *Manduca sexta*, a group of ~ 300 post-mitotic neurons (the EP cells) migrates onto a specific set of muscle band pathways on the gut surface. Previously, we showed that these neurons commence the expression of the guanyl nucleotide binding protein Go_α at the onset of migration. Using a semi-intact embryo culture preparation that allows access to the neurons and their pathways during migration, we have manipulated G protein activity in the EP cells to investigate whether this signaling molecule participates in the regulation of EP cell migration. Our results have demonstrated that stimulation of the G proteins in these cells, using AlF_4, $GTP\gamma S$, and mastoparan (which selectively activates G_o and G_i), caused a premature termination of EP cell migration. In contrast, inhibition of G proteins with $GDP\beta S$ and pertussis toxin, had no obvious effect. Since Go_α is associated with the modulation of intracellular Ca^{2+} in other systems, we have examined whether the effects of Go_α on EP cell migration involves Ca^{2+}-dependent processes. While EP cell migration will still proceed in [0] Ca^{2+} saline, manipulations that elevated intracellular Ca^{2+} caused a marked inhibition of migration. In addition, the inhibitory effect of mastoparan was prevented in [0] Ca^{2+}-saline, indicating that regulatory effects of Go_α on EP cell motility is Ca^{2+}-dependent. We are presently investigating whether constitutively active mutants of different G proteins can be expressed in the EP cells to modulate neuronal migration *in vivo*. Supported by NIH # NS35369.

216.14

HEPARIN-BINDING GROWTH-ASSOCIATED MOLECULE (HB-GAM) and N-SYNDECAN (SYNDECAN-3) IN DEVELOPING RAT HEART. J.O. Hiltunen, M. Kaksonen, R. Nolo, M. Scheinin*, M. Saarma and H. Rauvala. Inst. of Biotechnology, Univ. of Helsinki; Dept. of Pharmacol., Univ. of Turku, Finland

Heparin-binding growth-associated molecule (HB-GAM, pleiotrophin) is a cell surface- and extracellular matrix-associated protein, which has been shown to promote neurite outgrowth and synapse formation. It shares 50% sequence identity with midkine (MK), which promotes the growth of neuronal and PC12 cells. Syndecans are heparan sulfate proteoglycans regulating the biological effects of heparin-binding molecules. N-syndecan (syndecan-3) has been shown to function as a receptor for HB-GAM.

We studied HB-GAM and N-syndecan expression in developing rat heart by non-radioactive in situ hybridization using digoxigenin-labeled riboprobes. We also performed anti- HB-GAM and anti- N-syndecan immunohistochemistry to characterize protein localization and used neuron-specific antibodies to identify cells expressing these proteins.

Both HB-GAM and N-syndecan expressions were detected in the atrioventricular and the outflow tract regions at embryonic day 11 (E11). By E13 both messages were localized to regions undergoing epithelial-mesenchymal interactions.

At E16 mesenchyme the base of the heart expressed strongly HB-GAM message and was especially seen in the cells surrounding cardiac ganglion neurons, probably in Schwann cells. At the same time N-syndecan mRNA could be observed in cardiac ganglion neurons (anti-peripherin immunostaining). HB-GAM and N-syndecan expressions were the same at E20, except that the HG-GAM message was already declining. During day 13 N-syndecan expression was still detected in cardiac ganglion neurons.

These results suggest a role for HB-GAM and N-syndecan in development of cardiac ganglion neurons and also for non-neuronal cells in the atrioventricular region.

Juselius Foundation, Finnish Academy.

216.15

TGF-β1 AND MOTILE BEHAVIOUR OF THE OLIGODENDROGLIAL CELL LINE OLI-NEU: EVIDENCE FOR INVOLVEMENT OF DSD-1-PROTEOGLYCAN O. Schnädelbach* and A. Faissner, Dept. of Neurobiology, University of Heidelberg, D-69120 Heidelberg

DSD-1-Proteoglycan (DSD-1-PG) is a chondroitin sulfate proteoglycan which is produced by glial cells in vitro and co-localizes with glial cells in vivo. It carries the DSD-1-epitope, a particular glycosaminoglycan specifically recognized by monoclonal antibody (mAb) 473HD. In vitro, DSD-1-PG is upregulated on the oligodendroglial precursor cell line oli-neu by members of the TGF-β superfamily, as shown with mAb 473HD (Soc. Neurosci. Abstr. 21, 1312 (1995)). In the light of these observations we investigated potential roles of DSD-1-PG for glial cell behaviour.

A bioassay for migratory behaviour was designed where oli-neu cells migrate on preformed tracks of defined extracellular matrix (ECM) glycoproteins. When 4 ng/ml TGF-β1 were applied over 48 hrs, the migratory performance on laminin was decreased to 58% of control values. This effect was partially reversed by application of 50 µg/ml polyclonal DSD-1-PG antibodies and completely abolished by addition of 20 µg/ml mAb 473HD. Consequently, incubation of oli-neu with antibodies in the absence of TGF increased migration speed by 25% on laminin. No significant changes of migratory behaviour were observed on control ECM substrates under these conditions. Analogous results were obtained in a three-dimensional trans-filter migration assay within 8 hrs, rendering unlikely that anti-proliferative effects of TGF-β are the cause of decreased migration. Finally, in a short-term adhesion assay, TGF-β1 reduced the number of adherent oli-neu cells on laminin by 46 %, while the number of cells adhering to fibronectin was only slightly reduced.

We conclude from these findings that DSD-1-PG might contribute to the control of migratory behaviour of the glial cell line oli-neu on laminin.

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216.16

POSTNATAL DEVELOPMENT OF LAMININ- AND FIBRONECTIN-LIKE IMMUNOREACTIVITY IN THE RAT HIPPOCAMPUS *IN VIVO* AND IN ORGANOTYPIC CULTURE. L.Paulman*, L.S. Jones, L.Edwards, and M. Welsh, Dev Biol & Anat, Univ So Carolina, Sch of Med, Cola, SC 29208.

We have compared organotypic cultured and acutely prepared slices of rat hippocampus to examine expression of the extracellular matrix proteins laminin (LN) and fibronectin (FN). The sections were prepared using standard immunohistochemical techniques and polyclonal antibodies to FN, LN, and glial fibrillary acidic protein (GFAP). Tissues were fluorescently labeled and and viewed by confocal laser microscopy. The viability of the organotypic cultures was determined by standard extracellular field potential recording of random slices at all timepoints. *In vivo*, we found that expression of both FN- and LN-like immunoreactivity was localized mainly in vasculature at the earliest timepoints. By two weeks (in culture and in acute slices), some LN and FN staining was seen in the neuropil of the hippocampus, but was low compared to the vasculature. In the organotypic cultures, intense staining of vasculature, fibers and cells around the periphery of the slice was seen. This staining continued to be present up to 21d in culture, but was maximal at 2 weeks. The GFAP labeling of the acute slices at all ages demonstrated astrocytes with small numbers of processes which were moderately labeled. In the cultures intense labeling of astrocytes and a large number of processes was seen at all times in cultured tissue and on the adjacent membrane. As the expression patterns of LN and FN are similar in the acute and cultured tissues, although possibly higher in the cultures, it appears to be a good model for examining the postnatal expression of extracellular matrix. Support by NINDS NS27903.

216.17

ANALYSIS OF ECM FACTORS ON NG108-15 CELL FUNCTION & VIABILITY H. Bryant*, V. Kowtha, D. Stenger. Dpt. Physiol. USUHS, Bethesda, MD; CBMSE/NRL, Washington, DC 20375

NG108-15 (neuroblastoma x glioma) cells, cultured in defined, serum-free media (SFM containing N2 supplements) exhibit stable automaticity (the spontaneous occurrence of regenerative action potentials) following transient exposure to extracellular perfusates containing NH_4Cl. In the present study, we examine changes in morphology and electrical activity upon culturing these cells on extracellular matrix (ECM) factors in SFM. Matrigel™, which consists of laminin, entactin, collagen and heparan sulfate proteogylcan was tested first and it promoted cellular proliferation. Three singular ECM components (collagen IV, fibronectin and laminin) were then compared with control polystyrene (PS) and with Matrigel (added to cells after 3 days in vitro (div)). The singular components promoted NG108-15 cell differentiation (3 div). However, cells on laminin and fibronectin did not survive beyond 15 div. Cells on ECM with a final layer of Matrigel had more dividing cells than PS.

Summary of Results:

Proliferation: Fibronectin>Laminin>Matrigel>Collagen IV>PS
Neurite Outgrowth: Laminin>Collagen IV>PS>Matrigel>Fibronectin
Viability: PS~Collagen IV> Fibronectin>Matrigel>Laminin
Mean RP (10div): Collagen IV~PS>Fibronectin>Matrigel

Cells on Collagen IV exhibited stable automaticity (periods > 1hr). Moreover, an abundance of flat cells were present under ECM culturing conditions; these cells did not lift off under cellular stress (movement, rapid fluid flow). Overall, NG108-15 cells can be maintained on specific ECM factors which promote enhanced neurite development and increased membrane excitability.

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CELL DIFFERENTIATION AND MIGRATION V

217.1

ACTIVATED AKT ENABLES SURVIVAL OF DIFFERENTIATED NEURONAL CELLS.

E. M. Eves[1*], A. Bellacosa[2], P.N. Tsichlis[2], N. Hay[1] and M.R. Rosner[1]. [1]Ben May Inst., University of Chicago, Chicago, IL 60637 and [2]Fox Chase Cancer Center, Philadelphia, PA 19111.

PI-3 kinase has been suggested to mediate survival in neuronal cells. The proto-oncogene Akt (PKB, Rac p56)is a serine-threonine protein kinase that is a downstream target of PI-3 kinase. To test whether Akt acts as a survival factor in neuronal cells, we determined the effect of expressing c-Akt or its oncogenic counterpart v-Akt on the survival of conditionally (SV40-T^{ts}) immortalized rat hippocampal H19-7 cells. v-Akt enhanced survival of the differentiated H19-7 cells to an extent comparable to that observed with Bcl-2, and a small increase in survival was observed with c-Akt. When v-Akt and Bcl-2 were co-expressed in H19-7 cells, only a slight enhancement of survival beyond that conferred by each factor alone occurred. Furthermore, v-Akt expression did not significantly induce Bcl-2 or Bcl-xl_L expression in these cells. c-Jun NH_2-terminal kinase (JNK) has been suggested to mediate apoptosis in neuronal cells. Consistent with this possibility, JNK activity increases in differentiated H19-7 cells undergoing apoptosis. However, v-Akt did not suppress the increase in JNK activity. These results indicate that Akt can act as a survival factor for neuronal cells through a mechanism that is independent of induction of Bcl-2 or Bcl-x_L or inhibition of JNK activity. (Supported by NIH-NS 338-58)

217.2

MAH CELL LINES OVEREXPRESSING E1B19K STILL REQUIRE NGF FOR SURVIVAL AS POSTMITOTIC NEURONS.

M. Nakagawa, C.P.B. Sampson, G. Swanson* and A.M. Tolkovsky Dept. Biochemistry, University of Cambridge, Tennis Court Road, Cambridge, UK & Trends in Neuroscience, Cambridge, UK.

Postmitotic neurons cannot be stably transfected, making it difficult to manipulate signal transduction mechanisms and study their long-term effects on survival and differentiation. MAH cells, HNK+ sympathoadrenal precursor cells immortalised with v-*myc* (Birren & Anderson (1990) Neuron 4, 189), can be induced to differentiate into post-mitotic, NGF-dependent neurons by CNTF or FGF. However, proliferating MAH cells continually bud off apoptotic cells and their differentiation is not very efficient. Speculating that expression of survival genes might negate these apoptotic events and lead to an abundance of long-lived neurons, we created several stable MAH cell lines expressing h-*bcl2* or adenoviral E1B19K using retroviral vectors. As predicted, cells expressing *bcl2* (BMAHs) or E1B19K (EMAHs) did not produce apoptotic progeny and became resistant to death induced by cycloheximide, staurosporine or serum withdrawal. However, EMAHs were much more resistant than BMAHs to toxic insults, so we concentrated on their differentiation. No differentiation was obtained (after dexamethasone was withdrawn) unless NGF was added from the first day. Under these conditions, EMAHs were best differentiated into neurons using a combination of CNTF and high potassium. The poorest factor was bFGF, which caused huge colonies of differentiation-resistant cells to appear amongst the newly formed neurons. Interestingly, despite E1B19K expression, the EMAH-derived neurons still required NGF for their survival, neurons being maintained for ~2 months with NGF. Because it has been suggested that cells expressing high *myc* (e.g. neuroblastomas) are incapable of withdrawing from the cell cycle we investigated how EMAHs become post-mitotic. We report on the roles of some cell cycle and anti-cell cycle genes in this process.

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217.3

IDENTIFICATION OF MOUSE HOMOLOGUES OF EYES ABSENT (EYA), A DROSOPHILA GENE INVOLVED IN NEURONAL CELL DEATH.

Y. Tanno[1,2], T. Mori[1], S. Yokoya[1], T. Yamamoto[2], A. Wanaka[1*] [1]Dept. of Cell Science, Institute of Biomedical Sciences, [2]Dept. of Neurology, Fukushima Medical College, Fukushima 960-12, Japan

Eyes absent gene (*eya*) was originally identified by enhancer-trapping in the fruit fly. As its name implies, *eya* mutant shows massive cell death anterior to the morphogenetic furrow in the developing neural retina and this cell death occurs in an apoptotic fashion. *eya* activity, therefore, is thought to be required for the survival of eye progenitor cells and to influence the distribution of cells between differentiation and death. The *eya* gene encodes a protein with 760 amino acids which has little homology to known proteins and has no known functional domains. To elucidate the physiological significance of *eya*, we performed PCR on a embryonic mouse brain cDNA with fully-degenerated oligonucleotide primers which correspond to the homologous regions between Drosophila *eya* and human expressed sequence tag clones, and got two independent fragments with significant homology to *eya*. The expression domains of these genes in the developing mouse embryo and the structures of these will be presented and discussed.

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217.4

MODULATION OF INTRACELLULAR CALCIUM LEVELS OF THE WEAVER GRANULE NEURONS AFFECTS THEIR SURVIVAL.

Päivi Liesi* and Victor Krauthamer. LMCN, NIAAA, NIH, 12501 Washington Ave, Rockville,MD 20852 and FDA, CDRH 12721 Twinbrook Parkway, MD20852.

The cerebellar granule neurons of the homozygous weaver mutant mouse fail to migrate and die within the first two-weeks of postnatal life. The weaver gene is mapped in the chromosome 16 in the mouse, and recent evidence indicates that a point mutation in the GIRK2 potassium channel gene could be the weaver gene. P7-P9 normal and homozygous weaver granule neurons were cultured on a laminin substratum for 24-48 hrs. Within this time period, the normal granule neurons extended long neurites whereas the weaver granule neurons showed impaired neurite outgrowth. 10 nM BABTA-AM and 50-100 mM ethanol rescued the neurite outgrowth potential of the weaver neurons similar to verapamil, and MK-801. Calcium imagining studies were combined with the rescue studies to correlate the effects of different rescue agents with the modulation of calcium levels inside the weaver neurons. The results raise the question of whether reduction of intracytoplasmic calcium levels by calcium channel blockers (verapamil and MK-801), calcium chelators and moderate concentrations of ethanol is associated with the rescue of the neurite outgrowth potential of the weaver granule neurons.

This work was supported by NIAAA and FDA.

217.5

Role of Polyunsaturated Fatty Acids in Nervous System
Lisa Edsall, Martha Garcia, R.W. Peoples* and Hee-Yong Kim, Laboratory of Membrane Biochemistry and Biophysics, LMCN , NIAAA, NIH, Rockville, MD 20852.

Neuronal membranes are enriched with polyunsaturated fatty acids esterified in phospholipids, especially arachidonic (20:4n6) and docosahexaenoic (22:6n3) species. These polyunsaturates are thought to be essential for proper neuronal function. In order to substantiate the role of these polyunsaturates, we investigated the release of these polyunsaturated fatty acids along with their effects on the survival and proliferation of rat pheochromocytoma cells were examined. Upon stimulation with carbachol, neuronal cells preferentially released 20:4n6 in comparison to 22:6n3, suggesting that 22:6n3 as a membrane component rather than as a released free fatty acid may be of more physiological importance in neuronal membranes. When PC12 cells were subjected to serum free conditions to induce apoptosis in the presence of 1 to 25 uM 20:4n6 or 22:6n3, DNA fragmentation in cells exposed to 20:4n6 showed significant resistance to fragmentation, while the effect of 22:6n3 under these conditions was minimal. Growth and proliferation of neuronal cells were also influenced by fatty acids as evidenced by the significant inhibition of ^{3}H-thymidin incorporation by 20:4n6 in both Neuro-2A and PC12 cells. Alternatively, 22:6n3 exhibited a more proliferative effect, in some cases increasing thymidin incorporation by almost 40%. These results may suggest a functional dichotomy in the role of these two prevalent fatty acids in neuronal systems. Ready relase of 20:4n6 from neuronal membranes upon stimulation of PLA_2 may be of more importance to the signaling aspects of the cell, while 22:6n3 may be essential for maintaining cell integrity. Supported by the NIH intramural program.

217.6

TISSUE CULTURE FLASKS AS A VARIABLE AFFECTING THE VIABILITY AND INTEGRITY OF CELL LINES, L.M. Konopka*, B. Wong, S.M. Delisi, J.W. Crayton, Biological Psychiatry Section, Hines V.A. Hospital, Hines, IL. 60141.

Tissue culture work using well-described cell lines studied under well-characterized experimental conditions provides the advantages of preparation-reproducibility and a well controlled environment. Investigators tend to assume that standard, commercially-available reagents and disposable supplies contribute to the maintenance of highly-reproducible conditions. During the past year, we noticed that two cultured cell lines, AtT-20 pituitary corticotrophs derived from a mouse tumor line and rat derived lactotroph/gonadotroph GH3 lines both showed a progressive marked failure to grow and a lack of adhesion to the flasks. Systematic study of the various reagents involved, including all solutions, reagents, experimental conditions, and media suggested that the failure to grow coincided with a change in the culture flasks used. "Old" flasks were compared with "New" flasks obtained from the same manufacturer. Parallel, controlled studies using the same cell lines and identically-prepared reagents showed that "New" flasks failed to permit adequate adhesion of cells and within 24-72 hours led to distorted, moribund cells. Identically-treated cells grown in parallel with "Old" flasks showed the expected cell adhesion and growth. To date we have been unable to identify the differences in the two flasks that produced such different results. This work suggests that failures of cell lines may be related to subtle differences in materials generally expected to be highly standardized and uniform.

Assays of flask characteristics should be undertaken when the lot number or supplier is changed.

This study was supported by POTTS Foundation and Chicago Consortium for Psychiatric Research

PROCESS OUTGROWTH, GROWTH CONES, AND SPROUTING II

218.1

NEUROTROPHIC EFFECTS OF L-DOPA IN POSTNATAL MIDBRAIN DOPAMINE / CORTICAL ASTROCYTE COCULTURE, MA Mena, Johanna Bogulavsky, Irina Ryjak, Viviana Davila, & David Sulzer*. Dept Neurol & Psych, Columbia U & Dept Neurosci, New York State Psych Inst, NY 10032.

Parkinson's disease patients who receive L-DOPA achieve long-term benefit following treatment cessation (Nutt 1995, Neurology). We found that short-term L-DOPA elevates extracellular dopamine (DA) by increasing quantal size (Pothos et al., this meeting). To study long-term response, we used postnatal ventral midbrain neuron/cortical astrocyte cocultures in serum-free, glial-conditioned medium. L-DOPA (50μM 3 days post-plating) increased surviving TH$^+$ neurons by 166±9%. Although increase was attenuated at >100μM, there was no significant neurotoxicity at 400μM. L-DOPA increased TH$^+$ processes that extended >800μm by 177%, and TH$^+$ neurons with >3 primary processes by 162%. This contrasts with embryonic glial-free cultures, where 25μM L-DOPA is toxic (Mena 1993, NeuroReport) and glial-conditioned media protective (Mena 1996, NeuroReport). D-DOPA was not neurotrophic (50-200μM), implying a role for DA synthesis. Moreover, the D1/D2 agonist apomorphine (10μM) increased survival of TH$^+$ neurons by 150±16%. However, the decarboxylase inhibitor carbidopa (25μM) did not block the neurotrophic effect, indicating a DA-independent action. This paradox may be due to promotion of free radical scavengers. Pretreatment with the glutathione peroxidase inhibitor *L*-buthinone sulfoximine (3μM for 24h) alone had no neurotoxic effect, but blocked the neurotrophic action of L-DOPA. *N*-acetyl-*l*-cystine (250μM for 48h), which promotes glutathione synthesis, was similar to L-DOPA, increasing TH$^+$ neurons by 183±13%. Finally, L-DOPA, which upregulates glutathione (Han 1996, J Neurochem), did not induce quinone formation, whereas D-DOPA and apomorphine did. We are currently testing whether free radicals upregulate glutathione synthesis. Funded by the Parkinson's Disease Foundation and NIDA 10154 & 07418.

218.2

MOSSY FIBER INTERACTIONS WITH PLASMA MEMBRANES FROM TARGET CEREBELLAR GRANULE CELLS
M.S. Ward and C.A. Mason*. Departments of Pathology, Anatomy and Cell Biology, and Center for Neurobiology and Behavior, College of Physicians and Surgeons, Columbia University, New York, NY 10032.

Cerebellar granule cells purified and grown in monolayer culture inhibit the growth of neurites from explants of basilar pons, a major source of afferent mossy fibers (Baird et al., J. Neurosci. 12:619, 1992). Previous studies have shown this effect to be specific for pontine explants and dependent on NMDA receptor activity (Baird et al., J. Neurobiol. 23:579, 1992 and J. Neurosci. 16:2642, 1996). To investigate the role of target granule cell membranes in the regulation of afferent growth, we have developed a protocol for the preparation of highly enriched plasma membranes from purified granule cells or glia grown in primary culture. Using a two-step ultracentrifugation approach, we are able to prepare a membrane fraction with 30- to 40-fold relative specific activities of the plasma membrane markers 5'-nucleotidase and alkaline phosphatase. This fraction is applied onto a coverslip and used as a substrate for cell culture by the approach of Wizenmann et al. (Neuron 11:975, 1993), permitting the comparison of pontine mossy fiber growth on alternating lanes of membranes and/or laminin. With this approach, we are analyzing pontine axon length, fasciculation pattern, and branch formation on membrane stripes, and the potential role of adhesion molecules in target regulation of afferent growth.

Supported by NIH grants NS16951 (C.A.M.) and MSTP 5 T32 GM07367 (M.S.W.).

218.3

PREFERENTIAL BRANCHING OF CORTICAL NEURITES ON MEMBRANE STRIPES FROM APPROPRIATE LEVELS OF THE SPINAL CORD. E.Dent, J. Callaway and K. Kalil.* Neuroscience Training Program and Dept. of Anatomy, University of Wisconsin, Madison, WI 53706.

During development of the hamster corticospinal pathway *in vivo*, axons from both forelimb and hindlimb sensorimotor cortex extend the full length of the corticospinal tract. Nevertheless, interstitial collaterals branch only into targets at topographically appropriate levels of the cord. Previous studies *in vitro* showed that sensorimotor cortical growth cones respond to developmentally regulated membrane bound cues in the spinal cord. Therefore, in the present study we investigated whether membranes from different levels of the spinal cord would selectively influence branching of neurites from different topographic regions of the sensorimotor cortex. Alternating lanes of membranes from 8 day cervical and lumbar spinal cord were applied to filter supports and then transferred to glass coverslips to permit visualization of cortical neurites and their branches with phase microscopy. Explants from newborn hamster forelimb or hindlimb sensorimotor cortex were positioned on the membrane assays and after 48 hours neurite branching was assessed in fixed cultures. Branches were counted only if they grew at right angles from the cortical neurites and were at least 10u in length. Preliminary results showed that neurites from forelimb cortical explants branched equally well on membrane stripes from cervical and lumbar cord. In contrast, neurites from hindlimb cortical explants showed a marked preference for branching on membrane stripes from the lumbar cord. These results are consistent with the situation *in vivo* where hindlimb cortical axons must bypass cervical targets without branching, perhaps because of inhibitory target cues. Axons from forelimb cortex, on the other hand, may not need to recognize lumbar spinal targets because they regress from lumbar levels before target innervation occurs. To determine whether inhibitory molecules in the cervical cord prevent the growth of inappropriate collaterals from hindlimb axons, we are currently treating the membrane assays with PI-PLC. Supported by NIH Grant NS 14428 to K.K.

218.4

POSSIBLE ADHESIVE PROPERTIES OF ACETYLCHOLINESTERASE IN DEVELOPING CEREBRAL CORTEX. K.J. Claytor*, L.M. Amezcua, J. Yu and R.T. Robertson, Departments of Anatomy and Neurobiology and of Physical Medicine and Rehabilitation, College of Medicine, University of California, Irvine, CA 92717.

Recent work from this laboratory has investigated transiently expressed acetylcholinesterase (AChE) in developing cerebral cortex. AChE is expressed transiently by some thalamocortical axons during the time when the axons are growing into cortex and forming synapses with cortical neurons. The function of the transiently expressed AChE remains unknown. Recent evidence indicates that the AChE molecule includes a sequence homologous with cell adhesion molecules, suggesting that AChE may serve cell adhesion functions. The experiments presented here explored this possibility. Fluorescent microspheres (Covaspheres) were coated with recombinant AChE (kindly provided by Dr. Palmer Taylor of UCSD) and exposed *in vitro* to freshly cut sections of forebrain from early postnatal rat or mouse pups. The fluorescent microspheres coated with AChE bind non-randomly to slices of developing cerebral cortex, with a developmental pattern that mimics the position of AChE positive thalamocortical axon terminals. AChE coated spheres show preferential attachment to deep cortical layers in the first postnatal days, before layer IV differentiates from the cell dense cortical plate, and strong attachment to layer IV after layer IV differentiates. Microspheres coated with bovine serum albumin show only low level and random patterns of attachment. These data suggest that the transiently expressed AChE may serve an adhesive function to aid the developing thalamocortical axons' growth into cortex. *Supported by NIH grant NS 30109*

218.5

DENDRITIC DEVELOPMENT OF NEWLY-GENERATED GRANULE CELLS IN THE ADULT HIPPOCAMPUS OCCURS UPON CONTACT WITH RADIAL GLIA-LIKE CELLS. T. Seki*[1,2] and Y. Arai[1]. [1]Dept. of Anatomy, Juntendo Univ. Sch. of Med., Tokyo 113, Japan, [2]Dept. of Genetics, Case Western Reserve Univ., Sch. of Med., Cleveland, OH44106-4955

The granule cell layer of the hippocampal dentate gyrus possesses two immature aspects. Firstly, the granule cells are continue to be produced in the inner most region of the dentate granule cell layer even during adult period. We have found that newly generated granule cells of the adult dentate gyrus specifically express a highly polysialylated neural cell adhesion molecule (NCAM-H) that is known to be essential in neuronal development. Unlike mature granule cells, the NCAM-H expressing dendrites scarcely have dendritic spines, but spike-like or fan-shaped fine processes. Secondly, there are radial glia-like cells which are similar to radial glia in the developing cerebral cortex. The radial glia-like cells are located in the inner most region of the dentate granule cell layer and extends long radial processes which express a glial fibrillary acid protein (GFAP). Here we show that both NCAM-H expressing granule cells and GFAP-expressing radial glia-like cells decreased with aging. Further, the NCAM-H positive dendrites were found to be in close contacts with the radial processes of the glial cells by confocal laser scanning microscopy and immunoelectron microscopy. Occasionally, the NCAM-H expressing fine processes were surrounded by the glial processes. We assume that radial glia-like cells are associated with the development of the dendrites of newly generated granule cells in the adult dentate gyrus

218.6

DENERVATION IS INSUFFICIENT TO INDUCE COLLATERAL SPROUTING OF MYELINATED PRIMARY AFFERENTS IN THE ADULT SPINAL CORD. T.P.Doubell, H.Gill and C.J.Woolf. (Spon: Brain Research Association). Dept. of Anatomy & Developmental Biology, University College London, UK.

Using the transganglionic tracer B-HRP to map the central projections of rat sciatic myelinated A-fibres, it has been previously shown that peripheral nerve injury induces the sprouting of the central axons of myelinated primary afferents into lamina II of the dorsal horn, an area which normally only receives input from C-fibres (Woolf et al., Nature 355 (1992) 75-77).

We have found that the C-fibre specific neurotoxin capsaicin applied locally to the sciatic nerve also induces sprouting of A-fibres into lamina II (Mannion et al. (1995) Soc. Neurosci. Abtsr. 21,). Since nerve injury and capsaicin both induce transganglionic C-fibre atrophy, the production of vacant synaptic sites in lamina II may be a factor which initiates A-fibre sprouting in the dorsal horn.

The aim of this study was to test whether denervation alone is sufficient to produce A-fibre sprouting. The L4 spinal segment was partially denervated by sectioning the L4 segmental nerve or the L4 dorsal root, and we investigated, using B-HRP labelling, if remaining intact or peripherally axotomized myelinated sciatic afferents sprouted into the denervated areas.

Neither dorsal root nor segmental nerve section resulted in neighbouring intact myelinated fibres sprouting into lamina II and these lesions prevented the central terminals of injured axons from sprouting into the denervated area. We conclude that denervation alone does not produce sprouting and are examining whether a chemotropic factor released by C-fibres may be involved.

Supported by the MRC.

218.7

BASAL FOREBRAIN CHOLINERGIC PROJECTIONS TO CEREBRAL CORTEX: LACK OF TARGET SPECIFICITY IN ORGANOTYPIC SLICE CULTURES. J. Baratta*, D.H. Ha, J. Weiss, J. Yu and R.T. Robertson, Depts. of Anatomy and Neurobiology, Neurology, and Physical Medicine and Rehabilitation, College of Medicine, University of California, Irvine, CA 92717.

Previous studies have demonstrated that basal forebrain cholinergic neurons send their axons to the cerebral cortex in a topographically organized projection. At the extremes, cholinergic neurons of the septum send their axons to hippocampus while cholinergic cells in the substantia innominata and medial globus pallidus send their axons to lateral neocortex. Results of experiments presented here tested the hypothesis that this topographic organization results from target preferences of the cholinergic neurons. Tissue slices containing either medial septum or substantia innominata were grown together with slices of lateral neocortex and of hippocampus as organotypic triple cultures. After several days to 2 weeks, cultures were fixed and processed for AChE histochemistry to demonstrate growth of cholinergic axons. Cholinergic neurons from septum and from substantia innominata projected axons into both neocortex and hippocampus in organotypic patterns. The density of axons was greater in hippocampus than in neocortex, irrespective of whether the axons were derived from septal or substantia innominata tissue. Addition of exogenous nerve growth factor increased the number of AChE postive axons, but did not alter their apparent patterns of termination. These data suggest that basal forebrain cholinergic neurons can innervate any portion of the cerebral mantle, and the topographic organization seen in vivo does not result from chemospecificity of targets. *Supported by NIH grant NS 30109*

218.8

OBSERVATIONS ON THE MORPHOLOGY OF GROWING THALAMO-CORTICAL AXONS IN DEVELOPING RAT. K.Ishii*[1,2], E.G.Jones[1] and T.Shirai[2]. [1]Dept. of Anatomy and Neurobiology, Univ.of California, Irvine, CA 92715 and [2]Dept. of Anatomy, Yamagata Univ. Sch. of Med., Yamagata 990-23, Japan.

We examined the outgrowth of thalamocortical axons in developing rats and their distribution in the cerebral cortex with DiI and Biocytin labeling methods. All animals were anesthetized with sodium pentobarbital (Nembutal,i.p.) or by hypothermia. We placed crystals of DiI into the thalamus in the fixed brains from embryonic day(ED)16 to postnatal day(PD)1, labeled thalamocortical axons with DiI anterogradely, and observed the pathway under a fluorescence microscope. We also injected 5%Biocytin (Sigma) from a posterior direction into the right thalamus of ED19 and PD0 rats. After 12-24 hours,the rats were transecardinally perfused with 4%paraformaldehyde in 0.1Mphosphate buffer. Sections were cut in the coronal plane on a freezing microtome and reacted for Biocytin histochemistry. At ED16, thalamocortical axons were found in the intermediate zone laterally in the cerebrum. Their tips showed various growth cones. By ED18, they had arrived at dorsal areas, and formed bundles in the developing white matter. At ED18, their tips began to appear in layer VI of the cerebral cortex in the lateral and dorsal areas. At ED20, they were densely distributed in layer VI. At PD1, they had reached middle layers and had many horizontal branches there. Many of the tips continued to show growth cones and a few of them reached layer I. These findings show the morphology of thalamocortical axons as they arrive at the intermediate zone at ED16, as they enter into the deep layer of the cerebral cortex at ED18, and begin to be distributed in the deep and middle layers of the cerebral cortex at PD1. Supported by fellowship of Ministry of Education,Science and Culture in Japan(KI).

FORMATION AND SPECIFICITY OF SYNAPSES III

219.1

AGRIN ISOFORM EXPRESSION BY CHICK SPINAL CORD NEURONS IN CULTURE. L. S. Honig* and K. Scott. Department of Neurology, University of Texas Southwestern Medical Center at Dallas, Dallas, TX 75235-9036.

Agrin, a ~200kD glycoprotein, is expressed as several isoforms, through developmentally regulated alternative splicing. Only certain isoforms are active in synaptogenesis at the neuromuscular junction. This study examines the regulation of expression of these isoforms by neurons of the E6 embryonic chick spinal cord. Prior studies have shown that motor neurons (MN) in vivo principally synthesize "active" A4B19 and A4B11 (B+) isoforms during synaptogenesis, while other spinal neurons (SN) principally express "inactive" A4B0 (B-) isoform. We have cultured isolated embryonic spinal neurons and examined by PCR studies whether the pattern of isoform expression might be regulated by the presence of neurotrophic factors or by the influence of target tissue, i.e. muscle cells. MN cultured 2 -7days show increasing "active" B+ isoform production, especially A4B19. Surprisingly, cultured SN, which at the time of isolation express almost exclusively B- isoforms, also start expressing B+ isoforms during culture. A variety of neurotrophic factors (CNTF, BDNF, NT3, GDNF), which do not affect MN survival in our cultures, do not have marked effects on the neuronal patterns of agrin expression. Tissue extract (from E10 chick embryos) increases MN survival, as already demonstrated, but it does not alter the relative pattern of isoform expression during culture. Co-culture of motor neurons with myotubes, resulting in muscle AChR aggregation, does not cause apparent alteration of the isoform pattern. Thus, under our conditions, factors other than cell type, developmental stage and culture period that might influence neuronal agrin expression were not identified. The pattern of isoform expression of agrin may be a relatively autonomous cell function.
(Supported in part by an award from the UT President's Research Council.)

219.2

AGRIN INDUCES PHOSPHORYLATION OF MUSCLE-SPECIFIC KINASE (MuSK) IN MYOTUBE CULTURE. A. K. Y. Fu*, K. M. Chan, F. C. F. Ip, D. M. Valenzuela[1], D. J. Glass[1], G. D. Yancopoulos[1], K. W. K. Tsim and N. Y. Ip. Department of Biology, Hong Kong University of Science and Technology, Clear Water Bay, Kowloon, Hong Kong, and [1]Regeneron Pharmaceuticals, Inc., Tarrytown, New York.

A recently identified muscle-specific kinase, designated MuSK, was found to be highly expressed during muscle development, and localized to the neuromuscular junction in mature muscle, coincidental with the distribution of acetylcholine receptor (AChR). The expression of MuSK was low in proliferating myoblasts and was dramatically induced upon fusion into myotubes. In an attempt to identify the ligand that can activate MuSK, we have utilized the neuron-muscle co-culture system, i.e. NG108-15 and C2C12 cells, to examine the ability of various candidates to induce phosphorylation of MuSK. We found that conditioned medium from NG108-15 cells, as well as exogenous addition of agrin could induce phosphorylation of MuSK in C2C12 myotubes within 5 minutes; significant MuSK phosphorylation was still observed at 4 hours. Inactive form of agrin that could not induce AChR aggregates also failed to induce MuSK phosphorylation. The ability of agrin to induce MuSK phosphorylation was similarly observed with primary rat muscle cultures. Co-localization of MuSK and AChR was observed at the neuromuscular junction of the adult rat, but not during embryonic stages when clustering of AChR was evident. Taken together, our findings demonstrated that agrin could activate MuSK and suggest that agrin-MuSK interactions play an important role in the development of the neuromuscular junction.

219.3

DEVELOPMENTAL EXPRESSION AND UPREGULATION OF MUSCLE-SPECIFIC KINASE (MuSK) IN DENERVATED SKELETAL MUSCLE OF THE CHICK AND RAT. F. C. F. Ip*, A. K. Y. Fu, K. W. K. Tsim, J. Cheung, D. M. Valenzuela[1], D. J. Glass[1], G. D. Yancopoulos[1] and N. Y. Ip. Department of Biology, Hong Kong University of Science and Technology, Clear Water Bay, Kowloon, Hong Kong, and [1]Regeneron Pharmaceuticals, Inc., Tarrytown, New York.

A novel receptor tyrosine kinase specific for the skeletal muscle lineage, designated MuSK, was recently identified. The developmental profile of MuSK gene expression in various tissues of the chick and rat was examined by Northern blot analysis. Multiple MuSK transcripts were detected at abundant levels in skeletal muscles of the rat and chick, particularly during the stages of E10 to P2 in chick and E16 to P2 in rat. Whether these multiple transcripts represent different alternatively spliced forms of MuSK remains to be determined. Interestingly, a low level of MuSK expression could also be detected in different brain regions. Similar to that observed in denervated rat skeletal muscle, the expression of MuSK in skeletal muscle of postnatal chick was upregulated after nerve transection or crush. While MuSK expression remained elevated 60 days after nerve transection, it returned to control level 30 days after nerve crush. Similar pattern of MuSK upregulation following nerve injury could be observed in four different skeletal muscles examined. Taken together, our findings revealed similar profiles of expression for MuSK and AChR during development and following nerve injury.

219.4

FUNCTIONAL INTERACTION WITH THE ACHR IN C2 MYOTUBES IMPLIES A ROLE FOR SRC-FAMILY KINASES IN AGRIN'S SIGNALING PATHWAY. C. Fuhrer and Z.W. Hall*, National Institute of Mental Health, NIH, Bethesda MD.

In cultured mammalian muscle, agrin induces tyrosine phosphorylation of the β subunit of the nicotinic acetylcholine receptor (AChR). The time course and specificity of phosphorylation suggest that it could play a role in agrin-induced AChR clustering. To investigate the mechanism by which the AChR becomes tyrosine-phosphorylated we tested the ability of src-family kinases to interact with peptides derived from the cytoplasmic loop of the β subunit of the AChR. Using bacterial fusion proteins we found that src binds to and phosphorylates the N-terminal half of the β subunit loop containing a tyrosine-phosphorylation site. Immunodepletion of src abolishes phosphorylation, showing that src accounts for most of the β subunit tyrosine-phosphorylating activity in muscle extracts. The interaction of src with the β subunit is specific, as the corresponding peptides from the γ and δ subunits did not bind src, and because no binding was observed to the src-family members yes and fyn. To examine the association of endogenous AChR with src, receptors were isolated from C2 myotubes by affinity purification and tested for the presence of src-family kinases. Both fyn and, to a lesser degree, src were associated with the receptor. AChRs were also found to have tyrosine-phosphorylated β subunits. Our results are consistent with previous experiments showing that fyn is associated with tyrosine-phosphorylated Torpedo AChR (Swope and Huganir, 1994, J. Biol Chem. 269, 29817-29824). We suggest that the AChR of mammalian muscle is initially phosphorylated by src and subsequently interacts with fyn in a phosphotyrosine-dependent way. These interactions could play a role in agrin's signaling pathway. Supported by NIMH, Muscular Dystrophy Association, and Human Frontier Science Program.

219.5

THE DISTRIBUTION OF AGRIN IN THE SYNAPTIC CLEFT AT THE NEUROMUSCULAR JUNCTION IS CORRELATED WITH THE ARRANGEMENT OF ACTIVE ZONES IN THE AXON TERMINAL. M. Schwarz, P. Theodosopoulos, R. Marshall, and U.J. McMahan*, Dept. of Neurobiology, Stanford University, Stanford CA-94305.

Activity dependent exocytosis of ACh-containing vesicles in the axon terminal occurs along the sides of "active zones" in the presynaptic membrane. The muscle fiber's plasma membrane just opposite the active zones is infolded and AChRs in the plasma membrane are concentrated at the mouths and along the upper sides of the folds. Agrin, which is released by the axon terminal and then bound to the basal lamina in the synaptic cleft induces the formation and maintenance of junctional folds and AChR aggregates. As part of a long range study aimed at characterizing the mechanisms involved in the formation and maintenance of the postsynaptic apparatus we studied the distribution of agrin in the synaptic basal lamina and its relation to active zones at the adult frog neuromuscular junction. Agrin was labeled for EM by a gold conjugated antibody which recognized muscle as well as neural agrin, which is also concentrated in the synaptic cleft but has relatively little AChR-aggregating activity. We found that 68% of the gold particles were located in the synaptic cleft directly under the active zone; this area accounted for only 22% of the total area of the synaptic cleft. The distribution of grains at the active zone peaked at its edges, the sites of activity dependent exocytosis. The concentration of gold particles in the junctional folds was greater than in extra-active zone regions of the synaptic cleft, although it gradually diminished with distance from the mouth of the fold. These findings lead to the hypotheses that the release of agrin from axon terminals is activity dependent, that the release occurs at the same sites as the release of ACh, that the sites of action of neural agrin on the muscle fiber are at or near the mouth of the junctional folds and that neural agrin is stably concentrated in the basal lamina near its sites of action.
This study was supported by NIH grant NS14506.

219.6

LOCALIZATION OF UTROPHIN mRNA IN SKELETAL MUSCLE FIBERS AND ITS REGULATION BY AGRIN. A.O. Gramolini[1], J.M. Tinsley[2], G.S. Robertson[1], K.E. Davies[2], J. Cartaud[3] and B.J. Jasmin[1]*. [1]University of Ottawa, Ottawa, Canada; [2]John Radcliffe Hospital, Oxford, UK; [3]Institut Jacques Monod, Paris, France.

In contrast to the sarcolemmal distribution of dystrophin, utrophin accumulates at the postsynaptic membrane of the neuromuscular synapse. To explore the cellular and molecular mechanisms involved in maintaining this synaptic localization, we first performed in situ hybridization experiments. We showed that out of 375 neuromuscular synapses identified by acetylcholinesterase histochemistry, 313 (84%) displayed a precise co-localization between the presence of utrophin mRNAs and that of neuromuscular synapses. Abolition of nerve-derived electrical activity had only a modest effect on utrophin mRNA expression suggesting that the restricted presence of utrophin transcripts within the postsynaptic sarcoplasm is influenced locally by factors released from motor nerve terminals. In separate studies, we examined the developmental regulation of utrophin expression in C2 myotubes. Initially, we observed that utrophin transcripts are rare in myoblasts but that in multinucleated myotubes, levels of utrophin mRNA increase markedly. Through a sequential detergent/salt extraction procedure, we also noted that in myotubes, utrophin mRNAs are enriched within a cytoskeletal-bound polysomal fraction. Next, we treated myotubes with agrin purified from Torpedo electric tissue in attempts to identify putative extracellular cues involved in maintaining the synaptic accumulation of utrophin mRNAs. In addition to inducing acetylcholine receptor clusters which contained utrophin, agrin treatment significantly increased expression of utrophin transcripts. Utrophin mRNAs in myotubes treated with agrin were also found preferentially within the cytoskeletal-bound polysomal fraction. Together, these results show that utrophin transcripts are selectively expressed within the postsynaptic sarcoplasm of adult neuromuscular synapse and further suggest that agrin may contribute to the maintenance of this synaptic accumulation.
This work is supported by AFM and MRC of Canada and the UK.

219.7

ULTRASTRUCTURAL ORGANIZATION OF DEVELOPING ACETYLCHOLINE RECEPTOR AGGREGATES. D. D. Kunkel* and J. Stollberg, Békésy Laboratory of Neurobiology, University of Hawaii, Honolulu, HI 96822.

Acetylcholine receptor (AChR) aggregation is one of the key events in synaptogenesis at the neuromuscular junction. Aggregation begins shortly after contact by a motorneuron, and can be induced by several experimental manipulations including agrin - an extra-cellular matrix protein synthesized by motor neurons. Observations of AChR aggregation have been limited to the resolution of light microscopy. Thus, very little is known about the molecular structure of AChR aggregates. In order to examine the spatial arrangement of AChRs in developing aggregates we have employed high resolution scanning electron microscopy (SEM) and quantitative image analysis.

Cultured *Xenopus* muscle cells were stimulated with agrin, immuno-cytochemically labeled for AChRs using 12 nm gold particles, and observed using a field emission SEM. Backscattered electron images of the gold labeled AChRs were collected from random regions of membrane. Images were digitized and gold particle locations were ascertained in order to produce a list of their X-Y coordinates (+/- 2 nm). Using "nearest neighbor analysis" and "three point angle analysis we have shown 1) that small micro-aggregates of receptors occur within 2 hours of stimulation, and 2) that the receptors in these microaggregates are bound to an inflexible lattice with 10 (± < 1) nm spacing and hexagonal geometry. The data further indicate that formation of the lattice is the rate limiting step in receptor aggregation, rather than the diffusion rate of the receptor.

Supported by NIH grant NS26943 and Hawaii American Heart Foundation grant HIGS-17-95.

219.8

INTRACELLULAR CALCIUM FLUXES ARE REQUIRED FOR AGRIN-INDUCED AChR CLUSTERING

L.J. Megeath* and J.R. Fallon. Worcester Foundation for Biomedical Research, Shrewsbury MA 01545 and Dept. of Neuroscience, Brown University, Providence RI 02912.

Agrin is an extracellular matrix protein that induces the clustering of acetylcholine receptors (AChRs) and other postsynaptic molecules on muscle cell membranes. The signal transduction pathways by which agrin induces the clustering of AChRs are poorly understood, but may involve tyrosine phosphorylation of AChR subunits. We investigated the role of intracellular calcium fluxes in agrin-induced AChR clustering using BAPTA-AM, an agent that clamps intracellular calcium. BAPTA-AM inhibits agrin-induced AChR clustering on embryonic chick myotubes. Inhibition was dose-dependent and was 67% at 50 μM BAPTA-AM (n=7). BAPTA-AM treatment altered neither agrin binding nor AChR levels, and its effects were reversible. AChRs remained mobile in BAPTA-AM treated myotubes, as determined by their ability to be clustered by anti-AChR antibodies. Interestingly, agrin-induced tyrosine phosphorylation of AChR β subunits was unaffected by BAPTA-AM.

These results indicate that intracellular calcium fluxes are necessary for agrin-induced AChR clustering, and suggest that they are downstream of AChR phosphorylation in agrin's signaling pathway. These findings raise the possibility that the formation and maintenance of postsynaptic structures could be regulated by the interaction of such calcium fluxes with agrin's signal transduction mechanism.
Supported by MDA and NIH.

219.9

THE ROLE OF LAMININ IN THE ASSOCIATION OF ENDOGENOUS MUSCLE AGRIN WITH DYSTROGLYCAN J. E. Sugiyama, M.J. Ferns* and Z.W. Hall. National Institute of Mental Health, NIH, Bethesda, MD 20892.

The development of the postsynaptic specialization during synaptogenesis at the neuromuscular junction is thought to be organized by agrin, an extracellular matrix component produced by both nerve and muscle. Nerve-derived agrin induces acetylcholine receptors to cluster at the neuromuscular junction, but the functional role of muscle-derived agrin, a biologically less-active form, remains unclear. *In vitro* studies have shown that soluble forms of both nerve and muscle agrin bind to alpha-dystroglycan, a member of the dystrophin complex. Here, we show that endogenous agrin in muscle extracts is associated with dystroglycan and that this association is enhanced by the addition of laminin. Extracts of C2 myotubes were treated with EHS laminin and immunoprecipitated with an antibody against agrin. Following dissociation in SDS sample buffer, the proteins were separated by PAGE, transferred to nitrocellulose blots and probed with a beta-dystroglycan antibody. Agrin in the extracts was found to be associated with beta-dystroglycan. Surprisingly, the extent of association was increased in a dose-dependent manner by laminin, which is known to bind alpha-dystroglycan. Both heparin and calcium removal inhibited the enhanced association seen with laminin. These results indicate that laminin may play a role in assembly of the myotube extracellular matrix by enhancing or stabilizing the interaction between muscle agrin and the dystrophin-glycoprotein complex, thereby strengthening the linkage between the extracellular matrix and the cytoskeleton. (This work was supported by grants from NIH and MDA)

219.10

α-DYSTROGLYCAN BINDS HEPARIN, M.A. Bowe* and J.R. Fallon, Worcester Foundation for Biomedical Research, Shrewsbury, MA 10545 and Dept. of Neuroscience, Brown University, Providence, RI 02912

Synaptic function is critically dependent upon the coordinated expression and highly ordered interaction of an ensemble of cytoskeletal, transmembrane and extracellular components. α-Dystroglycan (αDG) is an extrinsic peripheral membrane protein found in several tissues, including muscle and brain. It is the major agrin binding protein in postsynaptic membranes from *Torpedo* electric organ. αDG forms a tight complex with the transmembrane protein ß-dystroglycan, which has been shown to bind the cytoskeletal components dystrophin and utrophin. The α·ßDG complex is therefore well-poised to form a key link between the cytoskeleton and the extracellular matrix at the synapse.

Proteoglycans have been implicated in regulating synaptic structure. For example, the glycosaminoglycan (GAG) heparin blocks both agrin- and nerve-induced acetylcholine receptor clustering on cultured neurons, and muscle cells lines deficient in GAG synthesis form abnormal post-synaptic specializations. Here, we asked whether the α·ßDG complex interacts directly with GAGs. We found that native α·ßDG complex solubilized from *Torpedo* electric organ bound to immobilized heparin. To map the heparin binding site, we made expression constructs encoding fragments of α- and ß-dystroglycan. Recombinant αDG also bound heparin, with the protein sequence responsible for binding located within the c-terminal half of αDG. Carbohydrate modification of αDG is not necessary for heparin binding. These findings suggest that GAGs may directly regulate dystroglycan's activity. [Supported by the NIH and ACS.]

219.11

EFFECTS OF INHIBITION OF AGRIN SYNTHESIS ON ADHESION, NEURITE OUTGROWTH AND GEPHYRIN CLUSTER FORMATION. G. Escher*[1], C. Bechade[2] & A. Triller[2]. [1]Inst. Anat., Univ. of Lausanne, 1005 Lausanne, Switzerland. [2]Biologie Cellulaire de la Synapse (INSERM CJF 94-10) Ecole Normale Supérieure, Paris, France.

Agrin, a synaptic basal lamina protein synthesized by motoneurons is involved in the aggregation of AchRs. Agrin is widely expressed in the CNS and might be involved in the formation of receptor-rich microdomains.

To approach this question, we used rat embryonic dorsal horn neurons kept in culture. These neurons express glycine receptors (GlyR) which form postsynaptic clusters. They are maintained as such by gephyrin, a protein anchoring the GlyR to the cytoskeleton, and necessary to the maintenance of GlyR clusters. We previously showed that agrin is synthesized in these cultures by almost all neurons and is targeted to axons. We now show that gephyrin clusters (present on somata and on dendrites) are almost always in front of agrin positive axons. This is compatible with a role of agrin in the clusterization of the GlyR.

To test this hypothesis, agrin expression was inhibited using two antisense phosphorotioate oligonucleotides; the corresponding shuffled oligonucleotides being used as controls. After 6 days in vitro, agrin-like immunoreactivity was markedly reduced by the daily addition of one or the other antisense oligonucleotides given at of 1 or 5 µM (agrin transcripts could still be detected by RT-PCR). Important morphological changes were observed: cells grouped together and formed aggregates, neurites started to fasciculate, and cells eventually detached from the bottom of the dish. In contrast, in control or untreated cultures, no such morphological changes were observed. Gephyrin clusters of the membrane however, were similar in treated, control or untreated cultures. As suggested by the recent unraveling of agrin as an heparan sulfate proteoglycan, our results support the idea that agrin plays a role in cell adhesion and neuritic growth.
Work supported by Swiss NSF 364.017.93 (G.E.) and by AFM grants (A.T.)

NEUROTRANSMITTER SYSTEMS AND CHANNELS: DEVELOPMENT OF INTRINSIC CELLULAR PROPERTIES—IONIC CURRENTS AND SYNAPTOGENESIS

220.1

DEVELOPMENT OF CELLULAR PROPERTIES OF NEURONS IN RAT SUPERFICIAL SPINAL DORSAL HORN . M.C. Jiang* and G. F. Gebhart, Department of Pharmacology, University of Iowa, Iowa City, IA 52242

It is well appreciated that the central nervous system, including the spinal cord, undergoes postnatal maturation. The goal of these experiments was to investigate the maturation of cellular properties of lumbo-sacral spinal neurons in the superficial dorsal horn.

Sprague-Dawley rats (both sexes) were divided into five groups according to age (1 and 20 d old). Transverse slices (400 µm) from the lumbo-sacral spinal cord were maintained at 34°C in oxygenated artificial CSF for intracellular recordings of neurons in a current clamp mode. Intrinsic properties were tested by intracellular current injection, 0.5 nA each step, at varying durations. Synaptic input was obtained by electrical stimulation of the attached dorsal root.

Neurons with stable resting membrane potential (at least - 55 mV) and greater than 55 mV amplitude action potential were studied. It was found that the threshold for action potential generation required greater depolarization in younger rats. The half-width of the action potential clearly showed an age-dependent decrease from 1.2 ms to 0.6 ms. The majority of dorsal horn neurons showed slow afterhyperpolarizations (AHP). Fast AHP and afterdepolarization (ADP) could also be found in older rats. Regarding spike frequency adaptation (SFA), three types of SFAs, fast, slow and non-SFA, could be seen at all ages. However, non-SFAs were more common in younger rats. The major component of the fast EPSP is NMDA receptor-mediated in younger rats and AMPA receptor-mediated in older rats. These results reveal that the nature of synaptic input and information processing in the superficial dorsal horn is different in young rats. Supported by NS 19912.

220.2

DEVELOPMENT OF ELECTRICAL EXCITABILITY IN ANTENNAL-LOBE NEURONS OF THE SPHINX MOTH *MANDUCA SEXTA*. A.R. Mercer[1,2], P. Kloppenburg[1], and J.G. Hildebrand[1]*. [1]ARL Div. of Neurobiology, Univ. Arizona, Tucson, AZ 85721, and [2]Dept. of Zoology, Univ. Otago, Dunedin, NZ.

Using whole-cell, patch-clamp recordings from antennal-lobe (AL) neurons *in vitro* and in semi-intact brain preparations, we have examined changes in cell membrane properties associated with the development of the AL neuropil. Development of whole-cell current profiles and cell excitability was examined in 2 morphologically distinct subtypes of *Manduca* AL neurons *in vitro*: PB neurons and RR neurons, identified previously as projection (output) neurons and local AL interneurons, respectively (Oland & Hayashi 1993, *J. Neurobiol.* 24:1170). Cells cultured from animals at stage 3 of the 18 stages of metamorphic adult development exhibit whole-cell current profiles that are dominated by large outward (K^+) currents. Although calcium spikes can be elicited from cells at this stage, only a small percentage exhibit sodium spikes. In cells taken from animals at progressively later stages of adult development, the percentage of spiking cells increases, and the waveform of sodium-based action potentials becomes shorter in duration and larger in amplitude. From stages 3 to 10, a significantly larger proportion of PB neurons exhibit sodium currents than RR neurons. By stage 14, sodium currents are apparent in almost all neurons, and spontaneous bursts of spiking activity are frequently observed. Although the appearance of sodium-based action potentials coincides temporally with the ingrowth of primary sensory afferent axons and the onset of formation of protoglomeruli in the AL neuropil, deafferentation early (stage 2) in metamorphic adult development does not affect the development of the whole-cell current profiles of RR neurons and PB neurons. Recordings from AL neurons in isolated brain preparations suggest that cells *in vitro* reflect well the membrane properties of same-stage AL neurons *in situ*. Changes in the biophysical properties of PB and RR neurons over time in culture, if they occur, are very slow. *[Supported by NIH grants AI-23253 (JGH,PK), NS-28495 (JGH) and ORG MFZB77 (ARM)]*

220.3

DEVELOPMENTAL TIME-COURSE STUDIES OF CYTOCHROME OXIDASE, NMDAR1, NITRIC OXIDE SYNTHASE AND NA^+/K^+ ATPASE IN PRIMARY NEURONAL CULTURES OF RAT VISUAL CORTEX. C. Zhang* and M.T.T. Wong-Riley, Dept. of Cellular Biology and Anatomy, Medical College of Wisconsin, Milwaukee, WI 53226

Our goal was to determine the developmental time-course of metabolic activity of neurons in glucose-free cultures. We also wished to know if such developmental process bore any relationship to possible changes in neurochemicals related to excitatory glutamatergic synapses. We prepared primary neuronal cultures from postnatal rat visual cortex. Cytochrome oxidase (C.O.) histochemistry, nitric oxide synthase (NOS), NMDA receptor subunit R1 (NMDAR1), and Na^+/K^+ ATPase immunocytochemistry were done, and the intensities of reaction product for these neurochemicals were quantitatively analyzed by optical densitometry. Neurons in cultures showed morphology similar to those *in vivo*. The size of cell bodies visibly increased during the first two weeks in culture, and reached plateau after the third week. Neurites lengthened with time and formed local network after the first week. C.O. activity and immunoreactivity for NOS, NMDAR1 and Na^+/K^+ ATPase were present in both cell bodies and neurites, and their levels increased progressively from 1 to 21 days in culture. Increases in optical densitometric values for these neurochemicals were not attributable to increases in cell size during development. Our results suggest that the regulation of metabolic activity in neurons may be related, at least in part, to developmental changes in levels of NOS, NMDAR1 and Na^+/K^+ ATPase, which, in turn, may be associated with excitatory synaptic interactions in this culture system.

(Supported by NIH grant EY05439)

220.4

EXPRESSION OF AN N-TYPE CALCIUM CHANNEL COMPLEX IN THE DEVELOPING RAT HIPPOCAMPUS. O.T. Jones', G.M. Bernstein, J.Francis, D.G.M. Jugloff, W. Wong, J.H. Eubanks, and L.R. Mills. Playfair Neurosci. Unit, TTH Res. Inst. & Dept. Pharmacol., U. of Toronto, Canada M5T 2S8.

The expression of voltage-dependent calcium channels (VDCCs) in neurons is critical for transmitter release, excitability and in establishing the cytoarchitecture of the CNS. However, a molecular and cellular dissection of the interplay between VDCC expression and CNS development is plagued by the diversity of VDCCs in brain. Such diversity arises via multiple genes encoding the structurally dissimilar α_1,α_2/δ and β subunits of the VDCC heteromer and to alternative splicing of many of the RNA transcripts.

We now describe the expression of a major VDCC complex implicated in neuronal migration - the N-channel - from embryonic to adult stages in rat hippocampus. Expression was measured using the following indices: selective ligand binding, the levels of mRNA and protein corresponding to the $\alpha_{1B}\alpha_2/\delta$ and β_3 subunits of this complex, co-immunoprecipitations and fluorescent imaging of cell-surface N-VDCCs. While α_{1B} and β_3 subunits are already present in the E18 hippocampus, the bulk of their expression occurs in the first two weeks following birth. Initial data suggests that the fraction of α_{1B} associated with β_3 rises in the first week of birth. Surprisingly, the expression of N-VDCCs is not even throughout the hippocampus but occurs in subfields CA3 and CA2 prior to dentate gyrus and CA1. In all regions N-VDCCs appear to be first expressed on the soma and only then in the dendrites.

These data have significant implication for the role of N-VDCCs in migration, synaptogenesis and pruning and in the establishment of neurotransmission in the hippocampus.

This work was supported by MRC and NSERC Canada, The Ontario Mental Health, Savoy and Bloorview Epilepsy foundations.

220.5

WEAVER GRANULE NEURONS EXHIBIT ABNORMAL DIFFERENTIATION OF VOLTAGE-DEPENDENT IONIC CURRENTS. R.R. Stewart*, J.M. Wright and P. Liesi. Laboratory of Molecular & Cellular Neurobiology, NIAAA, NIH, Bethesda, MD 20892-8205.

The properties of voltage-dependent ionic currents were assessed in wv/wv and +/+ granule neurons cultured from P7 mice on a laminin substrate for 1 day in RPMI 1640 medium. Both types of granule neurons extended bipolar processes on laminin. With external and internal solutions to measure sodium and potassium currents, it was found that, 11 of 12 +/+ and only 1 of 11 wv/wv granule neurons had detectable sodium currents which were blocked reversibly by 300 nM tetrodotoxin. A-type potassium current ($I_{K(A)}$), identified by its steady-state half-inactivation (-90±2 mV +/+ and -88±2 mV wv/wv; mean ± SEM, P =0.6) and rapid recovery from inactivation properties (range 16-34 ms), was present in 21 of 23 +/+ and 14 of 24 wv/wv granule neurons. For cells with detectable $I_{K(A)}$, the current densities of +/+ and wv/wv granule neurons were significantly different (31±4 pA/pF for +/+ (n=21) and 18±2 pA/pF for wv/wv (n=14); P=0.02). In addition, the slowly inactivating delayed rectifier potassium current that was blocked reversibly by 20 mM tetraethylammonium was detectable in all granule neurons and had significantly more current density in +/+ (62±3 pA/pF; n=34) than in wv/wv (42±5 pA/pF, n=24) granule neurons (P<0.001). Thus, wv/wv granule neurons exhibit abormal development of three types of voltage-dependent ionic currents. This work was supported by an intramural grant from NIAAA, NIH.

220.6

DURING POSTNATAL BRAIN DEVELOPMENT THE OBSERVED CHANGES IN Na^+ CHANNELS GATING PROPERTIES REFLECT CHANGES IN THE CHANNEL'S SIALIDATION LEVEL. D. Balbi, C.Castillo and E. Recio-Pinto*. Instituto de Estudios Avanzados, Caracas 1015A, Venezuela. Anesthesiology and Physiology Depts, Cornell University Medical College, New York 10021.

The gating properties of rat brain Na^+ channels were studied at three postnatal ages, day 0 (P0), 15 (P15) and adult (>P30) by using the planar lipid bilayer system. The midpoint potential of activation of BTX-modified channels changed from -58mV in P0 channels to -76mV in older channels. At negative potentials gating state changes were observed in all channels; at positive potentials they were observed in P0 channels but rarely in older channels. A long nonconductive state was displayed by most P0 channels but rarely by older channels. Previous studies have shown that neuraminidase treatment of Na^+ channels produces a depolarization shift of the activation curve and increases the frequency of long channel closures (Recio-Pinto et al., 1990, Neuron: 5:675-684). In order to investigate whether the observed development changes were due to changes in the sialidation level of the sodium channel, Western blots were done using forebrain plasma membrane preparations before and after neuraminidase treatment. Before treatment, the channels' apparent MW was 210kDa for P0 and 240kDA for P15 and adult Na^+ channels. After neuraminidase treatment the apparent MW of Na^+ channels decreased and became similar for P0 and adult channels. Therefore the increase in the Na^+ channel apparent MW during postnatal development could be accounted for by an increase in the channels' sialidation level. Neuraminidase treatment of adult channels resulted in a channel behavior resembling that of P0 channels. Therefore, during postnatal brain development the Na^+ channel function changes and those changes appear to reflect changes in the channels' sialidation level. An increase in the sialidation level of Na^+ channels is an additional *in vivo* cellular mechanism that contributes in defining neuronal excitability during brain development.

220.7

DEVELOPMENTAL REGULATION OF INTRINSIC MEMBRANE OSCILLATIONS IN RAT CA3 HIPPOCAMPAL NEURONS. F. Strata*, M. Atzori, E. Cherubini. Biophys. Lab, Int Sch Adv. Studies (SISSA), 34014 Trieste, Italy.

Intracellular recordings were used to investigate the mechanisms underlying intrinsic membrane oscillations in CA3 hippocampal neurons in slices obtained from newborn and adult rats. In adult rats, when cells loaded with intracellular cesium were depolarized to a potential ranging from -50 to -43 mV, they exhibited membrane oscillations (0.1-4 Hz). These oscillations were unaffected by tetrodotoxin (TTX, 1 µM). In TTX, the frequency of the oscillations was voltage dependent, varying from 0.1 Hz at -58 mV to 7 Hz at -40 mV. Below -58 mV membrane oscillations were blocked. K^+-channel blockers such as 4-aminopyridine (200-500 µM), tetraethylammonium (10 mM) and barium (0.3-1 mM) elicited plateau potentials with superimposed fast (4-7 Hz), small amplitude oscillations. In the absence of external calcium, membrane oscillations were blocked; they reappeared when extracellular calcium was raised to 0.1 mM. The calcium channel antagonist, Cd^{2+} (50-100 µM) irreversibly blocked membrane oscillations. A similar effect was obtained with nifedipine (1 µM) or nickel (20 µM); in this case the block was voltage-dependent. Moreover, the intrinsic rhythmic activity was reversibly abolished by carbachol (10 µM).

In newborn rats (P1-P5), in the same experimental conditions (intracellular cesium), spontaneously occurring membrane oscillations were not detected. Few oscillatory events however could be triggered by a depolarizing current pulse to a potential more positive than -20 mV. These rapidly faded during the sustained membrane depolarization.

These data suggest that during development the kinetic properties of Ca^{2+} channels in the hippocampus are substantially different from adult ones and this may affect information processing in early postnatal life.

220.8

TONICALLY ACTIVE DIVALENT CATION ENTRY INTO EMBRYONIC RAT CORTICAL CELLS *IN VITRO* H. Xian, D. Maric, I. Maric and J. L. Barker*. Lab. of Neurophysiology, NINDS, NIH, Bethesda, MD 20892

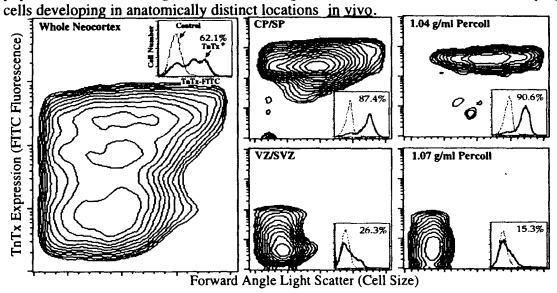
Cytosolic calcium (Ca^{2+}_c) is an important intracellular signal that regulates many cellular functions. Ca^{2+}_c is closely regulated by numerous membrane and intracellular mechanisms. We studied Ca^{2+} homeostasis in acutely dissociated embryonic cortical cells (E12-E19) using digital fluorescence microscopy and the Ca^{2+}-indicator fura-2. We found that the basal Ca^{2+}_c level largely depended on extracellular Ca^{2+} (Ca^{2+}_e). Removal of Ca^{2+}_e from perfusing saline decreased Ca^{2+}_c in a rapid and reversible manner. Cells with higher resting Ca^{2+}_c levels showed larger decreases in Ca^{2+}_c in Ca^{2+}_e-free, while cells with lower levels exhibited smaller decreases. Re-perfusion of cells with Ca^{2+}-containing medium (Ca^{2+}_e:1.8mM) elevated Ca^{2+}_c, which rebounded to levels higher than in control. We next examined the effects of Cd^{2+} and Mn^{2+} on Ca^{2+}_c to elucidate possible Ca^{2+} entry pathways. Cd^{2+} blocks voltage-dependent Ca^{2+} channels and has a higher binding affinity for fura-2 than Ca^{2+}. Perfusion of Cd^{2+} (>0.1mM) in the presence or absence of Ca^{2+} increased the ratio values for fluorescence emission intensities (I_{340}/I_{380}), but did not alter the fluorescence emission intensity at 360nm excitation. This effect of Cd^{2+} could not be reversed, even with long-term washing with Cd^{2+}-free control saline or EGTA-containing medium. Moreover, the L-type Ca^{2+} channel blocker nifedipine did not alter Ca^{2+}_c nor prevent the Cd^{2+}-induced effect. Addition of Mn^{2+} (0.5mM) in the perfusing saline decreased fura-2 emission intensity excited at 360nm (dye quenching), which was partially recovered following application of Cd^{2+}. These results indicate that there are tonically active Ca^{2+} entry pathways in plasma membranes of embryonic cortical cells, which are permeable to Cd^{2+} and Mn^{2+}, and contribute to basal Ca^{2+}_c levels.

220.9

TETANUS TOXIN C FRAGMENT IDENTIFIES ANATOMICALLY DISCRETE EMBRYONIC RAT NEOCORTICAL SUBPOPULATIONS IN SUSPENSION.

I. Maric*, D. Maric, and J.L. Barker LNP, NINDS, NIH, Bethesda, MD

In vitro studies into embryonic (E) neocortical development are often confounded by unavoidable loss of anatomical cytoarchitecture after cell dissociation. Here we show that fluorochrome-conjugated tetanus toxin C fragment (TnTx) relocates suspended cells in their anatomical context. Neocortex of E19 Sprague-Dawley rat embryos was microdissected into ventricular/subventricular zones (VZ/SVZ) and cortical plate/subplate (CP/SP) regions, then dissociated and reacted with TnTx-FITC. FACS analysis revealed two modal values of TnTx expression: $TnTx^{high}$ and $TnTx^{low}$ cells found predominantly in CP/SP and VZ/SVZ dissociates, respectively. Dissociates fractionated on continuous Percoll gradients according to their natural specific buoyant densities (BD) showed that $TnTx^{high}$ cells composed the most buoyant (BD ≤1.04 g/ml) while $TnTx^{low}$ and $TnTx^{-}$ cells constituted the least buoyant (BD ≥1.07 g/ml) populations. Thus, changes in TnTx binding and BD correlate in vitro, identifying cells developing in anatomically distinct locations in vivo.



220.10

ON-LINE RECORDING OF TETANUS TOXIN-IDENTIFIED EMBRYONIC RAT NEOCORTICAL DISSOCIATES REVEALS PHYSIOLOGICAL CORRELATES OF CELL PROLIFERATION AND DIFFERENTIATION.

D. Maric*, I. Maric, and J.L. Barker, LNP, NINDS, NIH, Bethesda, MD

We used tetanus toxin (TnTx) binding to discriminate and record functional transmitter receptor/ion channel properties among proliferative ($TnTx^{-}$) and differentiating ($TnTx^{low}$ and $TnTx^{high}$) subpopulations of rat neocortical cells in ventricular/subventricular zones (VZ/SVZ) and cortical plate/subplate (CP/SP) regions at embryonic day 19 (see I. Maric et al.). Neocortical dissociates were stained with fluorochrome-conjugated tetanus toxin C fragment and loaded with either a voltage-sensitive (oxonol) or Ca^{2+}-sensitive (Fluo-3) fluorescent indicator dyes. The physiological properties distributed throughout the entire neocortical dissociate were then profiled in ~2 minutes by randomly recording on-line identified TnTx expressing and non-expressing cells at ~1000 cells/second using a flow cytometer. Baseline recordings at room temperature revealed reproducible resting membrane potentials and Ca_c^{2+} distributions which varied according to TnTx-fluorochrome intensity and FALS (cell size) or SSC (cell complexity) levels. The majority (≥85%) of TnTx negative cells, with the lowest FALS or SSC, which we presume to be proliferating elements from VZ/SVZ, depolarized to 40mM K_o^+ and veratridine, a Na^+-channel agonist, but only a fraction (≤40%) of them depolarized to GABA and kainate, and none were affected by acetylcholine (ACh). Furthermore, none of the above depolarizing effects was associated with detectable elevations in Ca_c^{2+}. By contrast, the great majority of $TnTx^{high}$ cells, which accounted for ~20% of the dissociate and which we presume to be differentiating CP/SP neurons, were hyperpolarized by ACh and depolarized by the rest of above mentioned ligands. All of these agents triggered sizable increases in Ca_c^{2+} in the majority (≥70%) of these cells suggesting an expression of multiple transmitter receptors and ion channels on many CP/SP neurons. Interestingly, sequential Ca_c^{2+} elevations to ACh, GABA and kainate could be induced in these cells even when saturating concentrations of the agonists were allowed to accumulate, implying "multi-plexability" to these Ca_c^{2+} signalling pathways.

220.11

THE MECHANISM OF SPONTANEOUS CALCIUM OSCILLATIONS IN A SELF-ORGANIZING NETWORK OF CULTURED CORTICAL NEURONS.

Xiaoshu Wang* and Eric Gruenstein. Dept. of Molecular Genetics, Biochem & Microbiology, and The Neurosciences Graduate Pgm, Univ of Cincinnati Med School, Cincinnati, OH 45267

Rat cortical neurons cultured at high density form synaptically coupled networks in which clusters of cells undergo synchronized calcium oscillations. Although it is known that each calcium spike is the result of a burst of action potentials (APs), neither the underlying mechanism that gives rise to the AP bursts nor the reason the spikes are synchronized is understood. We have found that the calcium spikes are independent of intracellular calcium stores and thus depend solely on the influx of extracellular calcium. This occurs primarily via L-type voltage gated calcium channels (VGCCs) but also involves N- and T-type channels. Calcium spikes are regulated by at least 4 discrete K^+ channels including 3 voltage regulated channels which stimulate spiking and one calcium activated channel which inhibits. Initiation of calcium spikes requires AMPA/KA glutamatergic receptors, although NMDA receptors also augment the process. Termination of calcium spikes involves stimulation of inhibitory GABA receptors. Based on these observations we propose a model for the synchronized calcium oscillations in which the process begins with a spontaneous action potential that is triggered by the occurrence of several closely timed mini-EPSPs arising from the normal, release of glutamate at presynaptic membranes. As the synaptic density of the network increases, the likelihood that several APs will occur within a short period of time also increases. In this way the network generates a large number of nearly simultaneous 'secondary EPSPs' constituting a positive feedback loop which gives rise to a prolonged depolarization of the presynaptic membranes and a burst of APs. Each AP burst activates VGCCs and the resulting influx of calcium constitutes the rising phase of the calcium spike. Spikes are terminated by the combined activation of inhibitory GABA synapses and the slowly activating calcium dependent K^+ channels (I_{AHP}) which gradually repolarize the neuronal membrane. Since the I_{AHP} current inactivates very slowly, there will be a refractory period before the next calcium spike.

220.12

STABILITY OF CYCLIC ADP-RIBOSE ANALOGS,

J. A. Boland, W. K. Jones and L. M. Yunger*. Sigma Chemical Company, St. Louis, MO 63118.

Cyclic ADP-Ribose (cADPR) is a recently discovered cyclic nucleotide involved in calcium-induced calcium release in cells. NAD^+ is converted to cADPR by ADP-ribosyl cyclase, an enzyme we have isolated from the ovotestes of *Aplysia californica*. ADP-ribosyl cyclase will also catalyze the cyclization of NAD^+ analogs with purine bases other than adenine, such as guanine, hypoxanthine and adenines modified at C8. We have synthesized several cADPR analogs including cyclic inosine diphosphate ribose (cIDPR), cyclic guanosine diphosphate ribose (cGDPR), and 8-Bromo, 8-Azido and 8-Amino-cADPR. The effect of temperature and pH on shelf-life and solution stability for these various analogs will be shown. The sodium salts of cADPR, cGDPR, and 8-Bromo-cADPR are stable when stored as solids at -20°C, exhibiting little if any degradation in one year. Aqueous solutions (0.5 - 1 mg/ml, pH=7) hydrolyze at rates of 0-5% per week at 0-5°C.

Sigma Chemical Company

220.13

ARIA REGULATION OF NICOTINIC ACETYLCHOLINE RECEPTORS IN RAT INTERPEDUNCULAR NEURONS DEVELOPING IN VITRO

K. Wietasch and G. D. Fischbach*, *Department of Neurobiology, Harvard Medical School, Boston, MA 02115*

ARIA increases the number of nicotinic acetylcholine receptors (nAChRs) at the vertebrate neuromuscular junction. We have studied the effects of ARIA on AChR expression in CNS neurons, using the habenular-interpeduncular system of the rat. Several nAChR subunit transcripts are abundantly expressed in the interpeduncular nucleus (IPN). ARIA transcripts are expressed in all cholinergic nuclei in the CNS, including the medial habenula nuclei that provide the major input to the IPN. ACh-evoked ionic currents were characterized using patch clamp recordings. In neurons cultured from the IPN of newborn rats, three types of nicotinic currents could be distinguished in whole-cell configuration based on their kinetic properties and pharmacology[1]. Type I currents exhibited fast onset and rapid biphasic desensitization ($t_{10\text{-}90}$ = 2.4 ms; τ_1= 6.8ms, τ_2 = 168ms; n=73) and were blocked by methyllycaconitine (1nM) and α-bungarotoxin. Type II currents rose and desensitized more slowly ($t_{10\text{-}90}$= 8.3ms; τ_1 = 96.4ms, τ_2 =554ms; n=29) and were blocked by dihydro-β-erythroidine (10μM), mecamylamine (5μM) and hexamethonium (100μM). Type III currents exhibited the slowest rise and a slow monophasic decay ($t_{10\text{-}90}$ = 18.03ms; τ =348ms; n=42) and were completely blocked by mecamylamine (5μM) and partially blocked by hexamethonium (100μM). Some neurons were found to have a mixed fast and slow response. Single channel analyses suggest that at least two different types of AChRs were responsible for type II and III currents. Treatment of IPN cultures with recombinant ARIA (100pM human $ARIA_{177\text{-}246}$) lead to phosphorylation of a 185kD protein, suggesting that the cells express ARIA receptors. Immunocytochemistry indicated that IPN cells contain several ARIA receptors including erbB2, erbB3, and erbB4. Treatment of IPN cells with 100pM ARIA in the continued presence of either forskolin or 8-Br-cAMP was found to increase type I current-density. The effect of ARIA on type II and III currents is currently under investigation. NIH RO1-NS8226

(1) Alkondon, M. & Alberqurque, E. X., *J. Pharmacol. Exp. Ther.* 265, 1455-72 (1993).

220.14

REGULATION OF ACETYLCHOLINESTERASE EXPRESSION IN DEVELOPING RAT THALAMUS.

Z. Rakonczay, P. Hammond, R. Rao, and S. Brimijoin*. Dept Pharmacol., Mayo Clinic, Rochester, MN 55905.

Acetylcholinesterase (AChE) is often expressed on nerve cells before or during synaptogenesis and may play a role in development of the vertebrate nervous system (Layer, 1995). Regulation of AChE during ontogenesis remains poorly understood, however. We used a reverse transcriptase-polymerase chain reaction assay, standardized with reference to cyclophillin mRNA, to investigate transient expression of AChE in newborn rat thalamus. Relative densities of DNA bands were quantitated by computerized video densitometry of ethidium bromide stained gels. AChE activity was also measured biochemically and histochemically. Analysis focused on a non-cholinergic but AChE-rich area, the medial geniculate nucleus of the thalamus (MGN), where transient increases and decreases of AChE expression during postnatal development are especially obvious (Robertson & Yu, 1993; Brimijoin & Hammond, 1996). Whole thalamus was studied at 1, 3, 6, 9, 12, 16, and 22 days of age, and in adult rats; MGN was examined at 8 and 23 days. Levels of AChE mRNA in the thalamus rose steadily for about nine days and then decreased. The MGN showed high levels of AChE mRNA and enzyme activity at day 8 but at day 23 both measures of AChE expression were 55% lower ($p < 0.001$). This result could reflect reduced message stability. In vivo experiments were performed to assess that possibility by determining the decay of mRNA levels after treatment with actinomycin D. Overall, our data support the view that AChE up-regulation during neural morphogenesis may be an important feature of brain development. (Supported by grant NS 29646).

220.15

REDUCED VAChT mRNA EXPRESSION IN SEPTUM OF POSTNATAL RATS FOLLOWING PERINATAL LOW-LEVEL LEAD EXPOSURE. X.Sun, X.Tian, and J.B.Suszkiw*. Dept. of Molecular and Cellular Physiology, University of Cincinnati, Cincinnati, OH 45267-0576.

We previously reported that perinatal Pb exposure reduces the cholineacetyltransferase (ChAT)-mRNA expression in the septum of postnatal rat. In the present report we examined the effect of Pb-exposure on the vesicular Ach transporter (VAChT)-mRNA expression in postnatal rats. Rat pups were maternally Pb-exposed by giving 0.2% lead acetate in drinking water to dams. Septal tissue was homogenized in RNAzol™ B (1:20, w/v). Total RNA from both control and Pb-exposed animals was extracted, quantified at OD_{260}, loaded at 10, 5, and 2.5 µg onto nylon membrane in Dot-blot manifold apparatus, and hybridized with α-(^{32}P)UTP-radiolabeled VAChT RNA riboprobe or 21bp rat 18S rRNA probe, respectively. The blots were scanned and quantified with the aid of a phosphor imager SF (Molecular Dynamics). The VAChT mRNA signal was normalized to the corresponding signal obtained with a probe specific for the 18S ribosomal RNA. Relative to control levels, the VAChT mRNA levels in septa of PN7 and PN21 animals with Pb-exposure were significantly reduced by 32% and 29%, respectively. The results indicate that Pb alters the expression of both ChAT- and VAChT-mRNA. *This work was supported by NIEHS grant ES06365.*

220.16

DEVELOPMENTALLY REGULATED CHANGES ON THE PROPERTIES OF GLYCINE RECEPTORS IN CULTURED MOUSE SPINAL NEURONS. **Luis G. Aguayo*, Juan C. Tapia, Felipe A. Albarran, Jorge P. Roa. Labs. Neurophysiol. & Immunocytochem., University of Concepcion. Concepcion, Chile.**

We studied several neurophysiological properties of maturing glycine receptors in mouse spinal cord neurons cultured for various times: 3-7 days (early), 10-12 days (intermediate) and 17-24 days (mature). The glycine-activated Cl^- conductance, studied with whole-cell and gramicidin-perforated patch clamp techniques, increased about 6 fold during development and the current density increased about three fold. The sensitivity to glycine increased transiently from 39±2.8 µM in early cells to 29±1 µM in intermediate neurons. In mature neurons, the sensitivity to glycine was similar to that in early cells. The current decayed (desensitized) during the application of 500 µM glycine. The decay was single exponential and the time constant increased from 2212±139 ms in early cells to 4580±1071 ms in mature cells. Picrotoxin (10 µM) inhibited the current to a larger extent in early cells (46±6% of control). On the other hand, the current was potentiated by several concentrations of Zn^{2+} and ethanol to a larger extent in mature neurons. In addition, higher concentrations of Zn^{2+} (>100 µM) inhibited the current in all the neurons. The receptors were sensitive to strychnine even in the early neurons and the sensitivity of the receptors to the alkaloid (IC_{50}) increased from 22±3 nM in early neurons to 9±1 nM in mature neurons. In conclusion, several properties of developing spinal glycine receptors changed during neuronal maturation. This indicates that, similar to $GABA_A$ receptors, the function of these receptors are developmentally regulated. **Supported by FONDECYT 1950917 Grant.**

220.17

EXPRESSION OF GLYCINE RECEPTOR SUBUNITS AND GEPHYRIN DURING NEURAL DIFFERENTIATION *IN VITRO* **S. Heck, R. Enz[1], C. Richter-Landsberg[2], and D. H. Blohm. Univ. of Bremen, Dpt. of Biotechnology and Molecular Genetics, 28359 Bremen, [1]Max-Planck-Institute for Brain Res., Dpt. of Neuroanatomy, 69528 Frankfurt, [2]Univ. of Oldenburg, Dpt. of Biology, 26111 Oldenburg, Germany**

Inhibitory glycine receptors (GlyR) consist of α and β subunits and are anchored by gephyrin, which is spliced extensively in adult rat brain and necessary to form functional receptor microdomains. During rat brain development the predominant embryonic α2 subtype is replaced by α1 and α3 subtypes. Primary cultures of rat spinal cord neurons express only the α2 and β subunits. Retinoic acid induces the mouse EC cell line P19 to differentiate into neuronal and glial cell types. To characterize this process the expression of about 40 genes at various differentiation stages of P19 has been analyzed by RT-PCR, automatic sequencing and in part by non-radioactive *in-situ* hybridization. In case of GlyR and gephyrin, results were compared to primary cultures of rat hippocampal neurons, rat brain glial cells, and adult rat and mouse brain. GlyRβ and gephyrin are constitutively expressed in all cell types investigated. Interestingly, mouse GlyRβ contains an additional 48 bp in-frame insert. Gephyrin splicing pattern clearly changes during P19 differentiation. P19 cells express GlyRα1 and α2 subunits but no GlyRα3 transcripts during the 14 day differentiation period. Primary hippocampal neurons express GlyRα2 only while no GlyRα subunit transcripts were found in the glial cell types. In summary, P19 cells and primary hippocampal neurons but not primary glial cells express *in vitro* all transcripts known to be necessary for the generation of functional glycine receptors.

220.18

ALPHA-2 ADRENERGIC RECEPTOR DEVELOPMENT IN RAT BRAIN: AN AUTORADIOGRAPHIC STUDY. L.C. Murrin*, C.L. Coulter, H.K. Happe, D.B. Bylund, Dept. of Pharmacology, Univ. Nebraska Med. Ctr., Omaha, NE 68198-6260

Alpha-2 adrenergic receptors (A2AR) play an important role in many CNS processes, including learning and memory and pain perception. There is also evidence they help regulate development of some CNS regions, such as cerebral cortex. Relatively little is known about A2AR development. We initially examined the ontogeny of total A2AR in CNS using quantitative autoradiography. Sections from rat brain from 0, 5, 10, 15, 21 and 28 day old pups and from adults were labeled with 5 nM [^{3}H]RX821002, which has near equal affinity for all A2AR subtypes. Buffer was 50 mM sodium phosphate, pH 7.4, room temperature, and blanks contained 100 µM norepinephrine (NE). Sections were exposed to tritium sensitive film for 4-8 weeks. In many brain regions with the most dense labeling, receptor levels were relatively high at birth, ranging from 50-85% of adult levels, indicating a high level of prenatal development. These include the amygdaloid region, olfactory tubercles, striatum, lateral dorsal nucleus of the thalamus and frontal cortex I-III. By contrast receptor number in cerebellum was very high at birth and decreased to 10-20% of neonatal levels by adulthood. Thus there are major differences in receptor development from region to region. We also noted specific labeling of the corpus callosum and anterior commissure in the early postnatal period. This was seen with two radioligands and was inhibited by NE, suggesting specific labeling. This disappeared by adulthood. Development of A2AR in CNS displays several interesting and surprising features, suggesting dynamic involvement in many developmental processes. (Supported by the Ittner Foundation)

220.19

QUANTITATIVE ANALYSIS OF 5-HT$_{1A}$ RECEPTOR mRNA FROM EMBRYONIC RAT BRAIN BY COMPETITIVE RT-PCR

J. M. Lauder[1]*, J. Liu[1] and D. R.Grayson[2]. [1]Dept. of Cell Biology and Anatomy, Univ. of North Carolina School of Medicine, Chapel Hill, NC 27599, [2]Dept. of Psychiatry, Med. Coll. of Penn. and Hahnemann Univ., Pittsburgh, PA 15212

There is growing evidence that serotonin acts as a growth regulatory signal in CNS development and that this action is mediated by specific 5-HT receptors. We have quantified the expression of 5-HT$_{1A}$ receptor mRNA in embryonic rat brain using the highly sensitive method of competitive reverse transcriptase-polymerase chain reaction (competitive RT-PCR). Total RNA was prepared from pooled tissue samples from dissected E12, E14, E15, E16, and E18 rat brain regions. Absolute amounts of 5-HT$_{1A}$ mRNA transcripts were quantified by RT-PCR using specific primers and internal standards. The 5-HT$_{1A}$ cRNA internal standard was identical to the 5-HT$_{1A}$ target sequence except for a restriction site midway in the sequence. 5-HT$_{1A}$ subunit mRNA was co-transcribed and amplified with varying concentrations of internal standard. The concentration of target mRNA was determined using linear regression to find the "point of equivalence" where the ratio of internal standard to target RNA was equal to 1. Low levels of 5-HT$_{1A}$ transcripts were detected as early as E12 in the metencephalon (about 0.1pg/mg total RNA). By E14, levels had increased by 7 fold (about 0.7 pg/mg total RNA), and continued to increase until E16 (about 1.3pg/mg total RNA). At E18 the concentration was similar to E16, suggesting that a plateau had been reached. These results are consistent with expression patterns seen using immunocytochemistry with 5-HT$_{1A}$ receptor antibodies (gift of John Raymond). This study demonstrates that 5-HT$_{1A}$ receptor mRNA can be accurately measured in regions of embryonic rat brain using competitive RT-PCR. The presence of early prenatal expression of 5-HT$_{1A}$ receptors suggests that they may play important roles in prenatal brain development.

220.20

DIFFERENTIAL EXPRESSION OF THE α SUBUNIT OF SOLUBLE GUANYLYL CYCLASE IN THE DEVELOPING RAT BRAIN. R. Smigrodzki and P. Levitt*. Department of Neuroscience and Cell Biology, UMDNJ-Robert Wood Johnson Medical School, 675 Hoes Lane, Piscataway, NJ 08854.

In situ hybridization and DNA sequencing have been used to characterize a clone identified during a differential-display screen of the developing rat forebrain. Initial sequencing results indicate that the clone codes for an α subunit of the soluble guanylyl cyclase (sGCY). Its pattern of expression at embryonic days 15 and 18 in the rat forebrain resembles the α1 subunit in the adult, but is in stark contrast to the reported embryonic expression pattern of the β1 subunit. Strong expression is observed in the ganglionic eminence and striatum, developing medial habenula, olfactory tubercle and hippocampus. Somewhat weaker expression is present in the cerebral cortex and parts of thalamus. Broader expression is seen postnatally, but with very high levels in the same regions observed initially during embryonic development. The discrepancy between the expression of the α versus β subunits of sGCY indicates that the α subunit might perform a different function or associate with a different β subunit during development than in the adult animal. Supported by Fogarty Fellowship F05 TWO5141-02 and NIMH grant MH45507.

220.21

THE SWEAT GLAND INNERVATION OF TH -/- MICE UNDERGOES A CHANGE IN PHENOTYPE.

B.Habecker*[1], M.Rios[2], D.Chikaraishi[3], S.Roffler-Tarlov[2], S Landis[1]. [1] Dept. of Neurosciences, Case Western Reserve Univ. Cleveland, OH 44106, [2]Dept. of Neuroscience, Tufts Univ., Boston, MA 02111, [3]Dept. of Neurobiology, Duke Univ., Durham, NC 27710

The sympathetic innervation of rodent sweat glands undergoes a target-induced switch from expression of norepinephrine (NE) to acetylcholine (ACh) and vasoactive intestinal peptide (VIP) during postnatal development. Analysis of footpad extracts from sympathectomized rats indicates that production of the differentiation activity that induces this switch requires sympathetic innervation. Co-culture of sweat gland cells with sympathetic neurons stimulates sweat gland factor production which is blocked by adrenergic antagonists. Treatment of gland cells with forskolin also results in factor production. These findings indicate that NE can stimulate factor expression but do not eliminate the possibility that other nerve-derived molecules which increase cAMP could do the same. To test whether NE is necessary to induce production of the differentiation activity *in vivo*, we analyzed the gland innervation of mice lacking tyrosine hydroxylase (TH), the rate limiting enzyme in NE synthesis. The TH-null mutation was created using homologous recombination in embryonic stem cells and it caused embryonic lethality. TH-null animals were rescued by treating pregnant heterozygous mice with dihydroxyphenylserine from E8.5 until birth. Sweat glands, which develop postnatally, were not exposed to NE. The gland innervation of 3 wk old TH +/- animals contained TH-immunoreactivity (IR), whereas TH-IR was not detected in null animals. The gland innervation of both TH +/- and -/- mice were IR for the vesicular ACh transporter, a cholinergic marker, and VIP. Thus, sweat glands in mice lacking NE release a factor that causes the gland innervation to change phenotype. These results suggest that whereas NE stimulates factor production *in vitro*, it is not essential *in vivo*, and additional nerve-derived molecule(s) contribute to stimulation of factor expression.

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220.22

DEVELOPMENTAL PLASTICITY OF EXCITATORY SYNAPSES ON LUMBOSACRAL PARASYMPATHETIC PREGANGLIONIC NEURONS.

I. Araki* and W.C. de Groat. Dept. Of Pharmacol., Univ. Of Pittsburgh, Pittsburgh, PA 15261 and Dept. Of Urology, Univ. Of Kyoto, Kyoto, Japan.

In neonatal animals, micturition is mediated by a segmental somato-parasympathetic (PSYM) reflex activated when the mother licks the perineum. At approximately 3 weeks of age, this reflex is replaced by a spinobulbospinal PSYM reflex as the principal mechanism of excretion. This study was undertaken to examine the developmental changes in synaptic transmission between interneurons (INT) and PSYM preganglionic neurons (PGN) during the first three postnatal weeks in rats. PGNs in the L6-S1 spinal cord were identified by retrograde labeling with fluorescent dyes injected i.p. prior to the experiment. Whole cell patch clamp recordings were obtained from labeled PGNs in 120 μm spinal slices. Unitary synaptic currents were elicited in PGN at short latency (2 ms) by stimulating non-labeled single INT dorsal to PGN. The mean peak amplitude of glutamatergic EPSCs recorded at -60 mV was not different during the first and second postnatal weeks (39.5, n=14 and 37.4 pA, n=15, respectively), but was markedly reduced at three weeks of age (19.3 pA, n=14). When the spinal cord was transected at the T-10 segment on postnatal day 14, the reduction of EPSC magnitude did not occur at three weeks of age (38.2 pA, n=15). A quantal analysis showed that this developmental change in synaptic efficacy results from a reduction in the number of quanta released by single INT. The mean quantal size measured at -60 mV was 10.3 pA (n=14) at one to two weeks of age. A similar value was obtained in preparations from three week old rats with an intact or a transected spinal cord (10.1, n=11 or 10.4 pA, n=11, respectively). We conclude that this developmental synaptic modification is dependent upon the maturation of descending projections from the brain and may underlie the reorganization of excretory reflex pathways that occurs a few weeks after birth.

Supported by NIH grant DK 49430.

NEUROTRANSMITTER SYSTEMS AND CHANNELS: DEVELOPMENT OF EXCITATORY AND INHIBITORY RECEPTORS

221.1

INTRACELLULAR BLOCKADE OF GABA-A RECEPTORS AS A TOOL TO INVESTIGATE NEURONAL NETWORK ACTIVITY.

I. Khalilov [1], R. Khazipov [1], X. Leinekugel [1], J. Feger [2]* & Y. Ben-Ari [1]
1- INSERM Unite 29, 123, Bd de Port-Royal, Paris, 75674, France; 2- Lab. de Pharmacol., FaculténdePharmacie, 4, av.de l'Observatoire, 75006 Paris, France

An approach to isolate GABA and glutamate receptors mediated currents in the cell under investigation is proposed to study the neuronal network activity. Complete blockage of GABA-A receptors mediated currents was obtained by dialysis of the neurons recorded in whole-cell mode by internal solution in which Cl^- was substituted for F^-, known to be impermeable through GABA-A-channels, and Mg-ATP were omitted to induce rundown of GABA-A conductance. After 1-2 hours of such dialysis both GABA-A receptors mediated spontaneous synaptic currents and responses induced by GABA-Areceptors agonist isoguvacine (10μM) were completely blocked. AMPA-EPSCs were not affected during dialysis, whereas NMDA-EPSCs were reduced by about 20%. Giant depolarizing potentials (GDPs) which are present in CA3 pyramidal cells of neonatal hippocampus and represent an example of the neuronal network activity were used as a model to assay this technique. GDPs have been shown previouly as entirely GABA-A receptors mediated events. Dialysis by F^- / MgATP-free solution induced reduction of charge passing during GDPs and shift of the reversal potential from -46 to 2 mV. The dialysis-resistant component of GDPs was assumed as glutamate receptors mediated because of (i) about 0 mV reversal potential; (ii) outward rectification at potentials more negative than -20 mV, characteristic to NMDA receptors mediated responses; (iii) kinetics identity of individual events composing this component of GDP to glutamate-receptors mediated currents. It is concluded that in addition to GABA-A receptors synaptically activated glutamate receptors contribute to GDPs in CA3 pyramidal cells.

Supported by INSERM and Ministère de la Recherche et de l'Espace (MRE).

221.2

SYNCHRONOUS Ca^{2+} OSCILLATIONS CONTROLLED BY THE SYNERGISTIC ACTIONS OF $GABA_A$ AND NMDA RECEPTORS IN THE NEONATAL RAT HIPPOCAMPUS.

X. Leinekugel, R. Khazipov, I. Medina, I. Khalilov, A. Represa * & Y.Ben-Ari
INSERM Unite 29, 123, Bd de Port-Royal, Paris, 75674, FRANCE

In the developing brain, GABA and glutamate have trophic actions via changes in Ca^{2+} homeostasis. We used confocal microscopy and patch clamp whole cell recordings from hippocampal slices to study how GABA and glutamate affect Ca^{2+} homeostasis during physiological patterns of activity. In the neonatal hippocampus, GABA and glutamate have synergistic actions (see abstract Ben-Ari et al.) which generate repetitive giant depolarizing potentials (GDPs) (see abstract Khazipov et al.). P_{2-5} CA_3 pyramidal cells were loaded with the fluorescent dye Fluo3-AM to monitor spontaneous changes in $[Ca^{2+}]_i$ while an additional neuron was recorded in whole cell configuration to detect GDPs. The loaded neurons had spontaneous $[Ca^{2+}]_i$ oscillations synchronized with GDPs. In another series of experiments, we studied the cellular location of the NMDA component of GDPs. We loaded neurons with the fluorescent dye Fluo-3 via a patch pipette and measured changes in $[Ca^{2+}]_i$ during GDPs in voltage clamp mode. At a potential allowing for expression of NMDA component (-30mV), no Ca^{2+} influx could be observed in the soma during GDPs. These results suggest that Ca^{2+} influx through NMDA channels may be restricted to dendrites. This was confirmed in preliminary experiments with direct recordings from dendrites loaded with Fluo-3. We propose that the activation of $GABA_A$ receptors depolarizes different parts of the cell (soma and dendrites), activating voltage dependent Ca^{2+} channels and allowing for expression of NMDA receptor mediated responses. This could be a major factor for the induction of the synaptic plasticity controlled by GABA and glutamate in the neonatal hippocampus (see abstract McLean et al.).

Supported by INSERM and MRE.

221.3

MORPHOLOGICAL DEVELOPMENT OF STRATA RADIATUM AND ORIENS INTERNEURONS IN THE RAT CA3 HIPPOCAMPAL REGION

J.L. Gaiarsa, V. Tseeb, X. Leinekugel, R. Khazipov, I. Khalilov, M. Esclapez, B. Berger* and Y. Ben-Ari.
INSERM U29, 123 Bd. de Port Royal, 75014 Paris, FRANCE

Although biochemical and electrophysiological studies support the idea that the GABAergic system is well developed in the neonatal brain, the extent of dendritic and axonal arborization of interneurons has not been examined in detail. In the present study, interneurons of the hippocampal CA3 region were labeled intracellularly using the whole-cell patch clamp recording technique in rat hippocampal slices between postnatal day 2 and 6. The soma of the biocytin-labeled interneurons were located in the strata oriens and radiatum. Most of the labeled interneurons displayed immature features including dendritic as well as somatic thin, elongated spines and axonal growth cones. In spite of these immature features, the dendritic tree of neonatal interneurons was well developed. The axonal arborization appeared to be widely spread, extending relatively long distances from the soma, covering the entire width of the stratum radiatum. Numerous axonal collaterals were found to project along or into the CA3 pyramidal layer, occasionally entering the stratum oriens. A few axon collaterals also entered the stratum radiatum of the CA1 region.

Several studies have reported that the axonal arborization of interneurons exhibit a high degree of regional targeting in adult hippocampus. In contrast, in neonates, axons are widely distributed and do not display any terminal specificity. These observations suggest that axonal arborizations of interneurons undergo remodeling as development progresses.

Supported by INSERM

221.4

SYNCHRONIZATION OF INTERNEURON NETWORK IN NEONATAL RAT HIPPOCAMPUS.

R. Khazipov [1], I. Khalilov [1], X. Leinekugel [1], R. Miles [2]*& Y. Ben-Ari [1]
[1]*INSERM Unite 29, 123, Bd de Port-Royal, Paris, 75674, FRANCE*
[2]*INSERM Unite 261, Institut Pateur, 25, rue du Dr Roux, 75015 Paris, FRANCE*

In the neonatal hippocampus, physiological pattern of activity is characterized by GABA-receptors mediated giant depolarizing potentials (GDPs) (Ben-Ari et al., 1989). We found that GDPs result from the synchronous discharges of interneuron network. Dual patch-clamp recordings from pyramidal cells (PC) and stratum radiatum interneurons (IN) revealed that during GDP, INs fire burst of action potentials generated by interneuronal GDP (IN-GDP). IN-GDPs were mediated by GABA-A and glutamate receptors, since (i) reversal potential of IN-GDPs depended on intracellular Cl^-; (ii) at reversal potential of GABA-PSCs, IN-GDPs were composed of events with kinetics identical to glutamate-EPSCs; (iii) remaining after intracellular blockade of GABA-A receptors component of IN-GDPs reversed near 0 mV and revealed slope of negative conductance at MP < -20 mV, characteristic to Mg-block of NMDA channels. Pharmacology of evoked IN-GDPs revealed that most of excitation of INs is determined by cooperation between GABA-A and NMDA receptors. Thus, synchronous activation of interneurons during GDP implies synaptic mechanisms and is determined by cooperation of activities of (i) excitatory GABAergic connections between interneurons and (ii) recurrent glutamatergic connections to interneurons from the pyramidal cells. This cooperation is largerly based on the phenomenon of potentiation of NMDA receptors by depolarizing GABA, which attenuates voltage-dependent Mg^{2+} block and permeats the activition of NMDA-channels in neonatal interneurons (see Ben-Ari et al.).

Supported by INSERM and Ministère de la Recherche et de l'Espace (MRE).

221.5

SPONTANEOUS $[Ca^{2+}]_i$ INCREASES MEDIATED BY $GABA_A$ RECEPTOR ACTIVATION IN NEONATAL CORTICAL NEURONS.

D.F. Owens*, L.H. Boyce, M.B.E. Davis and A.R. Kriegstein, Dept. of Neurology, Columbia P&S, NY, NY 10032.

At early postnatal ages recordings from neocortical neurons demonstrated spontaneous postsynaptic currents (sPSCs) predominantly mediated by the activation of $GABA_A$ receptors. In recordings from P1-P4 slices, 72% of the cells had sPSCs. The sPSCs were not blocked by CNQX (10 μM) or AP-5 (100 μM), however, sPSCs were abolished with application of bicuculline methiodide (BMI, 10 μM). Furthermore, in individual cells, the reversal potential for averaged sPSCs matched the reversal potential found for muscimol-induced current. TTX also eliminated the sPSCs, indicating that they were action potential dependent. Using laser confocal imaging of cortical slices loaded with the Ca^{2+} indicator, fluo-3AM, brief spontaneous $[Ca^{2+}]_i$ increases were also observed at the same ages and were blocked by BMI and TTX. In experiments at P3 we found that 78% of the spontaneously active cells had $[Ca^{2+}]_i$ fluctuations that were blocked by BMI and 100% of these cells showed $[Ca^{2+}]_i$ increases in response to muscimol. Muscimol-induced increases were blocked by cadmium (500 μM), a voltage-gated Ca^{2+} channel blocker. These results indicate that $GABA_A$ receptors can depolarize neonatal cortical neurons and activate Ca^{2+} entry, and that spontaneous GABA-mediated events (presumably synaptic activity) can lead to $[Ca^{2+}]_i$ increases. $GABA_A$ receptor activation may therefore influence early neocortical development through the activation of Ca^{2+} dependent signal transduction pathways.

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221.6

GABA:ERGIC DEVELOPMENT IN THE EMBRYONIC CHICK TELENCEPHALON. P. Lundgren*[1], Å. Sellström[2] and M.-O. Mattsson[1]. 1) Dept. of Cellular and Developmental Biology, Umeå University, S-90187 Umeå. 2) Natl. Defence Research Establishment, S-90182, Umeå, Sweden.

The GABA:ergic phenotype is characterized by capacity to synthesize GABA (performed by glutamate decarboxylase, GAD), by the presence of the transmitter, and by mechanisms for release and re-uptake of GABA. The latter function is performed by activity of GABA-transporter proteins (GAT) located in the membrane. In order to establish the time-wise appearance of these characteristics, studies were done on material from the parahippocampal area of the developing chick embryo telencephalon.

GABA-immunoreactivity was found from embryonic day (E) 8 onwards. The expression was rapid, and to some degree transient, with a possible peak at E10/E11. mRNA for GAD65 as well as GAD67 was detected by means of in situ hybridization from E8. Early on, GAD67 mRNA was more prominent than GAD65 mRNA, with regard both to intensity and anatomical distribution. Immunostaining with 1440 antiserum (recognizing both GAD forms) revealed the same expression pattern as GAD67 mRNA. A homologue to the rat GAT-1 transporter was detected both on mRNA (Northern blots, in situ hybridization: from E8) and on protein (immunostaining; from E11) levels.

These data suggest that several GABA:ergic traits appear more or less simultaneously during a critical period from E8. The transient "overexpression" of GABA-immunoreactivity by E10/E11 suggests additional roles for GABA besides the normal inhibitory transmitter action during embryogenesis.

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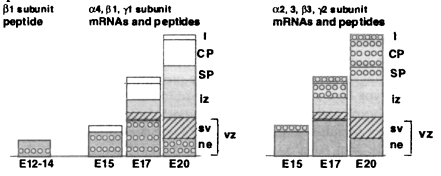
221.7

DIFFERENTIAL CHANGING CO-EXPRESSION OF $GABA_A$ RECEPTOR SUBUNIT mRNAs AND PEPTIDES IN THE DEVELOPING RAT NEOCORTEX. F.Lahjouji, W.Ma, A.Da Cunha*[1], W.Sieghart[2] and J.L.Barker Lab. Neurophysiology, NINDS; [1]Lab Cell Bilogy, NIMH, Bethesda, MD20892; [2]Dept. Biochemical Psychiatry, University Clinic for Psychiatry, Vienna,Austria..

The prenatal distribution of 8 $GABA_A$ receptor subunit transcripts and peptides (α1-4, β1, β3, γ1 and γ2) was determined in the cortex with *in situ* hybridization and immunocytochemistry. Our results reveal a close but not complete parallelism between the expression of the $GABA_A$ receptor subunit mRNAs and their encoded proteins. β1 immunoreactivity was first detected at E12 in cell bodies. By E14, β1⁺ processes emerged from the ventricular zone (vz) and extended perpendicular to the pial surface. These processes were also RC-1⁺ or nestin⁺. At E15, α4 and γ1 appeared, co-localizing with β1 in the vz until E19. At E20, this trio was also found together with all the other subunits in the cortical plate and subplate (see figure). The embryonic emergence and changing distributions of these subunits coincide with cellular proliferation, migration and differentiation, suggesting multiple roles of GABA throughout cortical development



221.8

$GABA_A$ AND $GABA_B$ RECEPTOR ACTIVATION AFFECTS CHOLINERGIC NEURONS IN THE DEVELOPING RAT MEDIAL SEPTUM, Y. Hong*, I.E. Mazzoni and R.L. Kenigsberg, Centre de Recherche, Hôpital Ste-Justine, Montréal, Québec, Canada H3T 1C5

GABA does not appear to function as an inhibitory neurotransmitter in the immature central nervous system but may play important roles in various other aspects of brain development. The present study was thus designed to examine some of the effects of the activation or blockade of GABA receptors on cholinergic cell development in the basal forebrain. The medial septal area from fetal rat (embryonic day 17) brain was isolated and cells dissociated and cultured in serum-free chemically-defined media. We found that the addition of GABA to these cultures for short periods of time (40 hours) slightly, but significantly increased the activity of choline acetyltransferase (ChAT). When present in the culture for extended periods of time (7 days), GABA markedly decreased ChAT activity. In contrast, bicucculline and picrotoxin significantly increased ChAT. The aforementioned responses were found to be identical in mixed neuronal-glial as well as pure neuronal cultures. Furthermore, muscimol mimicked the effects of GABA while baclofen increased rather than decreased ChAT activity. Interestingly, although muscimol-induced decrease in ChAT was still maintained in pure neuronal cultures, the cholinergic cell response to baclofen was lost. These results provide evidence for a role for GABA in the developing medial septum where GABA, tonically released under normal physiological conditions, may function to facilitate the maturation of $GABA_A$ receptors on neurons. These preliminary data also suggest that GABA may promote the differentiation of basal forebrain cholinergic neurons, presumably via the activation of $GABA_B$ receptors on glial cells. (Supported by MRC of Canada)

221.9

DIFFERENT INTRACELLULAR LOCALIZATION AND GABA RELEASE FROM NEUROBLASTOMA CELLS TRANSFECTED WITH GLUTAMIC ACID DECARBOXYLASE 65 AND 67 GENES. H. Asada*, Y. Kawamura, H. Kume, F.Y. Ji, K. Maruyama, and K. Obata, Lab. of Neurochemistry, Nalt. Inst. for Physiol. Sci, Okazaki 444, Japan.

γ-Aminobutyric acid (GABA) is a major inhibitory neurotransmitter in the central nervous system. It is synthesized from glutamic acid by glutamic acid decarboxylase (GAD). GAD has two isoforms, GAD65 and GAD67, which are encoded by two independent genes. Recent investigations have suggested that GAD65 is enriched in axon terminals but GAD67 is distributed throughout GABAergic cells bodies. GAD65 is anchored to Golgi-complex and thus will be transported to axon terminals. On the other hand, GAD67 is not associated with membranous component by itself and will be transported through axon accompanied with GAD65. The different roles of GAD isoforms can be investigated by over expression of each gene in non-GABAergic neuronal cells. In the present study, we used stably transfected neuroblastoma cells with GAD65 or GAD67 cDNA in order to examine intracellular distribution of GADs and release of GABA into the medium. In GAD65-transfected cells, GAD was localized in the cell organelle and in the neurites to their terminals and GABA was released spontaneously and with high potassium stimulation. On the other hand, GAD was distributed diffusely in the cell bodies transfected with GAD67 gene. GABA was contained in a high amount but was not released extracellularly. These results suggest the hypothesis that GAD65 not GAD67 is contributed to GABA release from presynaptic nerve terminals.

221.10

GLIAL CONDITIONED MEDIUM INDUCES CHANGES ON ENDOGENOUS AMINOACID NEUROTRANSMITTERS IN NEURAL PROGENITORS A.S.Herranz*, M.A.López-Toledano, C.Redondo, M.J.Casarejos, C.L. Paíno and E.Bazán. Depto. Investigación, Hospital Ramón y Cajal, INSALUD, 28034-Madrid, SPAIN.

Neural stem cells obtained from embryonic and adult rodent brain can be used as a source of neurons, oligodendrocytes and astrocytes for neural transplantation. Glial cells express neurotrophic and neurite growth promoting factors, including peptide growth factors, extracellular matrix proteins, cell adhesion molecules and neurotransmitters. The expression of these molecules may exert influences on development, neurite extension and survival of specific types of neural cells. The aim of this work is to study the change of endogenous content of free amino acids neurotransmitters in neural progenitors cells in response to glial conditioned medium (GCM). Progenitor cells, obtained from E 15 rat embryo striatal eminence, were cultured in DMEM:F-12 (1:1) supplemented with glucose, N2 components and 20 ng/ml EGF for a minimum of 5 passages, and then plated on poli-L-ornithine coated dishes or coverslips. Glial conditioned medium (GCM) was prepared from mesencephalic glial cells cultured for 24 in serum free defined medium (EF12). After 3 days postplating DMEM:F-12 medium was removed, replaced by GCM and maintained for 7 days. The endogenous amino acids content was analyzed by HPLC (OPA-precolumn derivatization and fluorimetric detection). Progenitors cells treated for 7 days with GCM show a significant increase in taurine, a moderate decrease in GABA, glutamine and serine and a decrease in isoleucine and leucine. No changes were found in aspartate and glutamate, as compared with controls. Since taurine is not provided in the culture medium, the increase of this amino acid might indicate that GCM promotes a selective differentiation of a specific type of cells with the biosynthetic machinery to produce taurine. Supported by FIS 93/555 and 94/0490.

221.11

FUNCTIONAL NON-NMDA GLUTAMATE RECEPTORS ARE PRESENT IN THE PREPLATE NEOCORTICAL ANLAGE. N. König*, M.J. Drian, N. Grimaud and M. Bardoul, EPHE Lab. Quantitative Cellular Neurobiology INSERM U336, University Montpellier II, 34095 Montpellier Cedex 5, France.

We have previously found cells bearing functional non-NMDA receptors permeable to divalent cations in the rat neural tube at very early stages of development (by embryonic day 11). These cells were located in the brainstem or the spinal cord. In the present study, we checked with two independent methods the presence of functional receptors in the neopallium at its earliest stage of organization: the preplate stage. First, we exposed slices of embryonic day 14 neopallium to AMPA/kainate receptor agonists and the desensitization blocker cyclothiazide in the presence of cobalt. After silver-intensification, a substantial number of labeled cells could be found in the lateral pallium. Most of these cells were located in a narrow layer above the matrix zone. Second, we dissociated tissue from the lateral and dorso-lateral day 14 neopallium, and analyzed (by dynamic Fluo-3 imaging) the modulation of cytosolic calcium after agonist application with or without cyclothiazide. Even in cultures examined shortly after plating, we consistently found responses to AMPA and kainate. As expected, the responses to AMPA were in most cells greatly enhanced in the presence of cyclothiazide. Surprisingly, this was also the case, in some cells, for kainate: the response to this agonist alone was in some cases hardly detectable, whereas a strong calcium increase was observed in the presence of cyclothiazide. Sister-cultures were maintained in vitro for up to 8 days. In these cultures, application of receptor antagonists dramatically reduced the number of surviving neurons, depending upon the dose and the timing of the treatment. Our results demonstrate (1) that functional glutamate receptors in the neopallial anlage arise earlier than hitherto reported, and (2) that receptor blockade may profoundly modify the fate of early neocortical cells. Work supported by: AFM, INSERM, IRME.

221.12

DEVELOPMENT OF EXCITATION AND INHIBITION IN THE RAT SUPERIOR COLLICULUS. J. Shi and M. Constantine-Paton* Department of Biology, Yale Univ, New Haven, Ct. 06520

We are studying the cellular and molecular mechanisms through which activity mediates central nervous system synaptogenesis using the visual layers of the rat superior colliculus. In these layers, AMPA and NMDA receptor subunits show a major upregulation between postnatal day (P)6 and P12 (Hofer et al. 1994, J. Neurochem. 62:2300). Retinocollicular map refinement is complete by P12 (Simon & O'Leary, 1992, J. Neurosci. 12:1212-1232) and eyes open at P14. Whole cell patch clamping in SC slices was conducted between P8 and P20 to investigate associated alterations in synaptic function.

Electrically evoked EPSCs were recorded in SC neurons as early as P8 but these decremented rapidly with successive activation. By contrast, TTX sensitive sEPSCs were robust in this early neuropil. sEPSCs from individual neurons held at their resting potential in magnesium free saline were averaged before during and after the slice was bathed in 50uM AP5. All averages sEPSCs had rise times <6msec and amplitudea at least three times greater than baseline. The difference in fall time between averages with and without AP5 was taken as an estimate of the NMDA receptor contribution to these spontaneous currents. The data indicate a high NMDA receptor contribution to these events between P8 and P10 with a large and rapid drop at P11. This is followed by a much slower decrement in the NMDA contribution through P20. We also observed an abrupt decrease in the average frequency of these sEPSCs between P17 and P19. This change appears to be due to the onset of $GABA_A$ receptor mediated inhibition. Bath application of bicuculline has little effect on the frequency of sEPSCs in the second postnatal week. After P17 bicuculline application significantly increases sEPSC frequency. Thus these data suggest that pronounced changes in excitatory transmission in the superficial SC occur prior to eye-opening and in close association with the refinement of the retinocollicular map. Synaptic inhibition develops later during a period where it could be influenced by pattern vision. Supported by NS32290 to MCP.

221.13

XENOPUS GLUTAMIC ACID DECARBOXYLASE TRANSCRIPTS ARE DEVELOPMENTALLY UPREGULATED IN THE EMBRYONIC BRAIN AND SPINAL CORD REGION. S. Watt and N.C. Spitzer*. Department of Biology and Center for Molecular Genetics, UCSD, La Jolla CA 92093.

GABA is the neurotransmitter in dorsolateral ascending interneurons in the *Xenopus* spinal cord. The developmental expression of GABA is both transcription- and Ca^{2+}-dependent, raising the possibility that expression of transcripts encoding glutamic acid decarboxylase (GAD), the GABA-synthetic enzyme, may be Ca^{2+}-dependent. To examine this hypothesis further, we cloned a gene encoding *Xenopus* GAD. Using PCR with degenerate primers to conserved sequences, a 479 bp DNA fragment was generated for screening of a *Xenopus* tadpole brain cDNA library. Following the isolation of a 2420 bp partial clone, a 780 bp 5' region of the gene containing the initiation of translation was amplified by anchored RT-PCR from total RNA of the spinal cord region. The two clones overlap by 263 bp and produce a 3.2 kb clone. From a 100 aa residue sequence in the region of the putative pyridoxal phosphate binding site, 92 are identical with rat GAD67. Appearance of GAD transcripts during development was examined by RT-PCR of total RNA from blastula (E10), and from the dorsal region at neural plate (E13-14), neural fold (E15-16), neural tube (E20-22) and tailbud (E30-34) stages. Expression is first detected at stages 20-22 when spontaneous Ca^{2+} spikes are first observed and persists at stage 30-34. *In situ* hybridization shows that transcripts are expressed in the brain of stage 32-34 embryos and in the spinal cord; transcripts are not detected at earlier stages of development. The Ca^{2+}-dependence of expression of these Xenopus transcripts is being investigated. Supported by NIH NS15918.

221.14

CO-LOCALIZATION OF NON-NMDA AND NMDA RECEPTORS AT EXCITATORY SYNAPSES ON EMBRYONIC *XENOPUS* SPINAL NEURONS. J. Rohrbough, E. Gleason* and N.C. Spitzer, Biology Dept., UCSD, La Jolla, CA.

Non-NMDA and NMDA receptors are co-localized at excitatory synapses in mammalian hippocampal (Bekkers & Stevens, 1989) as well as spinal neurons (Hori and Endo, 1992). The proximity of both receptors at individual synapses has important implications for synaptic plasticity. *Xenopus* spinal dorsolateral interneurons (DLis) receive excitatory input from primary sensory neurons (Sillar & Roberts, 1988, 1991). We made whole cell recordings of miniature excitatory postsynaptic currents (mepscs) in DLis to investigate the participation of non-NMDA and NMDA receptors at these synapses. At -70 mV in Mg^{++}-free solution containing TTX, strychnine, and bicuculline, 3 types of mepscs are present in most recordings from 4-8 d larvae (stages 42-48). ~70% are dual component mepscs, with a fast initial component followed by a variable slow decay in which single channel currents of 4-5 pA are occasionally resolvable; ~20% are fast, lacking a slow component; and a few (5-10%) are slow. Fast mepscs and the fast component of dual mepscs have rise times and decay time constants of about 400 μs and 1 ms, respectively, and are abolished by the selective AMPA receptor antagonist GYKI-53655 (25 μM), indicating they are mediated exclusively by AMPA-type non-NMDA receptors. Slow mepscs, the slow component of dual mepscs, and the background single channel noise are blocked by 1 mM Mg^{++} or 100 μM APV, indicating they are mediated by NMDA receptors. In 1 mM Mg^{++}, the NMDA receptor component is increasingly revealed at more positive holding potentials. These results indicate that both NMDA and AMPA receptors are co-localized at the majority of excitatory synapses on DLis at these developmental stages. These connections are presumed to be present by 30 hr (stage 26), when embryos first are motile in response to touch stimuli. In recordings from stage 27-38 embryos and larvae, all three types of mepscs are present though they occur with greater variability. However, ~50% are dual, ~50% are fast, and ~5% are slow, suggesting that AMPA receptor expression may precede that of NMDA receptors during development of excitatory synapses in the spinal cord. Supported by NS09603 (JR) and NS15918 (EG, NCS).

221.15

POSTNATAL MATURATION OF AMPA- AND NMDA-TYPE GLUTAMATE RECEPTORS IN MACAQUE MONKEY STRIATE CORTEX V. Meskenaite*[1], H. Kennedy[2], C. Dehay[2] and K.A.C. Martin[1]. [1]Institute of NeuroInformatics, Zuerich Univ/ETH, CH-8006, Switzerland; [2] Cerveau et Vision, INSERM U 371, 69500 Bron, France.

L-glutamate is a major excitatory neurotransmitter that is essential both in development and in many integrative functions of the cerebral cortex. The transmitter actions of glutamate are mediated by different types of receptors. We used subunit specific antibodies to investigate the glutamate receptor localization in V1 in postnatal development.

In neonates (6-21 days), both AMPA (GluR1, GluR2/3) and NMDA (R2A, R2B) receptor-immunoreactivity (IR) were densely present within thalamorecipient layers 4C and 6. In layer 3, only GluR2/3-IR postsynaptic profiles were dense in the cytochrome oxidase blobs.

By 5-6 months postnatal, the laminar densities of labelled synapses changed dramatically, mainly, due to an increase in pyramidal cell layers. In 5-6 month old monkeys, AMPA R-IR was strongest in layers 4B and 5, and denser than the neonate in layers 1-3. NMDAR2B-IR was elevated in superficial layers. NMDAR2A-IR showed a reversed laminar profile when compared to the neonate: all layers showed strong labelling, except thalamorecipient layers 4A and 4C.

Comparison of 5-6 months postnatal animals with adults showed that in general glutamate receptors matured last in layers 5 and 1-3. The increase in the NMDA receptor subunits in the superficial layers may account for the synaptic plasticity seen outside layer 4 in adults.

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221.16

MOLECULAR ANALYSIS OF GLUTAMATE DECARBOXYLASE EXPRESSION IN THE EMBRYONIC CHICK BRAIN. E. Wågberg, A-K Åhman and M-O Mattsson*. Dept. of Cellular and Developmental Biology, Umeå University, S-90187 Umeå, Sweden.

The aim of the present study was to investigate the expression of glutamate decarboxylases (GAD) in the developing chick embryo telencephalon, and how these correspond to GAD65 and GAD67 in mammals.

GAD enzyme activity is present as early as by embryonic day 3 (E3). However, Northern blot analysis as well as immunoblots can detect GAD mRNA:s and proteins from E8. Expression levels are probably very low prior to this developmental stage. Thus, RT-PCR analysis of GAD65 expression revealed a fragment (216 bp; 83% identical to known mammalian sequences) which showed that the GAD65 mRNA is found by E4. The transcript sizes in the chick are 3.9 kb (GAD65) and 5.6 kb (GAD67) respectively, differing in size from the ones reported in mammals.

Immunoblotting confirmed the presence of proteins corresponding in size to the mammalian GAD forms. The expression patterns of these proteins suggest that GAD67 may predominate during early development, whereas the levels of GAD65 are higher during late embryogenesis as well as in the adult brain. Presently we are sequencing cDNA clones (λZAP-II library from chicken E11 telencephalon) corresponding to both GAD isozymes. Our data will be instrumental in elucidating the functional roles for each GAD during development of the nervous system.

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221.17

A PROTEIN INVOLVED IN INHIBITION IS REGULATED BY NMDA RECEPTOR ACTIVATION IN VIVO. S.M. Aamodt* and M. Constantine-Paton. Biology Department, Yale University, New Haven, CT 06520.

The topographic visual map in the rat superior colliculus develops postnatally by the elimination of incorrectly positioned retinal ganglion cell arbors. Chronic local blockade of N-methyl-D-aspartate (NMDA) receptors from birth prevents this process. We have proposed that the highly correlated activity associated with map refinement might close the sensitive period by replacing a juvenile subtype of the NMDA receptor with an adult subtype that is less permissive of synaptic plasticity. This hypothesis predicts that chronic receptor activation with the agonist NMDA begun at postnatal day (P)8 should cause premature expression of the adult NMDA receptor subunit pattern. However, previous experiments indicated that this treatment paradoxically increases NR2B subunit mRNA, normally low in mature animals.

One possible explanation for this unexpected finding might be that chronic NMDA receptor activation induces compensatory changes in inhibitory circuitry, causing an overall decrease in activity levels. Therefore, quantitative western blotting was used to evaluate the effects of NMDA receptor activation or blockade on glutamate decarboxylase (GAD) protein, the synthetic enzyme for the major inhibitory transmitter gamma-aminobutyric acid (GABA). In untreated animals, GAD protein levels were undetectable at P0 and P6, increased steadily from P12 to P27, and then declined somewhat to an adult level comparable to that of P19. After chronic treatment from P8 with NMDA, GAD levels at P19 were elevated to 141 ± 17% (S.E.) of untreated sibling control GAD protein. That value was significantly different from GAD levels in siblings treated with the NMDA receptor antagonist 2-amino-5-phosphonovalerate (D-APV; 53 ± 6% of normal, $p<.01$ vs. NMDA) or with its inactive isomer L-APV (69 ± 9% of normal, $p<.02$ vs. NMDA, ns vs. D-APV). These findings suggest that chronic NMDA treatment either induces premature development of inhibitory circuitry or increases overall inhibition levels in the superior colliculus. In either case, the activity-dependent development of inhibitory connections might help close the normal sensitive period for synaptic refinement.

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221.18

AMPHETAMINE INDUCED DOPAMINE OVERFLOW IS GREATER IN NEONATAL THAN IN ADULT RATS.

O. Contreras* and L.Hernández. Laboratory of Behavioral Physiology, School of Medicine, Universidad de Los Andes, Mérida 5101-A, Venezuela.

The effect of amphetamine on strial dopamine overflow was tested in neonatal and adult anesthetized rats. Ten (5 days old) rats were anesthetized with ether and a microdialysis probe was stereotaxically lowered in the striatum. DA, DOPAC and HVA were monitored following a one mg/Kg amphetamine i.p. measured by high pressure liquid chromatography with electrochemical detection (HPLC-ED). The same procedure was repeated with ten adult rats. Dopamine increased ten folds in the neonatal rats whereas it increased only two folds in the adult rats. This results suggest that dopaminergic neurons are not under feed-back inhibition in neonatal rats. They also might be relevant to interpret the paradoxical effects of amphetamine observed in hyperactive children.

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221.19

CHARACTERIZATION OF LAN-1 CELL-LINE AS A MODEL OF DEVELOPMENTAL DOPAMINE-SEROTONIN INTERACTION. A.Zuddas*, V.Lilliu, C.Mancosu, C.Cianchetti. Child Neurology and Psychiatry, Dept. Neurosciences, University of Cagliari, Cagliari, Italy.

LAN-1 is a human neuroblastoma cell-line expressing tyrosine hydroxylase (TH), muscarinic M1 and M3 receptors, veratridine-sensitive sodium channels and lacking voltage-sensitive calcium channel. We have shown that LAN 1 is an heterogeneous cell-line with about 10% TH-positive cells: LAN-1 express the cellular machinery for the high affinity dopamine uptake, they are able to synthesize serotonin but not dopamine. Dopamine synthesis could be activated by the presence of high concentrations of tetrahydrobiopterine (THB 10 mM), which also decrease the 5HIAA levels in these cells (Zuddas et al. *Soc. Neuroscience* 21: 1780, 1995). Aim of this study was to further characterize the dopaminergic phenotype of this cell-line. In LAN 1, the high affinity [3H]DA uptake was not modified by the presence of THB: it was partially inhibited by 1 µM desipramine (87%), chlorimipramine (71%) or benztropine (68%). The presence in the culture medium of Retinoic acid (RA, 10 µM final concentration), a differentiation factor for LAN 1, did not modified DA uptake nor THB-induced dopamine synthesis. No veratridine- nor carbachol-induced [3H]DA release were observed in the different experimental conditions (THB, RA), indicating that LAN 1 cell-line could express both serotoninergic and monoaminergic phenotypes but it is only partially differentiated. To verify specificity, THB was also added to the culture medium of two other serotonin- but not dopamine-synthesizing cell-lines: TB, derived from human neuroblastoma, and Mes-c-myc, generated from E 11 mouse mesencephalic primary culture by infecting with a replication defective retrovirus. In both TB and Mes-c-myc, THB did not induced DA synthesis nor modified serotonin turnover. In both these cell-lines, no high affinity [3H]DA was detected with or without THB in the culture medium. Taken together these data indicate that THB-LAN 1 cellular model could be an useful and specific tool for studing dopamine-serotonin interaction during early CNS development.

221.20

DIFFERENT CRITICAL PERIODS FOR AFFECTING D1 AND D2 RECEPTOR EXPRESSION DURING DEVELOPMENT. W.S. Thomas*[1], B.S. Neal-Beliveau[2] and J.N. Joyce[1], Parkinson's Center, Sun Health Research Institute[1], Sun City, AZ 85372; Dept. of Psychology, Indiana Univ. Purdue Univ. Indianapolis[2], Indianapolis, IN 46202.

We have previously demonstrated that neonatal lesions of the dopamine (DA) system have different behavioral and neurochemical effects than lesions administered in the adult. P0/P1 lesions result in loss of D1 binding sites, as well as oral dyskinesias in response to the D1 receptor agonist SKF38393. In contrast, D2 binding is unaffected after 6-OHDA administration. Adult 6-OHDA lesions result in no change or a small decrease in D1 receptor number. However, D2 binding sites increase in number and Parkinson-like behavioral changes occur with lesions in the adult.

To determine when D1 and D2 receptors are vulnerable to lesions of the DA system, we lesioned the neostriatum at P0/P1, P7, P15 or P20 and examined DA receptor numbers at P90 with quantitative autoradiography. Using [3H]mazindol to verify the presence of a lesion, we then quantitated D1 binding sites using [3H]SCH23390 and D2 sites using [3H]spiroperidol. Our results demonstrate a significant reduction in D2 sites in the caudal corpus striatum following lesion at P7. Preliminary results indicate that D1 binding sites increase in number with lesions at P7. The results indicate that there are different critical periods for affecting expression of D1 and D2 receptors. On the basis of our findings to date, we hypothesize that the critical period for D1 receptors occurs earlier in development than that for D2 receptors. Supported by MH51413 (B.S.N.) and MH48813 (J.N.J.).

221.21

SPONTANEOUS DOPAMINE RELEASE IN ORGANOTYPIC SLICE CULTURE OF VENTRAL MESENCEPHALON MEASURED IN REAL-TIME S. J. Cragg*, C. Holmes, C. R. Hawkey and S. A. Greenfield. University Department of Pharmacology, Oxford, OX1 3QT, UK.

Spontaneous, synchronised neuronal activity is believed to play a part in the formation and refining of connections in the developing nervous system. Using fast-scan cyclic voltammetry (4Hz) at carbon-fibre microelectrodes, we have shown spontaneous pulses of release of dopamine (DA) from developing neurons within organotypic slice cultures of the ventral mesencephalon (VM). VM cultures containing dopaminergic cells of the ventral tegmental area and substantia nigra, were prepared from two-day-old Wistar rats and incubated (roller tube technique) for 20 to 30 days *in vitro*.

Site-specific patterns of pulses of DA efflux were observed, either phasic or more regular, and the inter-pulse intervals ranged from 2 to 122 secs (mean ± S.E.M. 20.8 ± 2.7 s), while the concentration of pulses of DA efflux ($[DA]_0$) ranged up to 2.05 µM (0.48 ± 0.02 µM). $[DA]_0$ was proportional to the preceding inter-pulse interval, whereas it was unrelated to the subsequent interval. A single spontaneous release spike could be mimicked in duration and concentration by a single electrical pulse (0.1ms, 18V) applied locally, suggesting synchronisation of release from local release sites during a spontaneous release pulse. Both spontaneous and evoked efflux were strongly Ca^{2+}-dependent and TTX-sensitive to varying degrees. Spontaneous DA efflux was inhibited by glutamate antagonists, partially by NMDA-receptor antagonism (10-30µM l-APV) and completely by AMPA-receptor antagonism (25µM CNQX); however release was unaffected by metabotropic-receptor modulation (1mM l-APIII). In conclusion, spontaneous oscillations of $[DA]_0$ occur from developing dopaminergic neurons in organotypic slice culture of ventral mesencephalon, but may be extrinsically modulated by ionotropic glutamate receptors. SJC is the 1995 Goodger Scholar and CH holds an MRC studentship.

221.22

MEMBRANES FROM THE INTERMEDIATE LOBE OF THE HYPOPHYSIS ENHANCE HYPOTHALAMIC DOPAMINERGIC DIFFERENTIATION IN PRIMARY CULTURE. **J. Niquet[1], A. Faivre-Bauman[2], C. Loudes[2], R. Ubieta[3], C. Kordon[2], and J.L. Charli[1]*. [1]Instituto de Biotecnologia, UNAM, Cuernavaca, Mor., Mexico ; [2]Centre Paul Broca, INSERM U159, Paris, France and [3]Centro de Ingenieria Genetica, La Habana, Cuba.**

Coculture of foetal rat hypothalamic cells with adult cells of the intermediate lobe of rat hypophysis (ILH), a target of dopamine (DA) neurons, specifically accelerate both morphological and biochemical dopaminergic differentiation (Mol. Cel. Neurosci. 4, 55, 1993). Since soluble components secreted by ILH cells could only partially mimic the effect of intact cells, we determined whether contact of ILH cells with hypothalamic cells contribute to DA differentiation. Crude membranes from 0.6x 10^6 ILH cells added just after seeding 6x 10^4 hypothalamic cells (F17) per cm^2 in serum free medium enhanced tyrosine hydroxylase (TH) levels 3 fold at 3-6 days in vitro (DIV). The result was preserved with 1M NaCl washed membranes but not if adenohypophyseal membranes were used. Addition of membranes and conditioned medium, both from 0.6x 10^6 ILH cells, produced an effect similar to that due to coculture with the same number of ILH cells (5 fold). At DIV 6, membrane treatment stimulated total DA neuritic length as efficiently as coculture. The level of neuron specific enolase was not modified. These results suggest that ILH membranes contain a factor which enhances hypothalamic dopaminergic differentiation in vitro. Partially supported by grants from EU (CI1*-CT93-0301) and DGAPA-UNAM (IN205993).

222.1

REGULATION OF BDNF AND CNTF AND THEIR SIGNAL TRANSDUCTION PATHWAYS IN THE ADULT RAT HIPPOCAMPUS AFTER EXCITOTOXIC LESION J.S. Rudge*, E.M. Pasnikowski, P.E. Mather, N. Cai, R.M. Lindsay, S.J. Wiegand. Regeneron Pharmaceuticals Inc. 777 Old Saw Mill River Road, Tarrytown, New York. 10591

Systemic injection of the excitotoxin kainic acid (KA) results in a well-defined loss of CA1 and CA3 pyramidal neurons in the adult rat hippocampus. We are interested in understanding how the neurotrophic factors - Brain-derived Neurotrophic Factor (BDNF) and Ciliary Neurotrophic Factor (CNTF) are regulated in the hippocampus after KA injection and how their expression impacts upon the evolution of the lesion. Thus, we have used specific antibodies to BDNF (generously provided by Dr. Qiao Yan, Amgen) and CNTF (Regeneron RG0036) in conjunction with Northern blot analysis to follow expression of these factors as the lesion evolves. BDNF-like immunoreactivity is markedly increased in the perikarya of CA1 pyramidal neurons within 6 hours of KA. By 24 hrs staining is also present in stratum oriens and radiatum, where BDNF-lir is transiently apparent on astrocytes. In contrast, increased CNTF immunostaining is not apparent until 3 days after KA and appears to be exclusively restricted to reactive astrocytes. This expression is sustained for up to 1 month after the lesion. Despite the clear, and, in the case of BDNF, rapid increase in trophic factor levels in the hippocampus, the pyramidal neurons subsequently die. To study whether increased expression of these factors initiates signal transduction following KA, we are in the process of quantitating the level of phosphorylation of the BDNF receptor TrkB and the β components of the CNTF receptor LIFRβ/gp130 by Western blot analysis.

This work was supported by Regeneron Pharmaceuticals Inc.

222.2

CHANGES IN AP-1 COMPOSITION CORRESPOND WITH BIPHASIC PROFILE OF NGF mRNA EXPRESSION AFTER HILUS LESION-INDUCED SEIZURES. R.C. Elliott* and C.M. Gall Dept. of Anatomy and Neurobiology, University of California, Irvine, 92717.

Nerve growth factor (NGF) expression is increased in many brain areas following seizures. Previous work has suggested that NGF transcription is regulated via an AP1 binding site in intron 1 of the NGF gene. To better define the relationship between AP-1 binding and activation of NGF transcription, the present study evaluated NGF mRNA levels, AP-1 binding, and AP-1 composition after hilus lesion (HL)-induced seizures in adult rats. *In situ* hybridization has shown that HL-seizures elicit a biphasic increase in the NGF mRNA content of dentate gyrus neurons (Lauterborn *et al.*, Exp. Neurol., 1994). The present solution hybridization analysis replicated this finding, showing a 5-fold increase in NGF mRNA at 4 h post-HL, a return to control levels at 10 h, and a second increase to 2.5-times control levels at 24 h post-HL. Electrophoretic mobility shift assay (EMSA) experiments determined that AP1 binding is highly elevated at 4 h post-lesion, then declines progressively through 10 and 24 h, but remains higher than control levels at all timepoints. Supershift analyses indicated that JunD is the predominant Jun-family member in the AP-1 complex at 4 and 24 h, while JunB is predominant at 10 h. Thus, while total AP-1 binding does not correspond with the biphasic increase in NGF mRNA after HL-seizures, dynamic changes in AP-1 composition may underlie changes in the capacity of the bound AP-1 complex to activate NGF transcription. (Supported by NS26748)

222.3

INCREASED GDNF mRNA EXPRESSION FOLLOWING INTRASTRIATAL INFUSION OF QUINOLINIC ACID J.L. Bizon*[1], J.E. Springer[2], and C.M. Gall[1], Depts. of [1]Psychobiology, University of California, Irvine CA 92717 & [2]Anatomy and Neurobiology, University of Kentucky, Lexington, KY 40536

Glial cell line-derived neurotrophic factor (GDNF) is a dopaminergic survival factor which can be retrogradedly transported from the striatum by mescencephalic neurons. GDNF mRNA levels in normal adult sriatum are very low. The present study investigates the possible regulation of striatal GDNF by intense activation of the N-methyl-D-aspartate (NMDA) class of glutamate receptors. Quinolinic acid (QUIN), an endogenous NMDA agonist, was infused (1 µL, 240 nM) into the right striatum of nine rats. Following sacrifice at 6,10, or 20 hrs after QUIN administration (n=3 per time point), striatal sections were processed for GDNF mRNA localization using *in situ* hybridization. Dramatic increases in striatal GDNF mRNA were observed on the side of the lesion throughout the entire rostrocaudal extent of striatum, including portions several millimeters away from the lesioned site. Densitometric analyses demonstrated significant increases in striatal GDNF mRNA ipsilateral to the lesion at all time points examined ($p < .002$ for 6 and 10 hr; $p < .02$ for 20 hr). Significant differences were not found between time points indicating that comparable levels of GDNF expression persist for at least 20 hr after NMDA receptor activation. The majority of the GDNF labeling after QUIN infusion appears diffuse suggesting expression by glial cells and/or medium sized neurons, although some large, intensely labeled neurons were also observed. The current results indicate that striatal GDNF mRNA is increased by NMDA receptor activation. Moreover, these findings suggest that normal GDNF expression may be regulated by cortical glutamatergic afferents and that upregulation of this factor under conditions of excitotoxicity may afford protection to responsive neurons in the nigrostriatal system. (Supported by AG00538 and MH14599)

222.4

ADRENAL HORMONES REPRESS SEIZURE ACTIVATION OF BDNF EXPRESSION AT SPECIFIC 5' PROMOTERS.

J.C. Lauterborn*[1], F.R. Poulsen[1], P.J. Isackson[2], and C.M. Gall[1]. [1]Dept. of Anatomy and Neurobiology, Univ. of Calif., Irvine CA 92717 & [2]Dept. of Biochemistry and Molecular Biology, Mayo Clinic, Jacksonville Fl 32224.

Work from this laboratory has shown that adrenal hormones repress seizure-induced increases in brain-derived neurotrophic factor (BDNF) mRNA and that adrenalectomy selectively potentiates the expression of BDNF exons I & II mRNA after a single epileptiform afterdischarge. In the present study, the effect of adrenalectomy on recurrent seizure-induced increases in BDNF expression from the multiple 5' promoters was examined using the hilus lesion (HL) paradigm, *in situ* hybridization, and cRNA probes that distinguish transcripts containing BDNF exons I - IV. Previous work demonstrated that HL-seizures recur from 2-10 h post-lesion and that, in adrenal-intact HL rats, total BDNF mRNA peaks at 6-12 h and returns to control levels by 24 h post-lesion. In agreement with these findings, hybridization to BDNF exon I - IV mRNAs were at control levels in HL rats killed 24 h post-lesion. In contrast, in adrenalectomized (ADX) rats killed at 24 h post-HL, exon I mRNA levels were 2 to 3-fold greater than in ADX-controls within stratum granulosum ($p<0.05$), CA1 stratum pyramidale ($p<0.01$) and neocortical layers 2/3 ($p<0.05$). In HL/ADX rats, exon II mRNA was markedly elevated (> 7-fold; $p<0.01$) and exon III mRNA was slightly elevated (1.5-fold; $p<.05$) in CA1 stratum pyramidale relative to levels in ADX-controls. Exon IV mRNA was at control levels in most fields but slightly reduced in superficial entorhinal cortex of HL/ADX versus ADX-control rats. These data demonstrate that adrenal hormones inhibit seizure activation of BDNF expression from specific 5' promoters and, thereby, differentially modulate activity-dependent BDNF expression across neuronal fields and cell types. (Supported by NS26748)

222.5

CORTICOSTEROID REGULATION OF BDNF, NT-3, TRKB AND TRKC MRNA EXPRESSION IN THE RAT HIPPOCAMPUS. M.J.M. Schaaf, R.W.M. Hoetelmans, E.R. de Kloet and E. Vreugdenhil*. Leiden/Amsterdam Center for Drug Research, Division of Medical Pharmacology, Sylvius Laboratories, P.O. Box 9503, 2300 RA Leiden, The Netherlands

Chronic high plasma levels of corticosterone are known to decrease the neuronal cell viability in the hippocampus and may therefore be involved in several neurodegenerative disorders. In the hippocampus, corticosterone binds to two receptors: the low affinity glucocorticoid receptor (GR) and the high affinity mineralocorticoid receptor (MR). It is argued that simultaneous MR and GR occupation endangers neurons in the hippocampus, whereas occupation of MR is protective. This may be the result of a regulatory role of MR and GR in the expression of neurotrophic factors. In order to study this role, we administered a low, medium and high dose of corticosterone s.c. to adrenalectomized rats and measured the mRNA levels of BDNF, trkB, NT-3 and trkC six hours after corticosterone administration by in situ hybridization. Our results show that BDNF and trkB mRNA levels in CA3 and dentate gyrus are significantly lower after administration of a high dose of corticosterone compared to those after a low dose. Furthermore, trkB mRNA levels in these regions are two-fold higher in the low dose group compared to the vehicle treated. Levels of NT-3 and trkC do not show significant regulation by corticosterone in our study. Taken together, our results indicate that a high dose of corticosterone (and thus simultaneous MR and GR occupation) leads to a decrease in BDNF and trkB mRNA levels. This may be an explanation for the low neuronal viability in these areas at high plasma corticosterone levels.

222.6

KAINIC ACID INCREASES THE EXPRESSION OF THE PROHORMONE CONVERTASES FURIN AND PC1 IN THE MOUSE HIPPOCAMPUS M. Marcinkiewicz*, A. Meyer, P. Chrétien, G. Massicotte and M. Chrétien. Laboratory of Molecular Neuroendocrinology, Clinical Research Institute of Montreal, 110 Pine Avenue West, Montreal, Quebec, Canada H2W 1R7

Prohormone convertases (PCs) belong to the mammalian family of subtilisin/kexin-like enzymes which have been implicated in the posttranslational processing of precursor proteins. Several PCs are produced in the CNS and PNS, and only a few specific precursor-substrates have been identified *in vivo*. PCs may be involved in intracellular processing of precursors for neuropeptides, hormones and neurotrophic factors, including nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF). To study the interrelationships between the convertases furin, PC1 and PC2, and the neurotrophins NGF, BDNF and NT-3, we compared their mRNA distribution in different tissues. We also examined their expression in the hippocampus of mice undergoing kainic acid-induced seizures. In this experiment, *in situ* hybridization (ISH) demonstrated that the levels of mRNA for furin, PC1 and BDNF increased maximally at 3 hrs after kainic acid administration, followed by a decline to normal levels by 96 hrs. NGF showed small changes, while NT-3 was downregulated with minimal expression levels between 3 to 12 hrs. Double ISH with radioactively-labeled riboprobes and digoxigenin-labeled riboprobes demonstrated colocalization of furin with NGF and BDNF in the mouse submaxillary gland, and of furin and PC1 with BDNF in the trigeminal ganglion. Based on colocalization studies and evidence of coordinate expression with NGF and BDNF, we suggest the involvement of furin in processing of proNGF, and of both furin and PC1 in processing of proBDNF. Supported by the Medical Research Council of Canada (MT12686) to MM.

222.7

VIP AND PACAP STIMULATE BDNF EXPRESSION BY POTENTIATING THE EFFECT OF GLUTAMATE. J.-L Martin*, P.J Magistretti and G. Pellegri. Institut de Physiologie, Université de Lausanne, Switzerland.

The regulation of BDNF expression by VIP was analyzed by Northern blot in primary cultures of neurons and astrocytes originating from the mouse cerebral cortex. VIP stimulates in a concentration- and time-dependent manner BDNF expression both in cortical neurons and astrocytes although the induction is larger in neurons. The neuropeptide PACAP, which shares a high degree of sequence identity with VIP, also stimulates BDNF expression. Similarly to the potentiation by VIP and PACAP of glutamate-induced *c-fos* expression (Martin et al., J. Neurochem. 65, 1-9, 1995), the potentiating effect of both peptides is completely inhibited by the NMDA receptor antagonist MK-801, while MK-801 is ineffective in cortical astrocytes. These results indicate that, in cortical neurons, up-regulation of BDNF expression by VIP and PACAP is indirect, being mediated by low concentrations of endogenous glutamate (~ 1 μM) acting on NMDA receptors. In this context, it is important to note that exogenously added glutamate (100 μM) stimulates BDNF expression to a lower extent than VIP or PACAP. These and previous data (Martin et al., J. Neurochem. 65, 1-9, 1995) indicate that, by stimulating cAMP formation, VIP and PACAP increase the "throughput" or "strength" of glutamate-containing circuits in the cerebral cortex.

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222.8

CHARACTERIZATION OF THE REGULATION OF DOPAMINERGIC TROPHIC FACTOR AND CYTOKINE GENE EXPRESSION ASSOCIATED WITH NEUROTOXIN-INDUCED PLASTICITY OF DOPAMINE NEURONS IN YOUNG AND AGING MICE. A. Ho* and M. Blum. Fishberg Res. Ctr. for Neurobiology. The Mount Sinai School of Medicine, New York, NY 10029.

It has been reported that young mice challenged with the neurotoxin MPTP, show a substantial recovery of striatal dopamine nerve terminals after one month whereas middle-aged mice do not. Also, exogenously applied bFGF and IL-1β can induce sprouting of dopamine neurons after a neurotoxin challenge. We have previously demonstrated that bFGF mRNA can be regulated by IL-1β, thus suggesting bFGF may mediate IL-1β induced dopaminergic sprouting. However, the regulation of dopaminergic trophic factor and cytokine gene expression in response to acute and long term neuronal injury remains to be elucidated in C57BL/6 mice, particularly in aging mice. Since it has been observed that there is an aging-associated decline in compensatory dopaminergic sprouting in response to neurotoxin-induced toxicity, possibly, the regulation of cytokine and trophic factor gene expression becomes altered with age.

In young mice treated with MPTP, we observed that aFGF mRNA in both dorsal and ventral striatum did not change within one week post-lesioned. In contrast, we observed that bFGF mRNA levels increased 2.7 fold in the dorsal striatum and increased a significant 1.4 fold in the ventral striatum within one week post-lesioned in young mice. In the middle-aged mice treated with MPTP, we observed that neither aFGF and bFGF mRNA levels in the dorsal striatum changed within two weeks post-lesion, thus suggesting there may be an aging-associated loss of the induction of trophic factor synthesis in response to neurotoxin-induced injury. Ongoing studies are invesigating the long term effects of MPTP on FGF gene expression and whether endogenous cytokine expression in response to neurotoxin-induced injury becomes altered in aging mice. (Supported by NIH grant AGO8538)

222.9

CHANGES IN CNTF RECEPTOR α mRNA EXPRESSION FOLLOWING ENTORHINAL CORTEX LESIONS. H.-D. Hofmann*, M.-Y. Lee, M. Kirsch, M. Frotscher and T. Deller. Institute of Anatomy I, Freiburg, Germany.

Recently, ciliary neurotrophic factor (CNTF) has been implicated in reactive processes occurring after brain lesion. To further investigate the role of CNTF, we have studied the expression of CNTF receptor α (CNTFRα) in a well defined lesion model (unilateral lesions of the entorhinal cortex, EC). Expression of CNTFRα mRNA was determined in EC-lesioned and sham-operated adult rats after survival times of 4 hours, 3, 7, 10, 14 days, 4 weeks and 6 months by in situ hybridization using a digoxigenin-labeled RNA probe. In sham-operated, as in normal animals, CNTFRα expression was restricted to neurons (superficial layers of EC, granule cells of dentate gyrus, pyramidal cell layer of the hippocampus proper). At 4 hours postlesion a hybridization signal appeared in the tissue surrounding the lesion site in the EC. At three days postlesion, CNTFRα expression was observed in areas where degenerating terminals of entorhinal axons are located. The response was massive in the ipsilateral outer molecular layer (OML) of the dentate gyrus and less prominent in the contralateral OML and the lateral septum. After 10 days, CNTFRα expression had returned to control levels. The number of labeled cells and their distribution in the OML strongly suggested that receptor expression had been induced in astrocytes. In addition, beginning at 7 days postlesion, a significant reduction of the hybridization signal was observed in granule cells of the ipsilateral dentate gyrus as compared to the contralateral side. Our results demonstrate complex changes in CNTFRα expression at the site of lesion, in areas of terminal degeneration and sprouting, and in deafferented neurons. Thus, CNTF may be involved in the regulation of glial responses related to degenerative processes and may also contribute to sprouting phenomena and transsynaptic effects after neuronal deafferentation. *Supported by DFG: SFB 505/A4 and Leibniz Program*

222.10

HEPARIN-BINDING EGF: MODULATION OF GENE EXPRESSION *IN VIVO* AND NEUROPROTECTIVE EFFECTS *IN VITRO* FOLLOWING KAINATE TREATMENT. L.A. Opanashuk*[1], R.J. Mark[1,2], M.P. Mattson[1,2] and K.B. Seroogy[1]. [1]Dept. of Anatomy & Neurobiology and [2]Sanders-Brown Ctr. on Aging, Univ. of Kentucky, Lexington, KY 40536.

We have recently identified mRNA for heparin-binding epidermal growth factor (HB-EGF), a newly described EGF receptor ligand, within several brain regions including neocortex, hippocampus, and piriform cortex. Because seizure activity has been associated with modulation of trophic factor expression in these regions, the present study addressed the possibility that HB-EGF mRNA is regulated in a similar manner. Adult rats received either a saline or 12 mg/kg i.p. injection of kainic acid and were monitored for convulsive behavior. Following various survival periods, brain tissue was processed for Northern blot or *in situ* hybridization analyses using cRNA probes for HB-EGF mRNA (cDNA kindly provided by J. Abraham, Scios Nova). An increase in hippocampal HB-EGF mRNA levels was detected at 3 hr and peak expression occurred at 12-24 hr following the injection of kainate. In dentate gyrus, hybridization was maximal in granule cells 3 hr following kainate administration with levels declining below control by 48 hr. Scattered HB-EGF mRNA-expressing somata were evident in the hilar region between 6 and 24 hr. Prominent hybridization was apparent in and around the pyramidal cell layers of CA1 and CA3 after 12 hr; however, by 24 hr labeling was restricted to surrounding cells. By 48 hr hybridization was sparsely distributed in the CA1 region. To determine whether HB-EGF may serve a neuroprotective role, hippocampal cultures were pretreated with 0-100 ng/ml HB-EGF and then challenged with 10 or 50 μM kainic acid. Evaluation of neuronal survival indicated that HB-EGF significantly attenuated toxicity in response to kainate. Taken together, these findings indicate that HB-EGF expression is differentially regulated in response to kainate-induced seizure activity and suggest that this molecule is a trophic mediator for cells within hippocampus. Supported by NIH fellowship NS10007 (LAO) and UK Med. Ctr. (KBS).

222.11

NEUROTROPHIN mRNA EXPRESSION IS ALTERED IN LOCUS COERULEUS FOLLOWING TRAUMATIC SPINAL CORD INJURY. X. Mu*, J.E. Springer, K.H. Lundgren, and K.B. Seroogy. Department of Anatomy and Neurobiology, University of Kentucky Medical Center, Lexington, KY, 40536-0084.

Traumatic injury to the spinal cord (SCI) results in damage to descending axons whose cell bodies are located in midbrain pontine nuclei. One such population of neurons are the noradrenergic cells of the locus coeruleus, which are thought to influence the control of certain spinal cord systems involved in locomotion. Recent studies have demonstrated that neurons in the locus coeruleus express the mRNAs for the neurotrophin BDNF and NT-3, as well as for their high affinity receptors, trkB and trkC, respectively. In addition, there is evidence that locus coeruleus neurons respond to the neurotrophic actions of some members of the neurotrophin family. In the present study, we used in situ hybridization histochemistry to examine the expression of BDNF, NT-3, trkB, and trkC mRNAs in the locus coeruleus at different times following traumatic injury to the spinal cord at level T10. Within 24 hours following SCI, the levels of NT-3 mRNA were significantly down-regulated at several levels of the locus coeruleus. Conversely, BDNF mRNA expression was found to be substantially upregulated in numerous locus coeruleus neurons at the same post-injury time point. Interestingly, the mRNA for trkB and trkC remain unchanged at the 24 hour time point compared to laminectomy control animals. These studies demonstrate that NT-3 and BDNF mRNA expression is dramatically altered in neurons whose axons, but not cell bodies, are directly damaged by traumatic SCI. The pattern of expression suggests that BDNF may function as a neurotrophic factor for neurons that are functionally compromised due to injury to their descending axons. Supported by a grant from the Kentucky Spinal Cord and Head Injury Trust (JES).

222.12

IMMUNOLOCALIZATION OF FGF-2 IN THE RAT OLFACTORY BULB DURING DEVELOPMENT AND SUBSEQUENT TO OLFACTORY NERVE LESION. M. C. Miller, K. R. Hendricks, J. N. Kott*, R. S. Morrison, M. E. Lee and L. E. Westrum, Dept. of Neurol. Surg., U. of WA, Seattle, WA 98195.

Basic fibroblast growth factor (FGF-2) is thought to play a role in brain development based upon its expression and the expression of its receptor in neural tissue. We are interested in the relationship of FGF-2 to the process of nervous system repair after injury. We have studied FGF-2 in the olfactory bulb (OB). This structure provides an ideal model in which to study the reinnervation process due to the unique regenerative capacity of the olfactory nerve (ON) throughout life. Initially, using immunohistochemistry, we examined the developmental localization of FGF-2 in the OB of embryonic day (E) 15, E19, postnatal day (PN) 1, PN5, PN28 and adult rats. Next, we determined if there were any changes in FGF-2 distribution in the adult OB subsequent to an ON lesion. Results showed that FGF-2 immunoreactivity: 1) was absent in the OB during early development; 2) became moderate in ON layer astrocytes and ensheathing cells at PN28 and 3) was strong in ensheathing cells of the ON layer along with astrocytes throughout the OB in adults. In response to injury, astrocytic immunoreactivity increased in intensity throughout the OB and neurons subjacent to the lesion site become moderately reactive. The increased FGF-2 immunoreactivity in astrocytes and its appearance in neurons supports the hypothesis that FGF-2 may have multiple functions in response to nervous system injury and may act via paracrine and autocrine mechanisms.

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222.13

CHANGES IN RETINAL bFGF EXPRESSION FOLLOWING CONTROLLED OPTIC NERVE CRUSH. J. Weise*, M.R. Kreutz, T.M. Böckers, C. Seidenbecher and B.A. Sabel. Inst. of Medical Psychology, Otto-v.-Guericke University, Magdeburg, Germany.

Basic Fibroblast Growth Factor (bFGF) is a potent member of the Fibroblast Growth Factor family which comprises nine rather different forms of FGF. Although present in many tissues, bFGF is of particular interest in the mammalian CNS because it acts as a mitogenic and neurotrophic factor on several neuronal populations. In addition, there is evidence that it exerts neuroprotective effects after ischemia and excitotoxicity both in vitro and in vivo.

In this study we examined the expression of bFGF in the rat retina in response to a controlled crush of the optic nerve (ONC). Changes in bFGF expression were assessed on both mRNA and protein levels at different time points after ONC. Up to 48 hours after ONC we observed a drop in hybridization signals below control levels in all retinal layers. A gradual increase of bFGF mRNA occured at 72 hours to 1 week after ONC, especially in the inner nuclear layer. Signals were still elevated 4 weeks after optic nerve injury. Immunocytochemistry against bFGF revealed a staining pattern corresponding to the hybridization results. bFGF staining was generally decreased 12 and 48 hours after ONC. However, beginning at 1 week and peaking at 4 weeks after injury, an increase in immunoreactivity was observed, primarily in the inner nuclear layer. Staining for bFGF was still detectable above control levels even at 10 weeks after ONC. These results suggest that long term upregulation of bFGF in Müller glia and astrocytes provides an important trophic support that may be in part responsible for the survival of a substantial number of retinal ganglion cells after traumatic optic nerve injury. Supported by DFG Graduiertenkolleg Magdeburg and BMBF Neurotrauma Magdeburg-Berlin TPA2 to M.R.K. and B.A.S.

222.14

ELEVATED BDNF mRNA EXPRESSION IN RETINAL GANGLION CELLS FOLLOWING OPTIC NERVE INJURY. H Gao*, X Qiao, F Hefti, JG Hollyfield, and B Knusel Andrus Gerontology Center, Univ. Southern California, CA; Div. Ophthalmology, Cleveland Clinic Foundation, Cleveland, OH

BDNF is the most abundant neurotrophic factor in the adult CNS. Recent studies have shown that exogenous BDNF promotes neuronal survival both in vitro and in vivo in neurodegenerative models. We have previously localized BDNF gene expression to retinal ganglion cells in the mouse. However, little is known about the function of endogenous BDNF in this location. To investigate whether BDNF may play a role in neuronal protection following ganglion cell trauma, BDNF expression was examined following optic nerve injury. The optic nerve in Sprague-Dawley rats was crushed intraorbitally posterior to the optic disc. For the controls, the optic nerve on the opposite side in each animal was similarly exposed but was not crushed. After intervals of 6 hrs to 6 weeks, eyes were processed for in situ hybridization using radio-labeled rat cRNA probes. In the normal rat retina, BDNF mRNA expression was present in a subpopulation of ganglion cells. Following the optic nerve crush, BDNF expression was significantly elevated in individual ganglion cells, and more ganglion cells demonstrated expression of BDNF than were observed in the controls, especially in the central retina. Elevated BDNF expression was first observed at 24 hrs, reached peak levels at 48 hrs, and declined to the basal level 2 weeks after crush injury. In control retinas without optic nerve crush, BDNF expression was localized to some ganglion cells, as was observed in normal eyes without surgery. These results indicate that the ganglion cells can up-regulate gene expression of BDNF in response to axonal injury and suggest that endogenous BDNF may contribute to a neuroprotective process following optic nerve injury.

This study was supported by research grants from NIA AG09793, AG10480, NINDS NS22933, Sankyo Co., Ltd, and National Parkinson Foundation.

222.15

CHANGES IN NGF PROTEIN LEVELS IN YOUNG BUT NOT AGED RAT BRAIN AFTER TOTAL IMMUNOLESION. J. Yu* and J.R. Perez-Polo. Dept. HBC&G, Univ. TX Med. Br., Galveston, TX 77555-0652.

In the present study we compared the effects of cholinergic deafferentation of the hippocampus, neocortex, and olfactory bulb on nerve growth factor (NGF) level and choline acetyltransferase (ChAT) activity in these areas after intraventricular injection of immunotoxin, 192 IgG-saporin which previously has been shown to be able selectively destroy cholinergic neurons in the basal forebrain. We showed that after total immunolesion with 3.4 μg 192 IgG-saporin ChAT activity was dramatically decreased in the basal forebrain and its target areas in both young and aged rats. NGF protein levels were significantly increased in the hippocampus, cortex, and olfactory bulb of young rats (see also J Neurosci Res 43:213-223) whereas no such responses were observed in aged rats at both two weeks and one month postlesion times. In addition, no changes in NGF and BDNF mRNA levels were observed in the hippocampus and cortex in aged rats as have been shown in young ones (Brain Res 705:247-254). Basal forebrain NGF levels were increased in both young and aged rats. These results suggest that aged animals response to cholinergic differently in terms of neurotrophin homeostasis. This is publication 52A from USPHS P01-A610514 awarded by NIA.

222.16

APPEARANCE OF FIBROBLAST GROWTH FACTOR(FGF)-9 IMMUNOREACTIVITY IN DAMAGED AXONS AND REACTIVE GLIAL CELLS IN RAT BRAIN FOLLOWING NEEDLE STAB INJURY. N. Otsuka[1,2], T. Todo[2], S. Nakamura[2], A. Aoyagi*[3], R. Machinami[1] and K. Ikeda[2]. [1]Dept.of Pathol., Fac.of Med., Univ.of Tokyo, [2]Dept. of Ultrastruct.and Histochem., Tokyo Inst.of Psychiat., [3]Dept.of Chem.Pharmacol., Fac.of Pharmaceut.Sci., Univ.of Tokyo, Japan.

FGF-9, originally isolated from a culture medium of a human glioma cell line as a growth-promoting factor for glial cells, is a newly identified member of FGF family. Recently, we found by immunohistochemistry that FGF-9 was present in neurons and astrocytes in human and rat brains. In this report, to see whether and how FGF-9 is induced in neural tissue by damage, we immunohistochemically examined brains of the rats, which had received cerebral needle stab injury, by using polyclonal antibodies raised against human recombinant FGF-9 or synthetic peptides of FGF-9(87-102 and 193-208, respectively). In result, two types of FGF-9 immunoreactivity were observed adjacent to or around the needle tract area; the staining in axons and/or dystrophic axonal swelling and the staining of reactive glial cells. At 15h following needle insertion, immunoreactivity to FGF-9 was discernible in swollen axons adjacent to the lesion. This immunoreactivity became stronger by time. On the other hand, immunostaining of the glial cells first appeared 50h after the injury. The intensity of staining became gradually stronger by time. By morphological criteria, the immunoreactive cells were considered to be mainly astrocytes. The damage-mediated FGF-9 appearance in degenerating axons and reactive astrocytes may have implications in repairs of the brain.

222.17

QUANTITATIVE AND QUALITATIVE ANALYSIS OF FGF-2 AND FGF RECEPTOR 1 mRNAS IN THE HYPOGLOSSAL MOTOR SYSTEM: REGULATION AFTER LESION. K. Huber, C. Meisinger, T. Janet*, C. Grothe. Institute of Anatomy, University of Freiburg, D-79104 Freiburg, Germany and University of Poitiers, F-86022 Poitiers, France (TJ).

In hypoglossal motor neurons, FGF-2 immunoreactivity (IR) shows a decrease 2 and 5 days after axotomy, after 11 days FGF-2 IR reappears. Iodinated FGF-2 is specifically retrogradely transported via high-affinity receptors (R) after injection into the tongue (Grothe & Unsicker, J. Neurosci. Res. 32, 1992; Grothe & Janet, J. Comp. Neurol. 353, 1995). To characterize physiological functions of FGF-2 in the motor system we have studied the expression and regulation of FGF-2 and FGFR1 mRNAs in the hypoglossal system 1 -28 days after lesion using RNase protection assay and *in situ* hybridization.

The FGFR1 mRNA in the hypoglossal nucleus is confined to motoneurons. The level of the FGFR1 mRNA shows no significant alterations 1, 2, 6, 10, 11, 14, 21, and 28 days after axotomy. The FGFR1 transcript level in the tongue, however, is up-regulated between 11 and 14 days after axotomy.

The weakly expressed FGF-2 mRNA is localized to the motoneurons. 10 and 11 days after peripheral nerve transection the FGF-2 transcript level is significantly enhanced. The expression level in the tongue is not altered.

In the hypoglossal nerve, crush lesion results in a significant increase of both transcripts, in a similar time course as we had previously shown for the sciatic nerve.

Together with previously reported data on survival effects of exogenous FGF-2 on hypoglossal neurons *in vitro* and *in vivo* the results strongly support the idea that endogenous FGF-2 is invovled during the regeneration events of motoneurons. (Supported by SFB 505/A2)

222.18

FURTHER EVIDENCE FOR AUTOCRINE TROPHIC SUPPORT OF RAT FACIAL MOTONEURONS BUT NOT RUBROSPINAL NEURONS AFTER AXOTOMY. N.R. Kobayashi*, K.C. Harrington and W. Tetzlaff Depts. of Zoology and Surgery, University of British Columbia, Vancouver, B.C. V6T 1Z4

We have previously reported that axotomized rat facial motoneurons increase expression of BDNF and its receptor, trkB, after axotomy, suggesting that these neurons receive trophic support in an autocrine manner. We are currently investigating whether other trophic factors known to act on motoneurons play a similar role. Using RT-PCR, we found an increase in FGF-2 mRNA in microdissected facial nuclei as early as 8 hours after facial nerve transection, which reached levels around 4 fold by 24 hours and was sustained throughout the period of investigation (14 days). In situ hybridization (ISH) demonstrated that this increase was seen in all axotomized motoneurons. In contrast to facial motoneurons, axotomized rubrospinal neurons undergo severe atrophy, which can be prevented by BDNF application to the red nucleus. Thus, in this study, we have tested the hypothesis that axotomized rubrospinal neurons fail to increase BDNF and trkB expression. ISH results indicate that only a few (less than 5 %) rubrospinal neurons increased BDNF ISH signals 3 days after axotomy. Decreased trkB mRNA expression was observed 7 and 14 days after axotomy . FGF-2 mRNA ISH signal was not detected in axotomized or contralateral rubrospinal neurons. Preliminary RT-PCR amplifications corroborate these findings by revealing a decrease in both BDNF and FGF-2 mRNA levels. These observations further support the hypothesis that rat facial motoneurons provide their own trophic support after disconnection from their target by axotomy. In contrast, axotomized rubrospinal neurons appear to lack autocrine support.

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222.19

BDNF UP-REGULATION IS LIMITED TO MYELINATING SCHWANN CELLS. H. Hyman Friedman, S.A. Singel, D.E. Playford*, G.M. Bray, and A.J. Aguayo. The Montreal General Hospital Research Institute, 1650 Cedar Ave., Montreal, P.Q., H3G 1A4.

Transection of a rat sciatic nerve, which contains a mixture of myelinated and nonmyelinated fibers, leads to a forty-fold up-regulation of BDNF mRNA. This increase in expression begins within three days of transection, increasing gradually to reach its peak only after three weeks. To determine if all Schwann cells were potential sources of this neurotrophin, we measured the levels of BDNF mRNA in the largely unmyelinated (>95%) cervical sympathetic trunk (CST) of rats. Both one week after crush and two weeks after transection, the level of BDNF mRNA distal to the site of injury was less than one tenth (0.1% BDNF/GAPDH) the level in transected sciatic nerves (2% BDNF/GAPDH). These results support the hypothesis that contrary to what happens in myelinated fibers, Schwann cells in unmyelinated nerve fibers do not up-regulate BDNF expression after axonal interruption.

Relevant to this observation, we have found that demyelinating nerve fibers of Trembler mice have a deficit in the up-regulation of BDNF mRNA following axotomy. Together, these experiments suggest that up-regulation of this neurotrophic factor may be limited to Schwann cells that have differentiated towards a myelinating phenotype.

(supported by the Multiple Sc lerosis Society of Canada)

222.20

DIFFERENTIAL REGULATION OF FGF-2 ISOFORMS IN SPINAL GANGLIA AND SCIATIC NERVE AFTER LESION. C. Grothe, K. Wewetzer*, C. Meisinger. Institute of Anatomy, University of Freiburg, D-79104 Freiburg, Germany.

In order to clarify functional roles of the endogenous FGF-2 in the peripheral sensory system we have studied the expression and regulation of the FGF-2 molecular weight isoforms in spinal ganglia (L4-L6) and in the proximal and distal nerve stump of the adult rat after sciatic nerve crush and transection injury using quantitative Western blot analysis. The regulation of FGF-2 at the protein level was correlated with alterations of the FGF-2 mRNA as determined by RNase protection assay.

In untreated dorsal root ganglia (DRGs) and sciatic nerve the 18kD, 21kD, and 24kD FGF-2 isoforms are expressed. The 18kD isoform often appears as a doublet. Seven days after crush or axotomy of the peripheral nerve all three FGF-2 isoforms display a significant increase in DRGs. The 24kD isoform, however, shows the highest increase. Under both experimental conditions (crush, transection), the alterations of the expression level of the different FGF-2 isoforms are very similar. In the proximal and distal nerve stump, all three FGF-2 isoforms are enhanced 7 days after crush, the proximal stump displays a stronger expression compared to the distal stump. The 21kD isoform shows the highest increase. Seven days after nerve transection in the distal nerve stump FGF-2 is not detectable. In the proximal stump the 18kD and 21kD isoforms show an approximately 8-fold increase and the 24kD isoform a 2.5-fold increase. Different expression of FGF-2 in the distal nerve stumps under regenerating and degenerating conditions is also found at the mRNA level.

The differential response of FGF-2 isoforms in DRGs and sciatic nerve suggests distinct physiological functions, a local reaction at the lesion side where neurite regrowth occurs and a trophic reaction for de-/regenerating neurons. (SFB 505/A2)

222.21

INDEPENDENT REGULATION OF IGF-II mRNA ISOTYPES IN MUSCLE DURING DEVELOPMENT AND DENERVATION. D.J. Marsh and D.N. Ishii*, Dept. of Biochem. and Molec. Biol. and Dept. of Physiol., Colorado State Univ., Fort Collins, CO 80523.

Insulin-like growth factor II (IGF-II) gene expression in muscle has previously been shown to be closely correlated with synaptogenesis and nerve regeneration in rats. This 36 kb gene has three promoters (P1, P2, and P3) that regulate the alternative usage of leader exons E1, E2, and E3. Here, we determined which promoters are likely to be active during development and nerve regeneration by studying the expression of IGF-II mRNA isotypes using exonic probes. All isotypes were developmentally down-regulated between 5-day-old and adult rats, however, E2 isotypes followed a different time course. The E3 isotypes showed the greatest up-regulation following sciatic nerve transection in neonates or crush in adults. By contrast, E1 and E2 mRNAs were up-regulated in adults but not neonates. E3 mRNAs were by far the most abundant IGF-II transcripts in muscle during development as well as denervation. These results show independent isotype regulation, and suggest that the P3 promoter may play a dominant role in regulating IGF-II gene expression during development and nerve regeneration. (Supported by grant RO1 NS24327-08)

NEUROTROPHIC FACTORS: EXPRESSION AND REGULATION—PHYSIOLOGIC AND PATHOPHYSIOLOGIC MECHANISMS III

223.1

INTRACELLULAR SORTING AND SITES OF DEPOLARIZATION-EVOKED NEUROTROPHIN RELEASE FROM HIPPOCAMPAL NEURONS. I. M. Krivko, D. W. Ethell*, J. Klose, R. Kolbeck and H. Thoenen. Dept. of Neurochem., MPI for Psychiatry, Am Klopferspitz 18A, 82152 Germany.

There is increasing evidence that neurotrophins (NTs) are not just target-derived trophic factors , but also act in specific aspects of neuronal plasticity. In order to understand the role neurotrophins play as mediators of neuronal plasticity we have employed confocal microscopy and transmission EM techniques to analyse intracellular sorting and sites of depolarisation-evoked NT6 release from CNS neurons. NT6 shares a common structure with other neurotrophins with the addition of a heparin-binding domain that causes the protein to stick to heparan proteoglicans on the neuronal surface. We modified NT6 to include a carboxy-terminal c-myc 9E10 epitope and transiently transfected E17 rat hippocampal neurons. Taking advantage of NT6's adherence to the cell surface, after release, we have been able to specifically localize the sites of activity dependent neurotrophin release from hippocampal neurons, using both anti-NT6 and anti-c-myc antibodies. NT6 release was found to be highly inducible by 50 µM glutamate with a patchy surface distribution. Further analysis confirmed the specificity of such release preferentially at neurite contact areas (potential synapses) and dendrite growth cones. Newly translated NT 6 was detected in membrane-confined, ER-like structures and sorting was followed through the Golgi apparatus to accumulation centers which directly corresponded with the sites of activity dependent neurotrophin release. Further, preliminary work with a c-myc BDNF construct has shown the same patchy intracellular distribution within hippocampal neurons.

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223.2

DIFFERENTIAL REGULATION OF ADULT AND NEONATAL CORTICAL ASTROCYTE FUNCTION BY DEPOLARIZING SIGNALS. J. Lackland*, H.Wu, I.B. Black, C.F. Dreyfus, Dept. Neurosci. & Cell Biol., UMDNJ/RWJMS, Piscataway, NJ 08854

To examine the regulation of astrocyte function in adult animals we have begun to grow Type 1 astrocytes from adult rat cerebral cortex for comparison with astrocytes from neonatal (P1) rats. Primary cultures of adult astrocytes were prepared (Schwartz et al, 1994) and grown for 4 weeks, at which time >98% of the cells were GFAP-positive. Cultures of P1 astrocytes were prepared (McCarthy & DeVellis, 1980) and grown for 3 weeks, with > 95% of the cells being GFAP-positive. No significant morphological differences were noted between the two culture types. In serum-free medium approximately 1/3 of the astrocytes were process-bearing while the majority were flat. Both types stained positively for GFAP, with the process-bearing cells staining more intensely. Previous work in our lab has shown that neonatal astrocytes in other brain areas respond to depolarizing signals with changes in morphology and/or neurotrophin mRNA expression. To examine the regulation of adult astrocyte function, confluent cultures were treated with KCl, stained for GFAP, and examined for changes in morphology. Exposure to KCl (6.25mM to 50mM for 4 or 24 hrs) did not significantly change the morphology of GFAP-positive cells in either adult or P1 astrocytes. The effects of depolarizing signals on neurotrophin mRNA expression were investigated. Both adult and P1 astrocytes express BDNF and NT-3 mRNA when grown in culture. In P1 astrocytes the expression of NT-3 mRNA was increased by treatment with KCl for 4hours. In contrast, in adult astrocytes, the expression of NT-3 mRNA was unaffected, suggesting that adult astrocytes are less sensitive to depolarizing signals than P1 cells. (Supported by NICHD: HD23315)

223.3

DIFFERENTIAL REGULATION OF NEUROTROPHIN EXPRESSION IN BASAL FOREBRAIN ASTROCYTES H. Wu*, I. B. Black, C. F. Dreyfus, Dept. Neurosci. & Cell Biol., UMDNJ/Robert Wood Johnson Med. Sch., Piscataway, NJ 08854.

Previous studies have demonstrated that neurotrophins are important for developing basal forebrain (BF) cholinergic neurons. NGF increases choline acetyltransferase activity, BDNF increases survival, and NT3 increases both survival and neurite outgrowth. Our initial studies indicated that BF astrocytes express these neurotrophins. To understand the role of neuron-glia interactions in BF development, we evaluated effects of neuroactive agents on neurotrophin expression in astrocytes from P1 rat BF.

Purified BF astrocyte dissociated cultures were prepared and neurotrophin mRNA levels were determined. NGF, BDNF and NT3 mRNAs were expressed by cultured BF astrocytes. To investigate regulation, effects of KCl, glutamate and carbachol were examined. KCl increased BDNF mRNA, but had no effect on NGF. The cholinergic agonist, carbachol, also increased BDNF expression, without changing NGF mRNA levels. Glutamate increased both NGF and BDNF. These stimuli had no significant effect on NT3 expression. However, in the presence of the thyroid hormones, T3 and T4, KCl increased NT3 mRNA 2-fold; glutamate and carbachol also elicited small but significant increases in NT3 mRNA levels.

Our data suggest that neuroactive agents differentially regulate BF astrocytic expression of neurotrophins. Neuron-glia interactions, therefore, may critically underlie the development and function of the BF. (Supp: NICHD:HD23315)

223.4

CALCIUM INFLUX THROUGH NMDA RECEPTORS PLAYS AN IMPORTANT ROLE IN THE INTERLEUKIN-6 INDUCED ENHANCEMENT OF CALCIUM RESPONSE TO NMDA IN DEVELOPING CNS NEURONS. Z. Qiu*, S. M. Conroy and D. L. Gruol, Dept. of Neuropharmacology, The Scripps Research Institute, La Jolla, CA 92037

Developing granule neurons chronically treated with interleukin-6 (IL-6; 5 ng/ml) show an enhanced calcium response to NMDA that involves an increase in calcium influx and release from intracellular stores (Qiu et al., J. Neurosci, 1995). Excessive extracellular calcium influx through NMDA receptor-gated channels and voltage-gated calcium channels (VSCC) could contribute to the increase in calcium induced calcium release from intracellular calcium stores in the IL-6 treated neurons. To understand the mechanism mediating the enhanced calcium response to NMDA, studies were carried out to differentiate the contribution of calcium through both pathways. IL-6 (5 ng/ml) was added to primary cerebellar granule cultures at 1, 4, 7 11 days in vitro (DIV), and its effects were assessed using Fura-2 based microscopic calcium imaging and the nystatin perforate-patch technique for electrophsiologic recordings. In IL-6 treated neurons the sensitivity to VSCC blockers of the calcium response to NMDA was altered in that N-type VSCC contributed to the IL-6 induced enhancement of calcium response to NMDA at early development stages, whereas there was no differences in the sensitivity to L- and P-type VSCC blockers. Neither control nor IL-6 treated neurons were sensitive to L-, P- and N-type VSCC blockers at late developmental stages. Current clamp studies showed that the depolarization to NMDA was enhanced in IL-6 treated neurons, which could contribute to the differences in the calcium response to NMDA between control and IL-6 treated neurons. Voltage-clamp studies showed that IL-6 increased the current response to NMDA. These results demonstrated that L-, P, and N-type VSCC were not involved in the enhanced calcium response to NMDA at late development stages. The effect of IL-6 could be due to an increase in the density of NMDA receptors or an increase in the permeability of the receptors to calcium. Supported by MH47680

223.5

CNTF INFLUENCES NGF EXPRESSION IN CULTURED ASTROCYTES

I. Semkova and J. Krieglstein, Institut für Pharmakologie und Toxikologie, FB 16, Philipps-Universität, Ketzerbach 63, 35032 Marburg, Germany*

CNTF and NGF are characterized as neurotrophic substances for different neuronal populations in CNS. However, a functional synergism between NGF and CNTF has been recently demonstrated. The physiological basis of functional relationship between NGF and CNTF is not well understood. Therefore, we investigated whether exogenous CNTF can influence the expression of NGF in cultured rat cortical astrocytes. Exposure to human recombinant CNTF (1 ng/ml to 0.1 pg/ml) increased the level of mRNA for NGF after 3 h of incubation. The increase in NGF message was followed by a corresponding increase in NGF protein (approximately 2-fold) secreted from the cells into the culture medium as determined 6 h later. Additionally, we found that at the same concentrations exogenous CNTF increased the level of mRNA coding for p75, a low affinity receptor for NGF and other neurotrophins. These results show that CNTF can influence the expression of NGF in cultured astrocytes and could alter the response to neurotrophins via upregulation of p75 receptor in these cells. Since CNTF is highly expressed in the lesioned brain the upregulation of NGF, if it occurs in vivo, can contribute to the neuroprotective activity of this cytokine.

223.6

Regulation of Neurotrophin Expression By Developing Basal Forebrain Oligodendrocytes. X. Dai*, L. D. Lercher, P. Qu, H. Wu, I. B. Black and C. F. Dreyfus, Dept. of Neuroscience & Cell Biol., UMDNJ / Robert Wood Johnson Medical school, Piscataway, NJ 08854.

Our previous studies indicated that basal forebrain (BF) oligodendrocytes express neurotrophins during development in culture and in vivo. To define regulatory mechanisms, we treated cultured oligodendrocytes with glutamate or KCl, and monitored morphological maturation and neurotrophin expression. Enriched oligodendrocyte cultures were established from postnatal day 1 BF. 48 hours after replating, cultures were exposed to glutamate (10 μM) or KCl (25mM) for another 48 hrs in the presence of tri-iodothyronine/thyroxine. Glutamate and KCL significantly increased the percentage of myelin basic protein-positive oligodendrocytes. To define effects on neurotrophin expression, a sensitive solution hybridization technique was used. Glutamate elicited a significant decrease in BDNF mRNA expression but had no effect on NT-3 expression. In contrast KCL elicited a significant increase in BDNF mRNA. These studies suggest that neuroactive molecules may regulate oligodendrocyte maturation and trophic function. (Supp: NICHD:HD23315)

223.7

BASIC FIBROBLAST GROWTH FACTOR AND PLATELET DERIVED GROWTH FACTOR INDUCE THE NEURON-SPECIFIC BRAIN DERIVED NEUROTROPHIC FACTOR mRNA IN RAT HIPPOCAMPAL CELL LINE, Y. Kim Kwon*, C. D. Stiles†, and S. Jin, Dept. of Biology, Kyunghee Univ., Seoul 130-701, Korea. †Harvard medical school, Boston MA 02115.

Brain derived neurotrophic factor(BDNF) is well known to promote neuronal survival and differentiation in central nervous system. BDNF mRNA is expressed in the highest level and the protein transduce the signal through trkB and MAP kinase in primary hippocampal culture. Basic fibroblast growth factor(bFGF) also facilitates neuronal survival in rat hippocampus but the mechanism is unclear. BDNF gene has 4 promoter which produce 8 alternative spliced transcripts, and 2 promoters are neuron-specific.

In the hippocampal precursor cell line, HiB5 which is immortalized using ts SV40 T antigen, bFGF promotes cell survival and differentiation. We found that the bFGF induced the neuron-specific BDNF transcripts including exon 1 but not exon 2 by using reverse transcription-polymerase chain reaction(RT-PCR) method with exon specific primers. Platelet derived growth factor(PDGF) also induces the neuron-specific BDNF mRNA. PDGF beta receptor mRNA and the protein were expressed when analyzed by northern and western blotting and the receptor protein were phosphorylated by PDGF BB.

These data are the first demonstration that neuronal growth factors such as FGF and PDGF regulate the expression of neuron-specific BDNF mRNA and suggest that they play a role in neuronal survival and differentiation through the action of BDNF.

This study is surported by SRC for Cell Differentiation and BIOTECH 2000 from KOSEF.

223.8

NGF TREATMENT INCREASES BDNF EXPRESSION IN TRK A IMMUNOREACTIVE DORSAL ROOT GANGLION CELLS AND IN THEIR CENTRAL TERMINATIONS WITHIN THE SPINAL CORD. J.V. Priestley, G.J. Michael, S. Averill, A. Nitkunan, G. Wotherspoon, M. Rattray, D.L.H. Bennett, Q. Yan and S.B. McMahon*. Divisions of Physiology and Biochemistry, UMDS, St. Thomas's Campus, London, SE1 7EH, and Amgen Inc., Thousand Oaks, California

Brain derived neurotrophic factor (BDNF) is present in dorsal root ganglia and increases in level following systemic NGF treatment and/or axotomy. However little is known about the dorsal root ganglion (DRG) cell types that express BDNF. We have combined immunocytochemistry and in situ hybridization to investigate this question.

In rat lumbar ganglia, 30-40% of trkA immunoreactive cells contained BDNF mRNA. 13 and 24 hours after 1 mg/Kg ip NGF, this percentage had risen to 60-75%. At the 13 hour time point, serial section analysis revealed that only 15% of cells expressing BDNF mRNA expressed trkC mRNA. Of these, half were also trkA immunoreactive. Less than 10% of the BDNF expressing cells expressed trkB mRNA. After systemic or chronic intrathecal (6μg/day, 14 days) NGF treatment, BDNF immunoreactivity increased in DRG cells and their central termination sites in the spinal cord. Analysis of the spinal cord staining revealed that the majority of BDNF terminals were also immunoreactive for CGRP, a known marker for trkA containing primary afferents. Ligation of dorsal roots showed accumulation of BDNF protein proximal, but not distal, to the site of ligation.

Our results show that NGF increases BDNF production in trkA expressing cells and that BDNF is anterogradely transported to the dorsal horn of the spinal cord. *Supported by the Medical Research Council, UK.*

223.9

EXAMINATION OF DRG NEURONS AND BEHAVIORAL TESTING IN MICE OVEREXPRESSING BDNF IN THE EPIDERMIS A.M. LeMaster*, J.E. Johnson, P.H. Kitzman, B.M. Davis and K.M. Albers. Dept. of Anatomy and Neurobiology and Pathology, Univ of KY Sch of Med., Lexington, KY 40536 and Dept of Neurobiology and Anatomy, Bowman Grey Sch of Med, Winston-Salem, NC 27157-1010

Brain-derived neurotrophic factor (BDNF) is important in the development of sensory neurons comprising the dorsal root ganglia (DRG). BDNF knockout mice have a 30% decrease in the DRG neuronal population. Other studies have shown exogenously applied BDNF promotes survival of DRG neurons. To elucidate the function of BDNF during development and in the maintenance of DRG neurons, we isolated transgenic mice that overexpress BDNF in the epidermis. Transgene expression was driven by promoter/enhancer elements of the human keratin 14 (K14) protein. In the present study, we examined the number of neurons expressing trk receptors, tyrosine kinase receptors that mediate the biological activity of neurotrophins. Uncorrected counts show no change in the number of trkA, trkB, or trkC expressing neurons in the DRG of K14-BDNF transgenic mice. However, increased numbers of trkA and trkB expressing neurons were found in trigeminal ganglia of K14-BDNF transgenic mice. In addition, elevated BDNF protein levels were found in the whisker pad skin, but not in the brain of K14-BDNF transgenic mice.

We also examined whether epidermal overexpression of BDNF coincided with changes in thermoceptive sensitivity. Midbrain infusion of BDNF was found to decrease nociceptive and thermoceptive sensitivity. Using the hot plate test, no change in thermoception was measured between control and K14-BDNF transgenic mice. Supported by NS33730 (KMA) and NS31826 (BMD).

223.10

THE EFFECTS OF OVEREXPRESSION OF NT-3 IN THE SKIN ON SENSORY INNERVATION OF THE CENTRAL NERVOUS SYSTEM. P.H. Kitzman*, B.M. Davis, A.M. LeMaster and K.M. Albers. Depts. of Pathology and Anatomy and Neurobiology, University of Kentucky. Lexington, KY 40536

The survival of a specific subpopulation of sensory neurons in both the trigeminal ganglia and the dorsal root ganglia has been shown to be dependent upon the availability of neurotrophin-3 (NT-3). Studies utilizing mice in which the NT-3 gene has been disrupted indicated that Ia projections do not innervate the spinal cord (Kucera et. al., 1995). However, systemic application of NT-3 at early developmental stages has been shown to inhibit the axon collateral growth in the gray matter of the dorsal horn (Zhang et. al., 1994). These studies indicate NT-3 may play a role in the development of sensory projections into the central nervous system. Our lab has isolated lines of transgenic mice that overexpress NT-3 in the epidermis. The expression of the NT-3 transgene begins at approximately E11 and continues throughout adulthood. The present study was undertaken to determine whether the overexpression of NT-3 in the skin alters the distribution of DRG sensory axons within spinal cord. Immunohistochemistry was performed on adult tissue using antibodies against BS-I-B4 (a marker for unmyelinated axons) and CGRP (a marker for peptidergic axons). At sacral levels there appears to be a decrease in CGRP immunoreactivity in the dorsal horn of the NT-3 mice when compared to control animals. At thoracic levels there was no obvious difference in either CGRP or BS-I-B4 immunoreactivity in the dorsal horn of the NT-3 mice when compared with the control animals. The lipophilic tracers DiI and DiA are also being used to determine overall changes in large-diameter axon distribution within the dorsal horn and to determine potential changes in the innervation between spinal segments. The effects of NT-3 overexpression on innervation of the trigeminal complex is also being studied. Supported by F32-NS09836-01 to PHK.

223.11

bFGF MODULATES THE EXPRESSION OF NGF, BDNF AND NT3 IN CULTURED HIPPOCAMPAL NEURONS. L. Ferhat, A. Represa, W. Ferhat, D. Zouaoui-Aggoun, Y. Ben-Ari, J. Ortiz*# and M. Khrestchatisky. Université René Descartes, Paris V, INSERM U-29, 123 Bd de Port Royal, 75014 Paris, France. # Fac. Pharmacie, Université Paris V, 4 Av de l'Observatoire, 75270 Paris Cedex 6, France.

Basic fibroblast growth factor (bFGF) is expressed in the hippocampus and has been demonstrated to promote neurotrophic effects on hippocampal neurons in vitro. We show that these neurons, even at the embryonic stage, express the mRNAs encoding the FGF receptors, bek and flg. We have characterized the effects of bFGF on the expression of NGF using RT-PCR, *in situ* hybridization and immunocytochemistry. In hippocampal neurons grown in the absence of serum, bFGF exposure induces an important elevation of NGF mRNA levels followed by a marked increase of NGF immunoreactivity. Combining *in situ* hybridization with a NGF probe and MAP2 immunocytochemistry we show that bFGF induction of NGF mRNA is localized in MAP2 immunoreactive neurons. We also show in primary cultures of hippocampal neurons that bFGF increases the steady state levels of BDNF mRNAs while it decreases those of NT3. These results suggest roles for bFGF in maintenance of connexions in the central nervous system, particularly the septo-hippocampal pathway, via the regulation of neurotrophic factor expression in neurons.

223.12

bFGF MODULATES THE EXPRESSION OF GENES ENCODING CYTOSKELETAL PROTEINS IN CULTURED HIPPOCAMPAL NEURONS: POTENTIAL INVOLVEMENT OF MAP2 VARIANTS IN MICROTUBULE ORGANIZATION AND STABILITY. W. Ferhat, L. Ferhat, A. Represa, D. Zouaoui-Aggoun, G. Charton, Y. Ben-Ari and M. Khrestchatisky. Sponsored by ENA . Université René Descartes, Paris V, INSERM U-29, 123 Bd de Port Royal, 75014 Paris, France.

Basic fibroblast growth factor (bFGF) is expressed in the hippocampus and has been demonstrated to stimulate differentiation of hippocampal neurons *in vitro*. In order to study the effects of bFGF on cytoskeletal organization in hippocampal neurons we used antibodies directed against MAP2, Tubulin and Actin. We show that bFGF altered the organization of microtubules but not microfilaments: the microtubules of neurons treated with bFGF are more fasciculated compared to controls. We also show that the cytoskeleton of neurons exposed to bFGF is more stable in the presence of destabilizing agents such as nocodazole. We have characterized the effects of bFGF on the expression of mRNAs encoding various cytoskeletal proteins using RT-PCR in cultured hippocampal neurons. bFGF does not modify the expression of mRNAs encoding α–Tubulin, β-Tubulin, α–Spectrin and Tau. However, bFGF produced a significant decrease of the expression of MAP2b and MAP2c mRNAs associated with a marked increase of NFM and MAP2d mRNA levels. Combining *in situ* hybridization with a MAP2d probe and MAP2 immunocytochemistry we show that MAP2d mRNA induction is selectively localized in neurons. Our data, associated with transfection experiments in non neuronal cells (Ferhat et al., J. Cell Science, in Press, 1996) suggests that MAP2d could be involved in stabilizing differenciated neurites. L.F is a recipient of a fellowship from AFM (Association Française Contre les Myopathies).

223.13

CYTOKINE-INDUCED SELECTIVE INCREASE OF HIGH MOLECULAR WEIGHT-bFGF ISOFORMS AND THEIR SUBCELLULAR KINETICS IN CULTURED RAT HIPPOCAMPAL ASTROCYTES. H. Kamiguchi*, K. Yoshida, K. Shimazaki, M. Inaba, H. Sasaki, M. Otani and S. Toya. Department of Neurosurgery, School of Medicine, Keio University, Tokyo 160, Japan.

The biological behavior of basic fibroblast growth factor (bFGF)-expressing cells, that is tissue regeneration or tumorigenic activity, seems to depend on the pattern of expression of bFGF isoforms. Previous studies have suggested that the increase of high molecular weight (HMW)-bFGF isoforms favors tissue regeneration, and that the over-expression of 18-kD isoform has tumorigenic potential. Cytokines such as interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α) and epidermal growth factor (EGF), which are considered to be supplied to the brain following injury, are probable factors responsible for up-regulation of bFGF expression in reactive astrocytes in the case of brain damage, however the effect of these cytokines on the expression of each bFGF isoform has not been elucidated. Western blot analysis revealed the expression of 18, 22 and 24-kD bFGF isoforms in cultured rat hippocampal astrocytes, and the expression of HMW-isoforms (22 and 24-kD isoforms) but not of 18-kD isoform was selectively increased by cytokines. Immunofluorescent analysis demonstrated that bFGF content in the cytoplasm of astrocytes is initially increased by cytokines followed by nuclear targeting and localization in agreement with the previous evidence that HMW-isoforms possess a nuclear targeting signal. Cytokine-induced selective increase of HMW-bFGF isoforms in astrocytes elucidated in the present study may be an important process of neuroprotection. Supported by grants from Keio University and The Ministry of Education, Science and Culture (No. 05557066, 05671183, 07771118).

223.14

REGULATION OF THE GENE EXPRESSION OF NEUROTROPHIC FACTORS AND RECEPTORS BY RETINOIC ACID IN LEUKEMIA CELL LINES. P. Xie*, W. M. Cheung, F. C. F. Ip, N. Y. Ip and M. F. Leung. Dept. of Biology, Hong Kong Univ. of Science & Technology, Hong Kong.

It has recently been shown that retinoic acid induced the expression of neurotrophin receptors in neuroblastoma and teratocarcinoma cells *in vitro*. To explore the potential involvement of neurotrophic factors in the actions of retinoic acid on leukemia differentiation, we examined the ability of retinoic acid to regulate the gene expression of various neurotrophic factors and their receptors in leukemia cells. We found that the expression of *trk* A, the high affinity receptor for nerve growth factor (NGF), was induced in K562 and KG-1 cells after exposure to retinoic acid. The expression of gp130, the shared receptor component of several cytokines, was moderately induced in K562 and HL-60 cells by retinoic acid treatment, and was dramatically induced in HL-60/S4 cells (a retinoic acid-supersensitive cell line derived from HL-60). In addition, the mRNA expression for NGF, neurotrophin-3 and neurotrophin-4/5 could be detected in some of the leukemia cell lines, but the expression of these neurotrophins was not affected by retionic acid treatment. In contrast, the profile of the changes in the expression of neurotrophic factors and their receptors induced by retinoic acid in neuroblastoma IMR-32 cells was different from that observed in leukemia cells. Taken together, our observations suggested that in addition to their pivotal roles in neuronal development, neurotrophic factors may also be involved in hematopoietic differentiation.

223.15

CYTOKINES STIMULATE NERVE GROWTH FACTOR SYNTHESIS IN REACTIVE ASTROCYTES, BUT NOT IN NORMAL ADULT ASTROCYTES. V. W. Wu and J. P. Schwartz*. Clinical Neuroscience Branch, NINDS, NIH, Bethesda, MD 20892

Cultured astrocytes synthesize a series of neuropeptides, transmitters, cytokines and growth factors. Injury to the brain transforms resting astrocytes to a reactive form and many of these same factors have been detected in reactive astrocytes in injured brain. In this study, we investigated whether cytokines can stimulate nerve growth factor (NGF) synthesis in reactive astrocytes. Reactive astrocytes cultured from 6-hydroxydopamine (6-OHDA)-lesioned rat brain display more immunohistochemical reactivity for GFAP than adult astrocytes from control brain. The NGF content of astrocytes cultured from control, saline-injected, and 6-OHDA-injected rat brain was measured by two-site ELISA. 6-OHDA lesion resulted in an increase of both NGF mRNA and protein in reactive astrocytes from both the ipsi- and contralateral side of striatum or cortex, with a larger increase on the ipsilateral side. Cytokines (IL-1ß, TGF-ß, IFN-γ) increased the content of NGF a further 3-4 fold in reactive astrocytes, but there was only a minimal effect in striatal and cortical astrocytes from control adult brain. This result suggests that the receptors for these factors may be turned on in astrocytes following injury, supporting the concept that cytokines play an important role during the process of brain injury.

223.16

MECHANISM OF VASOACTIVE INTESTINAL PEPTIDE-STIMULATED EMBRYONIC GROWTH: IGF-1, CYCLINS AND *FOS*. S. J. Servoss[1], G. W. Glazner, S. J. Lee, G. Gibney*, J. Y. Wu, D. E. Brenneman, and J. M. Hill, Lab. of Dev. Neurobiol., NICHD, NIH, Bethesda, MD 20892, [1]HHMI-NIH Research Scholar.

VIP treatment of whole mouse embryos in culture during neural tube closure and early neurogenesis results in dramatic growth with an increased rate of cell division through shortening of G1 and S phases of the cell cycle (Soc. Neuro. Abs. 21: 1546, 1995). These growth-regulating effects have been shown to occur indirectly through VIP-induced release of diffusible agents from the CNS, including activity dependent neurotrophic factor (ADNF). The influence of VIP treatment on gene expression of *fos* and the cell cycle regulators, cyclins D, E, A and B, was assessed in cultured E9.5 embryos, as was the potential role of IGF-1, an important neurotrophic factor and regulator of embryonic development, in VIP-stimulated growth. Competitive rt-PCR, using RNA extracted from cultured embryos, revealed a VIP-induced increase (400%) in *fos* message. Cyclin D and cyclin E mRNAs were increased with VIP treatment (198% and 268% of control, respectively), consistent with VIP-induced shortening of G1. A 232% increase in cyclin A message correlated with the shortening of S phase. Cyclin B transcript was elevated 258%, although the duration of G2 has been shown to be unaffected by VIP. Somite growth, size, and DNA and protein content were measured in embryos treated with IGF-1, anti-IGF-1, and combinations of VIP, ADNF, anti-ADNF, IGF-1 and anti-IGF-1. IGF-1 induced a significant dose-dependent increase in embryonic growth; suppression of endogenous IGF-1 by anti-IGF-1 inhibited growth. Both VIP- and ADNF-stimulated growth were blocked by anti-IGF-1, while anti-ADNF had no detectable effect on IGF-1-induced growth. These data suggest that both increased embryonic growth and shortening of the cell cycle duration associated with VIP treatment may be the result of transcriptional activation of multiple cyclins and *fos*. Furthermore, IGF-1 appears to act downstream from both VIP and ADNF in the regulatory pathway and mediates, at least in part, VIP-stimulation of embryonic growth.

223.17

DIFFERING EFFECTS OF GLUCOSE ON THE EXPRESSION OF CILIARY NEUROTROPHIC FACTOR AND ITS RECEPTOR.

D. E. Misek*, A. M. Hanash and X.-Q. Shu. Department of Internal Medicine, University of Michigan Medical School, Ann Arbor, Michigan.

Peripheral diabetic neuropathy is a significant and severe complication of diabetes mellitus, involving a multifactorial pathogenesis of functional, morphological and metabolic changes in the affected nerves resulting from hyperglycemia. Ciliary neurotrophic factor (CNTF) and its receptor have been shown to be present in the peripheral nervous system. Moreover, CNTF has been shown to be synthesized in Schwann cells and its function is thought to include the support of normal or injured sympathetic, sensory and motor neurons in the peripheral nervous system. Therefore, we examined the effects of glucose on the expression of CNTF mRNA, using the rat JS1 Schwannoma cell line as a model of Schwann cells, and on CNTF receptor mRNA, using human SH-SY5Y neuroblastoma cells as a model of peripheral cholinergic neurons. A 600 bp rat CNTF probe was generated by BamHI digestion of pCNGEX (a generous gift of Dr. Peter Richardson). This probe hybridized to a 1.2 kb mRNA. Northern blot analysis of total RNA extracted from rat JS1 Schwannoma cells, cultured in the presence of 5 to 50 mM glucose, revealed a maximal dose-dependent decrease of CNTF mRNA of 80% observed in 20 mM glucose after 5 days. However, this decrease in CNTF mRNA was rapid, reaching a 60% decrease within 8 hours after exposure to 20 mM glucose. An 800 bp probe (bases 699-1502) encoding CNTFR was generated by RT-PCR using oligonucleotide primers specific for the human CNTFR. This probe exhibited 100% homology to the human CNTFR, and hybridized to a single 2 kb mRNA. Northern blot analysis of total RNA extracted from SH-SY5Y neuroblastoma cells, cultured in the presence of 5 to 50 mM glucose, revealed a dose-dependent increase of CNTFR mRNA, with a 2.5 fold increase observed in 50 mM glucose after 5 days. However, this increase was rapid, reaching a 2-fold increase in 1 day. These data suggest that expression of both CNTF and its receptor may be altered by hyperglycemia, and may contribute to the functional and morphologic perturbations observed in diabetic neuropathy. Research supported by institutional funding.

223.18

PROTEASES REGULATE NERVE GROWTH FACTOR SECRETION FROM CULTURED VASCULAR SMOOTH MUSCLE CELLS. T.B. Sherer, J.M. Spitsbergen, and J.B. Tuttle*. Departments of Neuroscience and Urology, University of Virginia, Charlottesville, Va 22908

Nerve Growth factor (NGF) production in peripheral organs may play a role in the pathophysiology of hypertension, diabetes and cardiovascular, bowel or bladder obstructive disorders. We have been examining the cellular processes of NGF delivery and secretion in smooth muscle. NGF secretion was assayed via two-site ELISA of culture media. Inhibitors of extracellular proteases (aprotinin 0.7 to 7 TIU/ml) reduce NGF secretion from cells of vascular (VSMCs) and bladder origin. The mechanism of inhibition of NGF secretion was tested in VSMCs cultured from hyperactive (WKHA) and hypertensive (WKHT) inbred rat strains. Thrombin (3 μg/ml), a serine protease, increased NGF secretion rate over control by 218 ± 30% in the initial 4 hours and the effect was maintained throughout a 24 hour test period. Thrombin-induced increases in NGF secretion were prevented by actinomycin D and cycloheximide, suggesting RNA transcription and protein synthesis are required. Trypsin and collagenase also increased NGF secretion. Aprotinin blocked thrombin-induced increases in NGF as well as lowered basal secretion rates to between 47 ± 2.2 % and 93 ± 4.5 % of control during a 24 hour period. Aprotinin also reduced the stimulation caused by IL-1β. These results suggest that extracellular protease activity may regulate NGF secretion in smooth muscle. Proteases affect basal NGF production and interact with other endogenous regulators and may be involved in neuron-target dynamics. (Supported by a Research Grant from NIH and a School of Medicine Dean's Fellowship.)

223.19

1,25-DIHYDROXYVITAMIN D_3 REGULATES THE EXPRESSION OF $P75^{NGFR}$ AND GDNF.

P. Naveilhan[1,2], C. Baudet[1], D.Wion[1], M. Metsis[2], P. Brachet[1] and I. Neveu[2,*]. [1]INSERM U298, CHR d'Angers, 49033 Angers, France; [2]Laboratory of Molecular Neurobiology, Karolinska Institute, S-17177 Stockholm, Sweden.

Altered expression of neurotrophic factors and/or their receptors may contribute to some disorders in the nervous system. In order to characterize the factors that control their expression, we have analysed the effect of 1,25-$(OH)_2$ D_3 on the mRNA levels of neurotrophin receptors and glial cell line-derived neurotrophic factor (GDNF). RNAse protection assay did not show any significant changes in the levels of *trk* receptor mRNAs, but a marked increase in the expression of low-affinity neurotrophin receptor ($P75^{NTR}$) was observed in C6 glioma cells and not in primary astrocytes. Since long-term treatment of 1,25-$(OH)_2D_3$ induces cell death in C6 glioma cells, our findings support a possible role of $P75^{NTR}$ in 1,25-$(OH)_2D_3$-induced cell death. *In vivo* studies also indicate that 1,25-$(OH)_2D_3$ may contribute to the tissue-specific regulation of $P75^{NTR}$ since treatment of rats with 1,25-$(OH)_2D_3$ decreases $P75^{NTR}$ mRNA level in spinal cord but not in dorsal root ganglion or sciatic nerve. Our results also show that 1,25-$(OH)_2D_3$ is a strong inducer of GDNF. Treatment of C6 cells with 10^{-7} M 1,25-$(OH)_2$ D_3 for 48 h elicited an 18.5-fold increase in the level of GDNF mRNA and cotreatment with 1,25-$(OH)_2D_3$ and retinoic acid had additive effects. These data which constitute the first evidence of hormonal control of GDNF expression, suggest that 1,25-$(OH)_2D_3$ may participate in the regulation of GDNF *in vivo*.

These findings are consistent with a potential role of 1,25-$(OH)_2D_3$ in the regulation of neurotrophic factors and their receptors.

223.20

NICOTINE INTERACTS WITH ESTROGEN IN BRAIN IN REGARDS TO INSULIN-LIKE GROWTH FACTOR I mRNA EXPRESSION. S. Kito*, A. S.Shingo, J.Semba, R. Miyoshi[1], E. Shimizu, C. Kondo. The Univ. of the Air,Chiba, Japan 261, [1]Div. of Basic Med. Sciences, Royal Free Hospital Sch. of Med., London.

Recently it has been noticed that estrogen plays a role in memory and learning, while nicotine activates memory function at a short term level. In this paper we studied the cross talk between estrogen and nicotine from viewpoints of insulin-like growth factor I (IGF-I) mRNA induction in the hippocampus and cerebral cortex. Perfusion of 10^{-5} M nicotine caused an elevation of intracellular Ca^{2+} concentration in rat cultured fetal hippocampal neurons. It was also observed that subcutaneous injection of 1mg/kg nicotine induced c-fos expression in the rat hippocampus. Furthermore, a single injection of 1mg/kg nicotine induced IGF-I mRNA expression, though less than that observed by single injection of 500 μg/kg estradiol. The effects of coinjection of nicotine and estradiol were not additive and the IGF-I mRNA expression was intermediate between single injections of nicotine and estradiol. This means that a single injection of nicotine causes IGF-I mRNA expression, while nicotine inhibits estradiol-induced IGF-I mRNA expression. This mechanism was analyzed from viewpoints of AP-1 binding activity.

This study was partly supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture, Japan.

223.21

DIFFERENTIAL EXPRESSION OF FGF-2 ISOFORMS: REGULATION BY EXTRINSIC MOLECULES. C. Meisinger and C. Grothe*. Institute of Anatomy, University of Freiburg, D-79104 Freiburg

We had previously shown that the synthetic glucocorticoid dexamethasone (Dex) which is known to stabilize the endocrine phenotype of chromaffin cells induces an up-regulation of the FGF-2 mRNA level in PC12 cells and in the rat adrenal medulla *in vivo* (Meisinger et al., J. Biol. Chem., in press). Using quantitative Western blot analysis we studied the Dex effect on FGF-2 at the protein level.

Western blot analysis of PC12 cells and adrenal medulla using a monoclonal FGF-2 antibody revealed immunoreactive bands at 18, 21, and 22.5kD. After Dex treatment the FGF-2 protein level was significantly increased both *in vitro* and *in vivo*. This increase was confined to the 21kD FGF-2 isoform. Dex-effects on FGF-2 expression were not found in non-neuronal tissues, e.g. in adrenal cortex, skeletal muscle, and L6 myoblasts.

Neurite outgrowth of PC12 cells can be induced by K^+-depolarization. Treatment of PC12 cells with KCl revealed a significant elevation of the FGF-2 transcript level. The KCl-induced increase of the FGF-2 protein level was confined to the 22.5kD isoform.

These results suggest that the differential functions of FGF-2 could be related to the expression of different FGF-2 isoforms. Studies using PC12 cells which specifically over-express the low or high molecular weight forms are in progress to examine this hypothesis. (Supported by DFG Gr 857/8-2)

223.22

EXPRESSION OF TGF-β1, TGF-β3, AND IL-6 IN RAT PINEAL TISSUE: IN VIVO AND IN VITRO STUDIES. S-Y Tsai*, K. S. Schluns, P. T. Le, and J. A. McNulty Department of Cell Biology, Neurobiology and Anatomy, Loyola University Medical Center, Maywood, IL 60153

Recent studies have demonstrated that pineal microglia alter cultured pinealocyte differentiation. Although the mechanisms responsible for microglial mediation of pinealocyte differentiation are not known, a role for cytokines is postulated. Therefore we determined to study whether: 1) specific cytokines are expressed during development of the pineal gland; and 2) cytokines gene expression is modualted by norepinephrine (NE). The pineal glands were removed from Sprague-Dawley rats of different ages (postnatal day 1, 5, 10, 21, and 3-4 months) 4 hours after lights on (12:12 L:D cycle). The following groups of pineal glands were tested: 1) untreated controls frozen immediately on dry ice; 2) glands placed in organ culture for 3 h and treated with vehicle (10^{-5}M ascorbic acid) for 4 h; 3) glands placed in organ culture for 3 h and treated with norepinephrine (10^{-5}M NE) for 4 h. In a second experiment, dispersed pineal cells from 1-day-old animals were cultured for 7 days prior to NE (10^{-5}M) or vehicle (10^{-5}M ascorbic acid) treatment for 4 h. Total RNA was isolated from all tissues and analyzed for expression of IL-6 (rat cDNA, gift from Dr. Jack Gauldie), TGF-β1 and TGF-β3 (mouse cDNA) by RNA blot analysis. TGF-β1 and TGF-β3 mRNA were constitutively expressed in the pineal gland at all ages and in dispersed pineal cell cultures. IL-6 mRNA were expressed at day 21 and adult. NE stimulation enhanced TGF-β1 and IL-6 expression only in adult pineal glands. NE had no detectable effects on TGF-β3 expression in any of the groups studied. Developmental changes of TGF-β1, TGF-β3 and IL-6 genes expression suggests that these cytokines may play roles in pineal gland differentiation under sympathetic neural control. Studies are in progress to determine if microglia are the source of these cytokines.

223.23

CHARACTERIZATION OF TROPHIC FACTORS AND GENES WHICH EXPRESSED IN THE DOPAMINE-DEPLETED STRIATUM. I. Fujimoto*, K. Nakajima, Y. Shimano, H. Hida, A. Fukuda and H. Nishino. Dept. of Physiol., Nagoya City Univ. Med. Sch., Nagoya 467, Japan.

We have previously reported that the extract from dopamine (DA)-depleted striatal tissue (lesioned extract) had a trophic activity on cultured pheochromocytoma (PC12D) cells and fetal DAergic neurons. The action of the extract on promotion of neurite outgrowth of PC12D cells and fetal DAergic neurons were a concentration-dependent manner. To characterize the trophic activity, the factors were fractionated from lesioned extract using heparin column affinity chromatography and Sephadex G-100 gel filtration chromatography. The active fractions were further separated by ion-exchange HPLC. In other hand, differential display technique was applied to identify novel genes encording factors associated with trophic activity. Total RNAs from DA-depleted striatum and from the counter part of hemi-Parkinsonian model rats were amplified by RT-PCR using various sets of random primers. The amount of the amplified products were displayed on sequence gel, and compared. Some fragments expressed in lesioned side more than intact side, such as alpha-actinin, creatine kinase, human clone HFBEF54, etc. Characteristics of these genes in terms of trophic actions on DAergic neurons are discussed.

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223.24

MODULATION OF HUMAN NERVE GROWTH FACTOR PROMOTER ACTIVITY BY INTERLEUKIN-1β AND INTERFERON-γ. P.A. Baecker, R.O. Javid, A.N. Verity, C.J. Emmett, D.C. Solymar, R.M. Eglen and R.M. Johnson*. Dept. of Neurobiology, Inst. of Pharmacology, Neurobiology Unit, Roche Bioscience, Palo Alto, CA 94304.

We have cloned and sequenced the human nerve growth factor (hNGF) promoter. Consensus binding sites were found for transcription factors NF-κB, STAT1, EGF1 and C/EBP, in addition to the previously described sites for AP1 and SP1 (Cartwright, M. *et al* (1992). Mol. Brain Res. 15, 67-75). The NF-κB and STAT1 consensus binding sites were found to be in a novel, overlapping arrangement. NF-κB was activated by interleukin-1β (IL-1β) and the novel cognition enhancing drug, RS-66252, in the human glioblastoma cell line, T98G. Both IL-1β and RS-66252 elevated hNGF mRNA levels and enhanced secretion of hNGF from T98G cells. Interferon-γ (IFN-γ) activated STAT1 and inhibited hNGF production at the transcriptional level. Electrophoretic gel mobility shift assays employing an oligonucleotide containing the overlapping NF-κB and STAT1 sites, revealed NF-κB or STAT1 binding. A time course of hNGF promoter activity in response to 0.5 ng/ml IL-1β, showed maximal stimulation of ~65% over vehicle alone after 6 hrs of treatment. IFN-γ (500 U/ml) inhibited hNGF promoter activity in a biphasic time course, with an initial 15% decrease within one hour, followed by a further 20% decrease 6 hrs after treatment. Furthermore, IFN-γ was shown to partially reverse IL-1β stimulated hNGF transcription. The data suggests that IL-1β stimulates hNGF transcription through activation of NF-κB, while IFN-γ inhibits transcription through activation of STAT1. The close proximity of the NF-κB and STAT1 binding sites suggest mutually exclusive binding of these two transcription factors and reciprocal regulation of hNGF gene transcription.

NEUROTROPHIC FACTORS: RECEPTORS AND CELLULAR MECHANISMS I

224.1

LEVELS OF ^{125}I-IGF-I, ^{125}I-IGF-II AND ^{125}I-INSULIN RECEPTOR BINDING SITES IN THE HIPPOCAMPUS OF AGED MEMORY-IMPAIRED AND MEMORY-UNIMPAIRED RATS. R. Quirion, S. Doré, S. Kar, W. Rowe and J.-G. Chabot*. Douglas Hosp. Res. Ctr, McGill University, Montréal, Québec, Canada, H4H 1R3.

The insulin-like growth factors and insulin are localized in distinct brain regions and their respective functions are mediated by specific membrane receptors. High densities of binding sites for these growth factors are discretely distributed throughout the brain, including the hippocampal formation. IGFs and insulin, in addition to their growth promoting actions, are considered to play important roles in normal cell functions. We compared the anatomical distribution and levels of IGF receptors in young (6 month) and aged (24-25 month) memory-impaired (AI) and memory-unimpaired (AU) male Long Evans rats as determined in a Morris water maze task. Apparent levels of ^{125}I-IGF-I, ^{125}I-IGF-II and ^{125}I-insulin binding sites were evaluated in selective brain regions of young (n=7), AI (n=7) and AU (n=6) rats using quantitative receptor autoradiography. In the hippocampus, ^{125}I-IGF-I binding sites are concentrated primarily in the dentate gyrus (DG) and the CA_1-CA_3 sub-fields while high densities of ^{125}I-IGF-II binding sites are localized to the pyramidal cell layer, and the granular cell layer of the DG. ^{125}I-insulin binding sites are mostly found in the molecular layer of the DG and the CA_1 sub-field. No significant differences were found in ^{125}I-IGF-I, ^{125}I-IGF-II or ^{125}I-insulin receptor levels in any region of the hippocampus of young and aged animals. Furthermore, deficits in cognitive performance did not relate to altered level of these receptors in AI compared to AU rats. Other regions, including various cortical areas, were also examined and failed to reveal significant differences. It was very recently reported that the amount of IGF-I receptor mRNA was increased with age and positively correlated with learning deficits in AI rats (Stenvers *et al.*, Neuroscience 72:505,1996). However, IGF-I receptor protein levels are apparently not affected by the increases in mRNA levels. It thus appears that IGF-I, IGF-II and insulin receptor binding sites are not markedly altered during the normal aging process and their contribution to cognitive deficits in the aged rat is likely limited. Supported by the MRCC and The Alzheimer Society of Canada.

224.2

HABREC I, A NOVEL SERINE-THREONINE KINASE RECEPTOR WITH SPECIFIC CNS EXPRESSION PATTERNS. M. Lorentzon, L. Olson*, T. Ebendal§, A. Tomac. Dept. of Neuroscience, Karolinska Institute, S-171 77 Stockholm, Sweden; §Dept. of Developmental Neuroscience, Uppsala University, S-751 23 Uppsala, Sweden.

Known receptors for members of the transforming growth factor superfamily are of three types. Type I and type II are signaling receptors with intrinsic serine-threonine kinase activity. In order to find new members of this receptor family expressed in the CNS, a PCR-based cloning strategy was applied. Using cDNA from an adult rat habenula library and primers coding for highly specific regions of the receptor kinase domain, we found several type I and type II receptors. Partial cloning suggests that two of these receptors correspond to previously unidentified receptors, one type I (Habrec I) and one type II (Habrec II). In situ hybridization revealed a highly specific mRNA expression pattern of Habrec I in the rat CNS. mRNA expression was detected at embryonic day 19, persisted in the adult, where it was found e.g. in striatum, cortex, and hippocampus. Habrec I mRNA expression appeared predominantly neuronal. Treatment with kainic acid (12 mg/kg i.p.) led to a dramatic upregulation of Habrec I mRNA expression in gyrus dentatus 6 hr post injection. This upregulation was not diminished in rats coinjected with MK801 (2 mg/kg), suggesting that the upregulation was not NMDA receptor-mediated. Stereotaxic injections of 6-OHDA (8 μg/4 μl/4 min) were used to unilaterally lesion the dopaminergic nigrostriatal pathway and rats in which apomorphine induced more than 300 contralateral turns/hr were chosen. In situ hybridization did not reveal any change of the cellular expression of Habrec I mRNA in the dopamine-denervated striatum 1 day, 3 days, 1 week or 1 year after lesion.

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224.3

IMMUNOHISTOCHEMICAL LOCALIZATION OF INSULIN-LIKE GROWTH FACTOR II RECEPTORS IN MOUSE BRAIN. Y. Konishi[*1], S. Fushimi[1], D.-H. Chui[2], K. Takahashi[2], T. Tabira[2] and T. Shirabe[1]. [1]Department of Neuropathology, Kawasaki Medical School, Kurashiki 701-01, Japan. [2]National Institute of Neuroscience, NCNP, Tokyo 187, Japan.

We previously reported that insulin-like growth factor II (IGFII) is one of the neurotrophic factors for central cholinergic neurons in mice (Brain Res. 649:53, 1994). Although at the report we showed IGFII receptor immunoreactivity in primary neurons including cholinergic neurons cultured from the septal regions of embryonic mouse brain, we demonstrate here the precise localization of IGFII receptor immunoreactivity in adult mouse brain to clarify the target cells of IGFII. The immunohistochemical study was performed with indirect immunoperoxidase technique by using a rabbit anti-IGFII/mannose-6-phosphate (M6P) receptor antibody (provided by Dr. M. Himeno, Kyushu University, Fukuoka, Japan).

This antibody labeled cytoplasm of the cell bodies in many neurons throughout the brain, but did not that in astrocytes. The result was consistent with the autoradiographic localization reported by Lesniak et al. (Endocrinol. 123:2089, 1988). The localization of IGFII receptors was also examined with immunoelectron microscopy. Furthermore, to obtain the circumstantial evidence that IGFII acts on cholinergic neurons via its receptors to increase choline acetyltransferase (ChAT) activity, we performed double immunofluorescence staining with the anti- IGFII/M6P receptor antibody and anti-ChAT antibodies provided by Drs. B. Wainer, Emory University, Atlanta, GA and T. Ichikawa, Tokyo Metropolitan Institute of Neuroscience, Tokyo, Japan. The colocalization was imaged by laser scanning confocal microscopy. (Supported partly by the Funds from Kawasaki Medical School Research Project in Japan.)

224.4

PHARMACOLOGICAL CHARACTERIZATION OF THROMBIN (PAR-1) RECEPTORS IN RAT ASTROCYTES X. VIGÉ, Th. DEBEIR AND J. BENAVIDES* Synthélabo Recherche, CNS Research Department, BP 110, 92225 Bagneux Cédex, France.

The proteolytic action of thrombin on its receptor (Protease activated receptor-1 or PAR-1) results in a conformational change in which the new N-terminal sequence self-activates the receptor. Peptide analogs of this N-terminal sequence (TRAPs) are able to mimic the effect of thrombin and an extensive search has led to the definition of the structural requirement for the activation of thrombin receptors in several peripheral systems (platelets, endothelial cells). These studies have demonstrated the existence of species and cell type differences in the pharmacology of PAR-1. We have now characterized pharmacologically thrombin receptors in cultured rat astrocytes by using [^{3}H]-thymidine incorporation as an end-point.

Thrombin increases [^{3}H]-thymidine incorporation into DNA with an EC_{50} of 1 nM (+ 550 % at 100 nM thrombin). This effect is mimicked by TRAP-14 (EC_{50} = 3 µM, + 500 % at 100 µM) and a peptide containing non natural amino acids (H-Ala-Phe(p-F)-Arg-Cha-HArg-Tyr-NH_2), (EC_{50} = 0.8 µM, + 300 % at 30 µM). The effect of TRAP-14 (10 µM) on [^{3}H]-thymidine incorporation into DNA was significantly prevented (EC_{50} = 150 µM, Ki = 34 µM) by a peptide (LVR(D-cys)GKHSR) previously described as an antagonist in human platelet aggregation (IC_{50} = 30 µM). Finally, a peptide corresponding to the N-terminal sequence of trypsin-activated PAR-2 (SLIGRL) did not affect DNA synthesis up to a concentration of 100 µM.

These results demonstrate that rat astrocytes express PAR-1 receptors which are pharmacologically similar to those previously characterized in human platelets.

224.5

ACTIVATION OF THROMBIN RECEPTORS INFLUENCES THE MORPHOLOGICAL DIFFERENTIATION OF DOPAMINERGIC NEURONS IN VITRO. Th. DEBEIR, J. BENAVIDES AND X. VIGE* Synthélabo Recherche, CNS Research Department, BP 110, 92225 Bagneux Cédex, France.

Evidence has been provided for the involvement of thrombin receptors in the development and differentiation of the Central and Peripheral Nervous Systems. In situ hybridization studies have revealed high levels of thrombin receptor mRNA in the mesencephalon, suggesting that dopaminergic neurons may be an important target for thrombin's actions. In this work we have evaluated the effects of thrombin receptor activation, either by thrombin or by TRAP-14 (a 14 amino-acid agonist of thrombin receptor), on the differentiation of cultured rat dopaminergic neurons. Pure cultures of embryonic mesencephalic neurons were treated with different concentrations of thrombin or TRAP-14 the day after plating and tyrosine hydroxylase positive neurons (TH$^+$) were studied the 5 th day of culture. TH$^+$ cell counting, dopamine uptake and morphometric analysis were also performed. Neurotrophin 4, already demonstrated active in such a model, was chosen as a reference compound. NT4 (10 ng/ml) increased the number of TH$^+$ cells and dopamine uptake by +250% and +145 %, respectively, whereas thrombin (0.1 to 10 nM) and TRAP-14 (0.1 to 100 µM) did not affect significantly these two parameters. The results of morphometric studies are summarized in the table.

Treatment * p < 0.05 ** p < 0.01	Neurite lengths (% of control)		Segment lengths (% of control)	
	Primary	Secondary	Proximal	Distal
NT4 (10 ng/ml)	+ 57**	+ 66*	+ 140**	+ 16**
Thrombin : 10 nM	+ 13	- 42**	+ 38**	- 58**
TRAP-14 : 1 µM	+ 33**	- 45**	+ 41**	- 31**

These data demonstrate that thrombin receptor activation initiates a complex remodelling of synaptic organisation in central nervous system. Indeed, both thrombin and TRAP-14, favor the growth of primary neurites increasing proximal segments but reducing, in contrast to NT4, the length of distal elements which represent secondary neurites and primary endings.

224.6

C-RET mRNA IS STRONGLY EXPRESSED IN DOPAMINE NEURONS OF SUBSTANTIA NIGRA AND THE VENTRAL TEGMENTAL AREA IN ADULT RODENTS. **A. Tomac, B. Hoffer*, L. Olson. Dept. of Neuroscience, Karolinska Institute, S-171 77 Stockholm, Sweden and Dept. of Pharmacology, Univ. of Colorado Health Sciences Center, Denver, CO 80262.**

The receptor tyrosine kinase encoded by the c-ret proto-oncogene is expressed in developing nervous and excretory tissues, suggesting a functional role in several ganglia, the enteric nervous system, the central nervous system and the kidney during development (Pachnis et al., Development 119:1005, 1993). C-ret has been implicated in multiple endocrine neoplasias and Hirschprung's disease. Null mutations lead to absence of enteric neruons and renal agenesis (Schuchardt et al., Nature 667:318, 1994). The c-ret expression pattern and knockout effects parallel the distribution of GNF mRNA (Lindqvist et al., Soc. Neurosci. Abstr. 20:1101, 1994; Hellmich et al., Mech. Dev. 54:95, 1996), and effects of GDNF on ganglia (Ebendal et al., J. Neurosci. Res. 40:276, 1995). Here, we have used in situ hybridization to study the cellular expression of c-ret mRNA in further detail. We find a very high c-ret mRNA expression in the dopamine neurons of substantia nigra and the ventral tegmental area of adult mice and rats. In particular, neurons in pars compacta and substantia nigra express very strong signals. We have previously demonstrated potent actions of GDNF on the adult mouse dopamine neurons (Tomac et al., Nature 373:335, 1995). The present results, therefore, are compatible with the view that c-ret might act as a GDNF receptor in the adult nigrostriatal dopamine system. Further mapping of c-ret at the mRNA and protein levels is in progress.

Supported by the Swedish MRC and USPHS.

224.7

ACTIVIN TYPE IIA RECEPTOR mRNA EXPRESSION BY NEURONS OF THE AVIAN DORSAL ROOT GANGLION. K. Kos, and J.N. Coulombe * Dept. of Anatomy and Cell Biology, Uniformed Services University of the Health Sciences, Bethesda, MD 20814

Previous studies have suggested that activin serves as a target-derived neurodifferentiation factor regulating the expression of somatostatin in neurons of the avian ciliary ganglion (CG). Activin signalling in CG neurons appears to be transduced by the activin receptor type IIA (ActR type IIA) since these neurons express mRNA for the ActR type IIA, but not the activin receptor type IIB.

We examined whether dorsal root ganglion (DRG) neurons also express mRNA for the ActR type IIA. Oligonucleotide primers designed to amplify the chicken ActR type IIA were used in reverse transcription polymerase chain reactions (rtPCR). Total RNA was isolated from embryonic day 16 (E16) and post-hatch day 1 (P1) chicken lumbar DRG. The RNA was reverse transcribed and cDNA used as a template in rtPCR. ActR type IIA specific primers amplified a PCR product of the appropriate size for the type IIA receptor from both E16 and P1 cDNAs. It thus appears that mRNA for the ActR type IIA is expressed in the embryonic and post-hatch chicken DRG.

To examine what cell types within the DRG contain mRNA for this activin receptor, digoxigenin-labeled riboprobes from a cloned ActR type IIA fragment were used for in situ hybridization on cryostat sections of embryonic chicken DRG. Antisense strand, but not sense strand, ActR type IIA specific riboprobes hybridized to cells within these sections. Hybridization of this probe appeared to be specific for cells with a neuronal morphology. Moreover all of the neurons within the DRG appeared to hybridize with the ActR type IIA probe. These results suggest that activin may play a role in differentiation of DRG neurons.

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224.8

THE TGF-ß TYPE II RECEPTOR IN THE CENTRAL NERVOUS SYSTEM. **M. Böttner, K. Unsicker* and C. Suter-Crazzolara. Dept. of Anat. & Cell Biol., Univ. Heidelberg, INF 307, D-69120 Heidelberg, Germany.**

Members of the TGF-ß superfamily signal through heteromeric receptor complexes which consist of type I and type II serine-threonine kinases. The recently cloned TGF-ß type II receptor is abundant in many peripheral tissues, but attempts to detect expression in the CNS have failed so far. However, the potential ligands (TGF-ß2 and -3) for this receptor are expressed in the CNS and affect neurons and glial cells. To solve this apparent discrepancy, we decided to investigate the possibility that a novel type II receptor specific for these growth factors is expressed in the brain. We performed degenerate lift-off-PCR from rat CNS. Primers were targeted against the amino acid sequence of type II specific serine-threonine kinase domains. The resulting PCR products were cloned and sequenced. Initial experiments with E16 rat brain reveal that the predominant PCR products are the activin type II receptors. This shows the feasibility of our approach and the specifity for the type II receptors. In addition, we were able to amplify the known TGF-ß type II receptor. Thus, TGF-ß may signal in the CNS through the same receptor as in peripheral tissues. Furthermore, we are generating more RT-PCR products (also with other primer combinations), which may allow purification of novel type II receptors which are different from the ones obtained so far. Supported by DFG.

224.9

Localization of GDNFRα, a Putative Receptor for GDNF Ligand. Mark Armanini*, James Treanor, Arnon Rosenthal, and Heidi Phillips. Dept. of Neuroscience, Genentech Inc., 460 Point San Bruno Blvd., SSF, CA.

A high affinity GDNF binding protein (**GDNFR**α) was purified by expression cloning. The high affinity binding to GDNF ligand suggests **GDNFR**α as a putative GDNF receptor in vivo (Treanor, et. al., Soc. Neuro. Abs. 1996). We performed in-situ hybridization for the mRNA to **GDNFR**α in the E15.5 rat embryo, as well as early postnatal and adult rat brain. Localization of expression at these developmental stages suggests cell populations requiring GDNF in development, differentiation, or maintenance. In the E15.5 embryo, mRNA for **GDNFR**α is widespread in the CNS and in peripheral tissues. Peripheral expression includes developing structures of the urogenital system, smooth and striated muscle, and dorsal root ganglia. In the E15.5 CNS, **GDNFR**α is expressed in structures known to be responsive to GDNF, including the ventral midbrain, and ventrolateral spinal cord. In the P1 and adult CNS, **GDNFR**α expression also includes GDNF-responsive structures such as the substantia nigra and spinal cord motoneurons. Other areas positively labeled in the adult nervous system include the dorsal root ganglia. The expression of **GDNFR**α within all known GDNF-responsive neuronal popualtions suggests **GDNFR**α may be a physiological receptor for GDNF.

224.10

Isolation and characterization of a receptor for Glial Cell Line-Derived Neurotrophic Factor

James Treanor!, **Klaus Beck**! , Laurie Goodman!, Christa Gray@, Mark Armanini!, Heidi Phillips!, Franz Hefti!*, Audrie Goddard@, Alun Davies#, Chris Henderson+ and Arnon Rosenthal! Department of !Neuroscience and @Molecular Biology, Genentech, Inc., 460 Point San Bruno Blvd., South San Francisco, CA 94080; #University of St. Andrews, Bute Medical Building, St. Andrews, U.K.; +INSERM U.382, IBDM, Campus de Luminy - Case 907, 13288 Marseille, Cedex 09

Glial cell line-derived neurotrophic factor (GDNF) is a potent survival factor for midbrain dopaminergic, spinal motor and noradrenergic neurons which degenerate in Parkinson's disease, amyotrophic lateral sclerosis and Alzheimer's disease respectively. Despite the physiological and clinical importance of GDNF, little is known about its mechanism of action. Here we describe the isolation of a protein (designated GDNFRα) that binds GDNF with a high affinity and is expressed on GDNF responsive neurons. GDNFRα, like the receptors for CNTF and endotoxin, is anchored to the cell membrane by a glycosyl-phosphatidyl inositol (GPI) linkage. Furthermore, primary neurons lose their response to GDNF (but not to other neurotrophins) following treatment with phosphoinositide-specific phospholipase C (PIPLC) which specifically cleaves GPI-linked proteins. Thus GDNFRα is an essential mediator of GDNF signaling and is likely to be a ligand binding component in a multi-subunit GDNF receptor.

224.11

SIGNAL-TRANSDUCING SUBUNIT GP130 OF AVIAN NEUROKINE RECEPTORS: CLONING AND FUNCTIONAL STUDIES IN SYMPATHETIC NEURONS. Geißen, M., Heller, S., Pennica, D.# and Rohrer, H*. Max-Planck-Institut für Hirnforschung, Deutschordenstr. 46, 60528 Frankfurt, Germany. #Genentech Inc., South San Francisco, California 94080, USA.

The neurokines ciliary neurotrophic factor (CNTF), growth promoting activity (GPA), cardiotrophin-1 (CT-1) and leukemia inhibitory factor (LIF) display survival and differentiation effects on a variety of developing neuron populations. These effects are mediated through receptor complexes that share a common signal transducing subunit, gp130. Although functional receptors for CNTF/GPA and LIF seem to be widely expressed in the developing and adult nervous system, the physiological role of these receptors remains unclear. Temporal and spatial specific elimination of these receptors in the living chick embryo would provide insights into their physiological role. To this end, the avian equivalent of gp130 was cloned (the full length cDNA shares about 55% identity to its mammalian homologues) and an antisense approach was developed to interfere with its function in neurons. Sympathetic neurons serve as a model system for target derived differentiation events. Upon treatment with neurokines, sympathetic neurons become cholinergic which is reflected by the induction of Choline acetyl transferase (ChAT) and the neuropeptide VIP.

To interfere with the function of gp130, primary cultures of sympathetic neurons from E7 chick embryos were transfected with antisense expression constructs. Two antisense vectors and the corresponding sense vectors were used. It was found that in neurons expressing gp130 antisense RNA the ability to respond to neurokines acting through the GPA receptor complex (GPA) or through the LIF receptor (CT-1) were both significantly reduced (by about 40%). This effect was quantified by immunostaining for the neuropeptide VIP.

These results confirm that gp130 function is required to mediate the biological action of GPA and other neurokines on primary neurons. By the use of retroviral vectors, this approach can now be used to investigate the role of gp130 during development of the nervous system.

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224.12

THE SOMATOSTATIN RECEPTOR SUBTYPES, AND THEIR SIGNAL TRANSDUCTION SYSTEMS, THAT ARE EXPRESSED IN CEREBELLAR GRANULE CELLS. D.J.Wilson, Y. Sugita* and J.P.Schwartz. Clinical Neuroscience Branch, NINDS, NIH, Bethesda MD 20892

Somatostatin (SST) has been shown to function as a trophic factor for cerebellar granule cells (CGCs), stimulating neurite outgrowth and differentiation. A previous report suggested that a SST receptor (SSTR) was present for only 2 days in vitro (DIV) on CGCs prepared from postnatal day 8 rats, and that this receptor inhibited adenylate cyclase (AC) (Gonzalez et al, PNAS 1992). Using primers designed to distinguish among the SSTR subtypes, we find that CGCs express mRNA for subtype 1 only on DIV1, but that mRNA for subtypes 2, 4, and 5 is present from DIV1 to DIV 10 in culture, with decreasing expression over this time course. Although SSTR have been primarily associated with inhibition of AC, there is one report of stimulation in brain (Markstein et al, Neurosci. Lett. 1989). In CGCs, both SST-14 and the analog [des-ala^{1},des-gly^{2}, his^{4,5}, D-trp^{8}]-SST stimulate AC over the time course from DIV1 to 12. These results suggest that the trophic effect of SST on CGCs may be mediated by a SSTR, possibly subtype 2,4,or 5, which is positively coupled to AC.

224.13

Cyclin B and p34^{CDC2} kinase in the striatum of MPTP-treated mice. V. Parekh, P. Rowell* and M. Gupta, Depts. of Pediatrics, Pharmacology and Anatomical Sci. & Neurobiology, U of L Medical School, Louisville, KY.

Cyclin B and CDC2 kinase are key regulators during the proliferative stage of the cell-cycle. Previous studies from this laboratory have shown that MPTP treatment in mice can damage the nigrostriatal dopaminergic system and leads to an increase in the number of GFAP immunoreactive astrocytes in the striatum. Furthermore, young adult mice treated with MPTP show a complete recovery within six months after treatment, unlike aging mice treated in a similar manner. In order to determine if p34^{CDC2} kinase and its subunit Cyclin B are altered following MPTP toxicity, young adult and aging mice were given a single i.p. injection of MPTP (30mg/kg body weight), and sacrificed one hr later. Fresh striata were quickly dissected and placed in kinase buffer. Cyclin B andp34^{CDC2} kinase was determined using immunoprecipitation and Histone as a substrate. The results show an increase in phosphorylation of Cyclin B activity in the young adult mice compared to vehicle controls. Aging mice treated with MPTP did not show an altered Cyclin B activity. These data suggest age-related changes in cell-cycle regulating cyclins.

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224.14

GANGLIOSIDES ALTER NEURONAL MORPHOLOGY BY MODULATING THE ASSOCIATION OF MAP2 WITH MICROTUBULES AND ACTIN MICROFILAMENTS L.-J. Wang, R. Colella and E.J. Roisen*. Anatomical Sciences & Neurobiology, University of Louisville Medical School, Louisville, KY 40292.

Gangliosides are glycosphingolipids that are relatively abundant in neurons and are found in the outer leaflet of the plasma membrane. They enhance neuritogenesis of primary neurons and established neuronal lines. Our previous studies demonstrated that exposure to the ganglioside GM1 increased the microtubule network and promoted neuritogenesis of Neuro-2a neuroblastoma. Furthermore, a redistribution of MAP2 immunoreactivity from the perikaryon to the distal processes occurred following 24 hr GM1 treatment. Immunoelectron microscopy demonstrated that MAP2 was closely associated with the subcortical cytoplasm: more MAP2 label per unit area was found in processes and spines after GM1 treatment. We examined the immunolocalization of MAP2, actin and tubulin with confocal and electron microscopy to determine if GM1 affects the distribution and expression of actin microfilaments in this region. GM1 exposure caused a redistribution of actin from a uniform, subsurface, circumferential layer to specific foci in the processes and spines. These changes paralleled the redistribution of MAP2 from perikaryal regions to spines after ganglioside exposure. Computer-assisted morphological analysis of actin labeled with colloidal gold revealed more label on the GM1 treated cells ($p<0.0001$), especially in the areas close to the spines. Studies examining the effect of GM1 on actin synthesis demonstrate a dose dependent increase in actin mRNA and protein levels. Our data suggest that gangliosides enhance neuritogenesis and determine dendritic or axonal fate by modulating cytoskeletal elements through a collaboration of actin and MAP2 dependent mechanisms.

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224.15

CNTFRα-IMMUNOREACTIVITY IN THE PRIMATE CNS. Yaping-Chu, A. John MacLennan[1], Conwell Anderson*, and Jeffrey H. Kordower Department of Neurological Sciences, Rush Presbyterian-Luke's Medical Center, Chicago, IL 60612 and [1]Department of Neuroscience, University of Florida Brain Institute, University of Florida College of Medicine, Gainesville Florida, 32610.

Ciliary neurotrophic factor (CNTF) sustains the viability and phenotypic expression of a variety of neuronal populations including cranial and spinal motor neurons. The distribution of the ciliary neurotrophic factor receptor α (CNTFRα), which is essential for the trophic effects of CNTF to occur, is unknown in any primate species. The present study used a polyclonal antibody directed against CNTFRα to evaluate the distribution of CNTFRα-ir within the brain and spinal cord of Cebus apella monkeys. CNTFRα-ir was found exclusively within neurons. In the anterior horn of the spinal cord, virtually all motor neurons were immunoreactive for CNTFRα. Furthermore, a similar pattern of CNTFRα-ir was seen within all cranial motor nuclei with general somatic efferent function (III, IV, motor V, VI, motor VII, and XII cranial nerves). CNTFRα-ir was also seen on other regions involved with motor function including the Purkinje cells of the cerebellum, substantia nigra pars compacta, red nucleus, dorsal motor nucleus of X cranial nerve, and layer V of sensory motor neocortex. A few CNTFRα-ir were seen within the globus pallidus with concomitant terminal-like staining within the subthalamic nucleus. Autonomic regions such as the mesencephalic nucleus of the trigeminal nerve and the interomedial lateral cell column of the thoracic spinal cord also contained CNTFRα-ir neurons. CNTFRα-ir was observed within both the rostral (sensory) and posterior (autonomic) portions of the nucleus solitarius. Finally, dense CNTFRα-ir was observed within the pyramidal cell layer of the hippocampal formation and the granule cell layer of the dentate gyrus. The dense expression of this CNTFRα-ir within regions subserving motor, autonomic, and sensory functions suggests that CNTFRα supports many CNS regions with diverse functions. (Supported by a private donation)

224.16

NOVEL PATHWAY OF NUCLEAR TARGETING OF FGF-RECEPTOR 1 AND ITS ROLE IN CELL CYCLE CONTROL IN HUMAN GLIOMA CELLS. E. Mordechai[1,2], J. Moffett[1], L.N. Ligidakis[1], C.L. Yee[1], E.K. Stachowiak[1], P.A. Maher[3], M.K. Stachowiak[1,*]Division of Neurobiology, Barrow Neurological Institute, Phoenix, AZ 85013; [2]Immunosciences Lab, Inc., Beverly Hills, CA 90211; [3]The Scripps Research Institute, La Jolla, CA 92307.

Nuclear accumulation of FGFR1 coincides with the proliferation of human glial cells (Stachowiak et al. 1996; Mordechai et al. 1996). To determine how nuclear FGFR1 exerts its pleiotropic mitogenic effects, we have analyzed its structure-function relationship in human glioma cells. We have transfected the full length FGFR1 cDNA and FGFR1 missing the membrane insertion domain (FGFR1/-MID), as well as, FGFR1 missing the leader sequence domain (FGFR1/-LS) into glioma cells (SF-763) that do not express FGFR1. Immunohistochemistry and Western analysis of fractionated, transfected SF-763 cells demonstrate nuclear accumulation of FGFR1, FGFR1/-MID, or FGFR1/-LS with marginal extra nuclear localization. Consistently, nuclear localization coincides with enhanced FGFR1 associated kinase tyrosine kinase activity in the nuclear fractions of FGFR1 and FGFR1/-MID transfected SF-763 cells. Proliferation assays have demonstrated enhanced mitotic activity of SF-763 cells transfected with either FGFR1 or FGFR1/-MID, resulting from enhanced G_1 to S transition. Additionally, SF-763 cells transfected with FGFR1/-LS have the same basal mitotic activity as SF-763 cells transfected with CMV-Neo. In conclusion: (i) FGFR1 can accumulate in the nucleus without prior insertion into the cell membrane (ii) nuclear accumulation of FGFR1 coincide with enhanced mitotic cycle (iii) leader sequence domain is necessary for the mitogenic activity of nuclear FGFR1. (Supported by NIH - HL49376 and NSF-IBN9411226).

224.17

A RAS/RAF REGULATED POTASSIUM CHANNEL CONTROLS MUSCLE GENE ACTIVATION IN 10T1/2 CELLS EXPRESSING THE MUSCLE TRANSCRIPTION FACTOR MRF4.

T.L. Peña, S.F. Konieczny & S.G. Rane*. Dept. of Biol. Sci., Purdue Univ., W. Lafayette, IN 47907.

To understand how receptor tyrosine kinase (RTK) signaling controls cell growth and differentiation, we have focused on ion channels, specifically a small conductance Ca^{2+} activated K^+ channel, SK, as potential targets and physiological mediators of this signaling. Mitogenic peptide growth factors upregulate SK channel expression in fibroblasts and stimulate proliferation which is inhibited by the SK channel blocker, charybdotoxin (ChTX) (JBC 269:31183, 1994). Since cell proliferation and differentiation are ultimately effected via gene regulation, our goal is to determine how SK activity regulates these processes. We now present evidence, combining patch clamp techniques with ectopic overexpression of MRF4 in the 10T1/2 fibroblast cell line, that implicates SK channel activity in transcriptional regulation. In response to the mitogenic peptide bFGF, 10T1/2-MRF4 cells express high levels of SK, MRF4 is negatively regulated, and the cells proliferate as fibroblasts. When bFGF is withdrawn, SK levels decrease, and a myogenic program is initiated via MRF4, including expression of acetylcholine (ACh) receptor channels observed as ACh activated whole-cell currents. We now report that cells grown in bFGF and ChTX, i.e., high SK levels, but SK functional blockade, also express high levels of ACh receptor channels, consistent with removal of bFGF negative regulation of MRF4. These results suggest that bFGF negative regulation of MRF4 occurs indirectly through upregulation and activity of the SK channel. Thus, these experiments provide the first evidence linking ion channel activity to control of a defined transcriptional regulatory complex.

Support: Abbott Labs Fellowship (TP), R01AR41115 (SK), R01GM43462 (SR).

224.18

Increased epidermal growth factor-receptor (EGF-R) expression following MPTP treatment in mice. M. Gupta*, M. Demianova, A.Shum-Siu, and F.J. Hendler, Depts. of Anatomical Sci. & Neurobiology, Medicine, and Biochemistry, Henry Vogt Institute of the Brown Cancer Center, U of L Medical School, and the Louisville VAMC, Louisville, KY.

Epidermal growth factor-receptor (EGF-R) has been implicated in neuronal development as well as in mediating neurotrophic and neuromodulatory effects in the nervous system. The present studies were undertaken to examine if EGF-R is expressed in the nigrostriatal dopaminergic system and if its expression is altered following treatment with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). Young adult mice were given two injections of MPTP (15 mg/kg body weight s.c.) 24 hr apart and sacrificed 1 hr after the second injection. Mice were anesthetized, and perfused with 4% paraformaldehyde. Brains were removed and 8μm sections were cut through the striatum and substantia nigra (SN). EGF-R mRNA was detected using in situ RT-PCR. Sections were pretreated with trypsinogen and DNase. EGF-R cDNA was synthesized using an antisense oligonucleotide primer. The EGF-R cDNA PCR amplification with digoxigenin-dUTP was carried out in situ for 7 cycles using taq polymerase. EGF-R mRNA synthesis was detected using a monoclonal anti-digoxigenin antibody conjugated with alkaline phosphatase. The results show increased EGF-R expression in the striatum and SN of MPTP-treated mice, suggesting an increased synthesis of EGF-R in response to MPTP toxicity. Since EGF-R has been show to have a mitogenic effect and to activate other tyrosine kinases, including MAP kinase, these data suggest that MPTP treatment activates the signal transduction pathway. Supported by grants from the Jewish Hospital Foundation and NSF EPSCoR to MG.

224.19

DIVERGENT REGULATION OF NEURONAL PHENOTYPE BY NEUROTROPHINS IN SK-N-SH HUMAN NEUROBLASTOMA CELLS. S.S. Lesser* and D.C. Lo. Department of Neurobiology, Box 3209, Duke University Medical Center, Durham, NC 27710.

Neurotrophins help shape neuronal phenotype by regulating the development and maintenance of neuronal excitability through the expression of voltage-gated and ligand-gated ion channel expression. We have used the sympathetic-like human neuroblastoma cell line, SK-N-SH, to examine the differential effects of different neurotrophic factors within a single cellular context. SK-N-SH cells are normally responsive to NGF, but when cultures are treated with retinoic acid (RA), these cells become responsive also to BDNF, NT-3, NT-4. Previously, we have described the differential regulation of Na^+, Ca^{++}, and K^+ currents in SK-N-SH cells by the neurotrophins. NGF and NT-4 upregulate Na^+, Ca^{++}, and K^+ currents. BDNF regulates Na^+ and Ca^{++} currents only, and NT-3 regulates only K^+ currents.

In this study we examined the initial signaling events which may underlie down-stream differences in late gene expression. Using phospho-specific antibodies, we have assayed Trk, CREB, and ERK activation and have found that each signaling intermediate is induced by the neurotrophins. However, each neurotrophin induces phosphorylation of Trk, CREB, and ERK to similar levels and with similar time courses. Additionally we have examined the regulation of other late genes, such as GAP-43, which is also differentially regulated by neurotrophins in a pattern distinct from that for any of the voltage-gated ion channels. Thus, we conclude that no two neurotrophins have the same set of effects on late gene expression in RA-treated SK-N-SH cells, and that this divergence in regulating neuronal phenotype occurs despite similar early transduction events.

We would like to thank Regeneron Pharmaceuticals for their generous gift of neurotrophins. This work was supported in part by an award from the Ruth K. Broad Foundation, the Alfred P. Sloan Foundation, and NIH grant NS32742.

224.20

Preparation of a Cell-Free Translation System from PC12 Cells. M.Shibutani, E.Kim, P.Lazarovici, M.Oshima*, and G.Guroff. Section on Growth Factors, NICHD, NIH, Bethesda, MD 20892.

Until now, very few studies on translational regulation during neuronal differentiation have appeared. The postmitochondrial fraction (S10) contains the cellular components essential for translation, and a high salt wash (HSW) of the ribosomes is enriched in eukaryotic initiation factors. To investigate the phenomena linked with translational regulation as a possible mechanism during neuronal differentiation, we established a cell-free translation system utilizing an S10 extract from PC12 cells. The products synthesized from either firefly luciferase mRNA or PC12 cell poly(A) RNAs in the PC12-S10 extract were increased by the addition of the HSW from PC12 cells. Increases in the translation of luciferase mRNA by the addition of PC12-HSW were dose-dependent and also dependent on the time of incubation. The translation of human epidermal growth factor receptor mRNA could also be detected in the PC12-S10 extract translation system by immunoprecipitation. *N*-linked glycosylation of the translation products also was observed. The efficiency of translation was altered by the addition of Mg^{2+} or K^+, and optimization of these ions was necessary for each mRNA. The translation system made from PC12 cells, then, is capable of the synthesis of proteins of relatively high molecular weight and should be useful for analyzing mechanisms of translational control during proliferation and differentiation of cells from a neuronal lineage.

225.1

ACTIVATION OF MAP KINASES (ERKs) BY ESTRADIOL IN CEREBRAL CORTICAL EXPLANTS: CROSS-COUPLING OF THE ESTROGEN AND NEUROTROPHIN SIGNALING PATHWAYS. C. D. Toran-Allerand*, E. Mauri, C. Leung, M. Warren and M. Singh. Dept. of Anatomy & Cell Biology, Columbia Univ. Coll. P&S, New York, NY. 10032.

Estrogen and the neurotrophins have important roles in the developing and adult central nervous system (CNS). Our previous studies have demonstrated estrogen and neurotrophin receptor co-localization throughout the developing forebrain, suggesting a potential substrate for steroid/neurotrophin interactions. We are testing the hypothesis that estrogen/neurotrophin receptor co-expression leads to reciprocal regulation at the level of signal transduction, through cross-coupling of converging estrogen and neurotrophin signaling pathways whose end-points in the nucleus may be the very same genes. Postnatal day 2 cerebral cortex was maintained as organotypic explants in roller tube culture for 7 days. Following 24 hrs. of estrogen and neurotrophin deprivation, we investigated estradiol's activation of ERKs 1 & 2 (**E**xtracellular-signal **R**egulated **K**inase) and found rapid (by 5 min.) tyrosine phosphorylation of the ERKs (maximal at 30 min.) that persisted for at least 4 hrs. Although estrogen-induced phosphorylation implies activation (required for ERK nuclear translocation) in-gel kinase assays confirmed that phosphorylated ERKs were much more active in phosphorylating MBP as a substrate than untreated controls. In starting to define the pathways leading to ERK activation, we found that both estradiol and the neurotrophins (NGF, BDNF and NT-4/5, but not NT-3) induced tyrosine phosphorylation of the estrogen receptor. Conversely, and in keeping with the idea of reciprocal cross-coupling of the signal transduction pathways, we have found that estradiol also induced tyrosine phosphorylation of the *trk* receptors. Our results provide a novel and alternative developmental mechanism for estrogen action that would explain how estrogen and the neurotrophins could each regulate the same broad array of ERE (estrogen response element)- and non-ERE-containing genes. (Supported by grants from NIH (NIA), NIMH, NSF, and an ADAMHA RSA to DT-A).

225.2

PC12-E_2 Cells: A Novel Model System for the Study of Estrogen and Neurotrophin Interactions. M. Singh*, E. Mauri, C. Leung, M. Warren and C. D. Toran-Allerand. Dept. of Anatomy & Cell Biology, Columbia Univ. Coll. P&S, New York, NY. 10032.

We have previously reported that wild-type, differentiated PC12 pheochromocytoma cells express very low levels of estrogen receptors. Here we report that a PC12 cell variant, PC12-E_2 (Wu, Bradshaw, J. Cell. Physiol. 164: 1995), is estrogen responsive and expresses high estrogen receptor levels (≈50% of the levels observed in estrogen receptor over-expressing MCF-7 cells). Moreover, although tyrosine phosphorylation of the estrogen receptor appeared to be expressed constitutively, these cells responded to added estrogen. PC12-E_2 responsiveness to estrogen was determined morphologically and by measuring estradiol-induced ERK activation. Estradiol phosphorylated ERKs 1 & 2 on tyrosine within 15 min. In contrast, no estradiol-induced ERK stimulation was observed in wild-type, differentiated PC12 cells, suggesting that the estrogen receptor was integral to the response. Morphological studies revealed extensive and rapid NGF induction of neurite outgrowth (within 24 hrs.). While estradiol alone appeared to have little effect on neurite growth, the concurrent addition of estradiol and NGF elicited both a synergistic enhancement of neurite growth and apparent mitogenic activity. We are utilizing these cells to investigate the pathway(s) by which estradiol activates the ERKs. Preliminary co-precipitation experiments show an association between *src* and estrogen receptor, *src* with MEK (**M**AP Kinase/**E**RK-activating **K**inase), and MEK with B-*raf*. MEK, which phosphorylates the ERKs on tyrosine residues, is known to exist as a complex consisting of at least hsp90 and B-*raf*. Since both the unliganded estrogen receptor and *src* are also known to be associated with hsp90, these findings imply a direct association between the estrogen receptor and at least one component of the MAP Kinase cascade (MEK), suggesting a route by which estrogen could activate the ERKs directly. (Supported by Grants from NIH (NIA), NIMH, NSF, and an ADAMHA RSA to DT-A).

225.3

UP-REGULATION OF A NOVEL GENE TRANSCRIPT IN PC12 CELLS STIMULATED WITH NERVE GROWTH FACTOR AND MAST CELLS STIMULATED WITH STEM CELL FACTOR. S.-Y. Tam*, M. Tsai, and S.J. Galli. Depts. of Pathology, Beth Israel Hospital & Harvard Medical School, Boston, MA 02215.

We have recently used the technique of mRNA differential display to identify novel genes whose expression is differentially regulated in mast cells activated by IgE and specific antigen. Of the several differentially expressed cDNA probes identified, one clone hybridized to a 2.6 kb mRNA whose expression was rapidly increased in mouse mast cells that had been activated through the FcεRI for 30 min or 1 hr, but which returned to baseline levels after 2 hrs. Sequence analysis of an entire full-length cDNA clone revealed no significant homology to any known gene sequences, suggesting that the cloned cDNA may represent a novel gene transcript. To examine whether the activated expression of this novel gene transcript was specific for the FcεRI-dependent signaling pathway in mast cells, Northern analysis was performed to assess its expression in mouse bone marrow-derived cultured mast cells that had been stimulated with stem cell factor (SCF) and in PC12 cells that had been stimulated with nerve growth factor (NGF). In mouse mast cells stimulated with SCF (50 ng/ml), the mRNA levels of this novel gene were increased with a time course that was essentially identical to that observed with IgE and antigen stimulation. On the other hand, in PC12 cells stimulated with NGF (50 ng/ml), the mRNA expression of the identified gene was not affected 30 min after stimulation; however, its levels were significantly elevated 1 hr after stimulation and remained undiminished at 2 hr. These data thus indicate that the expression of this novel gene transcript can be differentially regulated in mast cells and PC12 cells via different types of ligand-receptor interactions. Experiments are in progress to test the idea that the gene product encoded by this novel transcript may represent a common signaling element mediating diverse functional responses in different cell types. SUPPORT: NIH Grants AI/CA-23990 and CA/AI-72074 and the Beth Israel Hospital Pathology Foundation.

225.4

DOWN-REGULATION OF CYCLIN F IN NERVE GROWTH FACTOR-TREATED PC12 CELLS. V.Movsesyan, M.Whalin, E.Broude, and G.Guroff*. Section on Growth Factors, NICHD, NIH, Bethesda, MD 20892

The PC12EY subclone was used to study the influence of nerve growth factor (NGF) on the cyclin F content during neuronal differentiation. Immunoblot analysis showed a marked decrease in cyclin F levels in PC12EY cells treated with NGF. This down-regulation, was prevented by the addition of K-252a. A smaller reduction of cyclin F was observed when cells were treated with fibroblast growth factor, but epidermal growth factor had no comparable effect. Time course studies suggest that the decline in cyclin F expression precedes the changes produced by NGF in the distribution of cells within the cell cycle (increase in cells containing 4C DNA), as shown by flow cytometry. Immunocytochemical staining showed largely perinuclear localization of cyclin F and confirmed the significant decrease of the immunoreactivity in NGF-treated PC12EY cells. These data suggest that cyclin F may be involved in NGF-mediated cell cycle events.

225.5

QUANTITATIVE CHANGES IN THE BINDING OF TRANSCRIPTION FACTORS TO RESPONSE ELEMENTS IN AXOTOMIZED AND NGF-TREATED DORSAL ROOT GANGLION NEURONS. J.D. Corness, T-J. Shi, T. Hökfelt*. Dept. of Neuroscience, Karolinska Institute, 17177 Stockholm, Sweden.

Transection of an axon is known to cause diverse changes in the protein make-up of the cell body, and it is believed that the axotomy-induced changes in peptide expression may ultimately be due, at least in part, to decreased availability of growth factors in the neuronal cell bodies. In this experiment we studied the influence of axotomy and nerve growth factor (NGF) treatment on the *in vitro* binding of transcription factors extracted from the dorsal root ganglia (DRGs). We have prepared whole-cell protein extracts of DRGs for quantitative binding analysis during electromobility shift assays (EMSA). DRGs from spinal cord levels L4 and L5 were taken from rats at various time points following sciatic nerve axotomy and compared to contralateral and unoperated controls. In addition, a group of axotomized rats was given 1μg NGF with 0.1% BSA through a capsule applied to the proximal nerve ending, and the protein extracts from these ganglia were compared to those of axotomized animals and normal controls. Binding of these extracts was carried out in the presence of labelled oligonucleotides for the response elements AP-1, AP-2, NFkB, Sp-1 and TFIID, as well as for selected elements within the rat galanin gene promoter. Preliminary results show that axotomy caused an increase in specific binding to the AP-1 probe which was counteracted by NGF treatment. For a probe which includes the galanin CRE and the sequence surrounding it, binding did not increase after axotomy; however, a specific complex binding to this probe was seen to be down-regulated by the growth factor treatment. (Supported by Swedish MRC 04X-2887.)

225.6

DIFFERENTIAL EFFECTS OF VASOPRESSIN ANALOGS ON GENE EXPRESSION OF NEUROTROPHINS IN RAT BRAIN Y. C. Du, A. W. Zhou, J. Guo and X. L. Yang[1]*. Shanghai Ins. of Biochemistry, [1]Shanghai Ins. of Physiology, Chin. Acad. of Sci., Shanghai, China 200031.

In situ hybridization and Northern blot assay were used to evaluate the effect of arginine-vasopressin (AVP) analogs on the transcription of genes for nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF) and neurotrophin 3 (NT-3) in the brain of adult Wistar rat (♂) at h-12 after injection (s.c. 1.3 μg/kg body weight in 0.5 mL saline). 1) The differential expression of neurotrophins was significantly found. NGF and BDNF, but not NT-3, were enhanced by AVP_{4-8} [I] in the cerebral cortex and hippocampus. 2) In the same conditions, behaviour-active AVP [II] resulted in a smaller effect and its behaviour-inactive homologue, oxytocin [III] did not. 3) Furthermore, additional four synthetic analogs of AVP_{4-8}, *i.e.* NLPR [IV], Ac-YQNC(C)PR amide [V], ZDC(C)PR [VI] and $(ZNCPR)_2$ [VII] were tested and the results showed that the NGF mRNA level increased significantly in the brain after s.c. injection of either [IV] or [V], while the other two had no positive response. 4) The enhancement of the NGF expression of [III] and [IV] was substantially inhibited by [VI] which was known as the most effective antagonist of AVP_{4-8}. In general, the neuropeptide potencies in enhancing NGF expression were well consistent with their ligand-receptor binding abilities and learning behavioral activities reported previously. According to the documented data on the differential distributions of NGF, BDNF and NT-3 mRNA in limbic system, the selective enhancement of these gene expressions undoubtedly indicated the involvement of NGF and BDNF, but not NT-3, in learning and memory via strengthening the basal cholinergic projection. It is postulated that these analogs may share with AVP_{4-8} the same signalling pathway in adult rat brain.

This study was supported by grants of The National Natural Science Foundation and Shanghai Joint Laboratory of Life Sciences.

225.7

THE AKT PROTO-ONCOGENE IN NGF AND PDGF SIGNAL TRANSDUCTION. Thomas F. Franke[1,2,+], Henryk T. Dudek[3], Alex Toker[2], Lewis C. Cantley[2], Michael E. Greenberg[3], and David R. Kaplan[1,+,*]. [+]ABL-Basic Research Program, NCI-FCRDC, Frederick, MD, 21702; [1]Montreal Neurological Institute, McGill Univ., Montreal, Quebec H3A 2B4;[2]Division of Signal Transduction, Beth Israel Hospital, and Department of Cell Biology, Harvard Medical School, Boston, MA 02215; [3]Division of Neuroscience, Children's Hospital, Boston, MA 02115.

We have previously shown that the kinase activity of the *Akt* proto-oncogene is induced by PDGF in fibroblasts in a manner dependent on the activity of PI 3-kinase (Franke *et al.*, Cell 81, 1995). Our recent results show that only one of the products of PI 3-kinase, PtdIns-3-4-P_2 is able to activate Akt when added to cells. This regulation is mediated by direct interaction of PtdIns-3,4-P_2 with the Akt pleckstrin homology (PH) domain. Thus, PtdIns-3,4-P_2 is sufficient to activate Akt by binding direclty to the Akt PH domain. We also extended our previous studies by examining the activity of Akt in biological relevant systems such as PC12 cells and primary neuronal cells. Akt activity in PC12 cells is induced rapidly and specifically by NGF, but not by EGF. Moreover, the tyrosine residue on TrkA regulating the activity of PI 3-kinase *in vivo* is also necessary and sufficient to induce Akt activation by NGF. In addition, we find that Akt activity in primary neuronal cells is regulated by factors inducing the activity of PI 3-kinase. A possible role of Akt as a downstream effector of PI 3-kinase in PI 3-kinase-dependent pathways such as neurite outgrowth and neuronal survival will be discussed.

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225.8

STUDIES ON THE REGIONAL DISPOSITION OF rhNGF AND RELATED NEUROTROPHINS FOLLOWING ICV ADMINISTRATION IN RODENTS. E. Escandon*, L. Rangell, G. Keller, E. Szonyi, and JL. Mendoza-Ramirez. Department of PK-Metabolism. Genentech, Inc. 460 Point San Bruno Blvd. South San Francisco, CA 94080.

Nerve Growth Factor (NGF), Neurotrophin 3 (NT-3) and Neurotrophin 4/5 (NT-4/5) are critical for the survival and maintenance of several neuronal populations in the peripheral nervous system. In the central nervous system these neurotrophins are also able to attenuate and prevent neuronal degeneration. Multineurotrophin 1 (MNTS-1) was the first neurotrophin mutant designed and produced by recombinant DNA technology with full NGF, NT-3 and NT-4/5 combined biological activities (Urfer et al. EMBO J. 13:5896-5909, 1994). Since these molecules do not cross the blood brain barrier, the objective of this study was to compare the specific brain regional disposition of NGF, NT-4/5 and MNTS-1 following a single intracerebroventricular (ICV) administration. Our results indicate that ^{125}I-rhNGF (100 ng/rat) delivered ICV is specifically incorporated by cholinergic neurons in the medial septum, the nucleus of diagonal band of Broca, and nucleus basalis of Meynert. A time course analysis following ICV administration indicated that ^{125}I-rhNGF remains in specific brain regions for as long as 72 hrs. The disposition of ^{125}I-rhNGF to the caudate-putamen, cerebellum, pons and specific uptake by the spinal cord will be discussed, as well as a comparative electron microscopy autoradiographic analysis with radiolabeled NGF, NT-5 and MNTS-1. (Commercial funding: Genentech, Inc.)

225.9

mRNAs FOR ONE, TWO OR THREE MEMBERS OF TRK RECEPTOR FAMILY ARE EXPRESSED IN SINGLE RAT TRIGEMINAL GANGLION NEURONS. M. Moshnyakov, U. Arumäe, M. Reeben*, and M. Saarma. Program of Molecular Neurobiology, University of Helsinki, Helsinki, FIN-00014, Finland.

trkA, trkB and trkC constitute a family of protein tyrosine kinase receptors that specifically bind neurotrophins with high affinity. We studied the expression of mRNAs for trkA, trkB and trkC in single rat trigeminal ganglion neurons at embryonic days 12 and 16 to determine, whether single trigeminal ganglion neurons express one trk family member or coexpress several of them. For that purpose we elaborated a sensitive technique of reverse transcriptase-polymerase chain reaction to detect all neurotrophin receptors in a single neuron. Expression of neurofilament light chain mRNA was used as a positive marker to confirm the recovery of mRNAs from single neurons. Neurofilament-positive samples were subsequently analyzed for the expression of mRNAs for catalytic trkA, trkB and trkC, and in some cases, low-affinity neurotrophin receptor (p75). We found neurons expressing one, coexpressing two, or even all three trk receptors. In both developmental stages, most of the analyzed neurons coexpressed two or three trk family members with the combination of trkB and trkC being the most often found. In many neurons analyzed, p75 mRNA was coexpressed with trks, but we also found neurons expressing only trks without p75, and a neuron expressing p75 alone. There were also neurons containing neither trk receptors nor p75. In this work, expression pattern of four neurotrophin receptors in single neurons was determined for the first time. Moreover, coexpression of three or even four receptors was demonstrated directly. This work was supported from the grant of the Academy of Finland, from the University of Helsinki, and from the grant of Sigrid Juselius Foundation.

225.10

NGF INDUCES NEURITE OUTGROWTH AND NUCLEAR TRANSLOCATION OF ERKs DESPITE INHIBITION OF ERK ACTIVITY. P. Kahle*[a], A. N. Verity[a], A. Meyer-Franke[a], J. L. Twiss[b], and E. M. Shooter[a]. Depts. [a]Neurobiology and [b]Pathology, Stanford Univ. School of Medicine, Stanford, CA 94305-5401, and [a]Dept. Neurobiology, Inst. Pharmacology, Roche Bioscience, Palo Alto, CA 94304-9819.

Nerve growth factor (NGF) causes growth arrest and differentiation of responsive neurons. A very prominent NGF signaling event is the activation of mitogen-activated protein kinases (MAPKs). These regulate immediate-early gene (IEG) induction via phosphorylation and activation of transcription factors, but the relative contribution of MAPKs to neuronal differentiation remains unclear. We have investigated effects of the cyclin-dependent kinase/MAPK inhibitor Olomoucine on NGF-mediated IEG induction and neurite outgrowth in PC12 cells. Olomoucine did not affect tyrosine phosphorylation nor nuclear translocation of extracellular signal-regulated kinases (ERKs) in response to NGF. Thus, phosphorylation of ERKs rather than activity determines nuclear import. NGF-stimulated activation of the 90kDa ribosomal S6 kinase was decreased by Olomoucine, revealing ERK-dependent and -independent pathways. Olomoucine abrogated ERK-dependent induction of the *β-actin* gene, while induction of the more complex IEGs *c-fos*, *NGFI-A*, *NGFI-B*, and *c-jun* was delayed. However, Olomoucine minimally affected *vgf* induction, which depends on activation of Ca^{2+}/cAMP response element binding protein (CREB) by phosphorylation on Ser-133. Indeed, NGF-induced CREB phosphorylation was not affected by Olomoucine. Finally, Olomoucine inhibited neither neurite initiation nor regeneration. Thus, ERKs appear to be dispensable for NGF-mediated neuronal differentiation due to the massive redundancy of NGF signaling. (Supported by fellowships from Roche Research Foundation, Swiss Nationalfonds, Ciba-Geigy-Jubiläumsstiftung (to P,K.), Deutsche Forschungsgesellschaft (to A.M.-F.), an NIH Training grant (HD0749, A.N.V.), NIH grants (NS01706 to J.L.T, NS04270 to E.M.S.), and by the State of California, Department of Health Services, under contract 93-18647).

225.11

MAPK IS PHOSPHORYLATED BY BDNF, NT-3, NGF, GDNF AND MIDKINE IN MOTONEURON-HYBRID CELLS.
Michikawa M.(1)*, Sanjo N.(2), Ouwada K.(2), Muramatsu H.(3), Muramatu T.(3) and Cashman N.R.(4) (1)Dept. of Dementia Res., National Institute for Longevity Science, Aichi, Japan. (2)Dept. of Neurol., Tokyo Med. and Dent. Univ., Tokyo Japan. (3)Dept. of Biochem. Nagoya Univ. School of Med. Aichi, Japan. (4)Montreal Neurological Institute and Hospital, Montreal, Quebec, Canada

Neurotrophic factors, such as Midkine, BDNF, NT-3, GDNF, CNTF are known to promote the survival of motoneurons *in vitro* or *in vivo*. However, their intracellular pathways in motoneurons have not been well investigated so far. A neuroblastoma x spinal cord hybrid cell line (NSC19: Cashman, 1992) was used for this study. Several established neurotrophic factors were added to the culture medium for 5 min at various concentrations, cultured cells were harvested and processed for western blot analysis using anti-MAPK antibody (erk2) or anti-phosphotyrosine antibody.

Among the factors tested, BDNF, NT-3, GDNF and Midkine, which are known to have neurotrophic actions on motoneurons, phosphorylated MAPK, but CNTF did not. Unexpectedly, MAPK of NSC19 cells was phosphorylated by NGF treatment, which has no trophic effect on motoneurons: showing motoneurons may have changed some characters by hybridization, however, NSC cell cultures are useful system for investigating the signal transduction systems of motoneurons.

225.12

ELUCIDATION OF SIGNALING PATHWAYS UNDERLYING ION CHANNEL INDUCTION DURING GROWTH FACTOR STIMULATED PC12 CELL DIFFERENTIATION.
M.D. Hilborn, J.D. Pollock*, R.R. Vaillancourt[1], & S.G. Rane. Dept. of Biol. Sci., Purdue Univ., W. Lafayette, IN 47907. [1]Nat. Jewish Cen. Immunol. Resp. Med., Denver, CO 80206.

There is a debate as to whether full neuronal differentiation of PC12 cells can be achieved by sustained Ras/ERK activity alone, or by Ras/ERK complemented by other signals. EGF transiently activates Ras/ERK and acts as a mitogen, but it produces morphological differentiation when combined with KCl or cAMP, the latter of which produces sustained Ras/ERK activity (Mark et al., JCB 130:701, 1995; Yao et al., JBC 270:20748, 1995). We have used whole cell patch clamp to ask if upregulation of calcium and sodium channels, hallmarks of *physiological* differentiation, can be achieved by sustained ERK activation (EGF+cAMP), or ERK activation with depolarization (EGF+KCl). We find that treatment of PC12 cell cultures for 4-7 days with EGF in combination with 0.5 mM cAMP or 60 mM KCl elicits extensive neurite outgrowth, but fails to upregulate sodium and calcium channel currents. These observations demonstrate that sustained Ras/ERK activity alone or in complement with cAMP is insufficient for ion channel upregulation. To identify the signals that are required for physiological differentiation, we are now examining ion channel upregulation in PC12 cell lines transfected with either wild-type PDGF receptor, or mutant receptors deficient for activation of distinct signaling pathways (Vaillancourt et al., MCB 15:3644, 1995). In response to PDGF, PC12 cells exogenously expressing the PDGF receptor extend neurites and show increased current densities comparable to those observed in NGF treated cells. By assessing the ability of mutant PDGF receptors to upregulate calcium channels, we hope to elucidate which specific signaling pathways, in addition to Ras/ERK, are necessary and/or sufficient for ion channel upregulation during neuronal differentiation.
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225.13

Staurosporine induces neurite outgrowth and stimulates pp60^{src} by trk- and ras-independent signal transduction pathway(s). P. Lazarovici*, M. Whalin, H. Jiang, D. Kaplan‡, and G. Guroff, Section on Growth Factors, NICHD, NIH, Bethesda, MD 20892 and ‡Montreal Neurological Institute, Montreal, Quebec, Canada.

Staurosporine, a microbial protein kinase inhibitor, is a potent, partial, NGF-functional agonist inducing rapid neurite outgrowth in PC12 cells, independent of protein kinase C inhibition, and dephosphorylation of tau, microtubule-associated proteins. In contrast to NGF-activated, trkA receptor-mediated signal transduction, the signalling pathways responsible for staurosporine-induced neurite outgrowth are unknown. We have used different PC12 cell lines, overexpressing or lacking trkA and dominant-negative ras or src, as model systems to examine the phosphorylation reactions by which staurosporine induces neurite outgrowth. Staurosporine (50 nM) treatment of PC12 cells resulted in a rapid activation of pp60^{src} and pp60^{yes}, but not Csk (C-terminal src kinase). Staurosporine does not activate trkA tyrosine kinase activity, ras-MAP kinase pathway, PLC-gamma or suc-associated neurotrophic factor-induced tyrosine phosphorylated target (SNT) tyrosine phosphorylations. Staurosporine-induced neurite outgrowth is not blocked by a dominant inhibitory mutation of ras or src. Furthermore, staurosporine inhibits S6 kinase activity in PC12 cells. These findings indicate a ras-independent signalling by staurosporine and suggest that the activation of the src family of kinases and the inhibition of S6 kinases, may reflect combinatorial novel signal transduction cross-talk pathways contributing to neurite outgrowth which are distinct from those emanating from the trkA receptor.

225.14

TWO DEFINED INDEPENDENT SITES RESPONSIBLE FOR NGF SPECIFICITY ANALYSED BY PURIFIED MUTANT NEUROTROPHINS.

Kias Kallander* and Ted Ebendal Dept. of Developmental Neuroscience, Box 587, Biomedical Center, Uppsala University, S-751 23 Uppsala, Sweden,

Despite the large sequence similarity (60 %) among the known neurotrophins within the NGF family, they display different activities on different subsets of neurons. Recent studies have shown that the various neurotrophins are ligands with high affinity for different receptors of the Trk family of tyrosine kinase receptors. In earlier studies we have defined five NGF-amino-acid residues giving NGF-like activity when exchanged into NT-3. The most efficient NGF-like transformation was obtained by the exchange of Pro-Val and Leu-Val-Gly in NT-3 to the NGF residues Val-Phe (residues#: 48, 49) and Gln-Ala-Ala (96-98). The mutated sites are situated in two b-loops at one end of the NGF molecule, forming a cleft that could interact specifically and with high affinity with the signalling NGF receptor complex. The other independent site, situated in the N-terminal of NGF, was defined by a series of mutations replacing NT-3 corresponding residues. In this case, the least altered molecule with NGF-like activity was obtained when a one amino acid replacement, from a Lys to a Pro (5) and an extra residue, a Phe (7), was inserted into the corresponding position in NT-3. Two mutants each with alterations in defined independent sites were transfected into CHO cells for production of neurotrophins and their expression amplified using methotrexate. The mutant neurotrophins were then purified by selective precipitation and ion-exchange chromatography and were eluted as single bands as judged on 10-20 % gradient SDS-PA gels. The activities of the purified mutant neurotrophins were compared with NT-3 and NGF by measuring survival of chicken sympathetic neurons, fibre outgrowth from sympathetic and Remak's ganglia and activation of the human TrkA and TrkC receptor. Also, the binding of the purified neurotrophins to PC12 cells alone, PC12 cells overexpressing TrkA and PC12 cells lacking endogenous TrkA (PC12nnr5) was analysed.

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225.15

BIOTINYLATION OF NERVE GROWTH FACTOR (NGF) FOR THE STUDY OF RECEPTOR-MEDIATED RETROGRADE TRANSPORT. J.U. Choh and J.R. Unnerstall*. Dept. Anatomy and Cell Biology, University of Illinois at Chicago, College of Medicine, Chicago, IL, 60612-7308.

Receptor-mediated retrograde transport of trophic factors like NGF from the nerve terminal to the soma is essential for neurotrophin-mediated signal transduction. To better study the dynamics of this process at the anatomical level, we have synthesized a biologically active biotinylated derivative of 2.5S NGF. A biotinylated NGF probe that retains biological activity, in conjuction with the appropriate avidin-conjugated markers, can improve the sensitivity and resolution with which the receptor-mediated NGF transport pathway is visualized. A similar strategy was first described by Rosenberg et. al. (*J. Neurochem.* **46**:641, 1986); however, replication of these procedures have not been reported. In contrast to this previous report, our approach utilized gentler labeling conditions and included a strepavidin affinity purification step. To biotinylate NGF (C-biotinyl-NGF), 2.5S NGF (1mg/ml) was incubated with 1.25 mM biotin hydrazide and 1.25% 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC) at 4°C overnight. C-biotinyl-NGF was purified from free-NGF with streptavidin-conjugated affinity chromatography. To remove excess biotin hydrazide and EDC, the eluent from the affinity chromatography was concentrated and exchanged with PBS without crosslinking substances. Biotinylation of NGF was confirmed using an enzyme-linked immunosorbent assay (ELISA) against PC12 cells or a plasma membrane preparation of PC12 cells labeled with C-biotinyl-NGF. Biotinylation of 2.5S NGF was further confirmed using Western blots probed with streptavidin-conjugated horseradish peroxidase. The biological activity and potency of C-biotinyl-NGF was confirmed by assessing its effectiveness in promoting neurite outgrowth from PC12 cells. We propose that the procedures utilized to produce C-biotinyl-NGF provide for the synthesis of useful probes for the study of receptor-mediated NGF transport. *(Funded by Campus Research Board, University of Illinois at Chicago.)*

225.16

NEUROTROPHIC ACTIVITY OF SYNTHETIC NGF PEPTIDE DERIVATIVES. F.M. Longo[1]*, M. Manthorpe[2], Y. Xie[1] and S. Varon[3]. [1]Dept. of Neurology, UCSF/VAMC, San Francisco, CA 94121, [2]Vical Inc., La Jolla CA 92121 and [3]Dept. of Biology UCSD, La Jolla CA 92093.

Synthetic peptides corresponding to β-hairpin loop regions of NGF were purified by HPLC and assayed for neurotrophic activity using dorsal root ganglion sensory neurons. Peptide conformation was constrained by chemical modifications. A peptide corresponding to one loop region had significantly greater survival-promoting activity than peptides corresponding to other loop regions. Assays of ten additional peptides of varying length corresponding to this region showed that peptide fractions P7 and P9 had 70-80% of NGF's maximum activity at concentrations of 125 and 250 µM. One group of derivatives was active in the 10-100 µM range. P7 peptides resembled partial agonists rather than non-specific trophic agents in that they were inhibitory instead of additive to NGF. Antiserum directed against the extracellular domain of the p75 NGF receptor (supplied by M. Chao) inhibited activity of NGF, P7 and P9 to a similar extent but had no effect on scrambled P7. The Trk receptor inhibitor K252a inhibited NGF but not P7. Ongoing studies will further elucidate structure-activity relationships. Taken together, these studies raise the possibility that NGF peptide derivatives support neuronal survival via a p75 receptor-mediated, Trk kinase-independent mechanism. These derivatives might contribute to developing agonists or partial agonists of NGF. Supported by NIA AG 09873 (FL) and the VA (FL).

225.17

STRUCTURE-FUNCTION ANALYSIS OF THE C-TERMINUS OF NGF. A.Kruttgen*, J.V.Heymach, P.Kahle, E.M.Shooter. Dept of Neurobiology, Stanford University School of Medicine, Stanford, CA 94305-5401

Nerve growth factor (NGF) is a member of the neurotrophin family and plays an important role in development and maintainance of the nervous system. Previous work has shown that the C-terminus of mNGF is important for its function, because deletion of residues 112-120 abolished its bioactivity. In the present study we performed a detailed structure-function analysis of this region by deleting stepwise each of the nine C-terminal residues as well as constructing 6 point mutants. The constructs were transiently expressed in COS-7 cells and quantified by ELISA as well as Western blotting. Biological activity was assessed by using PC12 cells in a short term response -TrkA autophosphorylation on tyrosine residues- as well as a long term response -neurite outgrowth scored after 3 days. Our results show that deletion of residues 116-120 as well as a double point mutant with ArgArg 118 119 changed to SerSer did not decrease NGF bioactivity. Further deletion of the carboxy-terminus led to strongly decreased stability of the mutant proteins -as measured by an ELISA recognizing only the native NGF- possibly because of approaching too close to cysteine 110 which is involved in the "cystine-knot-motif" providing rigidity to the molecule. Five point mutants covering the region between position 111 and 115 showed normal stability and decreased bioactivity at low ligand concentrations. This supports the idea that the C-terminus plays a role in structure and function of NGF.

(supported by NIH and DFG)

225.18

ROLE OF PI-3 KINASE IN NERVE GROWTH FACTOR-MEDIATED DIFFERENTIATION OF PC12 CELLS. M. Ashcroft[1]*, R.M. Stephens [1], and D.R. Kaplan [1,2]. (SPON: Brain Research Association). [1]ABL-Basic Research Program, NCI-FCRDC, Frederick, MD 21702, and [2] Montreal Neurological Institute, Montreal, PQ H3A2B4.

Nerve growth factor (NGF) treatment of responsive cells results in the stimulation of the activity of many intracellular signalling proteins and several transduction pathways. One of these proteins, phosphatidylinositol-3 kinase (PI-3K), has been implicated in the regulation of cell survival and neurite elongation processes in NGF-treated PC12 cells. To assess the role of PI-3K in NGF-mediated neuronal differentiation, we developed a system whereby endogenous PI-3K activity could be stimulated in an NGF-inducible manner in the absence of the activity of any other known signalling protein. We initially generated a mutant Trk/NGF receptor (Trk^{def}) which lacked sequences Y490, Y785 and KFG responsible for recognizing and activating the major Trk intracellular targets Shc, PLC-γ1, PI-3K and SNT. This receptor was kinase active but defective for NGF-induced responses when expressed in PC12 nnr cells, which lack endogenous Trk. Site-directed mutagenesis was used to introduce a PI-3 kinase binding consensus site (*YMDM*) originally identified in the PDGF receptor, into the kinase insert domain of Trk^{def}. Trk^{def} encoding the PI-3K site (Trk^{def}+PI-3K) was transfected into PC12 nnr cells and at least 25 stable clones were isolated and screened for expression levels. NGF treatment of the Trk^{def}+PI-3K-expressing cells resulted in the activation of PI-3K, however activation of any of the other known intracellular targets of Trk was not observed. In response to NGF, these cells underwent a rapid rate of neurite outgrowth compared to PC12 control cells. These results suggest that PI-3 kinase activity alone is sufficient for the initiation of stable neurite outgrowth in PC12 cells. We will use this system to examine the role of PI-3K in cell survival as a prelude to studies in primary neuronal culture. (Research sponsored by the National Cancer Institute, DHHS, under contract with ABL.)

225.19

REGULATION OF NEUROTROPHIN SIGNALING PATHWAY PROTEINS IN DOPAMINERGIC NEURONS BY DRUGS OF ABUSE. D.H. Wolf, E.J. Nestler,, and D.S. Russell* Division of Molecular Psychiatry, Depts. of Neurology and Psychiatry and the Interdepartmental Neuroscience Program, Yale University School of Medicine, New Haven, CT 06508

Chronic exposure to drugs of abuse induce a number of persistent biochemical and physiological alterations in the dopaminegic neurons of the ventral tegmental area (VTA). These include increases in tyrosine hydroxylase protein levels, decreases in neurofilament proteins, and a reduction in axoplasmic transport from theVTA.

Neurotrophins (NTs) are important mediators of induction and maintenance of neuronal differentiation in the brain. Expression in the VTA of NTs and Trk receptors persists in the adult. Drugs of abuse may exert part of their persistent effects via perturabation of the NTs, the Trks, or the intracellular signaling pathways they utilize. It has been shown that local infusions of NTs including BDNF, NT-3, and NT-4 can prevent or reverse some of the biochemical changes induced by chronic morphine or cocaine (Berhow, MT, et al., *Neuroscience*, 68:969, 1995). Evaulation of NT pathway signaling proteins in adult rat brain reveals that levels of some of these proteins are substantially enriched in the VTA compared with other brain regions. For instance, PLC-γ-1 is nearly 20-fold more abundant in VTA than hippocampus or frontal cortex. Chronic exposure to morphine regulates the levels of some of these proteins. After 6 days of morphine exposure, protein levels of PLC-γ-1 increased 30%, while levels of insulin receptor substrate (IRS) proteins decreased 25-35%. No significant changes were seen in levels of TrkB, PI-3-kinse, or proteins in the Ras/Raf/ERK signaling pathway. However, morphine exposure regulates the enzyme activities of proteins within this pathway.

Some of these changes are mimicked in PC12 and SHSY-5Y cell lines, and are useful to assist in elucidating molecular mechanisms. (supported by DA08227 and the Ribicoff Research Facilities, State of CT).

225.20

GENE EXPRESSION IN THE SPINAL CORD NEURONS AFTER SCIATIC NERVE INJURY OF THE ADULT ANIMALS. JK Cui*, YC Pan, ZZ Gu, S Shenaq, and PK Liu, Baylor College of Medicine, Houston, TX 77030

To demonstrate a dependence of spinal cord motoneurons on the communication with their targets, the expression of immediate early gene c-*fos* and neurotrophin genes in the lumbar (L3-L6) spinal cord neurons was examined in Sprague-Dawley rats (male ≥ 9-week old) with unilateral sciatic nerve transection. Using *in situ* hybridization, we detected the expression of c-*fos* mRNA in the motoneurons of the spinal cord segments within 45-min to 3-hr of peripheral nerve transection (n=4 in each time point). The expression of c-*fos* mRNA was also correlated positively with the expression of Fos antigen using immunohistochemistry, while no change in calbindin and parvalbumin antigens were noted. The expression of BDNF mRNA increased at 90 min after sciatic nerve transection. However, no detectable enhancement in the expression of NGF mRNA was observed. DNA fragmentation in neurons was observed using the incorporation of digoxigenin-dUTP by terminal transferase into 3'-OH terminals of DNA fragments in the ipsilateral section of the spinal cords 48 hr after nerve injury. Nuclei that exhibited DNA fragmentation were not observed in the spinal cord of the control animals. Lastly, we observed that the majority of astrocytes did not have DNA fragmentation. Because the detection of DNA fragmentation using this assay is one of early detections of apoptosis or programmed cell death, the result suggested we could detect early cell death in spinal cord, and indicated a target dependence of the neurons in the spinal cord after transection of sciatic nerve. [Supported by grants from the Vivian L. Smith Foundation for Restorative Neurology, the Kent Waldrep Institutes for Paralysis Research, the American Heart Association (94012700) and from the NIH (NS34810) to PKL].

HORMONES AND DEVELOPMENT: SEXUAL DIFFERENTIATION

226.1

HORMONAL REGULATION OF ANDROGEN RECEPTOR (AR) mRNA EXPRESSION IN THE NEONATAL RAT FOREBRAIN. M. McAbee* and L.L.DonCarlos. Neuroscience Graduate Prog & Dept. Cell Biol., Neurobiol., and Anatomy, Loyola University of Chicago, Coll. of Med., Maywood, IL 60153.

Testosterone and its metabolites masculinize the developing forebrain. Estrogen is the primary metabolite responsible for this process; however, there is evidence that androgens, via androgen receptors, also play a role in the organization of male-typical behaviors and neuroendocrine regulation. For that reason, we have studied the sites and regulation of AR in the developing forebrain. Previously, we showed that AR mRNA expression was greater in males than in females by postnatal day 10 (PND-10; PND-0 = day of birth), in the medial preoptic area (MPOA) and caudal bed nucleus of the stria terminalis (cBNST). In contrast, AR mRNA levels were not different in males and females in other regions (lateral septum, arcuate n., ventromedial n. of the hypothalamus, and premammillary n.). Gonadectomy on either PND-0 or PND-5 reduced AR mRNA expression in PND-10 males in the MPOA and cBNST but not in other areas. In the present experiments, male rats were gonadectomized on PND-0 and treated daily with diethylstilbestrol (2μg/day), dihydrotestosterone proprionate (10μg/day), testosterone proprionate (10μg/day) or oil vehicle. Intact males were treated with flutamide (40μg/day),vehicle, or were untreated. Rats were sacrificed on PND-10, and brains were removed and processed for standard in situ hybridization using an 35S-labeled riboprobe for AR mRNA. The results to date confirm our previous findings in neonatal rats and suggest a complex pattern of regulation of AR mRNA by gonadal steroid hormones. Supported by NIMH 48794.

226.2

ONTOGENY OF ANDROGEN RECEPTOR IMMUNOREACTIVITY IN RAT BRAIN. C. W. Malsbury* and D. Babstock. Dept. Psychology, Memorial University, St. John's, NF, Canada, A1B 3X9.

We have used the androgen receptor (AR) antibody, PG21, donated by Dr. Gail Prins, with immunohistochemistry to identify AR-immunoreactive (ARir) neurons in male and female rats of various ages. Apparently, PG21 binds much better to ligand-bound, than to unbound, AR. Because of this, male and female littermate pairs were either given a single ip injection of dihydrotestosterone benzoate (DHTB) 30 minutes before perfusion or left untreated before perfusion.

The results show that different brain regions begin to show ARir staining at different times during development. For example, in males the ventral premammillary nucleus (VPM) contains many ARir neurons with dense nuclear staining as early as postnatal day (PND) 1, even without DHTB treatment. In contrast, the posterior-dorsal medial amygdala (MePD) shows little staining at PND 1 or 4. However, by PND 14, MePD contains many ARir neurons in untreated males. Within the hippocampus, areas CA1 and CA3 contain numerous ARir pyramidal neurons in adult male rats. Although some ARir cells are seen in the hippocampus as early as PND 4, the adult pattern of staining is not seen until much later (after PND 14). For example in CA1, at PND 4 most ARir cells are seen just outside the pyramidal cell layer, while in the adult, most are confined to this layer.
Supported by NSERC Grant OGP0007907 to C.W.M.

226.3

ANDROGEN RECEPTOR IMMUNOREACTIVITY IN PUDENDAL MOTONEURONAL SUBPOPULATIONS OF THE ADULT MALE RAT. C. L. Jordan* Dept. Psych., UC Berkeley, CA 94720-1650.

Pudendal motoneurons (MNs) of the adult male rat are grouped in two distinct motoneuronal pools, the spinal nucleus of the bulbocavernosus (SNB), positioned medially, and the DLN, positioned dorsolaterally. Most SNB MNs innervate the sexually dimorphic bulbocavernosus (BC) and levator ani (LA) muscles, and a small number of SNB MNs innervate the sexually monomorphic anal sphincter (AS) muscle. DLN MNs innervate either the sexually dimorphic ischiocavernosus (IC) or the sexually monomorphic external urethral sphincter (EUS) muscles. Collins et al. (Brain Res., '92) report that BC, LA and IC MNs shrink in size after castration whereas AS and EUS MNs do not. This raises the question of whether AS and EUS pudendal MNs have androgen receptors (ARs). I combined Fluoro-Gold retrograde tracing with AR immunoreactivity to answer this question for normal adult male rats. I find that a large percentage of MNs within each subpopulation have AR+ nuclei, including MNs within AS and EUS subpopulations. However, a lower percentage of AS and EUS MNs have AR+ nuclei compared to the other subpopulations. Because androgen may act directly on pudendal MNs to regulate their size (Watson et al., Soc. Neurosci. Abstr., '96) the apparent androgen-insensitivity of AS and EUS MNs may reflect fewer AR expressing MNs in these groups, leading to smaller, less discernible average response to androgen. Future studies will address whether AS and EUS MNs with AR+ nuclei are androgen sensitive. Supported by IBN-9210229 and IBN-9309856.

226.4

SYNERGISTIC EFFECTS OF TESTOSTERONE METABOLITES ON DENDRITIC GROWTH IN A SEXUALLY DIMORPHIC RAT SPINAL NUCLEUS. E.M. Kurz*, K.A. Burke, M.R. Widows, and D.R. Sengelaub. Program in Neural Science, Indiana University, Bloomington, IN 47405.

Motoneuron dendrites in the spinal nucleus of the bulbocavernosus (SNB) grow exuberantly through postnatal day (P)28. This growth is steroid-dependent: dendrites fail to grow in males castrated at P7, but grow normally in castrates treated with testosterone (T). Treatment with either of the T metabolites dihydrotestosterone (DHT) or estrogen (E) alone supports dendritic growth in castrates, but not to normal male or T-treated castrate levels. Furthermore, in intact males treated with the E antagonist tamoxifen, dendritic growth is reduced to levels seen in DHT-treated castrates, suggesting that E is necessary for normal SNB dendritic growth. In this study, we tested the hypothesis that DHT and E act together to support development of SNB dendrites.

Dendritic morphology was assessed in normal males and males castrated on P7 and treated daily with DHT (dihydrotestosterone propionate, 2 mg/day) combined with low or high doses of E (estradiol benzoate, 5 μg or 100 μg/day) (n=4 per group). SNB motoneurons were retrogradely labeled with cholera toxin-HRP at P28, when dendritic length is normally maximal, and motoneuron morphology assessed in three dimensions (Eutectic NTS). Dendritic lengths in DHT+high E-treated castrates (mean=5855 μm) were like those of normal males (mean=5614 μm). In contrast, dendritic lengths of DHT+low E-treated castrates (mean=3836 μm) were significantly shorter than those of both normal males and DHT+high E-treated castrates. These results suggest that E and DHT act synergistically to support normal masculine SNB dendritic development. Supported by NIH NS24877.

226.5

Brain-Derived Neurotrophic Factor Regulates Expression of Androgen Receptor in Perineal Motoneurons. H.A. Al-Shamma* and A.P. Arnold, Dept. of Physiological Science and Laboratory of Neuroendocrinology, Brain Research Institute, UCLA, Los Angeles, CA 90095-1527.

In adult male rats, axotomy of the spinal nucleus of the bulbocavernosus (SNB) motoneurons causes a reduction in expression of nuclear androgen receptor (AR), which is reversed when the motoneurons reinnervate the target perineal muscles. The present experiments further characterize the role of target-derived factors in the regulation of motoneuronal AR. We studied AR immunoreactivity in SNB motoneurons one week after these motoneurons were either 1) silenced with TTX, 2) treated with an axoplasmic transport blocker, vinblastine, or 3) axotomized and treated with one of several neurotrophic factors. Unilateral activity blockade with TTX did not alter the density of AR immunoreactivity. In contrast, unilateral blockade of axoplasmic transport with vinblastine significantly reduced the number of densely labeled AR-immunoreactive motoneurons to 28% of that on the untreated control side ($p<0.0001$). These studies suggest that AR expression in the SNB is not dependent on neuromuscular activity, rather it is dependent on factors that are retrogradely transported to the SNB motoneurons. We tested different protein trophic factors as possible regulators of AR expression in the SNB. Unlike other trophic factors (CNTF and NT-4), treatment of severed SNB axons with gel foam soaked in brain-derived neurotrophic factor (BDNF, 2.9mg/ml in PBS) significantly blocked the axotomy-induced down regulation of AR immunoreactivity ($p<0.05$). These results indicate that protein trophic factors can regulate expression of steroid receptors in neurons.

This work was supported by National Institutes of Health grant MH10839 (H.A.A.).

226.6

DEVELOPMENT OF SEXUAL DIMORPHISM IN CNTF-RECEPTOR KNOCKOUT MICE. M.L. Howell*[1], T.M. DeChiara[2] G.D. Yancopoulis[2] and N.G. Forger[1]. [1]Neuroscience and Behavior, University of Massachusetts, Amherst, MA, and [2]Regeneron Pharmaceuticals, Tarrytown, New York.

The spinal nucleus of the bulbocavernosus (SNB) is a sexually dimorphic neuromuscular system in rodents. Motoneurons of the SNB are located in the lumbar spinal cord and innervate perineal muscles that attach to the base of the penis. During prenatal development, males and females have comparable numbers of SNB cells. Androgen secreted from the testes maintains these motoneurons in males whereas they die in females during the late prenatal and early postnatal period. The administration of testosterone can prevent the death of SNB cells in females. Recently, ciliary neurotrophic factor (CNTF) has been shown to mimic some effects of testosterone in development of the SNB system. Specifically, perinatal CNTF treatment prevents degeneration of SNB motoneurons and their target muscles. To examine whether the CNTF receptor is *required* for the normal development of sexual dimorphism in the SNB, we counted SNB motoneurons in mice with a null mutation of the alpha component of the CNTF receptor. CNTF receptor knockouts (CNTFR-/-) exhibit significant motoneuron death and do not survive beyond postnatal day 1 (DeChiara et al., 1995). SNB cell number was reduced in both male and female CNTFR-/- newborns as compared to their CNTFR+/+ counterparts ($p < 0.05$), as would be expected. However, the percent reduction in the SNB was greater than the reduction in the lateral horn. When expressed relative to motoneuron number in the lateral horn of the same spinal segments (# of SNB motoneurons divided by # of lateral horn motoneurons), CNTFR-/- males had significantly fewer motoneurons in the SNB than did CNTFR+/+ males ($p < 0.001$), and CNTFR-/- males did not differ significantly from CNTFR+/+ or CNTFR-/- females on this measure. Thus, deletion of the CNTF receptor may reduce or eliminate the development of a sex difference in the SNB.

Supported by NIH HD33044 and the Whitehall Foundation.

226.7

NEONATAL DEVELOPMENT OF THE SPINAL NUCLEUS OF THE BULBOCAVERNOSUS IN THE MONGOLIAN GERBIL: A LIGHT MICROSCOPIC THIONIN STUDY. G.S. Fraley* and C. Ulibarri, Department of Veterinary & Comparative Anatomy, Pharmacology and Physiology, College of Veterinary Medicine, Washington State University, Pullman, WA 99164-6520.

The spinal nucleus of the bulbocavernosus (SNB) of the Mongolian gerbil (*Meriones unguiculatus*) is located in the lumbosacral spinal cord in area X. The SNB innervates perineal musculature and is sexually dimorphic in that male gerbils have more and larger SNB motoneurons than do female gerbils. To examine the normal postnatal development of the gerbil SNB, groups of male and female gerbils were aldehyde perfused at ages from postnatal day 1 (PND1) through PND15 and PND25. Spinal cords were dissected, cryoprotected, and embedded in 5% gelatin. Spinal cords were then sectioned coronally (50 μm) on a cryostat, mounted on slides and stained with thionin. SNB motoneurons were counted by an individual unaware of the treatment groups. Statistical analyses were conducted using analysis of variance. At birth male gerbils had more SNB motoneurons than did female gerbils. The number of SNB motoneuron number increased over time. However, at all ages male gerbil pups had significantly more SNB motoneurons than did female gerbil pups (mean ± SEM; PND1 ♂ = 76.8 ± 7.3, ♀ = 36.6 ± 9.6; PND3 ♂ = 89.5 ± 16.5, ♀ = 48.3 ± 4.3; PND6 ♂ = 172.8 ± 9.1, ♀ = 55.5 ± 11.3; PND9 ♂ 235.3 ± 25.1, ♀ = 42.3 ± 6.8; PND12 ♂ = 244.5 ± 19.3, ♀ = 63.7 ± 8.3). The maximal number of SNB motoneurons seen in males at PND25 (250 ± 7.5) was lower than that reported previously for adult males. In contrast, the maximal number of SNB motoneurons seen in females on PND15 (58.7 ± 1.3) was equivalent to that reported in adult females. This study suggests that the developmental pattern in the SNB of gerbils is different than the developmental pattern in the rat. This research was supported by NSF (BNS-9112097) to CU.

226.8

POSTNATAL DEVELOPMENT OF 5α-REDUCTASE-CONTAINING CELLS IN THE RAT FOREBRAIN. Y. Tsuruo*, H. Yokoi and K. Ishimura, Dept. of Anatomy, Tokushima Univ. Sch. of Med., Tokushima, 770, Japan

Steroid 5α-reductase (5α-R) is the enzyme converting steroids with C-3 ketone and C-4,5 double bond to 5α-dihydro form. The enzyme is known to be preferentially localized in the white matter rather than the gray matter, and the enzyme activity is shown to be high during the first 1-2 weeks of postnatal life, when the active myelination occurs. To further investigate the localization and characteristics of 5α-R-containing cells, we raised a rabbit antibody against a rat 5α-R type-1, and the localization of 5α-R-containing cells was immunohistochemically examined in the rat brain. During the first week of postnatal life, positive immunoreaction was observed in small cells within the subventricular zone (SVZ) adjoining the lateral ventricle and in the neighboring white matter including the corpus callosum. Some of these cells were shown to be mitotic. Following several weeks, the intensity of immunostaining as well as the number of 5α-R-positive cells increased in the white matter. These cells appeared to consist of two morphologically different cells: one had a few fine processes and the other showed several radiating processes. By double immunostaining using antibodies against several glial markers, most of these 5α-R-positive cells were shown to be oligodendrocytes, and some were astrocytes. These results suggest that the cells containing 5α-R, mostly oligodendrocytes, are primarily generated in the SVZ early in the postnatal life, and gradually distributed to the white matter region in the forebrain during development.

226.9

INCIDENCE OF PYKNOTIC CELLS IN THE PREOPTIC AREA OF NEONATAL RATS. L.L. DonCarlos* & K. Schwenk. Dept. Cell Biol., Neurobiol., & Anat., Loyola Univ. Stritch Sch. Medicine, Maywood, IL 60153.

Exposure to the estrogenic metabolites of testosterone during the early postnatal period has permanent effects on rodent brain development. One effect of estrogen exposure is to alter the number of cells in specific regions of the preoptic area (POA), such as the central portion of the medial preoptic nucleus (MPNc), which is more cell dense in males, and the anteroventral periventricular nucleus, which is more cell dense in females. In the present studies, we examined the number and distribution of pyknotic cells throughout the perinatal period in order to determine both where and when cell death occurs in this region. Sprague-Dawley rat pups (postnatal day 0, 4, 7 and 10; PND 0=day of birth) were anesthetized and perfused with 10% formalin. Brains were embedded in paraffin and cut at 6 microns on a rotary microtome. Sections were stained in toluidine blue, dehydrated, cleared and coverslipped. A subjective analysis showed that the number of pyknotic cells in the medial POA as a whole was highest on PND 0 and 4, and more pyknotic cells were found in the caudal POA than in the rostral POA. Neurons of normal and pyknotic appearance were then counted within the caudal MPNc. The density of total cells decreased in the caudal MPNc with age and was not different in males and females. In contrast, the density of pyknotic cells in the caudal MPNc was highest on PND 7 in both males and females, and higher in females than in males. Further analyses of the distribution and relative abundance of pyknotic cells in the POA are in progress. Supported by NIMH 48794.

226.10

A REGIONAL SEX DIFFERENCE IN THE SIZE OF THE LOCUS COERULEUS OF THE SPRAGUE-DAWLEY RAT. D. M. Babstock*, C. W. Malsbury and C. W. Harley. Dept. Psychology, Memorial Univ. Newfoundland, St. John's, NF, Canada, A1B 3X9.

Previous studies indicate that the locus coeruleus (LC) can be divided into subareas depending upon dominant efferent projection zones. Ascending projections to the forebrain originate within the dorsal half and projections to the spinal cord and the cerebellum course from the ventral half of the LC. Using Sprague-Dawley rats, the present study analyzed sex differences in total LC area and in ascending and descending projection zones. Horizontal sections were stained with cresyl violet or for tyrosine hydroxylase and it was found that the dorsal ascending projection zone is larger in the male. One of the well-defined subareas within the dorsal half of the LC provides noradrenergic innervation of the hippocampus, a structure that exhibits various male-dominated sex differences in the rat, and post hoc analysis localized the sex difference to this area of the LC. No difference was found in total LC area. As well, a sex difference is indicated in the shape of the LC by a longer anterior-posterior extent in males, while females have a greater dorso-ventral extent.
Supported by NSERC Grants PGSB117079 (to D.M.B.), OGP007907 (to C.W.M.) and A9791 (to C.W.H.).

226.11

UNBIASED STEREOLOGICAL STUDY AND GOLGI ANALYSIS OF THE SEXUALLY DIMORPHIC NUCLEUS OF THE MEDIAL PREOPTIC AREA.
L. Xue, J. P. Andrade, M. M. Paula-Barbosa and M. D. Madeira*. Dept. of Anatomy, Porto Medical School, 4200 Porto, Portugal.

The effects of gonadal steroids on the anatomy of the sexually dimorphic nucleus of the medial preoptic area (SDN) have been much studied, both during development and in the adult rat. However, apart from volume estimations, precise morphometric analyses of this nucleus are still lacking. Thus, we have estimated the total number of SDN neurons, using the fractionator method, and the mean volume of its neurons, by applying the nucleator to vertical sections. In addition, we have performed a metric analysis of the dendritic trees of SDN neurons. We found that in males the total number of neurons was 2.3x higher and the mean cell volume 1.4x greater in males than in females, and that these differences accounted for the male/female differences in the volume of SDN, which we found to be 3x greater in males than in females. Conversely, the total dendritic length, the total number of segments, the dendritic density and the number of spines per unit length of bipolar and multipolar neurons were globally greater in females than in males. However, the differences were significant only for the number of terminal segments of bipolar and multipolar cells and for the dendritic density of the proximal part of the dendritic trees of multipolar cells.

These findings show that the sex differences in neurites are less robust than those present in neuronal numbers and volumes, which demands accurate studies at the ultrastructural level for a further characterization of the influence of gonadal steroids on the structural organization of the SDN.

This study was supported by JNICT, Project PECS/C/SAU/92/95

226.12

SEX DIFFERENCES IN NON-NMDA GLUTAMATE RECEPTOR BINDING IN NEONATAL RAT BRAIN. M.M. McCarthy*, K.Y.S Chan and J.G. Pfaus, Dept. of Physiology, Univ. of Maryland, Baltimore, MD and Dept. of Psychology, Concordia Univ., Montreal CA

The perinatal period of the rat constitutes a sensitive developmental window for steroid hormone mediated sexual differentiation of the brain. Changes in neurotransmission may be important regulators of differentiation. On the day of birth (PN 0), rats were divided into four treatment groups; 1) castrated males injected with testosterone propionate (TP, 50µg) or 2) oil vehicle, 3) females injected with TP, or 4) oil. On PN3, brains were collected and prepared for *in vitro* receptor autoradiography. Binding to the non-NMDA glutamate receptor was assessed by incubating fresh frozen slide-mounted tissue sections in [^{3}H]-glutamate in the presence of AP5 to block NMDA receptors and AP3 to block metabotropic glutamate receptors. Following appropriate washes, slides were exposed to tritium sensitive film for 8 weeks. Densitometry analysis of the films was performed using NIH Image. Results indicate that in the posterior portion of the ventromedial nucleus (VMN) there is significantly higher binding in males and females treated with TP (2-way ANOVA, $p<.01$). The same effect was seen, but to a lesser degree, in the medial preoptic nucleus ($p<.05$). In the anterior VMN, binding was significantly higher in males than females independent of hormone treatment ($p<.05$). There were no sex or hormone effects in the dorsolateral septal nucleus, cortex or most regions of the hippocampus. In a separate group of animals, immunocytochemistry for glutamate indicated a large number of glutamate immunoreactive neurons in the VMN on PN3, suggesting a population of glutamate interneurons exists in this brain region. By contrast, glutamate immunoreactivity in the preoptic area was broadly distributed. There were no obvious sex differences in the distribution of glutamate immunoreactive cells. In conclusion, steroid modulation of the glutamatergic system in the hypothalamus may be an important mediator of sexual differentiation of brain. *Supported by R29MH52716 (MMM) and MRC MT-13125 (JFP).*

226.13

DIFFERENTIAL EXPRESSION OF $GABA_A$ RECEPTOR SUBUNITS IN MALE AND FEMALE NEONATAL RAT BRAIN Aline M. Davis*[1], Silke Penshuck[2], Jean-Marc Fritschy[2], and Margaret M. McCarthy[1]. 1-Dept. of Physiology, University of Maryland, Baltimore MD 21201. 2-Institute of Pharmacology, University of Zurich, CH- 8057 Zurich Switzerland.

Sex differentiation of the rat brain is dependent upon the hormonal milieu during pre- and postnatal development. The mechanisms of differentiation are not well understood. We examined the role of the GABAergic system in mediating these differences and in establishing the sex specific phenotypes of the adult rat brain. GABAergic neurotransmission is well established early in development and promotes neurite outgrowth and cell survival in neonatal tissue. It is also regulated by steroid hormones in the adult. Evidence indicates pharmacological properties of the $GABA_A$ receptor vary with subunit composition, and that there is a developmental change in the ratio of $\alpha 2$ to $\alpha 1$ subunits in some brain areas. Therefore, we examined sex differences in subunit expression in neonatal rat brain.

Using an RNase protection assay (RPA), we have evaluated message levels for the $\alpha 1$ and $\alpha 2$ subunits in postnatal day 1 (PN1) rat brain. Brain regions were chosen for known content of GABAergic and/or estrogen receptor containing neurons. For both $\alpha 1$ and $\alpha 2$ we found sex differences in the ventromedial nucleus (VMN) and the arcuate nucleus (ARC). In the VMN, males had a higher abundance of mRNA for both subunits [ANOVA, $p<.01$]. However, in the ARC, levels were higher in females [ANOVA, $\alpha 1$ $p<.05$; $\alpha 2$ $p<.01$]. No differences were found in the medial preoptic area (mPOA), CA1 region of the hippocampus or the cingulate cortex (CTX). We also examined differences in $\alpha 1$ protein content by immunocytochemistry (ICC) in PN4 rats. The ICC data correspond with our RPA data, showing a more dense staining for $\alpha 1$ in the VMN of males [t-test, $p<.05$]. Therefore, we conclude that receptor subunit expression is sex dependent during the critical period, and that GABA plays a significant role in sex differentiation of the brain. This project was supported by NIH grant R29MH52716 to MMM.

226.14

POSTNATAL CHANGES IN ESTROGEN RECEPTOR, SUBSTANCE P AND CALCITONIN GENE-RELATED PEPTIDE IMMUNO-REACTIVITY IN RAT SENSORY NEURONS. S. Sarajari*, and M. M. Oblinger. Dept.Cell Biol. and Anat., Chicago Medical School, N. Chicago, IL 60064

In adult dorsal root ganglia (DRG), calcitonin gene-related peptide (CGRP), Substance P (SP) and estrogen receptor (ER) appear to be co-expressed in many of the small-sized nociceptive neurons. CGRP immunoreactivity (IR) is also seen in some medium and large-sized DRG neurons while SP-IR, as well as nuclear ER-IR, is largely restricted to the small sensory neurons in the adult. Recently we have found that estrogen treatment affects the levels of SP-IR (reduces) and CGRP-IR (increases) in DRG neurons. In the present study, we were interested in determining how the levels of SP and CGRP change in the DRG relative to ER during postnatal development. Lumbar DRGs were harvested from rat pups at postnatal (P) days 5, 10, 21 and 30; histological sections were stained with a variety of polyclonal and monoclonal antibodies. The results showed that nuclear ER-IR was present at the earliest times examined (P5) and was restricted to only the small neurons at all times examined (rather than being widely expressed at early times and then becoming more restricted in expression). SP-IR followed a largely similar pattern of change as ER. CGRP-IR at early postnatal times was similar to the adult pattern in that small, medium and some larger DRG cells were positive for this peptide. It will be of interest to determine how the establishment of peptide and ER expression in the DRG is related to gonadal steroid status.

Supported by NIH grant #AG13338 to MMO

226.15

DEVELOPMENT OF A SEXUALLY DIMORPHIC POPULATION OF PREPROCHOLECYSTOKININ mRNA CONTAINING NEURONS IN THE MEDIAL AMYGDALA OF THE RAT. D.S. Riding In* and R.B. Simerly. Division of Neuroscience, Oregon Regional Primate Research Center, Beaverton, OR 97006, and Department of Cell and Developmental Biology, Oregon Health Sciences University, Portland, OR 97201.

The posterodorsal part of the medial nucleus of the amygdala (MeApd) relays olfactory information to the hypothalamus and contains a hormonally regulated population of cholecystokinin (CCK)-containing neurons in adult rats. Moreover, the MeApd contains high densities of cells that express gonadal steroid hormone receptors, and both the MeApd and CCK have been implicated in the regulation of reproductive behavior and gonadotropin secretion. Because the MeApd is thought to sexually differentiate during the perinatal period, we used in situ hybridization to evaluate the pattern of preprocholecystokinin (PCCK) mRNA expression in the MeApd between embryonic day 18 (E18) and postnatal day 28 (P28). Very few PCCK mRNA containing neurons were found in the MeApd on E18, but a compact, sexually dimorphic population of cells was identified in the lateral part of the MeApd as early as postnatal day 0. By the second postnatal day (P2) male rats had approximately 4 times as many PCCK mRNA containing neurons in this cell group compared with that of females, and this difference was maintained through P28. To test the role of postnatal sex steroids in the regulation of this developmental pattern, newborn female rats were treated with testosterone during the postnatal period and levels of PCCK mRNA were measured in the MeApd. The results indicate that sex steroids have a profound influence on PCCK expression in the MeApd during the postnatal period, but that prenatal hormone exposure may also affect the number of neurons in the MeApd that can display the CCK phenotype.

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226.16

SEX DIFFERENCE IN GALANIN IMMUNOREACTIVITY IN THE FERRET PREOPTIC AREA/ANTERIOR HYPOTHALAMUS J.-J. Park*, S.A. Tobet and M.J. Baum Department of Biology, Boston University, Boston, MA 02215 and Department of Biomedical Sciences, The Shriver Center, Waltham, MA 02254

A sexually dimorphic male nucleus (MN) is seen in Nissl-stained sections of the dorsal preoptic area/anterior hypothalamus (POA/AH) of male ferrets, but not females, beginning as early as embryonic day (E) 37 of a 41-day gestation. In the POA/AH of ferrets we assessed the distribution of several neuropeptides previously reported to be sexually dimorphic in various rodent species, using immunohistochemistry. In males killed on E38, a dense cluster of galanin-immunoreactive (GAL-IR) cell bodies and processes was present in the dorsal POA/AH, beginning rostrally at the level of the diagonal band of Broca and extending caudally to include much of the MN-POA/AH and ending in the ventral bed nucleus of stria terminalis. In E38 females no GAL-IR cell bodies and processes were found in the dorsal POA/AH, although both sexes had a GAL-IR group in the ventral POA/AH. A sexually dimorphic group of GAL-IR cell bodies and processes was also seen in the dorsal POA/AH of adult males. Castration removed this dimorphic GAL-IR elements whereas administration of testosterone propionate (TP) reversed this effect. Administration of TP to ovariectomized adult females augmented the number of GAL-IR cell bodies and processes in the dorsal POA/AH, but not to the degree seen in males. Thyrotropin-releasing hormone, leucine-enkephalin and neurotensin IR elements were present in the POA/AH at E38 and in adulthood; their distribution was not sexually dimorphic. Arginine-vasopressin, oxytocin, vasoactive intestinal polypeptide, calcitonin gene-related peptide and somatostatin IR elements were not seen in the POA/AH of either fetal or adult ferrets. Our findings raise the question of whether galanin neurons in the dorsal POA/AH contribute to sexually allomorphic behavioral or neuroendocrine functions in this carnivore (Supported by HD21094; MH00392 and IBN94-10426).

226.17

ESTROGEN STIMULATES CCK AND PREPROENKEPHALIN mRNA IN THE FEMALE RAT LIMBIC-HYPOTHALAMIC CIRCUIT DURING THE PERIPUBERTAL PERIOD. K.L. Holland*, L. Abelson and P.E Micevych. Dept. of Neurobiology, Laboratory of Neuroendocrinology, Brain Research Institute, UCLA. Los Angeles, CA 90095.

CCK mRNA and peptide are expressed in regions of the rat central nervous system at neonatal ages. Within the limbic-hypothalamic circuit, however, the expression of CCK is delayed. Our developmental time course shows that during the neonatal period CCK mRNA levels are below the level of delectability in the medial preoptic nucleus (MPN), the principal nucleus of the stria terminalis (BNST) and the posterodorsal medial amygdaloid nucleus (pdMeA). Cells begin expressing elevated levels of CCK mRNA and peptide during the peripubertal period and in the adult, estrogen induces CCK expression within the interconnected cell groups of this circuit. The objective of this study was to determine if exogenous estrogen is able to induce the expression of CCK mRNA in the developing limbic-hypothalamic circuit. On PND 1, 10, 13, 18 and 23, female rats were subcutaneously injected with 25μg estradiol benzoate (EB), EB and 50μg progesterone or were untreated. All rats were sacrificed 48 hours after estrogen treatment and alternate brain sections were processed for CCK and PPE mRNA in situ hybridization histochemistry. EB induced PPE mRNA at all ages, while CCK mRNA expression was not increased by steroids until PND 20. Progesterone did not augment the estrogen effect on CCK or PPE mRNA levels. These results suggest that estrogen receptors are present and functional in the neonatal brain, as evidenced by the induction of PPE mRNA at all ages. Estrogen sensitive transmission within the limbic-hypothalamic circuit have not yet been established, or components of second messenger cascades which regulate estrogenic induction of CCK expression in the adult may not be functional before PND 20. Supported by NS21220 and T32-GM07185.

226.18

IDENTIFICATION OF ESTROGEN RECEPTOR TARGET GENES IN THE NEUROBLASTOMA CELL LINE SK-ER3, M. Garnier, D. Di Lorenzo$ and A. Maggi*, Inst. of Pharmacol. Sci., Milan-I 20133 $Hormonol. and Toxicol. Lab., Brescia Civic Hosp., Brescia-I 25100.

To study the activity of estrogen receptor (ER) in cells of neural origin, the ER expressing SK-ER3 cell line was obtained by stable transfection of ER in neuroblastoma cells. In these cells, estrogen (E2) causes growth arrest and differentiation. To identify the ER target genes relevant to the effects of E2 on the cell cycle and differentiation, we compared the mRNA patterns from E2-treated *versus* non treated SK-ER3 cells using the mRNA differential display PCR technique and combining different sets of oligo-(dT)11 (with M = A, G or C) and 10-mer arbitrary primers in the PCR reactions. The analysis of the radiolabeled amplification products on denaturating polyacrylamide gels evidenced 19 bands which were reproducibly differentially expressed. These products were reamplified, directly sequenced by cycle sequencing, labeled with digoxigenin and used as probes for Northern blots analysis to confirm their pattern of regulation following hormonal treatment.

Several genes appeared to be regulated in the neuroblastoma cells in response to E2. Among these genes, one appears to be essential for cell proliferation. Results will be discussed with emphasis on the role of the ER target genes in the effects of E2 on growth and differentiation of neuroblastoma cells.

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226.19

ORGANIZATIONAL EFFECTS OF ESTRADIOL ON D_2 AND 5-HT_2 RECEPTORS. J. Williams*, D. Bell, F. Bell, B. Knight, E. Knight, and B. Ludrick. Dept. Biol. Sci., Southeastern Oklahoma State Univ., Durant OK 74701.

Pertubations of perinatal brain sexual differentiation may be contributing factors in some cases of schizophrenia. During development, testosterone exerts organizational effects on brain differentiation. In early adulthood organizational effects of steroids may become manifested in the overt symptoms of schizophrenia. This study examined the organizational effects of gonadal steroids on the dopaminergic and serotonergic systems. Three day old F-344 rats were used. Male pups were castrated or sham operated and female pups were injected s.c. with 1 μg estradiol benzoate or vehicle. At 20 days of age animals were sacrificed. Immediately following sacrifice the brain was removed and the prefrontal cortex, striatum, and hippocampus were dissected out over ice and stored at -80°C until used in membrane preparations. Membrane preparations were used for Scatchard analyses of the D_2 and 5-HT_2 receptors. Organizational effects on receptor binding parameters appeared to be similar for castrated males/control females and for control males/estradiol-treated females. D_2 and 5-HT_2 receptors had a higher affinity (lower K_d) and greater receptor density (higher B_{max}) in the castrated male/control female groups relative to the control male/estradiol-treated female groups.

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226.20

DISRUPTION OF THE ESTROGEN RECEPTOR GENE INTERFERES WITH SEXUAL DIFFERENTIATION OF DOPAMINE NEURONS IN THE PREOPTIC REGION OF THE MOUSE. R.B. Simerly*[1,2], K.S. Korach[3], D.B. Lubahn[3] and M. Zee[1]. [1]Division of Neuroscience, Oregon Regional Primate Research Center, Beaverton, OR 97006 and [2]Department of Cell and Developmental Biology, Oregon Health Sciences University, Portland, OR 97201, [3]NIEHS, Research Triangle Park, NC 27709.

Although it has been known for some time that estrogen exerts a profound influence on brain development and the expression of sexually dimorphic functions, a definitive demonstration of the role of the estrogen receptor (ER) in sexual differentiation has remained elusive. In rats the anteroventral periventricular nucleus (AVPV) plays an essential role in mediating the preovulatory gonadotropin surge and contains a sexually dimorphic population of dopamine neurons that develops under the influence of perinatal sex steroids. This neurochemical sex difference is also present in adult C57BL/6J mice: female animals have a 4-fold greater number of tyrosine hydroxylase (TH) immunoreactive neurons in the AVPV relative to the number found in males. We tested the dependence of this neurochemical sex difference on a functional ER by comparing the number of TH-immunoreactive neurons in the AVPV of male wild type mice (WT) with that of male mice (ERKO) in which the ER had been disrupted by homologous recombination. The number of TH-immunoreactive neurons in the AVPV of ERKO mice was 3 times that of WT littermates suggesting that disruption of the ER gene prevented the hormonally induced loss of TH-immunoreactive neurons that normally occurs in males. Moreover, sexually dimorphic nuclei such as the bed nucleus of the stria terminalis and medial nucleus of the amygdala appear to be demasculinized in ERKO animals. These results indicate that a functional ER is essential for sexual differentiation of dopaminergic neurons in the AVPV, and perhaps plays a role in the development of other sexually dimorphic features of the mouse brain as well. Supported by NIH grants MH49236 and RR00163.

NEURONAL DEATH: INTRACELLULAR SIGNALS

227.1

THE ISOQUINOLINESULFONAMIDE-SENSITIVE PATHWAY IS INVOLVED IN NEURONAL APOPTOSIS INDUCED BY DIVERSE STRESSFUL STIMULI. **C.M. Cagnoli* and H. Maney. ASRI, Medical College of Pennsylvania and Hahnemann University, Allegheny Campus, Pittsburgh, PA, 15212.**

We previously reported that a group of isoquinolinesulfonamide kinase inhibitors, IQS (H-7, 1-(5-isoquinolinesulfonyl)-2-methylpiperazine; H-8, N-[2-(methylamino)ethyl]-5-isoquinolinesulfonamide; and H-9, N-2-(aminoethyl)-5-isoquinolinesulfonamide) prevents apoptosis triggered in neurons by the inhibition of phosphatases 1 and 2A with okadaic acid, and by the inhibition of protein kinase C with staurosporine and chelerythrine (*J. Mol. Neurosci*, 1996, in press). In the present study, we assessed the ability of IQS to prevent apoptosis induced in primary cultures of rat cerebellar granule neurons by diverse stressful stimuli. We selected three groups of stressors: a) treatment with ceramide C-2, a stimulator of stress-activated kinases; b) neurotoxins, i.e., beta-amyloid peptide and lead ion; and c) serum removal and potassium deficiency. Apoptosis was assayed by morphological appearance after nuclear staining with propidium iodide, DNA ladder, and by the in-situ TUNEL assay. Cell death was assessed by quantitative assay of membrane permeability to trypan blue dye. The IQS prevented apoptosis in all the above-noted models. It appears that the IQS-sensitive pathway is a common mechanism in different types of apoptosis. Because the anti-apoptotic action of IQS did not correlate with the known selectivity of these drugs in inhibiting protein kinases, nor could it be attributed to the antioxidative action of IQS, we propose that the IQS-sensitive pathway of neuronal apoptosis might involve the activation of a protein kinase not yet identified. Elucidating this mechanism may provide us with new strategies in the design of novel neuroprotective drugs.

227.2

STRESS-ACTIVATED PROTEIN KINASES, c-JUN, AND PROGRAMMED CELL DEATH IN THE DEVELOPING NERVOUS SYSTEM. J.R.Whitfield, A.Eilers, D.Lallemand#, L.L.Rubin* and J.Ham. Eisai London Research Laboratories, University College London, Gower Street, London, WC1E 6BT, UK and #Unité des Virus Oncogènes, Institut Pasteur, Paris, France.

When deprived of NGF, sympathetic neurons die by apoptosis and this death requires transcription and protein synthesis. We have studied the role of the Jun and Fos (AP-1) family of transcription factors in this process. We found that the level of c-Jun protein increased after NGF withdrawal and that c-Jun became highly phosphorylated on its transcriptional activation domain. Microinjection into sympathetic neurons of an expression vector for full length, wild-type c-Jun induced apoptosis. Furthermore, overexpression of a c-Jun dominant negative mutant protected sympathetic neurons against cell death after NGF withdrawal. These results suggest that the AP-1 family, and in particular c-Jun, plays a key role in neuronal cell death due to survival factor withdrawal.

We are currently studying the signal transduction pathways that lead to the activation of c-Jun after NGF withdrawal. One candidate for the kinase which activates c-Jun is Jun kinase (JNK, a stress-activated protein kinase), which phosphorylates c-Jun on serines 63 and 73 in the transactivation domain. In immune complex kinase assays we have found that Jun kinase activity increased when sympathetic neurons were deprived of NGF. Consistent with this, an antibody that specifically recognises c-Jun phosphorylated at serine 63 stained the nuclei of sympathetic neurons after NGF withdrawal. JNK is normally activated by SEK1 which in turn is activated by MEKK1. Microinjection of an expression vector for an activated form of MEKK1 induced the expression of c-Jun protein in the presence of NGF, and caused the injected cells to die by apoptosis. Co-injection of a SEK1 dominant negative mutant (SEK AL) inhibited MEKK1-induced cell death. These results indicate that an increase in the level of JNK activity is sufficient in itself to induce the expression of c-Jun protein and cell death.

Source of funding: Eisai Co. Ltd. (Japan).

227.3

BRIEF TREATMENT WITH PHORBOL ESTER PROTECTS CORTICAL NEURONS FROM APOPTOSIS INDUCED BY SERUM DEPRIVATION OR OXYGEN-GLUCOSE DEPRIVATION M.M. Behrens, U. Strasser*, B.J. Gwag, J.Y. Koh and D.W. Choi. Center for the Study of Nervous System Injury and Dept. of Neurology, Washington Univ. School of Medicine, St. Louis, MO 63110.

Several lines of evidence suggest a key role of protein kinase C (PKC) in mediating or modulating apoptosis. We examined the effect of the PKC activator, phorbol 12-myristate 13-acetate (PMA) on two forms of neuronal apoptosis in murine cortical cell cultures. Brief exposure to PMA (3-40 nM for 1 hr) produced a concentration-dependent reduction in serum deprivation-induced apoptosis in near pure neuronal cultures. A similar protective effect was also seen with brief exposure to 40 nM PMA against the neuronal apoptosis triggered by oxygen-glucose deprivation in the presence of NMDA and AMPA/kainate antagonists. This protective effect of PMA was selective for apoptosis; no protective effect was seen against the excitotoxic neuronal necrosis induced by oxygen-glucose deprivation in the absence of glutamate antagonists.

Supporting the possibility that PMA neuroprotection may be due to activation of PKC, no gross alteration in PKC α protein levels was observed at the end of a 1 hr exposure to 40 nM PMA. However, clearly further experiments will be necessary to determine the mechanisms responsible for this observed protective effect of brief PMA exposure.

Supported by a Roche Research Foundation Fellowship (US) and NIH grant 30337 (DWC).

227.4

UP-REGULATION OF PROTEIN KINASE C ISOZYMES UPON INDUCTION OF APOPTOSIS IN RAT CEREBELLAR GRANULE NEURONS. T. Ono, Y. Akita, K. Blomgren, A. Sato* and S. Kawashima. Department of Molecular Biology, The Tokyo Metropolitan Institute of Medical Science. 3-18-22 Honkomagome, Bunkyo-ku, Tokyo 113, Japan.

The primary culture of cerebellar granule neurons provides an excellent *in vitro* model to study the activity dependent regulation of survival which is a cellular basis of developmental plasticity. The presence of NMDA in the culture medium suppresses the apoptosis and greatly enhances the survival of cerebellar granule neurons. Apoptosis of the granule neurons can be induced by adding AP-5, a specific blocker of NMDA receptor, into the culture medium. Addition of H7 or TPA simultaneously with the induction of the apoptosis partially blocked the apotosis or enhanced the apoptosis, respectively, suggesting that protein kinase Cs were positively involved in the induction of apoptosis. In order to elucidate which protein kinase C isozymes were responsible for the accelation of apoptosis, we have employed western blotting analysis. Protein kinase C ßI, ßII, and ε were up-regulated and translocated from the cytosolic fraction to the membrane fraction upon the induction of apoptosis.

This work was supported in part by research grant from the Ministry of Education, Science, and Culture of Japan to T.O. (07780713).

227.5

ANNEXIN V LABELS NEURONS EARLY IN APOPTOSIS. G. Rimon, C. Bazenet, J. Taylor* and L.L. Rubin. Eisai Labs, University College, London WC1E 6BT, UK.

Neuronal apoptosis is a complex process that includes changes in nuclear and cytoplasmic structures. ICE-like proteases are thought to mediate at least some of these changes. However, substrates for these proteases have not yet been delineated completely, and the timing of activation of the different proteases has not been resolved, particularly in neurons. In order to address these issues, we have studied apoptosis in several different neuronal systems. For some studies, we have used NGF-differentiated PC12 cells, which die by apoptosis extremely rapidly and synchronously in response to treatment with staurosporine, a non-specific protein kinase inhibitor. One of the earliest changes (1-2 hours) that occurs following staurosporine addition is the appearance of high concentrations of surface phosphatidylserine, as indicated by intense labelling of live cells with fluorescent annexin V, a ligand for this phospholipid. Annexin binding occurs at a time when nuclear fragmentation is just becoming visible, as revealed by Hoechst staining. At this point, the plasma membrane is still intact since cells are not permeable to propidium iodide. Annexin V is a reliable marker of apoptosis since virtually 100% of cells with even mildly fragmented nuclei are labelled. Pretreatment with ZVAD-fmk, a peptide inhibitor of ICE-like proteases, prevents cells from becoming annexin V positive. Thus, an early event in apoptosis is the redistribution of phosphatidylserine to the outer leaflet of the plasma membrane by a mechanism that is dependent upon activation of ICE-like proteases.

Supported by Eisai Co., Ltd.

227.6

NEURONAL APOPTOTIC CELL DEATH MEDIATED BY MICROTUBULE NETWORK PERTURBATIONS B.Chromy, M.P. Lambert*, and W.L. Klein. Dept. of Neurobiology and Physiology, Northwestern Univ. Evanston, IL 60208.

Both colchicine and taxol can be used in asynchronous populations of proliferating cells to arrest the mitotic cycle. Recent experiments from several non-neuronal cell lines showed that these drugs also induce an apoptotic cascade. We have utilized these compounds in the neuronal CNS derived B103 cell line to study cell death and protein tyrosine phosphorylation. Cell death has been characterized first on the basis of morphological changes. Analysis via phase microscopy showed neurite atrophy and retraction as well as cellular rounding as early as two hours after treatment followed by membrane blebbing at 7-12 hours. Noticable cell death and disadhesion resulted within 24 hours. Ethidium homodimer-intercalated DNA fluorescence showed the occurrence of chromatin condensation and marginization in the dead cells. DNA fragmentation occurred by 20-24 hours, visualized as a 180-200 bp 'ladder' via DNA gel electrophoresis. The above data demonstrate both colchicine and taxol induce apoptotic B103 cell death. Quantitative measurements of apoptotic death were obtained via flow cytometry. Partial inhibition of apoptosis was caused by the protein synthesis inhibitor, cycloheximide, and by the specific protein tyrosine kinase inhibitor, genistein. Immunoprecipitation with tyrosine phosphorylated monoclonal antibodies revealed a striking increase in the phosphorylation of a ≈90 kDa cytoskeletally localized protein, roughly concurrent with the onset of DNA fragmentation. Although cycloheximide and genistein were able to fully block the induction of this ≈90 kDa protein, they did not completely block apoptosis. The lack of a complete inhibition could be due to apoptosis of a sub-population of cells in a distinct state of the cell cycle. Cells differentiated with Bt_2cAMP or arrested at G_0 through serum deprivation both underwent apoptosis after 24 hours of colchicine and taxol treatment. The G_2/M block conferred by these drugs thus may not be the only trigger for apoptosis. Since colchicine and taxol have opposite actions on microtubule integrity, yet both caused apoptosis in this system, disruption of the active flux of the microtubule network appears to be a crucial step for the initiation of apoptosis. At least two pathways leading to apoptosis appear to be initiated by the microtubule perturbations, one of which involves the hyperphosphorylation of a 85-90 kDa cytoskeletally localized protein.

Supported by grants to WLK from NIH and the Alzheimer's Association

227.7

TAU PHOSPHORYLATION OCCURS CONCURRENTLY WITH SELECT ALTERATIONS OF PHOSPHATASES AND KINASES DURING APOPTOSIS OF NEURONAL PC12 CELLS. P. K. Davis* and G. V. W. Johnson. Dept. of Psychiatry, Univ. of Alabama at Birmingham, Birmingham, AL 35294-0017.

Neuronal PC12 cells that are deprived of serum and nerve growth factor (NGF) undergo apoptosis. Although apoptosis normally plays an important role during development, it has been implicated in neurodegenerative diseases such as Alzheimer's disease (AD). Apoptotic cells shrink, and exhibit nuclear and chromatin condensation, and membrane blebbing. These events involve significant alterations of the cytoskeleton. Since the microtubule-associated protein tau has been identified *in vitro* as a mediator of microtubule stability, we examined the protein levels and phosphorylation state of tau during PC12 cell apoptosis. After 72 hours of serum and NGF deprivation, the majority of PC12 cells were apoptotic. During this time period, an increase in the phosphorylation of tau Ser/Pro sites within the epitope of the phosphate-dependent Tau-1 antibody was observed. Interestingly, in AD tau is hyperphosphorylated at the Tau-1 epitope, among others.

Although biochemical processes of apoptosis remain largely unknown, the involvement of cell cycle proteins such as cyclin-dependent kinases (cdks) during apoptosis have been reported. During PC12 cell apoptosis, cdc2 kinase levels and histone H1 kinase activity in $p13^{suc1}$ precipitates were significantly increased. Additionally, the catalytic subunit of protein phosphatase 2B (PP2B) significantly decreased concomitant with a large decrease in PP2B activity that occurred very early in the apoptotic process. As a consequence of decreased phosphatase and increased kinase activities during PC12 cell apoptosis, the phosphorylation state of several proteins including tau may be altered. Thus, the study of tau phosphorylation in neuronal apoptosis may provide pertinent information as to the mechanisms responsible for tau hyperphosphorylation in AD brain.

Supported by NIH grants N527538, AG12396, and AG06569.

227.8

EFFECTS OF TYROSINE KINASE ANTAGONISTS ON NGF-INDUCED SURVIVAL AND ACTIVATION OF TRKA IN SYMPATHETIC NEURONS, Cesar Sanz-Rodriguez, Eugene M. Johnson, Jr., and James L. Franklin*. Dept. of Molecular Biology and Pharmacology, Washington Univ. Sch. of Med., St. Louis, MO 63110

Binding of ligands to tyrosine kinase growth factor receptors causes phosphorylation of the receptors on tyrosine residues. A number of effector molecules then bind to these residues and become activated. In order to study how phosphorylation of the NGF tyrosine kinase receptor TrkA relates to NGF-survival-signaling, we first characterized the ability of four pharmacological agents (K252-a, staurosporine, 5'-S-methyladenosine, and herbimycin A) to cause inhibition of TrkA phosphorylation in sympathetic neurons exposed to NGF in culture. All of these antagonists caused a dose-dependent suppression of TrkA phosphorylation in response to NGF. We then tested the effect of these inhibitors on neuronal survival. Withdrawal of NGF caused complete loss of tyrosine phosphorylated TrkA within 2 hours of deprivation. K252-a, staurosporine, 5'-S-methyladenosine, and herbimycin A at concentrations which reduced tyrosine phosphorylation to levels comparable to NGF deprivation blocked the survival response to NGF. However, block of tyrosine phosphorylation of the receptor by as much as 90% did not suppress survival. These results suggest that only low levels of TrkA receptor phosphorylation are necessary to transduce the survival-promoting action of NGF.

Supported by a grant from the Ronald McDonald Foundation.

227.9

IGF-1 PROTECTS NEURONS FROM HYPEROSMOTIC-INDUCED PROGRAMMED CELL DEATH THROUGH THE PI-3 KINASE SIGNALING PATHWAY. C. van Golen* and E.L. Feldman. Neuroscience Program and Department of Neurology, University of Michigan, Ann Arbor 48109.

We are interested in the potential therapeutic value of insulin-like growth factor-I (IGF-I) in the treatment of neurological disorders such as diabetic neuropathy. We have used SH-SY5Y neuroblastoma cells, a clonal cell line structurally resembling developing neurons, exposed to hyperosmotic stress as an *in vitro* model of diabetic neuropathy. When these cells are exposed to hyperosmotic stress induced by mannitol, approximately 65% of them die by an apoptotic mechanism by 48 hours. We have previously shown that addition of IGF-I to the media will prevent the death of these cells.

IGF-I is known to activate at least two signaling pathways upon binding to its receptor, the MAP kinase pathway and the PI-3 kinase pathway. The current study was performed to investigate the potential role of the PI-3 kinase pathway in mediating IGF-I's protective effects. SH-SY5Y cells were plated at a 1 x 10^5cells/cm^2 density in 6 well plates. After 4 hr of serum deprivation, the cells were exposed to 600 mM mannitol ± 10 nM IGF-I and increasing concentrations of wortmannin, an inhibitor of PI-3 kinase activity. The number of apoptotic cells was then measured after 24 hr by staining the cells with propidium iodide and performing flow cytometry. We found that addition of 100 nM wortmannin reduces the number of IGF-I protected neurons by about half. Therefore, we conclude that the activation of the PI-3 kinase pathway through the IGF type I receptor is in part responsible for IGF-I's neuroprotective effects.

Supported by R29 NS32843 and grants from the American Diabetes Association and the Juvenile Diabetes Foundation International.

227.10

INHIBITION OF NEURONAL APOPTOSIS BY PI 3-KINASE DEPENDENT AND INDEPENDENT PATHWAYS. S.R. D'Mello*[1], S.P. Soltoff[2], K. Borodezt[1]. Dept. of Physiology and Neurobiology, University of Connecticut, Storrs, CT 06269 [1], and Div. of Signal Transduction, Harvard Medical School, Boston, MA 02115 [2]

Cerebellar granule neurons die by apoptosis when switched from culture medium containing elevated potassium (25mM KCl)) to medium with low K^+ (5mM KCl). Death in low K^+ can be prevented by insulin-like growth factor-I (IGF-1). To understand how IGF-1 inhibits apoptosis, we examined the role of phosphatidylinositol-3 kinase (PI 3-kinase), a signaling molecule shown by others to be required for NGF-mediated survival in the PC12 cell-line. In primary granule neurons, IGF-1 treatment leads to a robust activation of PI 3-kinase, as judged by lipid kinase assays and by Western analysis of tyrosine phosphorylated enzyme. Activation of PI 3-kinase occurs via tyrosine phosphorylation of the insulin receptor substrate (IRS-I) protein. PI 3-kinase is necessary for IGF-1 mediated survival. Treatment with two distinct inhibitors of PI 3-kinase, wortmannin and LY294002, reduces PI 3-kinase activation by IGF-1 and inhibits its survival promoting activity. Death resulting from a blockade of PI 3-kinase is accompanied by DNA fragmentation, a hallmark of apoptosis, and can be delayed by transcriptional and translational inhibitors. In sharp contrast to the necessity for PI-3 kinase in IGF-1 signaling, elevated K^+ does not activate PI 3-kinase and can maintain cell survival in the presence of PI 3-kinase inhibitors. Therefore, neuronal survival can be mediated by PI 3-kinase dependent (IGF-1) and independent pathways (elevated K^+).

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227.11

MOLECULAR MECHANISMS OF SURVIVAL AND APOPTOSIS IN CEREBELLAR NEURONS. H. Dudek[1], S.R. Datta[1], T.F. Franke[2], D.R. Kaplan[2], R.A. Segal[3], and M.E. Greenberg[1]*. [1]Div. Neuroscience, Children's Hospital, and Dept. of Neurobiology, Harvard Medical School, Boston, MA; [2]Montreal Neurological Inst., Montreal, Quebec; [3]Dept. Neurology, Harvard Medical School and Beth Israel Hospital, Boston, MA.

Using biochemical and molecular genetic approaches we have examined signal transduction pathways that may regulate survival of cultured cerebellar neurons. Activation of the MAP kinase pathway appears to be insufficient for survival; MAP kinase is strongly activated by agents that are poor survival factors, while insulin (which promotes survival) activates MAP kinase poorly. We have also directly tested the effects on survival of a number of factors in the Ras/MAP kinase pathway by using a modified calcium phosphate/DNA transfection protocol for primary neurons. These experiments indicate, for example, that expression of a constitutively active mutant of Ras is insufficient to prevent death. In contrast to these observations, insulin strongly activates the PI3 kinase pathway in these cells. Wortmannin, a PI3K inhibitor, blocks this activation and also induces death. This suggests that activation of this pathway is important for survival, and preliminary results suggest this effect may involve the protein kinase Akt. *funded by NIH grant CA43855 and ACS grant PF4059*

227.12

POTASSIUM-MEDIATED SURVIVAL OF RAT CEREBELLAR GRANULE CELLS MAY DEPEND ON PI-3 KINASE ACTIVITY T.M. Miller*, M.G. Tansey, E.M. Johnson, Jr. and D.J. Creedon Departments of Neurology and Molecular Biology and Pharmacology, Washington University School of Medicine, St. Louis, MO 63110.

Depolarizing concentrations of potassium promote the survival of many neuronal cell types, including cerebellar granule cells. The survival promoting effect of potassium is mediated by calcium entry through L-type calcium channels, which leads to a sustained rise in intracellular calcium. One possible mechanism whereby this increase in intracellular calcium promotes survival is by activating intracellular pathways involved in neurotrophic factor signalling. One such pathway is signalling through PI3-Kinase, which is involved in survival of PC12 cells (Yao and Cooper, *Science*, **267**: 2003. 1995). We have tested whether the survival promoting effect of potassium depolarization in cerebellar granule cells is dependent on the activity of PI3-kinase. Cerebellar granule cells maintained in the presence of serum and 25 mM extracellular potassium undergo apoptosis when switched to serum-free, non-depolarizing conditions. After 48 hours only 40% of the neurons remain viable as measured by calcein AM staining. Potassium depolarization blocks this apoptosis. The survival promoting effect of 25 mM potassium was negated by the addition of 30 μM LY 294002, an inhibitor of PI3-Kinase activity. The cell death induced by LY 294002 was similar to that caused by non-depolarizing conditions. In both situations the dying cells displayed nuclear condensation and the cell death in both cases was blocked by cycloheximide. This suggests that inhibition of PI3-kinase triggered programmed cell death. Cerebellar granule cells can also be maintained in serum-free media containing either 100 ng/ml IGF-1 or 800 μM cAMP. LY 294002 completely blocked the survival promoting activity of IGF-1, but had no effect on cAMP-mediated survival. These results suggest that PI3-Kinase activity is important for neuronal survival mediated by potassium, and IGF-1, but not cAMP. (Supported by NIH grant AG 12947.)

227.13

INHIBITION OF PI-3 KINASE INDUCES APOPTOSIS IN DRG NEURONS IN AN DEPENDENT MANNER **K.M.Rich*, J.X.Tong, M.A.Vogelbaum Department of Neurological Surgery, Washington University School of Medicine. St. Louis, MO 63110**

Dorsal root ganglion(DRG) neurons undergo apoptosis after withdrawal of nerve growth factor(NGF). This trophic factor-induced cell death is age dependent. During the first three weeks in culture, embryonic DRG neurons lose their dependence on NGF for survival. This loss of NGF dependence has been correlated with an increase in intracellular calcium levels during this period. NGF stimulates the tyrosine kinase *trk* receptor which in turn stimulates phosphatidylinositol-3(PI-3) kinase. Inhibition of PI-3 kinase with LY294002 (2-(4-morpholinyl)-8-phenyl-4H-1-benzopyran-4-one) induces apoptosis in sympathetic neurons (communication Dr. Doug Creedon). The ability of LY294002 to induce apoptosis in DRG neurons was examined.

Dissociated DRG neurons from rat embryos were maintained in cell culture for 5, 10, or 21 days at which time they were treated with LY294002. Neurons were stained with crystal violet after 72 hours and cell counts done to assess survival. Massive cell death (99% death) occurred in both 5 and 10 day-old DRG neurons with 25 μM LY294002. In contrast, 21 day-old DRG neurons showed only a 54% loss at concentrations of LY294002 up to 50 μM. Older DRG neurons (21 days in culture) were placed in medium with low calcium. Their intracellular calcium levels decrease to levels similar to the neurons grown in culture for only 5 days. Older neurons maintained in low calcium medium became very susceptible to treatment with LY294002 (50 μM). There was only 50% death in standard Ca^{2+} medium group compared with 97% death in neurons with lowered intracellular calcium. DRG neurons grown in cell culture for 10 days were treated with 50 μM LY294002 and stained with the fluorescent DNA-binding dye Hoechst 33258 after various time points. DNA condensation was found in 9% at 24 hours and 7.4% at 48 hours.

In conclusion, DRG neurons undergo apoptosis after inhibition of PI-3 kinase with LY294002 in a dose-dependent manner. This apoptotic response has similar characteristics with that found after NGF withdrawal. (Support NS NIH 29477)

227.14

CERAMIDE INDUCES APOPTOSIS IN MURINE CORTICAL NEURONS. C. H. Yeh* and D. W. Choi. Center for the Study of Nervous System Injury and Dept. of Neurology, Washington Univ. School of Medicine, St. Louis, MO 63110.

Recent studies have suggested that ceramide can serve as an intracellular mediator of mammalian cell apoptosis, for example that induced by tumor necrosis factor alpha, Fas ligand or ionizing radiation. To begin to examine the possibility that ceramide might also be a mediator of central neuronal apoptosis, we tested the hypothesis that addition of exogenous ceramide would induce apoptosis in cultured murine cortical neurons.

Mixed neuronal and astrocyte cultures exposed to 10-200 μg/ml ceramide (or 10-200 μM concentrations of the C2 synthetic analogue) for 24 hr developed concentration-dependent neuronal apoptosis associated with cell body shrinkage, membrane blebbing, chromatin condensation, DNA fragmentation, and cycloheximide sensitivity. The cell-permeable interleukin-1-β converting enzyme (ICE) inhibitor, Z-val-ala-asp-CH_2F (ZVAD, 100 μM), also blocked ceramide-induced neuronal death. Exposure to higher concentrations of C2 ceramide resulted additionally in glia death. Neurons in cultures nearly devoid of glia retained vulnerability to ceramide-induced apoptosis.

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227.15

ACTIVATION OF THE CERAMIDE-DEPENDENT SIGNAL TRANSDUCTION PATHWAY INDUCES APOPTOSIS IN CULTURED MESENCEPHALIC NEURONS. B. Brugg[1], P.P. Michel[1], Y. Bailly[2]*, Y. Agid[1] and M. Ruberg[1]. [1]INSERM U289, Hôpital de la Salpêtrière, 47, Bd de l'Hôpital, 75013 Paris. [2]CNRS UPR 9009, Centre Neurochimie, 5 rue Blaise Pascal, 67084 Strasbourg, France.

Recent evidence from our laboratory indicates that the degeneration of mesencephalic dopaminergic neurons of patients with Parkinson's disease occurs by the genetically programmed process known as apoptosis. These neurons express the low affinity receptor (p55) for the cytokine TNF-α, which, in patients, is synthesized by microglial cells in their vicinity. The recent description of the ceramide-dependent signal transduction pathway leading to apoptosis, in cells of the immune system, when activated by TNF-α suggests a possible mechanism for the death of dopaminergic neurons in parkinsonian patients. To test this hypothesis, primary cultures of rat mesencephalon were treated with cell-permeant ceramide analogs or liposome-delivered native ceramide, that can activate the signalling pathway downstream of the cytokine receptor.

After 7 days of maturation in vitro, ceramide treatment induced activation of the transcription factor NFκB/Rel and provoked dose- and time dependent apoptosis, reflected morphologically by neurite fragmentation, cell body shrinkage, chromatin condensation and fragmentation. Intranucleosomal DNA degradation could also be evidenced by gel electrophoresis. The death program could be antagonized by inhibition of protein synthesis, elevation of cyclic AMP levels, and appropriate concentrations of serum.

The signal transduction pathway which leads to apoptosis in immune cells following cleavage of ceramide from membrane sphingomyelin can be activated in neurons in vitro with the same consequence. The degeneration of such neurons in patients with Parkinson's disease could result from activation of this mechanism.

Supported by Bioavenir-France

227.16

ACTIVATION OF THE SPHINGOMYELIN PATHWAY INHIBITS PROLIFERATION OF IMMORTALIZED HYPOTHALAMIC GT1-7 CELLS M.A. Sortino*, V. Cardile[1] and P.L. Canonico[2]. Institutes of Pharmacology and [1]Human Physiology, University of Catania School of Medicine and [2]Chair of Pharmacology, University of Pavia School of Dentistry, Italy.

The sphingomyelin pathway, leading to ceramide generation, has ben related to cell cycle arrest and/or induction of programmed cell death by either serum deprivation or specific agonist receptor activation. To study this phenomenon we have used immortalized hypothalamic GT1-7 cells which are known to respond to serum starvation with reduced cell division and viability. Treatment of GT1-7 cells for different lenghts of time (6-48 h) with reported maximally effective concentrations of C8-ceramide and sphingomyelinase (10 μM and 200 mU, respectively) did not produce any change in cell proliferation and survival as assessed by either cell counting, MTT proliferative assay and LDH activity in the extracellular environment. No apparent sign of cell apoptosis was either evident by bisbenzimide nuclear staining or immunodetection of oligonucleosomes. However, a longer exposure (72 h) of GT1-7 cells to the same agents induced a significant, marked reduction of cell proliferation (59.2 and 41.5% for C8-ceramide and sphingomyelinase, respectively). Interestingly, the inhibitory effect of C8-ceramide was completely counteracted by the exogenous addition of phorbol ester (TPA, 100 nM, added during the last 24 h-incubation time) suggesting that also in GT1-7 cells a balance exists between the two pathways initiated by sphingomyelinase hydrolysis. Again this inhibitory effect was not accompanied by pronounced signs of apoptotic cell death.

Supported in part by a grant from CNR (95.04431.CT04) to MAS

227.17

RESTORATION OF ACID SPHINGOMYELINASE - DEPENDENT CERAMIDE GENERATION AND APOPTOSIS IN CELLS FROM NEURODEGENERATIVE NIEMANN-PICK DISEASE PATIENTS. L.A. Peña*, M.P. Santana, E.H. Schuchman, R.N. Kolesnick. Lab. of Signal Transduction, Memorial Sloan-Kettering Cancer Center, N.Y., N.Y. 10021.

An inherited deficiency of Acid Sphingomyelinase (ASMase) results in Niemann-Pick disease (NPD), a fatal neurodegenerative disorder. Neutral sphingomyelinase (NSMase) and ASMase are types of phopholipase C which cleave sphingomyelin into ceramide and phosphocholine. A novel ceramide-mediated signal transduction system has recently been described. Specific neurotrophic factor/cytokine receptors or irradiation of plasma membranes, activate the hydrolysis of sphingomyelin to generate ceramide. Ceramide serves as a second messenger stimulating specific kinases, phosphatases, and transcription factors that activate a final common pathway of apoptosis or cell growth.

Using EBV-transformed lymphoblasts as a model system, we show that induction of apoptosis is impaired in NPD cells. ASMase activity was restored to NPD cells by transient infection of normal copies of ASMase cDNA packaged in retroviral producing cell lines, or by stable transfection of ASMase cDNA in a mammalian expression vector. Furthermore, ceramide generation was restored, as measured by the DAG-kinase assay. When ASMase activity was restored to NPD cells, the ability of Fas antibody (200 ng/ml) and irradiation (20 Gy) to induce apoptosis was also restored, as quantitated by morphological changes in nuclear chromatin stained with Hoechst-33258. These data suggest (i) ASMase and not NSMase is critical for ceramide mediated apoptosis and (ii) gene transfer of ASMase into NPD cells can restore normal cell function and may be a potential therapy for NPD. (Supported by NIH CA57400 & CA61801.)

227.18

MATURE, "NGF-INDEPENDENT", AND IMMATURE, "NGF-DEPENDENT", NEURONS SHARE EARLY BUT NOT LATE PATTERNS OF GENE EXPRESSION AFTER NGF DEPRIVATION. C. van Rooyen, H.M. Tucker*, and S. Estus. Dept. of Physiology, Sanders-Brown Center, University of Kentucky, Lexington, KY 40536

We previously identified a temporal cascade of gene induction in a model of developmental apoptosis, i.e., neonatal rat sympathetic neurons undergoing NGF-deprivation. (Freeman, et al., Neuron 12 343 (1994); Estus et al., J. Cell Biol. 127: 1717 (1994). In this model, *c-jun, cyclin D1*, and *mkp-1* were induced beginning within 5 hours of NGF deprivation, while several genes were induced at 15 hours, including *c-fos, fos B, jun B* and *NGFI-A*. Neuronal death began thereafter and was largely complete by 72 hours. Significantly, the protein product of one of the induced genes, c-Jun, was necessary for apoptosis. To gain clarity into the mechanisms rendering neonatal "immature" neurons susceptible to apoptosis, we are evaluating whether similar genetic changes occur in "older", mature sympathetic neurons, wherein NGF-deprivation results in a protracted neuronal atrophy, with death occurring much later than 72 hours. Sympathetic neurons are maintained for up to 5 weeks in culture, then NGF deprived for various intervals, and RNA isolated. Quantitative RT-PCR data indicate that the induction of *c-jun* in immature and mature neurons is indistinguishable, whereas *c-fos* is induced only in immature neurons. These results suggest the existence of an apoptosis regulator that becomes active upon neuronal maturity and that acts temporally after c-Jun induction to inhibit apoptosis. Supported by NIH grants T32AG00242 and NS34370.

227.19

***c-jun* IS NOT NECESSARY FOR DEVELOPMENTAL CELL DEATH IN THE MAMMALIAN RETINA.** K.-H. Herzog*[1], S.-C. Chen[2] and J. I. Morgan[1]. [1]Developmental Neurobiology, St. Jude Children's Research Hospital, 332 N. Lauderdale St., Memphis, TN 38105; [2]Hoffmann-LaRoche, 340 Kingsland St., Nutley, NJ 07110.

Numerous studies have shown a correlation between c-*jun* expression and cell death. In addition, *in vitro* analysis suggest a causal role of c-Jun in programmed cell death. However, none of these experiments have assessed the contribution of c-Jun in the central nervous system (CNS) *in vivo*. To analyse the contribution of c-Jun to neuronal death, we have used mice that lack a functional c-*jun*-allele through homologous recombination. In the retina, a CNS structure, developmental cell death has been described in some detail. Unfortunately, cell death in the retina takes place in the first two postnatal weeks which is after the time at which c-*jun*-null mice die (~13d in utero). Therefore, to analyse neuronal tissue of c-*jun*-null mice a transplantation method was introduced. For the *in vivo* analysis of c-*jun* , retinas of either mutant or wildtype embryos were removed between E11.5 and E13.5 and allowed to develop in host rats until the theoretical stage of the transplanted eye was postnatal (P) days 6, P9 or P11. Analysis of transplanted eyes by TUNEL and examination of pycnotic nuclei revealed dying cells in -/- and +/+ retinas indicating that c-*jun* is not necessary for natural occurring cell death in the retina. To further assess the involvement of c-Jun, or an interacting factor in development we are currently constructing transgenic mice which either overexpress c-*jun* or a dominant negative form of c-*jun* in defined neuronal populations. These mice will be analysed for cell death and regenerative capacity.

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227.20

MOTONEURONS UNDERGOING APOPTOSIS HAVE INCREASED JNK (SAPK) ACTIVITY BUT NOT ERK1 ACTIVITY

M.A. Glicksman*, K. Walton, A. Basma, N.T. Neff, C.A. Dionne, A.C. Maroney Cephalon, Inc, West Chester, PA 19380

The activation of c-jun N-terminal kinase (JNK) is likely to play a critical role in apoptosis (Estus et al. 1994, Ham et al. 1995, Xia et al. 1995). In contrast, ERK1, has been shown to be non-essential for survival of superior cervical ganglion (SCG) neurons (Virdee and Tolkovsky 1995). In order to determine whether the signaling characteristics of apoptotic death in SCG neurons are paralleled in other cell types, JNK and ERK1 activities were examined in motoneurons undergoing apoptosis. Spinal cord cultures were enriched for motoneurons by metrizamide gradient centrifugation, and found to be approximately 75% immunoreactive for the low affinity neurotrophin receptor, a marker for motoneurons. In the absence of growth factors, motoneurons begin to die between 24-48h as measured by a fluorimetric viability assay. Apoptotic nuclei containing condensed chromatin were evident by Hoechst staining 48h after plating. JNK and ERK1 activities were measured in cell lysates by specific immunoprecipitation followed by *in vitro* kinase assays at various times after cell attachment. A 50% increase in JNK activity at 2 h and a 4-fold increase at 22 h was measured. The increase in JNK activity was not accompanied by changes in ERK1 activity suggesting that changes in ERK1 activity were not required for this process of apoptosis. Therefore, there are similar signaling events shared between SCG neurons and motoneurons undergoing apoptosis. In addition, motoneurons provide a relatively homogeneous neuronal population in which signaling and gene regulation during apoptosis can be studied.

227.21

CYCLIC AMP IMPROVES THE *IN VITRO* SURVIVAL OF MESENCEPHALIC DOPAMINERGIC NEURONS BY DECREASING APOPTOTIC CELL DEATH. X.Huang, W.M.Zawada, K.Prasad* and C.R.Freed. Div. Clin. Pharmacol., Dept. Radiology., Univ. Colorado School of Medicine, Denver, CO 80262.

In the search of a transplant cure for Parkinson's disease, agents which can improve midbrain dopamine (DA) neuron survival have received great attention. Although cyclic AMP (cAMP) has been shown to increase DA neuron survival *in vitro*, the mechanism underlying this effect has not been studied. We investigated the *in vitro* effects of dibutyryl cAMP (dbcAMP), a cAMP analog and 2-chloroadenosine (2-CAD), an adenosine agonist which is known to decrease cAMP levels at high micromolar concentrations, on the rate of apoptotic death of embryonic DA neurons from E15 ventral mesencephalon. Dopamine neurons were identified by tyrosine hydroxylase (TH) immunoreactivity and apoptosis was determined by characteristic nuclear chromatin condensation imaged by Hoechst 33258 fluorescent DNA dye. After 5 days in culture, 100 μM dbcAMP increased the number of surviving TH$^+$ neurons by 33%. This treatment also decreased the rate of the apoptosis observed in TH$^+$ neurons from 6% to 3%. By contrast, 100 μM 2-CAD decreased the number of TH$^+$ neurons 5-fold and increased the rate of apoptosis in these neurons from 7% to 40% on day 5 in culture. Previously, we have shown that a number of neurotrophic factors (IGF-I, bFGF, and GDNF) increase DA neuron survival by decreasing apoptotic cell death. By combining dbcAMP with bFGF and/or IGF-I, or GDNF, dopamine neuron survival was increased above that observed with the growth factor treatment. These results indicate that cAMP increases the survival of DA neurons by decreasing apoptotic cell death. Since cAMP effects on DA neuron survival are additive to those of bFGF and the combination of IGF-I and bFGF, cAMP is likely to act through a signal transduction pathway different from those employed by the growth factors. Supported by NS 18639, GM 07063, and the National Parkinson Foundation.

NEURONAL DEATH: ICE—RELATED RESPONSES

228.1

CHARACTERIZATION OF A cDNA HIGHLY ENRICHED IN CNS THAT SHARES SEQUENCE HOMOLOGY WITH THE ICE/CED-3 FAMILY W. P. Hynicka *and R. N. Pittman. Dept. of Pharmacology, University of Pennsylvania, Philadelphia PA 19104.

MUD 5 is one of several cDNAs isolated in a differential screen to identify messages upregulated during programmed cell death in terminally differentiated PC 12 cells following removal of NGF. Although the role of MUD 5 in programmed cell death is unclear it remains of interest because it is expressed almost exclusively in brain. MUD 5 is currently being characterized using a variety of biochemical, molecular and cell biological approaches. Northern blots indicate a single transcript of 1.4 kb that is expressed primarily or exclusively in brain. MUD 5 expression begins around postnatal day 4 and increases gradually until it reaches adult levels at postnatal day 25. *In situ* hybridization studies of adult rat brain localize MUD 5 mRNA to neurons in cortical and limbic system structures as well as areas of the cerebellum and brain stem.

The transcript has a high GC content, 62% overall, with some areas as high as 71%. Protein sequence analysis indicates that the N-terminal 210 amino acids of MUD 5 shares 25% sequence homology with Ced-3, ICE, and ICH-1s, including the QACR active site. Studies are currently under way to determine if MUD 5 has cysteine protease activity and whether it is a positive or negative regulator of programmed cell death. This work was supported by grant NS32465 from N.I.H.

228.2

MOLECULAR CLONING AND SEQUENCING OF A cDNA FOR RAT *NEDD2/ICH*-1. N.Sato, Y.Uchiyama#, D.M.Prevette* and R.W.Oppenheim. Dept. of Neurobiol. & Anat., Bowman Gray Sch. of Med., Winston-Salem, NC 27157. #Dept. of Cell Biol. & Anat.1, Osaka Univ. Med. Sch., Suita, Osaka, Japan.

Recent studies demonstrate that inhibition of interleukin-1β-converting enzyme (ICE) activity by expression of crmA or peptide inhibitors of the ICE family of cysteine proteases protect neurons from cell death. To furhter investigate the role of ICE family proteins in neuronal cell death, we have isolated cDNA clones for rat Nedd2/Ich-1, which was originally isolated as an mRNA expressed during early mouse embryonic brain development and which encodes a protein similar to ICE and the product of the C. elegans cell death gene ced-3. By use of primers based on the mouse Nedd2 DNA sequence, we first generated rat Nedd2 probes from total RNA of PC12 cells by RT-PCR. Then, a PC12 cell cDNA library (1×10^7clones) was screened using these probes. We isolated several cDNA clones and DNA sequencing of these clones showed these to be the rat homolog of Nedd2/Ich-1. Rat Nedd2/Ich-1 cDNA clones have an open reading frame of 452 amino acids. Predicted amino acid sequence of rat Nedd2/Ich-1 is highly similar to those of mouse (97.3%) and human (91.3%). The sequence QACRG containing the active Cys residue, which is necessary for the proteolytic activity of ICE family proteases, is also completely conserved in rat Nedd2/Ich-1. Rat Nedd2/Ich-1 also has several Asp residues in the presumptive amino and carboxyl cleavage regions similar to other ICE family proteins. This cDNA clone for Nedd2/Ich-1 should be useful for the investigation of neuronal cell death in the developing and adult rat.

228.3

ENZYMATIC PROPERTIES OF RECOMBINANT ICH-1L SUPPORT A ROLE IN MEDIATING APOPTOSIS. Sheryl L. Meyer*, Mark A. Ator, Stephen P. Trusko, Chrysanthe M. Spais, and Richard W. Scott. Cephalon, Inc., West Chester, PA 19380.

Considerable evidence exists demonstrating that the mammalian cysteine proteases homologous to the Ced-3 cell death protein of *C. elegans* are mediators of apoptosis. Family members can be classified into two subfamilies by amino acid homology; those homologous to the prototypic protease of the class, interleukin-1β converting enzyme (ICE), and those with homology to CPP-32. The substrate preference of the latter class implicates those enzymes in the cleavage of various proteins during apoptosis, including poly(ADP-ribose) polymerase (PARP). Because of the abundant expression of the homolog ICH-1L in the nervous system, we were particularly interested its enzymatic specificity. Recombinant truncated ICH-1L (a.a.143-435), CPP-32 and human ICE were expressed using the baculovirus system. All three proteins were processed to active heterodimers and caused premature cell death during expression. Kinetic parameters of partially-purified enzymes were measured with either the ICE-preferred substrate, Suc-YVAD-AMC, or the CPP-32-preferred substrate, Ac-DEAD-AMC, and the behavior of several diagnostic inhibitors was examined. The enzymatic properties of ICH-1L were very similar to CPP-32, suggesting that it belongs to that subfamily. It was also capable of cleaving PARP. These data support the hypothesis that activation of ICH-1L in the nervous system would contribute to apoptotic cell death. Cell-penetrant inhibitors of the CPP-32 subfamily would therefore be expected to ameliorate apoptotic events in neurodegeneration.

228.4

ACTIVATION OF ICE-LIKE PROTEASES AFTER ISCHEMIA. **R. J. DiRocco, V. R. Marcy, D. G. Flood, P. Dobrzanski, R. Siman, R. Scott, P. C. Contreras, M. Miller* and R. V. Bhat. Cephalon, Inc., Dept. of Pharmacology, West Chester, PA 19380.**

Members of the IL-1β converting enzyme (ICE)/CED-3 family of proteins have been implicated in neuronal cell death. The role of specific genes of this family in neuronal cell death, however, remains unclear. To address this issue, we have determined the expression and localization of the cell death genes, *Ice* and *Nedd-2* after seven minutes of global forebrain ischemia in gerbils. Chromatin condensation in the hippocampal CA1 pyramidal neurons was observed within two days, while internucleosomal DNA fragmentation and apoptotic bodies were observed by four days after ischemia. *Ice* mRNA and pro-ICE protein expression increased gradually over the four day period after ischemia. ICE-like immunoreactivity was not altered in neurons, but increased in microglia throughout the hippocampus. On the other hand, *Nedd-2*-like immunoreactivity increased in neurons two days after ischemia, prior to the onset of cell death in CA1 and diminished thereafter, as CA1 neuronal cell death progressed. These results suggest that *Ice* could indirectly contribute to tissue damage after ischemia via an inflammatory response. The role of *Nedd-2* in CA1 neuronal death after ischemia remains to be further evaluated.

228.5

Expression of an ICE-Related Protease (IRP) During Early CNS Development and Following Transient Global Ischemia. S. M. Paul[1,2*], X. Wu[1], Y. Su[1], D. Stephenson[1], B. Smalstig[1], K. Rash[1], J. Clemens[1] and B. Ni[1]
[1]Lilly Research Laboratories, Indianapolis, IN 46285; [2]Departments of Pharmacology, Toxicology & Psychiatry, IU School of Med., Indpls., IN 46285

The death of central neurons is widely recognized as a normal feature of vertebrate CNS development. Although the precise mechanisms underlying the fatal selection of neurons during development remains a mystery, previous studies suggest that their death occurs via apoptosis. We have recently cloned an ICE-like protease from rat brain which is quite homologous to human cysteine protease-P32 (CPP32) and which may represent the rat homologue of this ICE-related protease (IRP). A dramatic upregulation of the IRP mRNA is associated with K^+-deficiency-mediated apoptosis of cultured cerebellar granule neurons (see abstract by Ni et al.). We now report the developmental expression of this IRP and the selective protracted expression of the IRP transcript in CA1 pyramidal neurons following transient global ischemia. Expression of the IRP mRNA was determined during pre- and postnatal development of the rat CNS using *in situ* hybridization and Northern blot analyses. IRP mRNA in brain is predominately confined to pre- and early postnatal development (from E17 to PND5). IRP mRNA is selectively expressed in neuron-enriched regions of the developing brain, such as the cerebral cortex, hippocampus and cerebellum. IRP mRNA expression is dramatically decreased after PND10, since only trace levels of the IRP mRNA can be detected in most regions of the mature rat brain. Transient forebrain ischemia, which results in the apoptotic death of CA1 hippocampal neurons, induces IRP mRNA expression in hippocampal pyramidal neurons (CA1-4) at 24 hr following ischemia. However, a marked reduction in the expression of the IRP mRNA occurs in CA2-CA4 pyramidal neurons by 72 hr and thus a highly elevated expression of IRP mRNA remains confined to CA1 pyramidal neurons just prior to cell death. Our data suggest that this IRP, closely related to CPP32, may play an important role in the fatal selection of neurons during CNS development and may be responsible for the apoptotic death of CA1 hippocampal neurons following global forebrain ischemia.

228.6

ICE AND CYCLIN D1, BUT NOT BCL-2 mRNA EXPRESSION INCREASE FOLLOWING FACIAL NERVE AXOTOMY W. Tetzlaff, J.L. Vanderluit, K.C. Harrington, N.R. Kobayashi, J.D. Steeves* and J. Rossiter[1]
Depts. of Zoology and Surgery, U.B.C., Vancouver, B.C., Canada V6T 1Z4, [1]Queen's University, Kingston, Ontario, Canada K7L 3N6.

Motoneurons become more vulnerable to insult if previously axotomized. We are therefore examining whether this is reflected by a shift in the balance of death control genes after the initial axotomy. Interleukin-1-β-converting enzyme (ICE) and family members (nedd-2), *bax*, *bcl-xs*, *bad*, *bak* and cyclin D1 have been demonstrated to promote active forms of cell death whereas *bcl-2*, *bcl-xl*, and *bag* appear to have protective effects against cell death. In adult rats, the left facial nerve was transected at the stylomastoid foramen. Animals were sacrificed at 1 day (d), 2d, 3d, 7d and 14d following axotomy. The facial nuclei were microdissected for RNA extraction followed by reverse transcription and subsequent semi-quantitative (serial dilutions) polymerase chain reaction (PCR). 2d following axotomy, we found a 2-4 fold increase in ICE mRNA which remained elevated for at least 14 days. Similarly, a 2-4 fold increase in the mRNA of the cell cycle regulator, cyclin D1 was observed between 2 and 14d after axotomy. The level of mRNA expression for *bcl-2*, *bcl-xl* and *bcl-xs* did not change with axotomy. Thus, *bcl-2* and *bcl-xl* do not appear to respond to the increase in death promoting genes in this *in vivo* model. Preliminary data indicate an increase in mRNA, for *bax* and no changes for *bag* and *bad*. These data indicate a shift towards death promoting genes after an initial axotomy, which may explain enhanced susceptibility to a subsequent trauma.

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228.7

YVAD-CHO, AN ICE SPECIFIC INHIBITOR, CAN PREVENT MOTONEURON DEATH INDUCED BY AXOTOMY IN NEONATAL MICE. F. de Bilbao and M. Dubois-Dauphin* Dept. of Physiology, University Medical Center, 1211 Geneva, Switzerland.

When performed during the postnatal period, the lesion of the facial nerve induces death of facial motoneurons. We have previously reported that neonatal mouse lesioned motoneurons die by apoptosis within 72 hours, with a peak observed 28 hours after the lesion (de Bilbao and Dubois-Dauphin, 1996).

Several lines of evidence indicate that *in vitro* the ICE (interleukin 1β-converting enzyme) proteases family, the mammalian homologs of CED-3, are positive effectors of the cell death process. In order to determine if these proteases could be involved in the model of axotomy-induced cell death *in vivo*, we have administered a peptide inhibitor of ICE, YVAD-CHO, on the lesioned facial nerve of two days old mice (four injections of 10μl at 10μM). The effect of YVAD-CHO on motoneuron death was tested 24 hours after the lesion using the TUNEL method which labelled apoptotic DNA breaks *in situ*.

Our results show that YVAD-CHO can significantly prevent motoneuron death since 32% less TUNEL-labelled motoneurons were observed in treated mice when compared to control ($p<0.05$). These results indicate that ICE or ICE-like proteases may be involved in the cell death processes induced by an axotomy *in vivo*.

228.8

EXAMINATION OF THE ROLE OF THE ICE FAMILY CYSTEINE PROTEASES AND THEIR PUTATIVE SUBSTRATES IN MOTONEURON CELL DEATH L.Li, R.W. Oppenheim, and C.E.Milligan* Dept. Neurobiol. and Anat., Bowman Gray School of Medicine, Winston-Salem, NC 27157

We have previously shown that members of the ICE/Ced-3 family of cysteine proteases are involved in motoneuron(MN) cell death in the chick embryo. We are now attempting to identify the specific protease that mediates cell death. One candidate protease is Cpp-32, which cleaves the nuclear enzyme poly (ADP-ribose) polymerase (PARP) at a tetrapeptide sequence DEVD. We have used a well characterized primary tissue culture system that yields a pure population of MNs that survive in the presence of trophic support (muscle extract, MEX) and die in its absence. The death of MNs requires new gene expression and occurs by apoptosis. When a specific inhibitor of Cpp-32 (DEVD-aldehyde) is added to cultures of MNs deprived of MEX, their death is prevented. Initial western blots of E5.5, E8, E11 lumber spinal cord show that cleaved PARP is increased at E8, when MN cell death in lumbar spinal cord reaches its peak, and then decreased at E11 when MN death is complete. Experiments are in progress to determine whether naturally occurring MN death can be prevented in vivo with the Cpp-32 peptide inhibitor and to determine whether PARP and Cpp-32 are present in dying MNs. These results suggest that Cpp-32 may modulate motoneuron cell death following trophic factor deprivation.

228.9

GENETIC AND METABOLIC STATUS OF NGF-DEPRIVED SYMPATHETIC NEURONS SAVED BY AN INHIBITOR OF ICE-FAMILY PROTEASES. M. Deshmukh,[1] J. Vasilakos,[2] T. L. Deckwerth,[1] P. A. Lampe,[1] B. Shivers[2] and E. M. Johnson, Jr.[1]*
[1]Dept. of Mol. Bio. and Pharm., Wash. Univ. Sch. of Med., St. Louis, MO 63110. [2] Parke-Davis Pharm. Res. Ann Arbor, MI 48105.

Sympathetic neurons from embryonic rat superior cervical ganglion (SCG) undergo programmed cell death (PCD) when deprived of nerve growth factor (NGF). We have used an ICE-family protease inhibitor to examine the function of ICE-family proteases during SCG neuronal death and to assess the metabolic and genetic status of neurons saved by such inhibition. Boc-aspartyl(OMe)-fluoromethylketone (BAF), a cell-permeable inhibitor of the ICE-family proteases, inhibited ICE and CPP32 *in vitro* ($EC50 \sim 5\mu M$) and blocked Fas-mediated apoptosis in thymocytes ($EC_{50} \sim 10\mu M$). BAF also blocked the NGF deprivation-induced death of rat SCG neurons in culture ($EC_{50} \sim 10\mu M$). Compared to NGF-maintained neurons, BAF-saved neurons were smaller and maintained only basal levels of protein synthesis; readdition of NGF restored growth and metabolism. Although BAF blocked apoptosis in SCG neurons, it did not prevent the fall in protein synthesis or the increases in the expression of *c-jun*, *c-fos* and other mRNAs that occur during neuronal PCD, indeed the induction was augmented. This suggests that the ICE-family proteins function after these events during PCD. Commitment point experiments indicated that NGF and BAF rescue SCG neurons with an identical time-course, suggesting that both block apoptosis at either an identical or an adjacent event during neuronal PCD. Supported by NIH grant AG12947.

228.10

ICE-RELATED PROTEASES INVOLVED IN GROWTH FACTOR DEPRIVATION-INDUCED NEURONAL APOPTOSIS. D. Bozyczko-Coyne*, P. Dobrzanski, S. Meyer, T. O'Kane, S. Carswell, R.W. Scott and R. Siman. Cephalon, Inc., West Chester, PA 19380.

Apoptosis is a well-established mode of neuronal death essential to the development of the nervous system and recently implicated in multiple disorders of the adult CNS. With the identification of interleukin-1β converting enzyme (ICE) and a family of related cysteine proteases (IRPs) as contributors to apoptosis of a variety of non-neuronal cells, there is interest in discerning the role of specific proteases and their protein substrates in neuronal apoptosis. Using NGF-differentiated PC12 cells as a model, we sought to (i) determine whether ICE or particular IRPs are important for NGF deprivation-induced apoptosis, (ii) define the time at which proteolysis becomes crucial for the committment to apoptosis, (iii) identify proteins degraded during apoptosis, and (iv) develop a potential histochemical marker for apoptotic proteolysis. Immunoblot and rtPCR analyses revealed that the IRPs Nedd2/ICH-1 and CPP32 were expressed in NGF-differentiated cells, whereas ICE was not. Following NGF deprivation, each of two active site-directed inhibitors of Nedd2/ICH-1 and CPP32 promoted survival of PC12 cells, even when their application was delayed 16 hours after NGF withdrawal. Survival-promoting activity of the protease inhibitors was observed for at least 4 days based on measures of membrane integrity and mitochondrial function. Using an antibody designed to react specifically with a preferred IRP cleavage domain, two protein fragments of ~46 kDa and ~35 kDa were detected by immunoblotting following NGF deprivation. These two fragments co-migrated with products generated when PC12 extracts were incubated with recombinant versions of either ICH-1 or CPP32. When applied to immunohistochemistry, the antibody specifically labelled apoptotic bodies of degenerating PC12 cells. These data implicate either of the IRPs Nedd2/ICH-1 or CPP32 in NGF deprivation-induced apoptosis. IRP-mediated proteolysis is a late molecular event in the neuronal death pathway. An antibody specific for IRP-mediated proteolysis detects two substrates of potential importance to neuronal death, and may be a useful histochemical marker for neuronal apoptosis.

228.11

ACTIVATION OF A CPP32-LIKE ASPARTASE IN NAIVE AND NEURONAL PC12 CELLS UNDERGOING APOPTOSIS FOLLOWING WITHDRAWAL OF TROPHIC SUPPORT. L. Stefanis*, D.S. Park, C.Y.I. Yan, S.E. Farinelli, C.M. Troy, L.A.Greene. Dept. of Pathology and Neurology and Center for Neurobiology and Behavior, Columbia University College of Physicians and Surgeons, New York, N.Y. 10032.

Inhibitors of ICE (Interleukin-1β Converting Enzyme) and other members of the ICE/ced-3 aspartase family inhibit apoptotic cell death in a variety of models, including naive and neuronal PC12 cells and sympathetic neurons following withdrawal of trophic support. However, the inhibitors used are not specific for individual aspartases and the particular family members that are involved in each paradigm are unknown. In the current study, we used specific substrates to differentially measure ICE-like and CPP32-like activities in lysates of naive and neuronal PC12 cells following withdrawal of trophic support. We detected a substantial increase in CPP32-like, but not ICE-like enzymatic activity. This increase was rapid and occured well before morphological signs of cell death. A variety of agents that promote survival in this model, including ATA, NAC, bcl-2 and a number of cell cycle blockers, inhibited the induction of the CPP32-like activity, placing their action at a point further upstream in the apoptotic pathway. Actinomycin-D, an inhibitor of transcription, blocked the induction of the CPP32-like activity in neuronal PC12 cells only, raising the possibility that ongoing or novel transcription may be required for the aspartase activation in neuronal cells. Jun kinase activation temporally preceded the aspartase activation. When applied to living cells, zVAD-FMK, a pseudosubstrate aspartase inhibitor, blocked the activity/activation of the aspartase, but at a concentration about one order of magnitude lower than that required to promote survival. This would imply that the CPP32-like aspartase activation correlates with cell death, but is not the main determinant of apoptosis in this model.

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228.12

DIFFERENTIAL EFFECTS OF SERUM AND POTASSIUM DEPRIVATION ON APOPTOSIS AND CPP32 mRNA EXPRESSION IN RAT CEREBELLAR GRANULE CELLS. B.A. Eldadah*, A.G. Yakovlev, and A.I. Faden, Georgetown Institute for Cognitive and Computational Sciences, Washington, DC 20007.

Apoptosis appears to contribute to delayed neuronal death after traumatic or ischemic brain injury. One of the mechanisms leading to apoptotic cell death may involve a withdrawal of trophic support from neurons through a reduction in growth factors or transmitter-mediated neuronal activity. Primary cultures of cerebellar granule cells serve as an *in vitro* model of apoptosis that can be induced by the deprivation of serum or potassium ions, which are thought to provide trophic support to these cells.

The progression of cell death in granule cell cultures was examined after deprivation of serum or potassium alone and in combination. DNA fragmentation associated with apoptosis was assayed by agarose gel electrophoresis, using a modified resin-based DNA extraction technique, and *in situ* end labeling. In addition, semi-quantitative RT-PCR was used to measure changes in the mRNA levels of CPP32, an ICE-like cysteine protease known to cleave the DNA repair enzyme poly-(ADP-ribosyl) polymerase.

Our results show that, in cultures deprived of both serum and potassium, DNA fragmentation increased markedly by 12 hours after withdrawal, while in cultures deprived of either serum or potassium alone, fragmentation was increased only moderately at the same time point. Furthermore, RT-PCR showed significantly different profiles of CPP32 mRNA expression for each condition. Serum deprivation caused an early peak in CPP32 mRNA levels, while potassium deprivation led to a delayed rise in expression. Combined serum and potassium deprivation produced a progressively increasing pattern of expression. Taken together, these results show an additive effect of serum and potassium deprivation on apoptotic death in cerebellar granule cells and may suggest two pathways for the mediation of programmed cell death. (Supported by CDC CCR306634)

228.13

ACTIVATION OF THE CED3/ICE-RELATED PROTEASE, CPP32, IN CEREBELLAR GRANULE NEURONS UNDERGOING APOPTOSIS BUT NOT NECROSIS. R. C. Armstrong, T. J. Aja, S. Gaur, K. D. Hoang, T. Oltersdorf*, X. Bai, D. S. Karanewsky, L. C. Fritz, and K. J. Tomaselli. IDUN Pharmaceuticals, La Jolla, CA, 92037.

Neuronal apoptosis occurs during nervous system development and possibly in neurodegenerative diseases and following brain injury. Inhibition of Ced3/ICE-related proteases has been shown to inhibit neuronal apoptosis *in vitro* and *in vivo*, suggesting a pivotal role for these cysteine proteases in neuronal apoptosis. We have studied the activation of the Ced3/ICE-related protease, CPP32, in two *in vitro* models of mouse cerebellar granule (Cb Gr) neuronal cell death: low K+/serum deprivation-induced apoptosis and glutamate-induced necrosis. Pretreatment of Cb Gr neurons with an irreversible inhibitor of Ced3/ICE family proteases, ZVAD-fmk, specifically inhibited Cb Gr apoptosis but not necrosis, indicating a selective role for Ced3/ICE proteases in Cb Gr apoptosis. Extracts prepared from apoptotic, but not necrotic, Cb Gr neurons contained a protease activity that cleaved the tetrapeptide substrate DEVD-amc. Affinity labelling of the protease activity with an irreversible Ced3/ICE protease inhibitor (ZVK(biotin)D-fmk) identified two putative protease subunits, p20 and p18, that were present in apoptotic but not necrotic Cb Gr extracts. Western blotting with antibodies to the C-terminus of the large subunit of mouse CPP32 (anti-CPP32) identified p20 and p18 as processed subunits of the CPP32 proenzyme. Anti-CPP32 specifically inhibited the DEVD-amc cleaving activity, verifying the presence of active CPP32 in the apoptotic Cb Gr extracts. These results demonstrate that the Ced3/ICE homolog, CPP32, is processed and activated during Cb Gr neuron apoptosis. The lack of CPP32 activation during Cb Gr neuron necrosis suggests that proteolytic processing and activation of Ced3/ICE proteases are specific biochemical markers of apoptosis.

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228.14

CASCADE OF POTASSIUM DEPRIVATION-INDUCED APOPTOSIS OF CEREBELLAR GRANULE NEURONS J. B. Schulz*, M. Weller, T. Klockgether. Department of Neurology, University of Tübingen, 72076 Tübingen, Germany

Potassium (K^+) deprivation-induced apoptosis of cerebellar granule neurons requires new mRNA and protein synthesis. Using a fluorogenic substrate (DABCYL-YVADAPV-EDANS) for interleukin 1β-converting enzyme (ICE) we show that K^+ deprivation of cerebellar granule neurons induces cycloheximide-sensitive ICE-like protease activity. A peptide inhibitor of ICE-like protease activity, Ac-YVAD-chloromethylketone, prevents K^+ deprivation-induced apoptosis as assessed by staining with fluorescein diacetate. Further, reactive oxygen species (ROS) are essential mediators of K^+ deprivation-induced apoptosis of cerebellar granule neurons since neuronal death is also blocked by superoxide dismutase (SOD), N-acetyl-L-cysteine and free radical spin traps. Using fluorescent assays we show that ROS production after K^+ deprivation is blocked by actinomycin D, cycloheximide, and Ac-YVAD-chloromethylketone, suggesting that ROS act downstream of gene transcription, mRNA translation and ICE activation. Taken together, we show that new mRNA and protein synthesis, activation of ICE-like proteases, and ROS production are sequential events in K^+ deprivation-induced apoptosis of cerebellar granule neurons.

228.15

Cloning and Expression of a Rat Brain ICE-Related Protease (IRP) and Role in KCl Deficiency-Mediated Apoptosis of Cultured Cerebellar Granule Neurons

B. Ni[1]*, X. Wu[1], Y. Du[1], E. Hamilton-Byrd[1], P. K. Rockey[1], P. Rosteck Jr.[1], G. G. Poirier[2], Y. Su[1] and S. M. Paul[1,3]. [1]Lilly Research Laboratories, Lilly Corporate Center, Indianapolis, IN 46285; [2]PARP Metabolism Group, Laboratory of Molecular Endocrinology, Centre Hospitalier de l'Université Laval Research Center and Laval University, Sainte-Foy, Quebec, Canada, G1V 4G2; [3]Departments of Pharmacology, Toxicology and Psychiatry, IU School of Medicine, Indianapolis, IN 46202.

Several members of the ICE (interleukin-1ß converting enzyme) family of proteases have recently been implicated in the intracellular cascade mediating the apoptotic death of various cell types. It is unclear, however, whether ICE-like proteases are involved in apoptosis of mammalian neurons and if so, how they are activated. Here we report the cloning of an ICE-related protease (IRP) from rat brain which is almost identical to the previously characterized human cysteine protease - P32 (CPP32). Overexpression of this IRP cDNA in Hela cells results in cell death accompanied by strong internucleosomal cleavage of DNA, a typical feature of apoptosis. In cultured cerebellar granule neurons, reduction of extracellular K^+ reliably induces apoptosis and stimulates overexpression of this IRP mRNA. The latter is especially prominent 4 hr after switching from high K^+ to low K^+ medium. Expression of IRP mRNA was maintained at this level for at least 8 hr followed by apoptotic cell death and internucleosomal DNA fragmentation. Induction of this IRP mRNA and cell death are completely blocked by adding depolarizing concentrations of KCl $\leq$ 90 min after switching to low K^+ medium (i. e., before the commitment point for apoptosis). Finally, the antibody c-2-10 to poly(ADP-ribose) polymerase (PARP) detected cleavage of this death substrate beginning 8 hr after changing from high K^+ to low K^+ medium, coinciding with the time-course of induced expression of the IRP gene. Our data suggest that overexpression of this IRP, closely related to CPP32, may be involved in the neuronal death cascade in mammalian neurons.

228.16

INHIBITION OF ICE-RELATED CYSTEINE PROTEASES PREVENTS NEURONAL APOPTOSIS T. Lynch[1], J. P. Vasilakos[2], K. Raser[2], K. M. Keane[2], and B. D. Shivers[2]*. [1]Dept. of Neurology, University of Michigan, Ann Arbor, MI 48106; [2]Neuroscience Therapeutics, Parke-Davis Pharmaceutical Research, Division of Warner-Lambert Co., Ann Arbor, MI 48105

Members of the interleukin-1ß converting enzyme (ICE) family of cysteine proteases have been implicated in the regulation of apoptosis. To determine whether these cysteine proteases play an integral role in the low potassium-induced apoptotic death of cerebellar granule neurons (CGN), we tested the effects of a peptidomimetic (active-site) inhibitor, boc-aspartate-fluoromethylketone (boc-Asp-CH_2F), which selectively inhibits ICE and ICE homologs. Primary rat CGN (7 days in vitro) were incubated in 5 mM potassium (K^+) in the presence or absence of boc-Asp-CH_2F at selected time intervals of 6, 18, 24 and 48 hours. Apoptosis was assessed by propidium iodide staining, scanning electron microscopy and DNA fragmentation on agarose gels. Expression of ICE/homologs was analyzed by northern blotting. A single, exogenous application of 20-100 μM boc-Asp-CH_2F blocked apoptosis for up to 48 hours. In addition, neurons treated with boc-Asp-CH_2F and subsequently supplemented at 18 hours with 25 mM K^+ remained viable until 48 hours, suggesting that treated cells retain their ability to respond to trophic factors. The control peptidic fluoromethylketone (boc-Thr-CH_2F), and inhibitors to calpain I (Ac-Leu-Leu-norleucinal), calpain II (Ac-Leu-Leu-normethioninal), and cathepsin B (Z-Phe-Ala-CH_2F) did not prevent apoptotic death at comparable concentrations. Unlike cycloheximide, boc-Asp-CH_2F did not inhibit protein synthesis. Although northern blot analysis did not detect ICE expression in CGN, the homologs Nedd2 and CPP32 were found to increase following induction of apoptosis, making these proteases potential mediators of death in these neurons.

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228.17

POLY(ADP-RIBOSE) POLYMERASE ACTIVATION IS NOT REQUIRED FOR NEURONAL DEATH IN DEVELOPING RAT RETINA. L. Colombaioni.* Istituto di Neurofisiologia del CNR, 56100 Pisa, ITALY.

In neonatal rat retinas, isolated and cultured in defined medium, is possible to induce ganglion cell apoptosis by serum withdrawal (Colombaioni *Soc. Neurosci. Abstr.* 1994, 1995). Recent studies have demonstrated that in many cellular systems cysteine proteases, closely related to the interleukin converting factor (ICE), are critically involved in apoptosis onset. A potential intracellular substrate for ICE-related proteases is the nuclear enzyme poly(ADP-ribose)polymerase (PARP). This enzyme is involved in DNA repair and, when fully activated, can deplete intracellular energy stores to an extent which leads cell to death. Therefore the possibility exists that the proteolytic activation of PARP by ICE-like proteases is a key step in neuronal apoptosis. In the present study, to evaluate the role of PARP activation in serum-dependent death of retinal ganglion cells, the protective effect of an inhibitor of PARP, the 3-aminobenzamide (3AB), was tested. Retinas from newborn P0-P3 rats were cultured in serum free medium. 3AB, added at concentrations ranging from 0.1 to 1mM, did not prevent ganglion cell death, indicating that apoptosis of these neurons is not determined by a PARP-dependent energy depletion. Supported by Italian National Research Council grant.

228.18

ICE-LIKE PROTEASE INHIBITORS AND CERAMIDE PROMOTE SYMPATHETIC NEURON SURVIVAL FOLLOWING NGF WITHDRAWAL IN VITRO. Y. Wen, G. DeGuia, Y. Bai, X.-F. Tian, R. P. Hart, and G. M. Jonakait*, Dept. of Biological Sciences, Rutgers University, Newark, NJ 07102

Cultured neurons derived from neonatal superior cervical (sympathetic) ganglia (SCG) require NGF for survival in vitro. After 10 days in vitro, withdrawal of NGF and addition of antibodies to NGF (Genentech, Inc.) results in the apoptotic death of 54.4 ± 3.1% of the neurons as determined by bisbenzimide staining of nuclei. Because of the homology of ICE-like proteases to the cell death gene *ced-3* and because adult SCG express mRNA for both ICE and its family member CPP-32, NGF was withdrawn in the presence of a pan-ICE inhibitor, Z-VAD-fmk (50 μM). Only 14.3 ± 1.56% of neurons died. A cathepsin B inhibitor (Z-FAD-fmk) had little or no protective effect. By contrast, a granzyme B inhibitor (Z-AAD-cmk) enhanced NGF-induced cell death and killed almost 35% of the neurons even in the presence of NGF. Therefore, ICE-like proteases may be involved in mediating the neuronal cell death resulting from NGF withdrawal.

Because of the possible involvement of ceramide signalling in ICE-mediated cell death, we tested soluble C2 and C6 ceramide and found that both *protected* neurons from NGF withdrawal. A specific inhibitor of phosphatidylcholine-phospholipase C (D609), expected to block ceramide signalling, paradoxically saved neurons from NGF withdrawal. Supported by NINDS and NIMH.

NEURONAL DEATH: p53 AND Bcl FAMILY

229.1

Expression of p53 and several growth factors in denervated striatum after exitotoxic cortical lesion in rats. M. Isono*, Y. Wakabayashi, M. Fujiki, K. Asakuno, T. Mori, S. Hori. Dept. of Neurosurgery, Oita Med. Univ., Oita, Japan 879-55

Ablation of the sensorimotor cortex might induce degenerative changes in the dorso-lateral (DL) striatum. We have been studying the mechanisms of neuronal death underlying these degenerative changea in a surgical cortical ablation model (M. Isono et al, abstract, 25th annual meeting of Society for Neuroscience). In this study, we investigated molecular changes including apoptotic procedure in denervated striatum using animal models of exitotoxic cortical lesion. In this model, degenerative changes might progress relatively slowly compared to those in previous models. In adult male Wistar rats, N-methyl-D-aspartate (5 mg each) was applied extradurally over the sensorimotor cortex. These procedure resulted in progressive degenerative changes of the sensorimotor cortex. Immunoreactivities of p53, bFGF, c-fos protein and GFAP in the denervated ipsilateral DL striatum were examined by ABC method and compared to those of contralateral striatum. Also, DNA fragmentation was investigated by TUNEL method. The observation period ranged from 3 days to 3 months. According to the progression of cortical degeneration, expressions of bFGF, c-fos protein and GFAP became prominent in ipsilateral DL striatum. Interestingly, prominent expressions of p53 were also observed, especially 2 weeks after the operation, quite few cells TUNEL-positive cells were found at the same time. These data suggest not only the potential to regenerate in denervated striatum, but also the possibility that the apoptotic procedure might be involved in denervation-induced degeneration.
(partly supported by a grant from the Ministry of Education, Science and Culture of Japan)

229.2

EVIDENCE FOR p53-MEDIATED MODULATION OF NEURONAL VIABILITY. H. Xiang, D. Hochman, H. Saya, P. Schwartzkroin, R. Morrison*, Dept of Neurol Surg, Univ of Washington, Seattle, WA 98195, Dept of Oncology, Kumamoto Univ. Sch. of Med., Kumamoto 860, Japan

A role for p53-related modulation of neuronal viability has been suggested by the finding that p53 expression is increased in damaged neurons in models of ischemia and epilepsy. These findings were recently confirmed with the demonstration that mice deficient in p53 ("knockout" mice) exhibit almost complete protection from seizure-induced brain injury, whereas wild-type mice display significant neuronal cell loss in the hippocampus and other brain regions. Because the p53 "knockout" mice used in the latter study expressed a global p53 deficiency in all cell types, it was not possible to conclude that protection was conferred by the exclusive absence of p53 in neurons. Therefore, in the present study, we determined whether p53 expression in isolated neurons is directly coupled to a loss of viability. Primary cultures of hippocampal or cortical neurons were derived from animals containing p53 (+/+, +/-) or those deficient in p53 (-/-). p53 deficient neurons appeared identical to wild-type neurons with respect to morphology, neurofilament expression and resting levels of intracellular calcium. Neurons containing at least one copy of p53 were severely damaged by treatment with kainic acid or glutamate. Cell damage was assessed by direct cell counting and by nuclear morphology following propidium iodide staining of DNA. In contrast, neurons deficient in p53 (-/-) exhibited little or no damage in response to excitotoxin treatment. Despite their divergent outcomes, both p53 (+/+) and p53 (-/-) neurons demonstrated sustained elevations in intracellular calcium levels triggered by glutamate exposure. Restoring p53 expression to p53 deficient neurons, using adenovirus-mediated transduction, was sufficient to promote neuronal cell death, even in the absence of excitotoxin. These results demonstrate a direct relationship between p53 expression and loss of viability in CNS neurons.

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229.3

KAINATE INDUCES RAPID BUT REVERSIBLE NEURONAL DAMAGE IN HIPPOCAMPAL CA1 PYRAMIDAL CELLS IN p53-DEFICIENT MICE. Y. Kinoshita*, H. J. Wenzel, M. Kliot, C. Kinoshita, P. A. Schwartzkroin and R. S. Morrison. Department of Neurological Surgery, University of Washington, Seattle, WA 98195-6470.

The tumor suppressor gene, p53, is activated in response to DNA damage and causes growth arrest and/or apoptosis depending on the cellular context of the injury. We previously reported that kainate induces extensive cell loss in hippocampal subregions CA1 and CA3 in wild-type mice; apoptotic cell death was confirmed in CA1 (3-4 days), but not in CA3 degenerating neurons. In contrast, the majority of p53 (-/-) mice displayed no signs of CA1 cell damage at 3-12 days after kainate. However, based on an analysis of much earlier time points, we found that a fraction of p53 (-/-) mice showed clear and extensive morphological damage in CA1 pyramidal neurons 8 hr to 2 days following kainate injection. The peak of damage occurred at day 1 with more than 50% of p53 (-/-) animals showing severe damage. Cresyl violet staining showed that many damaged neurons in p53 (-/-) mice had condensed nuclei, shrunken cell bodies and loss of Nissl substance. They showed light and limited staining with the terminal transferase-based TUNEL method (preferentially stains double-strand breaks) and more intense and frequent staining with the DNA polymerase-based in situ nick translation method (preferentially stains single-strand nicks). The staining pattern of the damaged neurons observed with these methods differed from the typical pattern of delayed neuronal cell death seen in the CA1 region of wild-type mice. Despite the extent of neuronal damage, we did not detect a proportionate degree of neuronal cell loss in p53 (-/-) mice at 4 or 7 days; the number of surviving neurons was similar to that of nontreated control animals, and Fink-Heimer silver impregnation revealed little or no evidence of neuronal degeneration at these time points. Since the recruitment of new-born neurons is highly unlikely in the adult CA1, this observation suggests that the damaged neurons seen at earlier time points in p53 (-/-) mice are not dying but are actually recovering from the initial damage.

229.4

P53 EXPRESSION INDUCES APOPTOSIS IN HIPPOCAMPAL PYRAMIDAL NEURON CULTURES. J. Jordán*[1], M.F. Galindo[1], R.P. Roos[2], J.M. Leiden[3], G.D. Ghadge[2] and R. J. Miller[1]. [1]Pharmacological and Physiological Sciences, [2]Neurology, [3]Medicine, Univ. of Chicago, 947 E. 58th Street, Chicago, IL 60637.

Previous investigations on dividing cells, including neuronal precursors, have demonstrated the importance of p53 in mediating apoptosis caused by irradiation or genotoxic agents. We investigated the effects of p53 on rat hippocampal pyramidal neurons in culture by using an adenovirus that expresses p53 (Adp53; courtesy Schering-Plough Res.Inst.). p53 protein was not detectable in these neurons by Western blot under control conditions, but, 48 h following Adp53 infection, it was clearly detectable. Neuronal death increased greatly during the 72 h period following infection, with death of about 75% after 3 days. 48 h after Adp53 infection about 80% of the neurons were positive for TUNEL staining and displayed chromatin condesation using Hoechst 33348, indicating that p53 causes post-mitotic neurons to undergo apoptosis. We also examined the effect of the cyclin kinase inhibitor p21 and a constitutively active non-phosphorylatable form of retinoblastoma gene product (HΔpRb) by expressing these proteins following infection with recombinant adenoviruses. The 2 proteins did not have a major effect on neuronal viability. When cultures were exposed to X-rays (500 cGy) at 11 days *in vitro*, some neurons showed immunostaining for p53 5 h following irradiation. We investigated the possibility that p21 or HΔpRb expression could modify the apoptosis induced by either irradiation or p53 overexpression. Cultures were infected 3 days prior to x-ray irradiation or Adp53 infection. Overexpression of HΔpRb protected neurons from irradiation induced damage but was unable to prevent death when we reinfected the cultures with Adp53. Under similar conditions, p21 overexpression was unable to protect cells from death due either to irradiation or p53 overexpression. TGF-β1 was ineffective in preventing death due to Adp53 infection but did block irradiation induced death. These results suggest that p53 may mediate some forms of neuronal death, distinct from its actions related to cell cycle arrest, and provide support for its role in neurodegenerative diseases. PHS Grants DA02121, DA02575 and MH40165

229.5

P53 AND BAX ARE INVOLVED IN THE CELL DEATH PATHWAYS OF MOUSE TASTE RECEPTOR CELLS. Q. ZENG and B. OAKLEY* Department of Biology, University of Michigan, Ann Arbor, MI 48105

We investigated immunocytochemical expression patterns of the tumor suppressor p53 and BCL-2 family proteins in adult and developing mouse taste buds and gustatory epithelium in vivo. An antibody against p53 reacted with the nucleus and apical cytoplasm in 10-20% of adult mouse taste cells. Bax, known to be an inducer of cell death and a downstream target of p53, was evenly expressed within 10-20% of elongated taste cells of which some had recognizable apoptotic morphology. Double-labeling revealed taste cells with both p53 and Bax. BrdU labeling indicated Bax-positive taste cells were at least 5 days old. These lines of evidence are consistent with p53-promotion of bax and cell death. However, p53 was not essential for Bax-expression in taste cells. Postnatal day 3 mice had 0-2 Bax-positive cells per taste bud, but the first p53-positive taste cell was not evident until postnatal day 4. This suggested some Bax-expression may be independent of p53. Bax-independence was confirmed by the observation of numerous Bax-expressing taste cells in adult p53-gene null mutant mice. Squamous epithelial cells of the tongue did not express p53 or Bax.

Support: NIH

229.6

INCREASED EXPRESSION OF p53-TARGET GENES, BAX AND GADD-45 IN RAT BRAIN FOLLOWING EXCITOTOXIC LESION. P.E. Hughes*, T. Alexi, T. Yoshida, S.S. Schreiber and B. Knüsel. Andrus Gerontology Center and Depts of Neurology, Cell and Neurobiology, Univ. of Southern Calif. and Dept. of Physiological Science, UCLA, Los Angeles, CA.

In various cell lines p53, induced by DNA damage, has been shown to function to halt cell cycle progression (perhaps by increasing p21/WAF1 expression) and under certain circumstances to induce programmed cell death or apoptosis. Previously we have shown that excitotoxic lesioning of rat brain with intrastriatal injection of quinolinic acid (QA) increases p53 mRNA and protein in injured brain regions and that the expression of p53 mRNA clearly precedes DNA damage detected by TUNEL. Therefore, rather than being induced as a result of DNA damage it is possible that the expression of p53 may be involved in initiating neuronal apoptosis and therefore DNA fragmentation. Since p53 is a known transcription factor we have examined the expression of two p53-responsive genes, Bax (Bcl-2 associated X protein) and Gadd-45 (growth arrest- and DNA damage-inducible gene-45) in rat brain lesioned by intrastriatal QA. Here we show increased expression of Gadd-45 (preceding p53 expression) and down-regulation then re-expression of Bax (following p53 expression) in rat brain following intrastriatal QA. These results suggest that p53 may initiate neuronal apoptosis perhaps by regulating the ratio of Bax/Bcl-2.

229.7

THE DNA DAMAGE-INDUCIBLE GENE GADD45 IS OVEREXPRESSED IN THE RAT BRAIN FOLLOWING KAINIC ACID-INDUCED EPILEPTIC SEIZURES R.L. Zhu*, J. Chen, K.L. Jin, R.A. Stetler, R.P. Simon, and S.H. Graham, Dept of Neurology, University of Pittsburgh, Pittsburgh, PA 15261

Generation of oxygen free radicals and oxidative DNA-damage have been implicated in the pathogenesis of brain injury due to epileptic seizures and other neurodegenerative diseases. However, the molecular events that are associated with DNA oxidation and repair in these settings remain unclear.

GADD45 is one of the five GADD (growth arrest and DNA damage-inducible) genes that were originally identified by their induction in UV-irradiated Chinese hamster ovarian cells. GADD45 is induced by many DNA-damaging stimuli in a variety of mammalian cells. Recently, it has been found that GADD45 protein can bind to the nuclear protein PCNA and stimulates DNA excision repair. This study has examined the expression of GADD45 mRNA and protein using in situ hybridization, Northern blotting, Western blotting and immunohistochemistry in rat brains after kainic acid (KA)-induced seizures.

Both GADD45 mRNA and protein are expressed at very low levels in control brains. At 2-4h after KA injection, expression of GADD45 mRNA was remarkably increased in a variety of neuronal cell populations including hippocampal CA1-3 pyramidal neurons and dentate granular cells, neurons in the cortex especially the piriform cortex (Pr), caudate putamen (CPu), and the thalamus (Tl). At 8-16 h, mRNA was mainly increased in CA1-3 neurons and Pr but subsided in other regions. At 24h, mRNA was still moderately increased in CA1 neurons but decreased below control levels in CA3 and Pr. At the protein level, increased GADD45 expression was detected in neurons in CA1-3, DG, CPu, Tl, and cortex at 4-8 h. At 16-24 h, GADD45 protein was persistently increased in CA1 neurons and dentate gyrus; but decreased in CA3 and Pr. Histology revealed that sustained cell loss occurred in CA3 and Pr.

These results suggest that increased expression of DNA-repair genes such as GADD45 may be an important mechanism by which neurons recover from injury after epileptic seizures.

229.8

CELL DEATH INDUCED BY NIGERICIN IS SUPPRESSED BY OVEREXPRESSING BAX IN MN9D CELLS WHEREAS STAUROSPORINE-MEDIATED APOPTOSIS IS ACCERELATED. Jae H. Oh[1], Karen L. O'Malley[2], Stanley Krajewski[1], John C. Reed[1] and Young J. Oh[1]. [1]Dept. of Biology, Yonsei University, Seoul 120-749, Korea, [2]Dept. of Anatomy & Neurobiology, Washington University School of Medicine, St. Louis, MO 63110, USA, and [1]La Jolla Cancer Research Foundation, Cancer Research Center, La Jolla, CA 92037, USA

Bax has been shown to heterodimerizes with Bcl-2 and counters the protective effects of Bcl-2 in a hematopoietic cell line. Recent studies, however, suggest that Bax may subserve more diverse functions depending on the cell type and/or the apoptotic stimuli. To test the hypothesis that Bax function might vary depending upon the cell death inducing stimuli in a given cell type, we established and characterized a murine dopaminergic neuronal cell line (MN9D) stably expressing murine (MN9D/mBax) or vector alone (MN9D/Neo). Previously, we have shown that both the proton/potassium antiporter blocker, nigericin, and protein kinase inhibitor, staurosporine, induced apoptosis in the MN9D. In either paradigm, cell death could be attenuated by the overexpression of Bcl-2. In these experiments, MN9D/Neo and MN9D/mBax cells were treated with either 1.0 uM staurosporine or 0.1 uM nigericin and subsequently assayed using MTT method. Overexpression of Bax in the MN9D cells suppressed cell death induced by nigericin (86.6 ± 7.8% vs 22.0 ± 7.2% in 24 hrs of incubation) in a manner comparable to that seen with the MN9D/Bcl-2 cell line. In contrast, overexpression of Bax accerelated apoptotic cell death following treatment with staurosporine (27.0 + 4.6% vs 62.3 + 5.7% in 12 hrs of incubation). In both experiments, the magnitude of DNA fragmentation corresponded to the extent of cell death. Thus, depending upon the apoptotic stimuli, Bax subserves at least two and opposite functions in MN9D cells: acceleration or repression of apoptosis. (supported in part by grants from Ministry of Education and MH45530)

229.9

UPREGULATION OF BAX PROTEIN IN RETINAL GANGLION CELLS AFTER OPTIC NERVE LESION. S. Isenmann*, C. Wahl, S. Krajewski[1], J. C. Reed[1], and M. Bähr. *Neurologische Universitätsklinik, Hoppe-Seyler-Str. 3, D-72076 Tübingen.* [1]*La Jolla Cancer Research Foundation, La Jolla, CA, USA.*

We have investigated Retinal Ganglion Cell (RGC) death after crush lesion to the rat optic nerve (ON). Retina sections were examined 30 minutes to 105 days after lesion. Morphologic features suggested that at least part of RGC death occurs by apoptosis. Quantitative evaluation of TUNEL staining for DNA cleavage showed TUNEL positive cells between 3 and 8 days after proximal ON crush, with a peak at 6 days. No TUNEL positive RGCs were seen in control retinae and in retinae examined 55 and 105 days after crush lesion.

Bcl-2 is known to be a key regulator of apoptosis and has great potential to suppress cell death. Bax is a Bcl-2 homologue with strong Bcl-2 antagonistic, pro-apoptotic potential. To test whether proteins of the Bcl-2 family are involved in RGC death after lesion, we carried out immunocytochemical stainings using Bcl-2 and Bax antisera. Bcl-2 protein was readily detected in the majority of cells in the RGC layer in control retinae and during the first hours after optic nerve crush. Signal intensity decreased markedly within several days after lesion. In contrast, Bax was expressed at varying levels: while in most RGCs immunoreactivity was faint to moderate, scattered cells were strongly stained even in normal adult retina. The level of Bax expression increased as soon as 30 minutes after lesion, with more RGCs strongly positive for the protein. Increased Bax immunoreactivity was detected for at least two weeks after lesion.

We carried out double labeling studies for TUNEL and Bax in order to examine if RGCs undergoing DNA fragmentation were the ones that had upregulated Bax expression. However, in none of the sections processed, co-localisation of the two signals was detected. Thus, it seems likely that Bax expression precedes DNA fragmentation.

Our results suggest that regulation of Bcl-2 family proteins is involved in the induction of RGC death in response to ON lesion. These findings might bear consequences for future therapeutic strategies to prevent apoptotic cell death by modification of the ratio of anti- and proapoptotic Bcl-2 family proteins.

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229.10

Bax is required for naturally-occurring cell death in developing mouse lumbar dorsal root ganglia and motor pools. F.A. White[1]*, C.M. Knudson[2], S.J. Korsmeyer[3], and W.D.Snider[1] Dept. of Neurology, CSNS[1], Dept of Pathology[2], and Dept. of Medicine[3], Washington Univ., St. Louis, MO, 63110.

The bcl-2 family of apoptosis regulators contains both death inhibiting and death promoting members. Recent targeted deletion studies have identified bcl-x, a death inhibiting member, as a critical regulator of neuronal survival at early developmental stages. The function of the death promoting members, bax, bad, and bak, in relation to neuronal survival during development is unclear. To clarify the influence of these death promoting proteins in the developing nervous system, we examined the degree of apoptosis occurring in the well-characterized embryonic mouse lumbar spinal cord and dorsal root ganglia (DRG) in mice with a *bax* null mutation (Knudson et al., Science, 270:90). We compared *bax*(-/-) and *bax*(+/+) mice between embryonic day 13.5-15.5 using the TUNEL method.

In lumbar DRGs of E13.5 *bax*(+/+) animals, 41+/-4 cells/section (n=3) exhibited apoptotic figures while *bax*(-/-) mice exhibited virtually no cell death in DRG (n=4). At E14.5, *bax*(+/+) animals had on average 64 cells per DRG section, versus *bax*(-/-) animals which exhibited an average of less than 1 apoptotic figure per DRG section. Similiar to the DRG results, the ventral horn of E14.5 *bax*(+/+) mice exhibited 16 apoptotic figures per section while *bax*(-/-) animals exhibited an average of less than 1 apoptotic figure per spinal cord section. Lumbar DRGs of E15.5 *bax*(+/+) and *bax*(-/-) animals exhibited an average of 19+/-7 apoptotic figures per section (n=2) and less than 1 apoptotic figure per section (n=5), respectively. In sharp contrast, the occurence of cell death in the differentiating hindlimb, dermomyotome, and viscera did not appear to be affected in *bax*(-/-) mice, although there appeared to be higher degree of apoptotic figures in the developing gonads. These results show that the expression of bax, a death promoting bcl-2 homologue, is required for programmed cell death of sensory and motor neurons during normal development. Surprisingly, bax functions appear to be essential primarily for the nervous system as death in many other tissues occurs normally. Supported by MDA and NIH.

229.11

CHOLINERGIC NEURON-SPECIFIC OVEREXPRESSION OF BAX IN ChAT-BAX TRANSGENIC MICE J.D.Cooper*, T.M.Michaelidis, G.Tzimagiorgis, M.Sendtner†, M.Meyer and H.Thoenen

Department of Neurochemistry, Max-Planck-Institute for Psychiatry, 82152 Martinsried, Germany and †Neurological Clinic, University of Würzburg, 97080 Würzburg, Germany.

Bax has been shown to promote cell death in vitro by antagonizing the anti-apoptotic action of bcl-2. Bax mRNA and protein levels are up-regulated in neurons which are destined to die after various insults. However, the physiological role of bax in the mechanisms which influence neuronal survival remains unclear. To study the effect of overexpressing bax in defined populations of neurons we have generated transgenic mice in which expression of bax is driven by the promoter region of the murine choline acetyltransferase (ChAT) gene. We have previously shown that a 4kb EcoRI/HindIII fragment of the 5' flanking sequences of the murine ChAT gene confers NGF inducible expression of reporter genes in transiently transfected PC12 cells but not NIH 3T3 fibroblasts. This ChAT promoter fragment was linked to a construct containing coding and endogenous intronic sequences of the mouse bax gene. Pronuclear injections of this ChAT-bax construct have generated several lines of transgenic mice. Regional analysis of the CNS of ChAT-bax transgenic mice by RT-PCR and Western blot revealed high levels of transgene mRNA and bax protein in areas which contain large numbers of cholinergic neurons. In situ hybridization and immunohistochemical analysis confirmed that bax mRNA and protein are specifically expressed in cholinergic neurons. We observed a rostro-caudal gradient in the level of bax expression with highest levels in the spinal cord and facial nucleus and lower levels of expression in the cholinergic basal forebrain. The in vivo effects of increased levels of bax in the cholinergic system will be presented. (JDC Funded by a fellowship of the Human Capital and Mobility Program of the EC).

229.12

CELL DEATH GENE EXPRESSION & BAX KNOCKOUT EFFECTS IN THE DEVELOPING BARREL NEURAXIS. K.E. Good, H.-X. Dong, B.L. Schlaggar, J.J.A. Arends, C.M. Knudson, S. Korsmeyer, F.A. White, W.D. Snider* & M.F. Jacquin. Neurology & Center for the Study of Nervous System Injury, Washington Univ. Sch. Med., St. Louis, MO 63110.

It has previously been hypothesized that programmed ganglion cell death is necessary for pattern formation in the whisker-barrel neuraxis (Henderson et al., *J. Neurosci.*, '94). The bcl-2 gene family which regulates apoptosis has both death inhibiting (bcl-2, bcl-x) and death promoting (bax, bad, bak) members. Therefore, imunohistochemical patterns of cell death gene expression bear upon the cell death hypothesis as it pertains to pattern formation. In rats on postnatal days (P) 0-5, Bax expression occurred in the cortical plate, subplate and underlying white matter, and it diminished from P7-15. On P7, Bcl-2 was expressed in cells of cortical layers IV, V, VIa, the subplate and underlying white matter. On P0, p53 was expressed in the cortical plate, subplate and underlying white matter, whereas c-jun and c-fos were not. In the rat brainstem, Bax and Bcl-2 staining occurred in the P5-10 trigeminal root/tract, possibly in oligodendrocytes. Nucleus principalis was heavily labeled for Bax prior to birth, for p53 at birth, and for Bcl-2 on P4-5; few if any cells expressed c-jun or c-fos at birth. These staining patterns, in general, support the above-stated cell death hypothesis. However, in 3 adult mice homozygous for Bax gene deletion, whisker-related patterns were normal in the brainstem, thalamus and cortex, as determined by cytochrome oxidase histochemistry. Insofar as other studies suggest that programmed cell death is ameliorated in Bax knockout mice, the apparent normal development of barrel-like patterns fails to support this hypothesis. Ganglion cell numbers are currently being assessed in Bax knockout mice that have normal whisker-related patterns in the CNS. Supported by NIH DE07734, NS17763.

229.13

APOPTOSIS OF *BCL-X*-DEFICIENT TELENCEPHALIC CELLS IS REDUCED BY INSULIN-LIKE GROWTH FACTOR I (IGF-I) *IN VITRO*. K.S.Shindler* and K.A.Roth‡. Depts. of Pathology and ‡Molecular Biology and Pharmacology, Washington University School of Medicine, St. Louis, MO 63110.

Targeted disruption of the *bcl-x* gene causes massive cell death in the intermediate zone of the embryonic central nervous system (CNS), suggesting that Bcl-x plays a critical role in the regulation of immature neuron survival. This massive death is recapitulated *in vitro* by dissociated neuroepithelial cells from the embryonic day 12 telencephalon of *bcl-x*-deficient mice grown in basal media for 48 hours. *bcl-x*-deficient cells cultured in chemically-defined serum-free media (ITS), or in media supplemented with fetal calf serum (FCS), however, do survive and differentiate into neurons, indicating that factors present in ITS and FCS may promote *bcl-x*-independent anti-apoptotic pathways. To determine the ability of known trophic factors to stimulate these pathways, telencephalic cells from *bcl-x*-deficient, heterozygous, and wild-type littermates were cultured for 48 hours in basal media in the presence or absence of factor. Neurotrophin-3, neurotrophin-4/5, and brain-derived neurotrophic factor all failed to rescue *bcl-x*-deficient cells from increased cell death. In contrast, IGF-I demonstrated a significant survival effect, as fewer apoptotic cells, identified by clumped nuclear bisbenzimide staining, and more viable neurons, identified by microtubule-associated protein 2 immunoreactivity, were present in IGF-I treated cultures than in untreated cultures. Not all cells were rescued, however, as IGF-I treated *bcl-x*-deficient cultures did not contain as many neurons nor as few apoptotic cells as heterozygote and wild-type cultures. These results indicate that IGF-I can promote the survival of immature telencephalic neurons independent of the cell death inhibitor Bcl-x.

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229.14

BCL-XL OVEREXPRESSION IN TRANSGENIC MICE PROTECTS FACIAL MOTONEURONS AGAINST AXOTOMY-INDUCED CELL DEATH. A.Sh. Parsadanian*, C.R. Keller-Peck, J.L. Elliott, J.C. Harding, R.W.Gerfen, W.D.Snider. CSNSI, Dept. of Neurology, Washington Univ. Sch. of Med., St.Louis, MO 63110.

Bcl-x is a death-inhibiting member of the Bcl-2/Ced-9 family and is expressed in a long and short form. Like Bcl-2, in vitro the long form (Bcl-xL) is a potent inhibitor of apoptosis after trophic factor deprivation. In contrast to Bcl-2, Bcl-xL is required for neuronal survival during embryonic development. Also unlike Bcl-2, we have shown that Bcl-xL expression is upregulated in the postnatal period. In order to explore further the role of Bcl-xL in neuronal cell death in vivo and better understand the mechanisms by which it acts, we generated transgenic mice overexpressing Bcl-xL under the control of the α-tubulin-1 promoter (A.Gloster et al., J.Neurosci., 1994,14, 7319-7330). Twelve transgenic founders were obtained and analysed by Southern blot and in situ hybridization. The copy numbers and Bcl-xL mRNA expression pattern are varied between different lines. Lines 7194 (60 copies), 7199 (20 copies) and 7193 (80 copies) have been choosen for detailed analysis. Line 7194 shows high expression of the transgene in the cerebral cortex, CA1 and CA2 region of hippocampus, but not in CA3 region or dentate gyrus. It is not expressed in the cerebellum and is expressed in only a few motoneurons in the facial motor nucleus. In the line 7199, Bcl-xL is expressed at intermediate levels in cortex, hippocampus and brainstem including facial nucleus motoneurons. In the line 7193 Bcl-xL is expressed at high level in cerebellar granular cells (but not in Purkinje cells) and in motoneurons in brainstem, and to a lesser extent in hippocampus and cortex. In order to test whether Bcl-xL can prevent neuronal death in the postnatal period in vivo, we unilaterally transected facial nerve of neonatal wild-type and transgenic mice. Seven days post-axotomy the number of facial motoneurons were counted. In the line 7199 about 30% of the motoneurons survive in the lesion site vs. 7% in contralateral nucleus. The effect of the Bcl-xL is more dramatic in the line 7193. About 50% of the motoneurons survive 7 days after axotomy, consistent with the prominent expression of Bcl-xL in facial nucleus in this line. Our results show that Bcl-xL, like its homolog Bcl-2, can significantly protect neurons in vivo from apoptosis. Supported by NIH , MDA.

229.15

INTRACELLULAR LOCALIZATION OF BCL-2, BCL-XL AND BAX IN PC12 CELLS AND RAT CENTRAL NEURONS. T. Gotow[1], S. Waguri[1], Y. Ohsawa[1], T. Watanabe[1], Y. Tsujimoto[2], E. Kominami[3] and Y. Uchiyama*[1]. Dept. of Cell Biology and Anatomy I[1] and Biomedical Research Center[2], Osaka Univ. Med. Sch., Suita, Osaka 565, Japan and [3]Dept. of Biochemistry, Juntendo Univ. Sch. of Med., Tokyo 113, Japan.

Bcl-2 and its related proteins, BclxL and Bax, play essential roles in the regulation of apoptotic cell death, although it still remains unknown how they control the death process. To elucidate their regulation mechanism, we need to define which cellular components are targets for these proteins, since the Bcl-2 localization has been reported without consensus to be in association with certain organelles. We applied subcellular fractionation and immunocyto-chemistry including a cryothin-colloidal gold method with various antibodies against human, rat, mouse and chicken Bcl-2, Bcl-xL or Bax to PC12 cells transfected with or without the human *bcl-2* gene and normal rat central nervous tissue. With confocal laser microscopy, immunoreactivities for these three proteins showed the identical distribution, similar to that of Rhodamine 123, being prominant in perikarya and neurite tips in PC12 cells. The immuno-fluorescence was faint in wild PC12 cell, significantly enhanced in NGF-treated cells and much stronger in *bcl-2*-transfected cells. In the central nervous tissue, immunoreactivity was conspicuous in neuronal perikarya. Fractionation of *bcl-2*-transfected PC12 cells demonstrated that Bcl-2 was almost equivalent in distribution to mitochondria and ER/membrane fractions. With electron microscopic quantification of PC12 cells, immunogold particles were selectively associated with inner mitochondrial and ER/vesicular membranes, and hardly with any other membrane system. Such association of the reactivity to these organelles including synaptic vesicles was also observed in neurons and glial cells. The results suggest that Bcl-2 and other two proteins are preferentially integrated into inner mitochondrial and ER/vesicular membranes, where they contribute to the regulation of dying processes by interacting with each other and also being related with the organelle membrane functions.

229.16

CLONING AND EXPRESSION OF THE HUMAN BCL-2 RELATED PROTEIN, BAD W.A. Horne*, J.-L. Diaz, S. Ottilie, J.L. Chang, S. Gaur, K.M. Tuffo, L.C. Fritz and T. Oltersdorf IDUN Pharmaceuticals, La Jolla, CA 92037

Bcl-2 related proteins act upstream of ICE family proteases (Chinnaiyan et al., JBC 1996) to regulate a final common pathway for programmed cell death. Some of these proteins are present in neurons and may be involved in the regulation of neuronal survival. In particular, Bcl-x_L functions as a cell death suppressor (Gonzalez-Garcia et al., PNAS 1995), whereas Bad and Bax may act as cell death promoters (Yang et al., Cell 1995; Krajewski et al., J. Neuroscience 1995). The mechanism by which they act is thought to be related to their ability to form homo- and heterodimers. The pro-apoptotic effect of Bad may arise from its ability to displace bound Bax from Bcl-x_L.

To characterize the interactions of Bad with other Bcl-2 family members, we chose to clone human Bad. To isolate the human cDNA we searched the EST database (NCBI) with mouse protein sequence using the BLAST network service. This yielded a partial cDNA sequence homologous to mouse sequence which was then used to screen human cDNA libraries. Overlapping cDNAs were isolated from substantia nigra, bone marrow, and placenta. A full length *bad* clone isolated from placenta was used in further studies.

We found that the human *bad* cDNA encodes a 168 a.a. protein (18.4 kDa) that is 77 percent identical to mouse Bad. The N-terminus of the human protein, however, is 42 a.a. shorter. Like mouse Bad, the protein lacks a C-terminal anchor sequence, and is weakly homologous to other Bcl-2 family members in its putative BH1 and BH2 domains. There is a consensus site for A-kinase phosphorylation in the BH1 domain.

To characterize its interactions with other Bcl-2 family members, we expressed human Bad as 6His and GST fusion proteins in bacteria, and as LexA and B42 fusion proteins in yeast. We found that Bad binds with high affinity to Bcl-x_L (app. Kd =10 - 20 nM), but weakly to Bcl-2. It does not dimerize with with Bax, Bcl-x_S, or itself. Interestingly, Bad does bind to a mutated version of Bcl-x_L that doesn't bind Bax. Funded by IDUN Pharmaceuticals.

229.17

OVEREXPRESSION OF HUMAN BCL-2 IN MIDBRAIN DOPAMINERGIC CELLS USING A TYROSINE HYDROXYLASE PROMOTER. J. Liu*, J.P. Merlie†, and K.L. O'Malley. Dept. of Anat. & Neurobiol., Dept. of Mol. Biol. & Pharm†., Washington Univ., Sch. of Med., St. Louis, MO 63110

Oxidative stress is thought to contribute to several CNS disorders including Parkinson's Disease. For example somatic mitochondrial mutations as well as excess production of reactive oxygen species may be associated with the death of midbrain dopaminergic neurons. Several studies have shown that the cell death repressor protein, Bcl-2 blocks the effects of reactive oxygen species and thus protects cells from oxidative stress. In order to determine whether Bcl-2 could protect these cells in vivo, we used the rat tyrosine hydroxylase (TH) promoter to target Bcl-2 expression to midbrain dopaminergic cells. Previously we have shown that 2.5 kb of the rat TH promoter will direct foreign gene expression in the midbrain (Liu et al., Neurosci. Abs., 1994). In this study, three independent lines were analyzed which all showed cell type-specific bcl-2 gene expression in a sub-population of A8-A10 neurons. Bcl-2 immunoreactivity was found in the cytoplasm at a slightly different focal plane from that of TH probably due to its known association with membrane structures. It was also in the terminals of those A10 or medial A9 neurons which project to the ventral/medial part of the posterior half of the olfactory bulb. During the first three weeks of postnatal development, there are two waves of naturally occurring cell death in the substantia nigra. To determine whether overexpression of Bcl-2 affected the final number of neurons, we compared the A8-A10 cell numbers in control littermates and heterozygous bcl-2 transgenic animals at P21. To date, we have observed a small but consistent increase of dopaminergic neurons in animals that overexpress human Bcl-2. These studies suggest that Bcl-2 may play a developmental role in achieving adult numbers of midbrain dopaminergic neurons. Currently, we are investigating whether overexpression of Bcl-2 can attenuate the effects of midbrain dopaminergic neurotoxins such as MPP^+ or 6-OHDA. (Supported in part by NIH 45330)

229.18

INHIBITION OF BCL-2 TRANSCRIPTION WITH ANTISENSE OLIGONUCLEOTIDES POTENTIATES MPP+ INDUCED APOPTOSIS. C. P. Fall* and J. P. Bennett. Department of Neurology, University of Virginia School of Medicine, Charlottesville, VA 22908.

MPP+, the active metabolite of the Parkinsonism-inducing neurotoxin MPTP, inhibits complex I of the mitochondrial electron transport chain resulting in an increase in intracellular reactive oxygen species. MPP+ treatment causes apoptosis in cultures of the catecholamine-like SY5Y neuroblastoma cell. The protein bcl-2 has been shown to protect neurons from apoptosis resulting from endogenous and exogenous free radicals. Bcl-2 seems to function as the negative regulating species in a stoichiometric molecular switch which controls the ability of a cell to enter the apoptosis pathway.

Motivated by the search for a means to rescue nigral neurons from death in Parkinson's disease, we tested whether decreasing the level of bcl-2 would increase susceptibility to MPP+ induced apoptosis. SY5Y neuroblastoma cells were incubated with phosphorothioated antisense or sense oligonucleotides directed against the translation initiation site for bcl-2 mRNA and then exposed to MPP+. Cells treated with antisense oligos and MPP+ showed an increase in apoptosis over those treated with sense oligos and MPP+. Antisense oligos alone caused a slight increase in apoptosis over baseline. These data suggest that bcl-2 may play a role in MPP+ neurotoxicity.

C. P. Fall is supported by a Dean's Fellowship Grant, Univ. of Virginia.

229.19

IRON INDUCES CELL DEATH IN A CNS-DERIVED DOPAMINERGIC CELL LINE. J. Lotharius*, A. Uhland-Smith and K. O'Malley. Department of Anatomy and Neurobiology, Washington University, St. Louis, MO, 63110.

Parkinson's disease (PD) is a progressive neurological disorder characterized by a marked degeneration of dopaminergic neurons in the striatum. It has been suggested that environmental factors could be involved in the etiology of the disease. The effect of endogenous as well as exogenous toxins in the aging brain may also be exacerbated by the irreversible accumulation of somatic mitochondrial mutations. Such phenomena may lead to increased oxidative stress in response to toxic insults. Additionally, increased iron deposits have been found in post-mortem brains of afflicted persons. Iron has been shown to stimulate free radical formation and promote the autoxidation of dopamine. Because Bcl-2 has been shown in some systems to protect cells from oxidative stress, we tested whether Bcl-2 could protect a CNS-derived dopaminergic cell line, MN9D, from reagents involved in free radical production. Previously, we have shown that Bcl-2 protects these cells from MPP^+ induced cell death but not 6-OHDA (Oh et al, 1995, Neurobiol. Dis., 2:157-167). In this study we tested the hypothesis that Bcl-2 could protect these cells from iron toxicity. Both MN9D/Neo (control) and MN9D/Bcl-2 cells were treated with increasing concentrations of ferric chloride (50-1000 μM). Exposure of MN9D/Neo cells to iron for 24 h led to cell death with a mean lethal dose of 100 μM. Morphologically, iron-induced cell death did not resemble apoptosis inasmuch as no condensed nuclei or membrane blebbing was observed. Cells transfected with Bcl-2 were not protected from iron toxicity. In contrast to the previous 6-OHDA studies, the addition of 1 μg/ml cycloheximide did not block iron-induced cell death. Thus, in the MN9D cells, Bcl-2 did not block cell death mediated by factors contributing to oxidative stress.

Supported by MH45330.

229.20

ORDERING THE EFFECTS OF BCL2, AN ICE-FAMILY PROTEASE INHIBITOR, AND OTHER SURVIVAL AGENTS ON C-JUN KINASE ACTIVATION IN SERUM/NGF DEPRIVED PC12 CELLS. D.S. Park*, L. Stefanis, C.Y.J. Yan, S.E. Farinelli, and L.A. Greene Dept. of Pathology and Center for Neurobiology and Behavior, Columbia Univ. College of Physicians and Surgeons, New York, NY, 10032.

Previous studies indicate that activation of c-Jun kinase (JNK) is necessary for apoptosis of trophic-factor-deprived PC12 cells. Here, we determine the order in which known survival agents, including, BCL2, inhibitors of the interleukin-1 converting enzyme (ICE) family of proteases, blockers of transcription, and a variety of small molecules with differing modes of action, block apoptosis relative to JNK activation. Overexpression of BCL2 promotes PC12 cell survival and blocks JNK activation caused by trophic factor withdrawal. Similarly, aurintricarboxylic acid (an endonuclease inhibitor), *N*-acetylcysteine, the NO generator DETA•NO, 8-bromo-cGMP, and 8-(4-chlorophenylthio)-cAMP promote survival and inhibit JNK activation. In contrast, zVAD-fmk (a permeant ICE-family inhibitor), actinomycin-D, the G1/S cell cycle inhibitor deferoxamine, and the CDK inhibitor flavopiridol, all promote survival after trophic factor withdrawal, but do not attenuate the activation of JNK. These findings indicate that 1) BCL2 and a number of survival-promoting agents act upstream of JNK; 2) ICE-family protease actions, regulated genes required for cell death, and certain cell cycle blockers lie either downstream of JNK or on independent pathways required for apoptotic death. This work was supported in part by grants from the NIH-NINDS, March of Dimes, ALS foundation, and Aaron Diamond foundation. D.S.P. is the recipient of an Aaron Diamond Foundation Fellowship. S.E.F. was supported, in part, by an NRSA from the NIH-NINDS.

MOTOR SYSTEMS: DEVELOPMENT AND REGENERATION I

230.1

NMDA AND METABOTROPIC RECEPTOR mRNA EXPRESSION IN THE RAT STRIATUM FOLLOWING DECORTICATION DURING DEVELOPMENT. C. H. Lam*, and A. F. Sadikot. Division of Neurosurgery, Montreal Neurological Institute, McGill University, Montreal, Quebec

Glutamate receptors are thought to play an important role in central nervous system development. When the course of growth is altered by deafferentation, glutamate receptor mRNA expression may be altered. We use the striatum as a model due to a stereotypic temporal pattern of excitatory amino acid receptor mRNA expression and because of its major excitatory input from the cortex. In-situ hybridization using 35-S oligonucleotides generated from a published sequence (Wullner et al., 1994) was used to study the expression of two excitatory amino acid receptor families. Sprague-Dawley rats were decorticated at 4 days. At post-natal day 14, 21, and 30, they were sacrificed and hybridized using probes for NMDA and metabotropic receptor mRNAs. R1(ins) and R1(del) isoforms were found to be increased, particularly in the sensorimotor area of the striatum. 2A expression was equivocal while 2B, 2C, and 2D did not increase. The metabotropic receptor subtype M4 increased in a generalized manner throughout the striatum, but M1, M2, M3, and M5 were not increased. Alterations in message expression were seen as early as P21. Changes in subunit composition due to decortication may suggest a role for development of excitatory amino acid receptor mRNA expression in the striatum. (Supported by MRC of Canada).

230.2

FEWER AND SMALLER CEREBELLAR PURKINJE CELLS IN OLD SEDENTARY RATS AS COMPARED TO RATS SUBJECTED TO LONG-TERM PHYSICAL EXERCISE. J. O. Larsen*[1,2,3], M. Skalicky[4] and A. Viidik[5] [1)]Institute of Pathology, Aalborg Hospital, Denmark. [2)]Center for sensory-Motor Interaction, University of Aalborg, Denmark. [3)]Stereological Research Laboratory, Bartholin Building, University of Aarhus, DK-8000 Aarhus C, Denmark. [4)]Institute of Physiology, University of Veterinary Medicine, Vienna, Austria. [5)]Institute of Anatomy, University of Aarhus, Denmark.

The effects of long-term physical exercise on the number and volumes of Purkinje cell perikarya have been evaluated with unbiased stereological methods. Male Sprague-Dawley rats were trained from the age of 5 months to the age of 23 months. The training program consisted of running in a treadmill, with a horizontal floor speed of 20 m/min., for 20 minutes, twice a day for five days a week. The total number of Purkinje cells was estimated with the optical fractionator technique, and the volumes of the Purkinje cell perikarya were estimated with the vertical rotator technique. The runners were found to have 13 % more, and 10 % larger Purkinje cells compared to the sedentary controls, $2P = 0.02$ and $2P = 0.02$, respectively, while the mean weight of the cerebellum was the same in the two groups.

The extent to which neurogenesis occurs after the age of 5 months is not yet known, and it is not clear whether the observed difference of the Purkinje cell population is due to an increased formation of new Purkinje cells or a decreased loss of Purkinje cells in the rats subjected to physical exercise.

This study was supported by grants from The Danish Technical Research Council, The Danish Medical Research Council and The Danish Insurance Industry research project "Aging, Life and Fitness".

230.3

CONTROL OF BODY SUPPORT AND TRANSPORT BY THE LUMBAR AND THORACIC APPENDAGES IN LOCOMOTION AND ITS INITIATION BY NORMAL, TRANSECT, AND TRANSPLANT RATS. V. Graziani, M.R. Davies, W. Kargo, M. Shibayama, A. Book*, M. Murray, A. Tessler, and S.F. Giszter. Department of Neurobiology and Anatomy MCPHU, Philadelphia, PA 19129.

Previous work in our laboratories (Miya et al. 1994, Smeraski et al 1995) has shown that compared to normal rats, transplant recipient rats show a number of significant differences. Many of these are simplifications of the locomotor pattern. Others are compensatory adjustments due to absence or insufficiency of normal pathways. One such adjustment appears to be a prolonged overlap of stance phases in transplant recipients. During this overlap period there could be significant elastic force compensations occurring through the closed kinematic chains comprised of hindlimb, axial segments and forelimbs. It was unclear to what extent such mechanisms could form a basis for the initiation of locomotion, and for the fore-limb to hind-limb coupling in the transplant animals recovered locomotory function.

Previous work examined kinematics and muscle activation patterns. In this study we focussed on the ground forces and center of pressure variations in rats as they initiated locomotion or locomoted steadily over two six axis force transducers instrumenting either wide or narrow runway platforms. We predicted that if initiation pathways have reached the lumbar spinal cord in transplant recipients the parasagittal or axial components of the initiating ground force vectors of both forelimbs and hindlimbs should be of the same direction and approximate magnitude as in normals. In contrast, if initiation is a result of lumbar motion driven by forelimbs dragging the hindlimbs (rather like a treadmill) then ground force vectors will be of opposing signs. We observed that transplant recipient rats which showed good locomotion may show nearly normal ground forces during initiation. Initiation in the poorest walkers showed opposing ground force vectors in the forelimbs and hindlimbs. Support: ASRI grant to SFG, NIH HD01127-02 to VG and NIH 5P01 NS24707.

230.4

ORGANIZATION OF CORTICAL MOTOR REPRESENTATIONS IN NORMAL, TRANSECT AND TRANSPLANT RATS. W. Kargo, M.R. Davis, M. Murray* and SF Giszter. Dept Neurobiology and Anatomy , MCPHU, Philadelphia, PA 19129.

The rat neonatal transplant model uses transplants of fetal tissue or cell lines into fully transected neonatal spinal cord. Operated rats can show considerable motor function as adults (Miya et al. 1995, Smeraski et al. 1995). It has also been reported that corticospinal tract (CST) neurons cross transplants and enter lumbar cord in the rat neonatal transplant model (Bregman et al. 1996). The targets of the CST neurons, their cortical origins, and their relative role in recovery has not been established physiologically. In this study we examined cortical motor representations in normal, transect and transplant recipient rats as revealed by focal microstimulation. We also related features of the maps to a previously established data base of kinematic and muscle activity patterning during locomotion on a variety of tasks.

Animals were anaesthetized, the skull immobilized in a stereotaxic apparatus and the skull and dura opened to reveal the cortical surface. Mapping was performed with 50uA trains of 200usec pulses at 333Hz in the manner described by Wise and Donoghue, and Donoghue and Sanes. 16 pairs of electromyogram wires were used together with observation and muscle palpation to examine the organization of motor output. Mapping was performed across both granular and agranular cortex. Maps in normal rats closely matched published findings. We also found: (1) transplant and transect rats possesed no motor representation of the legs or tail i.e. we were unable to elicit any motor responses in these structures. (2) The trunk axial representation was greatly enlarged in transplant and transect rats as might be expected from published work. (3) Transplant rats which were considered 'good' walkers could have richer and more finely grained axial motor responses (4) Some bilateral forelimb responses were observed in transplant rats. We thus believe that CST projections that enter transplants and project caudal to the transplant region terminate predominantly on axial motor structures. Supported by ASRI grant to SFG and NIH 5P01 NS24707.

230.5

A SIMPLE TECHNIQUE FOR ORGANOTYPIC CULTURE OF 'THIN' SLICES: SPINAL CORD AND BRAINSTEM. C.P. Parsley*, K.W. Cheng, E.S.S. Ling, and S. Hochman. Dept. of Physiology, University of Manitoba, Winnipeg, MB., CANADA, R3E 0W3.

Existing approaches used for explant culture are the 'roller tube' (Gähwiler et al *TINS* 11:484, 1988) or 'interface' methods (Stoppini et al *J. Neurosci. Methods* 37:173, 1991). While powerful, both approaches have limitations. For example, the roller tube method is technically demanding, and although the interface method is a simpler approach, the membrane inserts are expensive with electrophysiological and imaging studies being difficult to perform. We have found that a reduced slice thickness provides sufficient oxygenation to allow explants to be cultured directly on the bottom of collagen-coated petri dishes.

Under sterile conditions, the dissected brainstem-spinal cords from E-15 rat fetuses were cut into transverse slices (100-200 µm thick), transferred to 35 mm petri dishes, coated with collagen, and then incubated at 37^{0}C in 5% CO_2 - 95% O_2 atmosphere in 600 µl media. Viable explant cultures have been obtained for at least 21 days *in vitro*. Live/dead cell indicators revealed predominantly living cells within the slices and in regions of cell migration. Neurons and astrocytes could be identified immunohistochemically and explants were observed to extend many processes. In order to test the usefulness of this approach, we incubated spinal cord explant cultures in serum-free Neurobasal™ Media and assessed the actions of various trophic factors on process outgrowth. Initial results with trophic factors suggest that CNTF, BDNF, and NT-4/5 enhance neuritic extensions whereas outgrowth in the presence of NT-3 or NGF were not statistically different from controls. Future studies will capitalize on this approach for electrophysiological studies in regeneration. *Supported by the Canadian Neuroscience Network. Trophic factors were generously supplied by Regeneron Pharmaceuticals, Inc.*

230.6

CORRELATES OF MUSCLE ATROPHY IN GRASSHOPPERS A.S. Clinton* and the late E.A. Arbas. ARL, Division of Neurobiology, University of Arizona, Tucson AZ 85721.

In the grasshopper *Barytettix psolus*, hind-limb autotomy induces atrophy in a group of proximal metathoracic leg muscles that are neither damaged nor denervated during the loss of the more distal leg segments. Damage to the leg nerve triggers the process trans-neuronally. This system provides a model for the study of regulatory interactions between identified neurons and their synaptic partners.

Within the group of 16 affected muscles, extent of atrophy varies both between muscles and between fibers within a single muscle. For example, discrete bundles of fibers in both muscle (M) #120 and M. #121 exhibit a pronounced resistance to atrophy, while remaining fibers degenerate completely. In contrast, M. #119 uniformly degenerates. In these 3 muscles as well as the remaining 13, differences in muscle atrophy are correlated with both innervation pattern and histochemical fiber type. Immunohistochemical staining reveals the presence of a proctolin-like substance associated only with muscle regions that exhibit resistance to atrophy. These same regions also receive inhibitory innervation, as demonstrated by the presence of GABA-like immunoreactivity. An additional correlate of muscle atrophy is derived from the histochemical staining of several of these muscles for myosin ATPase activity. In all muscles examined to date (7 out of 16), regions that are resistant to autotomy-induced atrophy contain slow type fibers. In contrast, the staining pattern in those muscle regions that degenerate severely is characteristic of fast/intermediate type fibers. Histochemical analysis of myosin ATPase activity in M. #120-124 from *B. psolus*, 5 weeks following autotomy, reveals that slow type fibers are maintained, while many of the fast/intermediate fibers degenerate. (Supported by NIH T32 NS07363 and NSF IBN 9210394).

230.7

TRANS-NEURONALLY INDUCED MUSCLE ATROPHY: HISTOLOGICAL AND PHYSIOLOGICAL CHANGES. K.E. Personius, R.F. Chapman* and the late E.A Arbas. ARL, Div. of Neurobio., Univ. of Arizona, Tucson AZ 85721.

When the grasshopper, *Barrytettix psolus*, sheds a hind-limb certain thoracic muscles, which are neither damaged nor denervated, exhibit severe atrophy. Previous studies indicate that muscle atrophy is triggered trans-neuronally by the severing of the leg nerve (which does not supply the thoracic muscles) during autotomy (Arbas & Weidner, 1991). We use this system to study changes induced in healthy muscle following remote nerve damage. This study used histochemical fiber typing and intracellular recordings to study changes in tergotrochanteral muscle #133b,c.

Muscle #133b,c contains only "fast" muscle fibers. 1 day post-autotomy no atrophy is seen; however, the percentage of fibers showing spontaneous miniature potentials (mEJP) is increased compared with controls. Atrophy is evident by day 3 and other physiological changes begin to occur. Some muscle fibers are depolarized compared with controls and membrane excitability is increased, with muscle fibers supporting post-inhibitory rebound spikes. By 10 days, atrophy is severe, but the number of fibers is the same. Muscle fibers are further depolarized and the percentage of fibers showing enhanced membrane excitability continues to increase. The frequency of mEJP is increased compared to control, indicating a pre-synaptic change at the neuromuscular junction. 15 days post-autotomy only a few atrophied fibers continue to stain for myosin ATPase. Interestingly, in the remaining fibers, resting membrane potential and the percentage of fibers showing mEJP are restored towards control values.

The rate of muscle atrophy is not constant. By day 15, a gradation of fibers exists: some which atrophy more slowly and others which are highly depolarized and have lost all contractile apparatus. Supported by NSF IBN 9210394 and APTA Post-Professional Scholarship.

230.8

OVEREXPRESSION OF MYOGENIN INDUCES A SHIFT FROM GLYCOLYTIC TO OXIDATIVE METABOLISM IN SKELETAL MUSCLE OF TRANSGENIC MICE. K. Gundersen*[1], M.M. Chi[2], Simon M. Hughes[3] and O.H. Lowry[2]. [1]Departments of Biology and Neurophysiology, Univ. of Oslo, NORWAY, [2]Dept. of Pharmacology and Mol. Biol., Washington Univ., St Louis MO, [3]The Randall Institute, King's College London, UK.

Myogenin is a member of a group of transcription factors that has been implicated in the initial differentiation of muscle cells in the developing embryo, but myogenin has also been implicated as a mediator of changes that occur in permanent, fully differentiated muscle cells. Thus, myogenin is thought to be involved in acetylcholine receptor up-regulation after paralysis, and recent data has indicated that the level of myogenin expression parallels changes in contractile properties.

We ligated myogenin cDNA to the myosin light chain 1/3 promoter and enhancer and inserted this construct into the genome of transgenic mice. This transgene confers tissue specific expression of myogenin in post mitotic fast muscle cells, and the expression apparently did not interfere with muscle differentiation. In fast muscles the levels of oxidative enzymes (acetoacetyl-CoA thiolase, β-hydroxyacyl-CoA dehydrogenase, citrate synthase and malate dehydrogenase) were increased 2-6 fold, while the levels of glycolytic enzymes (glycogen phosphorylase, glycerol-3-phosphate dehydrogenase, pyruvate kinase and lactate dehydrogenase) where reduced to 40-90 % compared to the levels in wild type animals. Preliminary fibertype analysis with specific antibodies against myosin heavy chain indicated that the changes in enzyme levels came about without major changes in fibertype composition.

It is well known that changes in activity, either experimentally, by electrical stimulation, or by physical training, can alter muscle properties. Thus, extreme changes in the pattern of activity can induce complete changes in fibertype, defined as a complete substitution of the myosin iso-enzyme. Milder forms of training lead to changes in the composition of metabolic enzymes not accompanied by change in fibertype. Our data suggest that myogenin could be a mediator of this type of activity dependent muscle plasticity. *Sup. by the Norwegian Research Council and the MRC.*

230.9

SATELLITE CELLS AND MUSCLE FIBER PROLIFERATION IN TOADFISH SONIC MUSCLE. K. E. Loesser and M. L. Fine*. Depts. of Biology, Mary Washington Col., Fredricksburg, VA 22401 and Virginia Commonwealth Univ., Richmond, VA 23284-2012.

Sonic muscle fibers on the oyster toadfish *Opsanus tau* swimbladder proliferate throughout adult life and have an unusual radial morphology: alternating ribbons of sarcoplasmic reticulum (SR) and myofibrils surround a central core of sarcoplasm. Large fibers in adults form multiple cores, fragment and appear to divide into smaller, more energy efficient units. In this study we examined the embryonic formation of sonic muscle and used electron and light microscopy to describe the morphology and incidence of satellite cells (SC) developmentally. Muscles fibers form late in the larval period from myoblasts with a heterochromatic nucleus. The cells do not appear to fuse into myotubes but enlarge and differentiate myofibrils in a single circular patch. The SR differentiates from the outside inward, separating the myofilaments which are variable in number and often exceed the 8 layers of thick filaments in adults. The % SC decreases from a high of 80% in larvae to a low of 1% in adults although the adult average is 10%. No embryonic type fibers in the process of differentiating myofibrils were seen in adults. Small immature fibers, which had not yet formed the central core, had a complete radially organized contractile cylinder, suggesting that splitting rather than SCs is the major source of new fibers in adults. Supported by NIH DCO 1083.

230.10

HINDBRAIN SEGMENTATION: ONTOGENY OF PERMANENT RHYTHMIC MOTOR ACTIVITIES IN THE CHICK EMBRYO. G. Fortin, A. Le Métayer and M. Denavit-Saubié*. Institut Alfred Fessard, C.N.R.S., Gif /Yvette 91198, France.

Respiration in vertebrates, is a permanent rhythmic motor behaviour generated centrally within hindbrain neuronal networks. During embryogenesis, the hindbrain (rhombencephalon) neuroepithelium becomes partitioned into a series of 8 reiterated compartments (rhombomeres) along the antero-posterior axis. We have shown, using an isolated hindbrain preparation, that this segmentation process whereby cells are given positional information and neural fates determined, is accompanied in its latest stage (E4) by the appearence of permanent rhythmic motor activities coactive on all branchiomotor nerves. These activities between E4 and E8 consist of short (10-40s) episodes of cyclical burst discharges separated by long (1-4min) periods of silence.

On the same preparation, a single electrical shock applied to the ventral aspect of the neural tube can evoke a full episode only when the stimulus falls within a 10-20s permissive time window preceding the normal occurrence of an episode.

Whole cell recordings combined to biocytin labeling indicate that 100% of the neurones so far recorded (n=40) are rhythmically modulated independent of their axial position. Distinct functional phenotypes are present: « motoneuron-like » cells discharging in phase with the motor nerve, « inhibited » cells discharging in phase opposition with the motor nerve, « pre-active » cells discharging prior to the motor nerve. All these neurons remain silent throughout the period separating episodes. One cell category however, fires bursts of action potentials throughout the silent period of the nerve. Given the permissive period of excitability preceding episodes, such cells could initiate the discharge of « pre-active » cells and in turn the onset of episodes.

Ubiquitary rhythmic patterns of activity in the hindbrain could rely on the early specification of gating properties within neuronal assemblies.(Supported by C.N.R.S. and Fondation pour la Recherche Médicale).

230.11

THYMOCYTE WINGED HELIX GENE IS ESSENTIAL FOR THE NORMAL DEVELOPMENT OF SPINAL MOTOR COLUMNS. C.-L. Dou*[1], X. Ye[2], G. Balas[1], D. Hirsh[3], and E. Lai[1], S. C. Li[2]. [1] Cell Biology Program, Memorial Sloan-Kettering Cancer Center, New York, NY 10021; [2] Dept. of Pediatrics and [3] Dept. of Biochemistry, Columbia University, New York, NY 10032.

Winged-helix (WH) transcription factors play important roles during embryogenesis. Thymocyte winged-helix (TWH) gene is a new member of this rapidly growing gene family. During neurogenesis, TWH is highly expressed in the neuroepithelial cells and in a subset of differentiated neurons. Its unique pattern of expression in the developing central nervous system suggesting a function in neural development. To learn the function of TWH, we have generated mice containing null mutation of TWH and analyzed the mutant phenotype.

About 80% of homozygous mutants die within the first postnatal week. Those surviving show retarded postnatal growth. Mutants also display motor deficits as indicated by muscle weakness and a clutching reflex characteristic of mice with motor neuron disease. In situ hybridization studies show that TWH is expressed in subpopulations of spinal motor neurons. The mutant animals have about same total number of Islet-1 positive motor neurons as their heterozygous littermates at E13.5, however, the number in the medial motor column (MMC) is reduced by birth. Interestingly, the number of neurons expressing TWH in the lateral motor column (LMC) is increased in mutants indicating a shift of TWH-expressing neurons from MMC to LMC. These results suggest that TWH plays an important role in the normal migration and/or segregation of motor neurons and the deficit in motor function may be due to a failure of misplaced motor neurons to make synaptic connections with their correct peripheral targets. Supported by the March of Dimes Basil O'Connor Starter Scholar Award.

230.12

Anatomy of the Dorsal-Ventral Motor Neurons of *Drosophila*

Yi-an Sun,* James Nagy and R. J. Wyman.

Department of Biology, Yale University, New Haven, CT 06511

Flight in *Drosophila* is powered by one large wing depressor muscle (the dorsal-longitudinal muscle, DLM) and three large wing elevator muscles, the dorsal-ventral muscles. The location and morphology of the motor neurons innervating the DLM is described in Sun and Wyman (1996, J. Comp. Neurol. In Press).

The three muscles are arrayed DVM I, II & III from anterior to posterior; they are composed of 3, 2 and 2 muscle fibers respectively. HRP was injected into each muscle fiber separately and the motor neuron was back-filled. Each of the seven muscle fibers is innervated by a single and separate motor neuron. Surprisingly, the anterior-posterior location of the motor neuron cell bodies does not correlate with the position of the muscles.

The cell bodies of the motorneurons (DVMns) to DVM I sit anterior, ventral and lateral in the T2 hemiganglion.Their primary neurites fasciculate and run straight dorsally to the surface. Here they loop postero-medially and then run anteriorly again to arborize near the midline, mostly ipsilaterally. The somas of DVMn II lie at the posterior edge of the T2 neuromere about midway in the D-V plane. Its primary neurite runs anteriorly. The cell bodies of DVMn III lie on the dorsal surface and anterior to those of DVM I. The primary neurite runs posteriorly. The dendrites of all 7 MNs ramify in the same dorsal-anterior region, which is slightly posterior to, but overlaps with, the ramification of the DLM dendrites.

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230.13

GLYCINE- AND GABA-MEDIATED SPONTANEOUS MOTOR ACTIVITY IN THE RAT FETUS *IN VITRO*. H. Nishimaru, M. Iizuka, S. Ozaki, & N. Kudo*. Department of Physiology, Institute of Basic Medical Sciences, University of Tsukuba, Tsukuba, Ibaraki 305, Japan.

Spontaneous motor activity in the fetal period are widely observed in various kinds of mammalian species. However, the neuronal mechanism mediating these early motor activity remain unknown. To examine this issue, we recorded motoneuronal activity from the lumbar ventral roots in an isolated spinal cord preparation from rat fetuses. Spontaneous bursts that were synchronized in the corresponding ventral roots on each side were observed periodically (0.5-5.0 min intervals) between embryonic day (E)14.5-E17.5. Kynurenate (4 mM), a glutamate receptor blocker, failed to block spontaneous bursts at E14.5-E15.5, while it completely abolished them at E17.5. On the other hand, glycine receptor antagonist strychnine (10 μM) completely blocked spontaneous bursts at E14.5-E15.5. At these stages, Bicuculline (10-40 μM), a $GABA_A$ receptor antagonist, reduced the amplitude of the spontaneous burst. Brief glycine (250 μM-2 mM) applications evoked excitatory responses that resembled the spontaneous bursts in their time course and amplitude at E15.5. These glycine-induced responses were not observed under Ca^{2+}-free saline, suggesting that they were synaptically evoked. These synaptic responses were not blocked by kynurenate (4 mM), but they were abolished by strychnine (10 μM). These results indicate that glycine receptors and $GABA_A$ receptors mediate the earliest spontaneous motor activity in the embryonic spinal cord.

231.1

TAU mRNA ISOFORM EXPRESSION FOLLOWING SCIATIC NERVE INJURY IN THE ADULT RAT. Christopher B Chambers* and Nancy A Muma, Dept. of Pharmacology, Loyola University Chicago, Maywood, IL 60153.

Microtubule-associated proteins (MAPs) play a key role in regulating neuronal microtubule assembly/disassembly and therefore provide a balance between two opposing forces in neuronal processes: stability and plasticity. Regenerating axons display a high level of plasticity, similar to that seen during development. The expression of tau proteins, one of the major MAPs present in axons, developmentally regulated such that only one tau isoform containing 3 microtubule-binding domains is expressed during development. This isoform has a lower ability to bind microtubules than do the adult tau isoforms which have 4 microtubule-binding domains, consistent with the high degree of plasticity in the developing nervous system.

We hypothesized that following sciatic nerve injury in the adult rat, tau gene expression would be altered to reflect that seen during development. We have used both regenerative permissive (nerve crush) and non-permissive (nerve transection) conditions and the reverse transcription-polymerase chain reaction (RT-PCR) to detect and quantitate alterations in tau isoform expression in the lumbar spinal cord (L4/L5 region) at various time points following nerve injury. The tau primers were designed to span exon 10 to allow amplification of fetal and adult tau isoforms. Primers specific for H3.3 histone gene were included in the amplification step to allow simultaneous amplification of a constitutively expressed gene as an internal control for amplification. Southern blot analysis of the PCR products with oligonucleotide probes specific for either the 3 or 4 repeat isoforms indicate that the tau primers generate both isoforms using cDNAs generated by reverse transcription of total RNA extracted from rat lumbar spinal cord as the template. Data will be presented indicating what alterations in tau gene expression occur following sciatic nerve injuries which either do or do not allow for successful regeneration.

(Supported by NS30460)

231.2

TIMING OF C-JUN PROTEIN INDUCTION IN RAT DORSAL ROOT GANGLIA AFTER SCIATIC NERVE TRANSECTION IS DEPENDENT ON LESION DISTANCE: SUPPORT FOR THE INVOLVEMENT OF RETROGRADE TRANSPORT IN C-JUN REGULATION. A.M. Kenney and J.D. Kocsis*. Dept. of Neurology, Yale Univ. Sch. of Med., New Haven, CT 06510 and Neuroscience Res. Ctr, VA Medical Center, West Haven, CT 06516

One of the earliest detectable molecular indicators of response to sciatic nerve crush or transection is the up-regulation of the immediate early gene c-jun mRNA and protein in L4/L5 DRG neuronal cell bodies. The lesion-induced signal which upregulates c-jun is not known, although studies using capsaicin, vinblastine, and colchicine suggest that a retrogradely transported molecule is involved. However, these compounds may have injurious effects which could independently influence c-jun regulation. We have used a surgical method to determine whether a transported molecule or another kind of signal, such as electrical discharge or cessation of ambient electrical activity, underlies the upregulation of c-jun protein. The sciatic nerve was transected 1 cm from the L4/L5 DRGs, at the convergence of the L4 and L5 spinal nerves, or 5 cm from the L4/L5 DRGs, at the midthigh level. We used western blotting to study the relative levels of c-jun protein in transected and sham operated contralateral DRGs during the first 24 hours after injury. Immunostaining of frozen sections was used to localize c-jun protein to neurons. Elevated levels of of c-jun protein were detectable 2-3 hours after the more proximal nerve transection, whereas increased c-jun protein was not apparent until 12 hours after the distal transection. The different rate of c-jun induction after proximal transection as compared to distal transection supports the hypothesis that a retrogradely transported molecule is responsible for c-jun induction after injury, as an instantaneous signal would lead to c-jun upregulation within a similar time frame regardless of injury site.

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231.3

IDENTIFICATION OF GENES ASSOCIATED WITH NEURONAL REGENERATION BY DIFFERENTIAL DISPLAY PCR

E.M.Hol*, F.-W. Schwaiger, A. Schmitt and G.W. Kreutzberg
Max-Planck-Institute for Psychiatry, Dept. of Neuromorphology, D-82152 Martinsried, Germany

Peripheral nerve injury leads to morphological, metabolic and molecular changes in the severed neurons and the surrounding glial cells. Neuron-glia interactions and changes in gene expression in these cells take part in the program that controls nerve regeneration. In search for novel genes associated with neuronal regeneration, changes in gene expression were analyzed in rat spinal cord 72h after sciatic nerve crush and in rat facial nucleus 72h after facial nerve axotomy.

The DNA fragments obtained with the differential display PCR (dd-PCR), were cloned, sequenced and their expression pattern was screened by in situ hybridization. Up to now we have isolated and sequenced 63 DNA fragments from spinal cord and facial nucleus. About 70% of these gene fragments showed no homology at all with sequences in the gene bank. Other gene fragments isolated showed a moderate to high homology with genes like stearyl CoA desaturase, S3 ribosomal protein, 12S rRNA, human pBX2 gene, chicken c-maf, hox4.4/4.5, NADH ubiquinone oxidoreductase, non-muscular myosin light chain, connexin 43, mouse elongation factor 2, human colon mucosa protein, ribosomal protein L7, homeobox protein R1a and pyruvate kinase. In situ hybridization revealed that 20% of the novel gene fragments were differentially expressed. We found neuron specific as well as glial specific genes.

In conclusion, we successfully identified novel regeneration associated genes by means of the dd-PCR technique.

231.4

CLONING OF AN AXOTOMY-INDUCED GENE WHICH IS SPECIFICALLY EXPRESSED IN INJURED MOTONEURONS. S. Kiryu*, N. Morita, H. Kiyama. Dept. of Neuroanatomy, Biomedical Research Center, Osaka Univ. Med. Sch., Suita, Osaka, 565 JAPAN.

To gain an insight into the molecular events during peripheral nerve regeneration, we compared differences in expression of mRNAs between axotomized and normal hypoglossal nuclei by means of differential display PCR(DD-PCR) coupled with in situ display. DD-PCR and subsequent in situ hybridization gave us several candidate gene fragments.

Among them, one clone showed unique expression pattern of the mRNA. A slight increase in the mRNA was detected initially on the injured hypoglossal nucleus 1 day after the nerve cut. The hybridization signal markedly increased to a peak level during the following 3 days and gradually decreased to control level over the following 4 weeks. However, no expression of the mRNA was observed in any other region of the rat brain. Searching of the nucleotide database, DDBJ, revealed that it had no similarity with other known genes. It is therefore suggested that this novel gene is highly implicated in nerve injury or regeneration event.

231.5

ENHANECD EXPRESSION OF 14-3-3 FAMILY MEMBERS DURING PERIPHERAL NERVE REGENERATION. K.NAMIKAWA, Q.N.SU, H.TOKI, and H.KIYAMA*, Dept of Neuroanatomy, Biomed.Res.Center, Osaka Univ. Med. Sch., 2-2, Yamadaoka, Suita, Osaka, 565,Japan

Differential display-PCR (DD-PCR) was used to isolate cDNA fragments whose mRNA expression are increased or decreased in mouse hypoglossal motoneurons after axotomy. In situ hybridization (ISH) confirmed that the expression of some fragments derived in DD-PCR have been up-regulated following nerve injury, and one of them was found to be highly homologous to rat γ subtype of 14-3-3 protein which is a Raf-1 kinase activator. To examine which subtypes of 14-3-3 members are involved in nerve regeneration, we used rat β, γ, η, θ, and ζ subtype of cDNA which have been already cloned in rat. Among this family members, β, γ, θ, and ζ subtypes were up-regulated. Especially, dramatic increase of θ and ζ subtype mRNA expression were detected within a few days after nerve transection and continued until 4 and 3 weeks, respectively. Recently some studies demonstrated that β and ζ subtype of 14-3-3 have been found to bind Raf-1 and function as Raf-1 kinase activators in vivo. Taking these findings into consideration, enhanced expression of 14-3-3 family members after nerve injury suggests that Raf-1 is likely to be activated during nerve regeneration. The present results together with our previous finding (MEK and ERK up-regulation after axotomy) suggest that enhancement of Raf-ERK signaling pathway machinery is probably crucial for nerve regeneration.

231.6

P53 INDEPENDENT CYCLIN G EXPRESSION IN MOTOR NEURONS AFTER NERVE INJURY. N. Morita*, S. Kiryu and H. Kiyama. Dept. of Neuroanatomy, Biomed. Res. Center, Osaka Univ. Med. Sch., Suita, Osaka 565 Japan.

An increase in cyclin G expression after nerve injury was demonstrated by differential display PCR (DD-PCR), carried out to compare differences in expression of mRNAs between axotomized and normal hypoglossal motoneurons in the rat. The nerve injury dramatically upregulated the expression of cyclin G mRNA in the motoneurons during nerve regeneration.

Since cyclin G has been shown to be a transcription target of p53, we further examined the cyclin G expression in p53 deficient mouse. Cyclin G was upregulated in motoneurons of p53 deficient mouse after nerve injury, and constant expression of cyclin G was also observed in some regions of the brain and in some other non-neuronal tissue of p53 deficient mouse. These results suggest that the expression of cyclin G, at least in the nervous system, is not regulated by p53 predominantly, and that there may be an alternative regulatory factors or pathways for cyclin G expression in the nervous system.

231.7

CHANGES IN GENE EXPRESSION IN DORSAL ROOT GANGLION CELLS FOLLOWING SCIATIC NERVE LESION OR DORSAL RHIZOTOMY

M. E. Rott*[1], M. Bähr[1] and J. D. Steeves[2]. [1]Dept of Neurolgy, Univ. Tübingen, Germany and [2] Dept. of Zoology, UBC, Canada.

We have used the differential display reverse transcriptase polymerase chain reaction technique (DDRT-PCR) to screen for differentially expressed genes following axonal injury which may be critical for regeneration of injured axons. It is thought that altered expression of intrinsic neuronal factors may be important determinants for axonal regeneration. In order to identify such genes, we compared gene expression following regenerative and non regenerative axonal injury in hatchling chicks subjected to either a sciatic nerve lesion or a dorsal rhizotomy, respectively. Total RNA was purified from lesioned DRGs as well as contra lateral control DRGs and analyzed by DDRT-PCR. Several hundred differentially expressed cDNA bands were detected, eluted from the gels, re-amplified and cloned. Sequence analysis of these cDNAs identified both known and unknown genes. To confirm the differential expression of cDNA clones chosen for further analysis, sequences were used as probes against total amplified cDNA from lesioned and control DRG RNA. A wide range of gene expression was observed for these clones in terms of magnitude and pattern of expression, up-regulation after sciatic lesion and/or dorsal rhizotomy as well as down regulation after one or both lesion paradigms. We have now selected a few of these clones for further analysis by developmental Northern blots as well as in situ hybridizations. Those cDNAs that give an expression pattern consistent with that expected for a regeneration associated neuronal factor, will be candidates for continuing cloning and sequence analysis studies.

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231.8

DIFFERENTIAL REGULATION OF A NOVEL FABP (DA11) IN AXOTOMIZED NEURONS FOLLOWING SCIATIC NERVE CRUSH. C.A. Molina†, Y. Liu*, A.A. Welcher*, M. De Leon*†

† Center for Molecular Biology and Gene Therapy and Dept of Physiology, Loma Linda, CA, 93350 ; *Amgen Inc., Amgen Center, Thousand Oaks, CA 91320; Department of Anatomy and Human Pathology, Loma Linda CA 92350.

We previously reported the cloning and characterization of DA11, a novel Fatty Acid Binding Protein (FABP) isolated from rat dorsal root ganglia (DRG) cDNA Library (De Leon, et al 1994). In addition we have demonstrated the expression and localization of DA11 protein in the DRG after Sciatic nerve transection (Molina et al 1995). The present study used immunocytochemistry (ICC), *in situ* hybridization (ISH), Western and Northern blots to examine the expression and localization of DA11 protein in the DRG and spinal cord after sciatic nerve crush. Adult male Sprague-Dawley rats received a left ipsilateral (I) sciatic nerve crush and right contralateral (C) sham manipulation at the mid thigh region of the sciatic nerve. Lumbar (L_4-L_5) spinal cord segments and respective I and C DRG were harvested and their proteins and total RNA extracted for analysis. Western blot results showed a significant increase in DA11 protein peaking 7 days following crush in the I DRG as compared to the C or naive controls. Subsequently, DA 11 protein in the I DRG decreased to control levels 35 days after injury. Northern blot analysis showed an up-regulation of DA11 mRNA in the I DRG as compared to naive control. The increase was apparent 3 days after the crush peaking at 7 days, and further after returned to normal levels by 28 days after nerve crush. Additional animals were surgically manipulated as above, perfused after 7 days, and processed for ICC with DA11 antisera. Results from the ICC demonstrated mostly cytoplasmic localization of DA11-like immunoreactivity in the C DRG neurons, with very few neurons exhibiting any nuclear staining. In contrast, the immunoreactive pattern of the I DRG showed a marked elevation of DA11 protein in the nucleus of DRG neurons as well as the large motor neurons of the ventral horn of the spinal cord. Our data suggest that DA11 is regulated during nerve repair and regeneration. This work was supported by NIH grant HD3807-Supplement.

231.9

AXOTOMY-INDUCED ALTERATIONS IN HEAT SHOCK PROTEIN (HSP) 70 PROTEIN LEVELS IN HAMSTER FACIAL MOTOR NUCLEI (FMN). G.A. Newfry[1,2] and K.J. Jones*[1,3] [1]Dept. of Cell Biol. & Anatomy, Chicago Medical School, North Chicago, IL 60064, [2]Dept. of Physical Therapy, Univ. IL at Chicago, Chicago, IL 60612, and [3]Dept. of Cell Biol., Neurobiol., & Anatomy, Loyola Univ. Chicago, Maywood, IL 60153.

In the adult hamster, facial nerve axotomy at the stylomastoid foramen induces a reactive sequence of events in the FMN that is geared toward neuronal survival and regeneration. Both axotomy and heat shock result in alterations in nucleolar morphology. General trauma has been found to induce hsps in mammalian brain. Based on evidence that the experimental overproduction of hsps accelerates nucleolar recovery after heat shock, hsps may be important in the recovery from axotomy-induced nucleolar alterations in neurons. Previous work has shown that axotomy induces hsp 70 mRNA within 30' in FMN of adult hamsters, with a return to control values by 12h. In this study, the ability of facial nerve transection to induce hsp 70 in FMN of hamsters of varying ages, which have attained differential nucleolar development and post-trauma survival capacities, was analyzed by western blotting. Postoperative (po) times were 2-24h. Each group had at least 3 animals. Ten µg of protein, from control and injured FMN, were electrophoresed. Paired gels were run for each experiment; one was Coomassie stained, the other transferred to Nytran membrane. Studies used a 1:1000 dilution of a rat antibody which recognizes denatured constitutive and induced members of the eukaryotic hsp 70 family (gift of Dr. S. Lindquist). Blots were developed with the Elite ABC kit (Vector Labs), and DAB/H_2O_2. In the adult FMN, in which 100% of the neurons survive axotomy, increased levels of hsp 70 protein were observed by 12h po. In the young FMN, in which the neurons are unsuccessful in post-trauma survival, decreased levels of hsp 70 protein were observed after injury. The results provide the first evidence that survival and regeneration after peripheral axotomy are correlated with hsp 70 protein levels. Supported by an APA grant (KJJ).

231.10

REGULATION OF LIM-TYPE HOMEOBOX GENES AFTER PERIPHERAL NERVE INJURY

F.-W. Schwaiger, E.M. Hol, A. Schmitt, and G.W. Kreutzberg*

Max-Planck-Institute for Psychiatry, Department of Neuromorphology, D-82152 Martinsried, Germany

LIM-type homeobox genes are believed to play an important role in motoneuron differentiation. The fate of motoneuronal projections depends on the LIM code, a combination of several LIM-type homeobox proteins characterising a given motoneuron. Injury (e.g. axotomy or nerve crush) of peripheral nerves prevents normal nerve cell function, possibly leading to changes in the expression of some LIM genes or reactivation of the embryonic LIM code.

We investigated two models of peripheral nerve injury (facial nerve transection and sciatic nerve crush) in rat. mRNA was isolated from facial nucleus and from spinal cord (T12-L1). After reverse transcription to cDNA the amount of Cyclophilin A as an internal standard was determined to estimate the amount of cDNA. A semiquantitative PCR for GAP-43 was used to validate the PCR conditions and showed GAP-43 to be upregulated by 560% in the facial nucleus and 340% in the spinal cord after nerve injury. Using primers for the semiquantitative PCR of the five known LIM-type homeobox genes (LIM-1, LIM-2, LIM-3, ISLET-1 and ISLET-2), we found a significant downregulation of ISLET-1 and possibly other LIM-type homeobox genes in both peripheral nerve injury models. This might indicate a "growth state" of regenerating motoneurons.

231.11

ISOLATION OF A NOVEL REGENERATION SPECIFIC GENE BY MODIFIED DIFFERENTIAL DISPLAY. Holly D. Soares* and James I. Morgan. Department of Developmental Neurobiology. St: Jude Children's Research Hospital, Memphis, TN 38101.

Outcome following sciatic nerve axotomy is dependent upon developmental age. For example, axotomy performed during the neonatal period results in cell death of axotomized neurons. However, primary motor and sensory neurons can survive sciatic nerve transection when axotomy is performed during adulthood. The present study utilizes neonatal and adult sciatic nerve axotomy as a model to examine the molecular mechanisms underlying a neuron's commitment to either a cell death or survival/regeneration program. Utilizing a modified version of differential display we have isolated a novel 5kb transcript which we have termed Reg1 for regeneration specific gene 1. Northern and *in situ* analysis show that Reg1 is up-regulated in axomotized regenerating adult motor neurons, but not in axotomized degenerating neonatal motor neurons. Further *in situ* analysis shows that Reg1 is not expressed in muscle, kidney, lung, pancreas or small intestine, but is constitutively present in granule cells of the hippocampus and cerebellum. Finally, in chronically transected neurons, Reg1 is still up-regulated at two weeks after axotomy. However two weeks following sciatic nerve crush, Reg1 gene expression has returned to normal levels. These data suggest that Reg1 is regulated by neuronal regeneration. In summary, Reg1 is a novel regeneration specific gene whose expression is localized to the nervous system. This work was supported by ALSAC institutional funding.

231.12

STEROID HORMONE AUTORADIOGRAPHIC LOCALIZATION OF ESTROGEN RECEPTORS IN HAMSTER BRAINSTEM AFTER FACIAL NERVE AXOTOMY. D.R. Sengelaub*[1] and K.J. Jones[2,3]. [1]Program in Neural Science, Indiana Univ., Bloomington, IN 47405, [2]Dept. of Cell Biology, Neurobiology & Anatomy, Loyola Univ. Chicago, Maywood, IL 60153 and [3]Rehab. Res. & Dev., Hines VA Hospital, Hines IL 60141.

We have demonstrated that administration of testosterone propionate (TP) to hamsters at the time of facial nerve crush axotomy at the stylomastoid foramen will accelerate both functional recovery from facial paralysis and the rate at which facial nerve regeneration occurs. Further work has shown that these effects appear to be androgen receptor-mediated. However, we have recently discovered that estrogen (E) may play a role in the steroid-mediated augmentation of peripheral nerve regeneration. While the presence of estrogen receptors (ER) in the facial motor nucleus (FMN) has been seen transiently in the developing rodent, ER have not been shown to be present in the adult FMN. In this study, we used steroid autoradiography to examine the hypothesis that E influences facial nerve regeneration via classically mediated steroid hormone action. Three groups of adult hamsters were used: normal males, castrated males + unilateral facial nerve axotomy, and castrated males + unilateral facial nerve axotomy + TP (1 s.c. capsule). Postaxotomy survival time was 10 days. Two days prior to sacrifice, normal males were castrated and T capsules were removed from implanted, castrated + axotomy animals. Hamsters were injected with tritiated E (s.c.; 1.5 mCi/g body weight) and killed 1.5 hrs later. Preliminary results (11 weeks exposure) revealed that while E accumulation was apparent in positive control nuclei (e.g., hypothalamus), no labeling was detected in the FMN. Thus, E effects on facial nerve regeneration do not appear to involve classically mediated steroid action at the FMN. Supported by NIH NS28238 (KJJ).

231.13

TNF-α AND IL-1 ACTIVATE MAP AND SAP KINASE IN HUMAN NEUROMA FIBROBLASTS. G. Lu, R.W. Beuerman,* S. Zhao, G. Sun, D.H. Nguyen, S. Ma, D.G. Kline LSU Eye Center and Dept. of Neurosurgery (DGK, SZ), LSU Medical Center, New Orleans, LA 70112

Tumor necrosis factor alpha (TNF-α) and interleukin-1 (IL-1) induce activation of mitogen-activated protein kinase (MAPK) and stress-activated protein kinase (SAPK), which locate at parallel signal transduction pathways and are involved in cell cycle G_0/G_1 transition and cellular proliferation in human fibroblasts. These cytokines are released by macrophages during the early inflammatory phase of nerve injury. In our study, fibroblasts cultured from surgically removed human neuromas were serum starved and stimulated with TNF-α, IL-1 (a and b), phorbol 12-myristate 13-acetate (PMA), or platelet-derived growth factor-AB (PDGF-AB), a potent activator for MAPK, individually or in combination. Myelin basic protein (MBP) and human *c-jun* (1-169) glutathione S-agarose transferase (GST) fusion protein were used as substrates in the protein kinase assay. The results showed that TNF-α and IL-1 produced a rapid activation of MAPK, with a peak at 15 minutes for TNF-α stimulation, and a peak at 30 minutes for IL-1 (a and b) stimulation. Increases in MAPK induced by TNF-α and IL-1 were similar to those induced by PMA and PDGF-AB. TNF-α and either IL-1a or IL-1b were synergistic in the activation of MAPK. In addition, TNF-α and IL-1 markedly increased activation of SAPK, whereas PMA and PDGF-AB were much less effective. The results of our study suggest that TNF-α and IL-1 may promote fibroblast proliferation by strongly stimulating these kinases after nerve injury and that this mechanism may play a role in forming the incipient neuroma. *Support:DAMD17-93-V-3013; EY04074, EY02377, NEI*

231.14

INCREASE IN GAP43 PROTEIN IN SCIATIC NERVE INJURY AND NEUROMA FORMATION. S. Zhao, Q. Ma, R. W. Beuerman, G. Lu, D.G. Kline*. Departments of Neurosurgery (SZ, DGK) and Ophthalmology, LSU Medical School, New Orleans, LA 70112.

The current study was designed to determine the expression of the neuronal growth-related protein GAP43 after sciatic nerve lesion and early stage of neuroma development. Sciatic nerves of adult rats were severed below the sciatic nerve notch and the severed ends were treated in one of three ways: (1) perineurium and endoneurium were sealed by a bipolar coagulator and buried in the muscles; (2) perineurium was tied with #6 suture; and (3) no treatment. The nerves were harvested at 2 days, 4 days, 14 days, and 1 month post surgery. Specimens of nerve (0.5 cm) were removed from the severed ends and total proteins were extracted. The results of Western blot showed that beginning 2 days post surgery, GAP43 protein was expressed in all the severed nerves. At 14 days after surgery, the nerves that were tied with suture tended to yield more GAP43 than the nerves in the other two groups. Further study is needed to determine the roles of GAP43 in nerve regeneration and neuroma development in the early stages after nerve repair or injury.

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231.15

CHANGES IN UBIQUITIN IMMUNOREACTIVITY IN THE QUAIL CILIARY GANGLION AFTER POSTGANGLIONIC NERVE CRUSH. M.E. De Stefano*, R. Squitti and G. Toschi. Dip. Biologia Cellulare e Sviluppo, Università "La Sapienza", P.le A. Moro 5, 00185-Roma, Italy.

Ubiquitin (Ub) is a highly conserved eukaryotic protein involved in several cell processes, such as the intracellular breakdown of damaged proteins in stress conditions. We studied, by light microscopy, the immunocytochemical localization of Ub in the quail normal ciliary ganglion, and measured the changes in immunolabelling intensity, after postganglionic nerve crush, in experimental and contralateral ganglia. The numerical values represent an arbitrary absorbance unit (gray scale value from 1 to 260) obtained using an image analyzer. In normal ganglia, both neuronal populations -ciliary and choroid neurons- show Ub immunoreactivity (Ub-ir). The immunoreaction product is evenly distributed in the cell cytoplasm and labels the nucleus intensely. Six hours after crushing the postganglionic ciliary nerves, we observed a slight, but significant increase (3.61±0.26) of Ub-ir in the cytoplasm of the ciliary neurons compared with normal ganglia (1.94±0.07). The immunopositivity reaches a peak 1 day after surgery (11.17±0.66), and then decreases after 3 (5.50±0.17) and 6 (4.51±1.11) days, when the nuclei appear strongly immunolabeled and highly eccentric. In the contralateral ganglia, Ub-ir is always significantly lower than in the corresponding treated ganglia. Our data suggest that the early increase in cytoplasmic Ub-ir in ciliary neurons, subjected to a reversible chromatolytic process after postganglionic nerve crush (De Stefano *et al*, 1994), is related to the targeting and degradation of damaged proteins. The strong nuclear positivity observed 6 days after crushing may, on the other hand, be related to high histon ubiquitination, involved in maintaining the structure of transcriptionally active chromatin. *Supported by Fondazione Cenci Bolognetti to MEDS and by MURST.*

231.16

EXPRESSION OF NEUREGULINS AND THEIR PUTATIVE RECEPTORS, ERBB2 AND ERBB3, IS INDUCED DURING WALLERIAN DEGENERATION S.L. Carroll*, M.L. Miller, P.W. Frohnert, S.S. Kim, and J.A. Corbett Div. of Neuropathology, Washington U. Sch. of Med., St. Louis, MO 63110.

Schwann cell dedifferentiation and proliferation is a prerequisite to axonal regeneration in the injured peripheral nervous system. The neuregulin (NRG) family of growth and differentiation factors may play a particularly important role in this process as these axon-associated molecules are potent Schwann cell mitogens and differentiation factors in vitro. We have examined Schwann cell DNA synthesis and the expression of NRGs and their receptors, the erbB membrane tyrosine kinases, in rat sciatic nerve, sensory ganglia and spinal cord 0-30 days post-axotomy. Analysis of NRG cDNAs from these tissues revealed several novel splice variants and showed that cells endogenous to injured nerve express NRG mRNAs. A selective induction of mRNAs encoding the GGF subfamily of NRGs occurs in Schwann cells, beginning 3 days post-axotomy. The onset of GGF mRNA accumulation and Schwann cell DNA synthesis coincide precisely. In later stages of Wallerian degeneration, however, Schwann cell mitogenesis markedly decreases while elevated GGF expression persists. Of the four known erbB kinases, Schwann cells express both erbB2 and erbB3 receptors over the entire interval studied. Expression of erbB2 and erbB3 is coordinately induced in response to axotomy, indicating that Schwann cell responses to NRGs may be modulated by changes in receptor density. Thus, in contrast to the concept of NRGs as axon-associated mitogens, our findings argue that NRGs produced by Schwann cells within the adult nerve itself induce Schwann cell proliferation during Wallerian degeneration, probably acting via autocrine or paracrine mechanisms.

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231.17

GLIAL FIBRILLARY ACIDIC PROTEIN (GFAP) EXPRESSION IN THE HAMSTER FACIAL MOTOR NUCLEUS (FMN): EFFECTS OF AXOTOMY AND STEROIDS. K.J. Jones[1,2], N.B.Kinderman[1,2], and M. M. Oblinger*[3]. [1]Dept. of Cell Biology, Neurobiology & Anatomy, Loyola Univ. Chicago, Maywood, IL 60153, [2]Rehab. Res. & Dev., Hines VA Hospital, Hines, IL 60141, [3]Dept. of Cell Biology & Anatomy, Chicago Med. Sch., North Chicago, IL 60064.

It has been shown that GFAP, the astrocyte-specific intermediate filament, increases in response to neural injury and that gonadal steroids regulate GFAP expression in the brain. Work in our laboratory has established that gonadal steroids accelerate recovery from facial paralysis following facial nerve injury in adult male hamsters. In this study, we examined the expression of GFAP mRNA levels in hamster FMN following facial nerve severance at the stylomastoid foramen. Adult gonadectomized male hamsters received right facial nerve axotomies, with the left side serving as internal control. Half the animals were also given TP at the time of injury. At 6h , 1, 2, 4, 7 or 14 d postoperative (dpo), the animals were killed and the brains quickly removed and frozen. *In situ* hybridization was performed with a ^{33}P-labeled GFAP cDNA probe. Slides were processed for autoradiography and quantitative analysis was performed using a computerized image analysis system. The results indicate that GFAP mRNA levels are significantly increased in the axotomized FMN of both TP-treated and nonTP-treated animals, relative to controls, by 2 dpo and remain elevated throughout the 2-week time period analyzed. However, between 2-7 dpo, GFAP mRNA levels were significantly *reduced* on both the injured and the control sides in the TP-treated animals, relative to the levels in the corresponding FMN from the nonTP-treated animals. These results are consistent with previous work identifying a preservation of synaptic input to the FMN after injury plus TP and suggest that a component of the mechanism by which steroids accelerate axonal regrowth after injury may involve GFAP. Supported by NIH grants NS28238 (KJJ) and AG13338 (MMO).

231.18

GONADAL STEROID REGULATION OF CYTOSKELETAL GENE EXPRESSION IN AXOTOMIZED HAMSTER FACIAL MOTONEURONS (FMN). P. D. Storer*[1], S. M. Drengler[1], M. M. Oblinger[2], and K. J. Jones[1,3]. [1]Dept. of Cell Biol., Neurobiol., and Anat., Loyola University, Chicago, Maywood, IL 60153, [2]Dept. of Cell Biol. and Anat., Chicago Med. Sch., North Chicago, IL 60064, [3]Rehab. Res. and Dev., Hines VA Hospital, Hines, IL 60141.

Axotomy of facial motoneurons(FMN) induces cytoskeletal mRNA expression as a component of the neuronal regenerative response. Testosterone proprionate (TP) can act as a trophic factor by augmenting select elements of the injury response, leading to accelerated nerve regeneration. In this study, we tested the hypothesis that TP treatment can affect the mRNA levels of α_1-tubulin and β_{II}-tubulin, two key cytoskeletal proteins involved in neuronal regeneration. Previous findings from northern hybridization studies showed that TP treatment differentially augments tubulin gene expression in axotomized FMN. Here, adult, gonadectomized male hamsters received right facial nerve axotomy, with the left side serving as internal control. Half the animals received TP implants at the time of injury. At 0.25, 2, 4, 7, or 14 days postoperative (dpo), animals were killed and the brains quickly removed and frozen. *In situ* hybridization was performed using ^{33}P-labeled cDNA probes to α_1-tubulin and β_{II}-tubulin. Slides were processed for autoradiography and quantitative analysis was performed. Results demonstrate that α_1-tubulin and β_{II}-tubulin mRNA levels increase significantly in axotomized FMN by 2 dpo relative to controls, and remain elevated through 14 dpo. In the TP treated animals, the induction of both cytoskeletal mRNAs by axotomy was enhanced, relative to axotomized non-TP animals. With TP, mRNA levels increased as early as 0.25 dpo, and remained significantly higher than non-TP levels through 14 dpo. The ability of TP to modulate these cytoskeletal mRNA levels confirms that TP can influence the cell response to injury, and further supports a role for TP in recovery from peripheral nerve damage. Supported by NIH grant NS28238 (KJJ) and AG13338 (MMO).

231.19

CYTOSKELETAL PROTEIN EXPRESSION IN LONG-TERM AXOTOMISED FACIAL AND SCIATIC MOTONEURONS. T. Petrov*, S. You, S.L. Cassar, W. Tetzlaff, T. Gordon. Div. Neurosci. Univ. of Alberta, Edmonton, Canada T6G, 2S2 and Dept. Zool. & Surgery, UBC, Vancouver, B.C. V6T 1Z4

This comparitive study of the expression of cytoskeletal proteins in axotomised rat facial and sciatic motoneurons determines whether reduced regenerative success after prolonged axotomy is due to a reduced capacity to maintain expression of regeneration associated genes and/or to respond to a refreshment axotomy. Facial or sciatic nerves were transected unilaterally and 1 to 12 months later, the contralateral nerves were transected 7 days prior to sacrifice and the experimental nerves cut a second time. There were four clear differences in the effect of prolonged axotomy on facial and sciatic motoneurons: 1) a 2.5 fold increase in actin mRNA at 7 days declined rapidly to control levels within 1 month of axotomy (facial) but more gradually within 6 months after sciatic axotomy, 2) increase in actin and alpha-tubulin mRNA in response to a second axotomy reached the levels of acutely axotomized sciatic but not facial motoneurons, 3) downregulation of neurofilament after the first axotomy was further enhanced by second axotomy in facial but not in sciatic motoneurons and 4) many facial but not sciatic motoneurons showed dramatic atrophy 1 year after axonal injury which was reversed by the second axotomy. Thus injury-induced upregulation of tubulin and actin is not maintained in long term axotomized motoneurons but can be induced again by a second injury. The second axotomy also compromises axonal size in facial motoneurons by a further downregulation of neurofilament. Both types of motoneurons respond to the second axotomy, but the weaker response of chronically injured facial motoneurons might indicate that their regenerative capacity is compromised as early as 6 months following chronic disconnection from their targets. It remains to be determined whether sciatic motoneurons show a weaker response with more prolonged axotomy or whether the differences are due to different distance between the cell body and the injury site in the two models. (Supported by Canadian MRC and NCE).

231.20

CHANGES IN MAP1B PHOSPHORYLATION DURING REGENERATION OF AXOTOMIZED SCIATIC NERVE IN ADULT RAT

D. Ma, T. Connors and I. Fischer*
Department of Neurobiology and Anatomy, The Medical College of Pennsylvania and Hahnneman University, Philadelphia, PA 19129

MAP1B is a major component of the neuronal cytoskeleton during early stages of development particularly in growing axons. In normal adult rat the phosphorylated MAP1B isoform, recognized by the monoclonal antibody 1BP (Boyne et al., J. Neurosci. Res. 40:439, 1995), was present in axons of DRG neurons, but not in their cell bodies. In contrast polyclonal antibodies that recognize all isoforms of MAP1B detected the protein in DRG neurons as well as their central and peripheral branches. Changes in MAP1B and its mRNA were analyzed in DRGs and their axonal projections using Western blots and RNase protection assays. The analysis showed that after axotomy there were no significant change in the levels of MAP1B and its mRNA in DRGs, their central branches and the proximal parts of peripheral branches. However, in the distal parts of sciatic nerve phosphorylated MAP1B was reduced as early as 3 days after sciatic nerve injury probably due to the Wallerian degeneration of axons. The levels of phosphorylated MAP1B in the distal sciatic nerve increased between 1-3 months after the injury concurrent with the arrival of regenerating axons to this region. We reasoned that MAP1B, which is transported into the growing distal-end of the axons with the slow axonal transport fraction, is phosphorylated in axons and may be required in facilitating axonal growth both during development and nerve regeneration. We propose that the relatively high levels of MAP1B and its mRNA in adult DRGs (Ma et al., J. Neurochem. 66:s64, 1996) provide structural plasticity to peripheral neurons and may correlate with their ability to regenerate without inducing this protein. Supported by NIH grant NS24725, NS 24707 and a fellowship from AHERF neuroscience center.

TRANSPLANTATION: FUNCTIONAL

232.1

HYPERTROPHY OF BRANCHING IN AXONS REGENERATED FROM ALLOTRANSPLANTED NEURONS IN THE CRAYFISH. KM Krause* and B Arietta, Department of Natural Sciences, St Thomas Aquinas College, Sparkill, NY 10976.

The branching pattern of transplanted neurons which reinnervate the superficial flexor muscle (SFM) of the crayfish *Procambarus clarkii* was examined using scanning electron microscopy (SEM). The 3rd and 4th abdominal ganglia, with attached SFM nerve, were isolated from one animal and transferred to the denervated field of another individual. Earlier electrophysiological work had shown that these transplanted neurons were able to accurately reform specific connections, yet only 65% of host muscle fibers displayed functional synapses suggesting feeble regrowth. (Krause and Velez, J. Neurobiol. 27:154-171, 1995) However SEM micrographs made 8-15 weeks after surgery reveal that the SFM axons from transplanted ganglia in fact exhibit robust regrowth with extensive branching. The fact that specificity remains intact despite hypertrophy suggests that the morphological alterations observed do not represent a failure to eliminate inappropriate connections, but a failure to coordinate the release of the proper signaling molecules from the pre- and post synaptic cells. Axons which were allowed to regenerate following simple axotomy did not show abnormal amounts of sprouting. Thus extensive sprouting is not a normal part of the regeneration response, but results from exogenous influences unique to transplanted neurons. *Funded by St. Thomas Aquinas College Fund*

232.2

PRENATAL ENGRAFTMENT OF CNS STEM-LIKE CELLS: AN APPROACH TO THERAPY FOR CAPRINE β-MANNOSIDOSIS. K.L. Lovell*, C. Mitchell-Herpolsheimer, N.K. Ames, E.Y. Snyder, Depts. Pathol. & Large Animal Clin. Sci., Mich. State Univ., E. Lansing, MI; Depts. Neurol. & Pediatr., Children's Hosp., Harvard Med. School, Boston, MA.

In caprine β-mannosidosis, inherited deficiency of the lysosomal enzyme β-mannosidase is associated with severe clinical manifestations at birth, cytoplasmic vacuolation and dysmyelination. However, a goat homozygous for the β-mannosidosis mutation, recently documented as a natural hematopoietic chimera, showed late postnatal onset of mild neurological symptoms and normal myelination. Studies demonstrated that a small amount of normal enzyme supplied prenatally significantly ameliorated the disease (Jones et al., J.Inher. Metab.Dis. 16:1012,1993). The present report describes studies targeted toward prenatal intracerebral therapy using two types of normal CNS cells: caprine fetal primary cells and the C17-2 murine neural stem-like cell line transduced with the lacZ gene (Snyder et al., Cell 68:33,1992; Snyder et al., Nature 374:367,1995; Lacorraza, et al., Nature Med. 2:424,1996). Cells were injected intracranially into two 60-day-gestation fetuses, during the immunotolerant period. Animals were sacrificed 8 days post-injection for analysis of transplanted cells, either by fluorescence to detect fast blue-labelled cells, or by incubation for 16 hours in X-gal. Donor cells were visualized in brain sections of both recipients. The activities of β-mannosidase and β-hexosaminidase were measured in media from cultured cells. Both cell types secreted lysosomal enzymes into the media. To our knowledge, this is the first report of a prenatal intracerebral xenograft (mouse to goat) of CNS stem-like cells during the immunotolerant period. These results reinforce the potential efficacy of prenatal therapy for lysosomal storage diseases characterized by early neurodegeneration. Support: NS33911 to M.Z.Jones & NS34247,NS33852, APA & PVA to E.Y.S.

232.3

INTRINSICALLY LABELED RELAY NEURONS OF HOMOTOPIC OLFACTORY BULB TRANSPLANTS ESTABLISH ORGANOTYPIC SYNAPSES WITH HOST NEURONS. G. Sekerková[1,2], Z. Katarova[1], E. Mugnaini[2*], E. Joó[3], J.R. Wolff[4] and G. Szabó[1]. Biological Research Centre, Institute of [1]Biochemistry and [3]Biophysics, 6701 Szeged, Hungary, [2]Northwestern University, Institute of Neuroscience, Chicago, Il, 60611 and [4]University of Göttingen, Dept. Anatomy, 37075 Götingen, Germany,

Embryonic olfactory bulb (OB) of transgenic mouse line Tg(GAD67*lac*Z7.5)1, in which the glutamic acid decarboxylase/*lac*Z transgene is strongly expressed in nearly all the mitral and tufted cells, was transplanted into unilaterally bulbectomized neonatal non-transgenic mice. Transplants were evaluated by β-galactosidase enzyme- and immunohistochemistry at the light and electron microscopic level. After 2 months survival the transplant developed into a bulb-like structure that occupied the space between lamina cribrosa and forebrain. The typical laminar organization of the OB was not present in this newly formed bulbar structure. The OMP-positive glomeruli were found irregularly distributed throughout the transplant. The grafted relay neurons, identified by the presence of blue reaction product filling their whole cytoplasm, were scattered in the neuropil of the transplant individually or in small groups. They showed long-term postoperative survival, received afferent axodendritic synapses from regenerated host olfactory terminals within newly formed glomerular arrays, established typical local circuits with unlabeled granule cells, and extended their axons into the piriform cortex where they synapse onto dendrites of host neurons. Our data show that homotopically transplanted relay neurons of the OB are able to integrate into the host olfactory pathway and indicate possible functional recovery. (Supported by OTKA T016971, OTKA T020638, NS09904 and Volkswagen Stiftung #I-7-777)

232.4

Transplantation of EGF-responsive neural stem cells derived from GFAP-hNGF transgenic mice attenuates excitotoxic striatal lesions. M.K. Carpenter*[1], C. Winkler[2], R. Fricker[2], S.C. Wong[1], C. Greco[1], D. Emerich[1], E.-Y. Chen[3], Y. Chu[3], J. Kordower[3], A. Messing[4], A. Björklund[2], J. P. Hammang[1]. 1) Cell and Molecular Neurobiology, CytoTherapeutics, Inc., Providence, RI 02906; 2) University of Lund, Sweden; 3) Rush Presbyterian Medical Center, Chicago, Illinois; 4) School of Veterinary Medicine, Madison, Wisconsin, 53706.

EGF-responsive neural stem cells isolated from the adult and embryonic striatum propagated in vitro, have the capacity to differentiate into both neurons and glia. Current strategies for the genetic modification of neural stem cells must overcome a number of problems including toxicity, long-term gene stability, transfection or infection efficiency and specificity. The use of transgenic mice in which promoter elements direct the expression of various genes may be an improved method for the genetic modification of these stem cells. We have generated transgenic mice in which the human GFAP promoter directs the expression of human NGF. EGF-responsive stem cells generated from these transgenic animals proliferate and form neurospheres, which appear identical to stem cells generated from control (non-transgenic littermate) animals. Upon differentiation, the transgenic stem cell-derived astrocytes express hNGF as indicated by ELISA. Previous studies have shown that local cell-based delivery of NGF will protect against QA lesions. We transplanted approximately 250,000-500,000 undifferentiated GFAP-hNGF or littermate control stem cells into the striatum of adult rats. One week after transplantation the rats received a striatal QA lesion (225 nmol). Two weeks post-lesion the animals were sacrificed and the brains were evaluated. Animals which received control cells showed comprehensive lesions of the striatum, whereas animals which received GFAP-hNGF stem cells show much smaller lesions. Qualitative histological assessment further demonstrated a sparing of ChAT, GAD and NAPH-d-positive neurons within the striatum of rats receiving GFAP-hNGF stem cells. These data suggest that transplanted GFAP-hNGF stem cells survive, differentiate, secrete NGF and can protect against excitotoxic lesions.

232.5

XENOGENEIC ENGRAFTMENT OF PORCINE FETAL LATERAL GANGLIONIC EMINENCE CELLS INTO THE RAT HIPPOCAMPUS: A POTENTIAL THERAPY FOR EPILEPSY

D.B. Jacoby, C. Lindberg, J. Ratliff, J. Dinsmore.* Diacrin, Inc., Charlestown, MA 02129.

Considerable research has demonstrated that intracerebral engraftment of fetal neural cells can potentiate the severity of epileptic seizures in animal models. Noradrenergic locus ceruleus neurons have been used to reinnervate the hippocampus and normalize basal and seizure-induced noradrenaline levels in the host brain. However, while some reports are promising, others have demonstrated only mild anticonvulsant effects. Recently, the inhibitory neurotransmitter gamma aminobutyric acid (GABA) has been shown to suppress seizures, thus raising the possibility that GABAergic tissues such as fetal lateral ganglionic eminence (LGE) cells may provide a regulated source of GABA. Porcine fetal LGE cells have previously been shown to survive and integrate in the adult rat striatum, but work by others has shown poor survival of fetal striatal cells outside of the striatum. We now address whether ectopically placed porcine LGE cells can survive and integrate in the hippocampus, a central regulator of epileptic activity. Porcine LGE cells were stereotaxically transplanted into the hippocampus of adult rats. To enhance graft survival, animals received immunosuppression with cyclosporine A (10 mg/kg/day, s.c.), or cells were treated with F(ab')2 antibody fragments directed against MHC class I antigen prior to engraftment. Our preliminary data demonstrate that fetal pig striatal grafts survive long term within the hippocampus.

These studies were supported by Diacrin, Inc.

232.6

COMPETITIVE INTERACTIONS FOR ADULT OLFACTORY BULB FOLLOWING ALLOGRAFTING INTO ADULT RAT STRIATUM: IMPLICATIONS FOR BRAIN DYNAMICS.

N.H. Liou*, J.J. Yu, J.C. Liu and F.W. Chang. Departments of Biology and Anatomy, National Defense Medical Center, P.O. Box 90048-502, Taipei, Taiwan, Republic of China.

Allografts derived from donor adult organ had long been used to overcome chronic abnormalities in human. Besides that, embryonic central nervous system (CNS) tissue such as substantia nigra (SN) have proven to be feasible as graft secreting dopamine and exhibited promising effect on the functional recovery from rat or human model of Parkinson's disease. However, various issues concerning practical use for allografting embryonic tissue still remained to be determined. Based upon those studies, instead use fetus tissue and/or cells we raise an issue expanding the avenue of allografts to explore fundamental biological issues and applications through the use of adult CNS tissue implanted in the adult host CNS. Documented evidence indicated that striatum is the area integrating as well as coordinating input information originated from general and special sensation. Adult olfactory bulb (AOB) that organized in the extent of less laminated structure and connected with outside in a relatively simple circuits. Under normal situation, there is no direct connection between these two structures. Hence, AOB is chosen from donor to determine whether its allografted adult tissue can survive robustly with increasing time after transplantion in the adult host striatum. Consequently, patterns of nerve innervation following grafting in the striatum and cell-cell interactions between graft and host tissue are necessary to examine if the well-integrated phenomenon can be achieved. Under this rational process, functional evaluation such as rotational behavior was also being tested. Results suggest that neurons of AOB still survive at the time tested four months after grafting. Striatal nerve innervation under retrograde tracing studies display increasingly isolated phenomenon following grafting compared to that pattern exhibited before grafting. EM observations reveal apparently consecutive characteristics of reorganization surrounding graft area. Further experiments are underway to extend the notion of brain dynamics in the adult mammalian CNS.

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232.7

IMPROVEMENT OF CHOLINERGIC EXPRESSION IN SEPTAL TRANSPLANTS BY PURIFYING CHOLINERGIC FETAL NEURONS,

J. Cadusseau, L. Verneuil and M. Peschanski* INSERM U 421 Faculté de Médecine Créteil FRANCE

Transplants of fetal septal-diagonal band tissue have been reported to supply neurons which mature and grow when implanted into a denervated host parenchyma. However the ability to reinervate the target is limited, due to the presence of heterogenous cell populations and a low number of cholinergic neurons. Attempts to enrich septal transplants in cholinergic neurons have used immunopanning. At developmental age E17 the septal areas of rat embryos contain many neurons expressing the low affinity NGF receptor (LNGFr) and in the adult septum nearly all the LNGFr positive neurons are choline acetyltransferase (ChAT) positive. We used antibodies against the LNGFr to purify the cholinergic neurons of the E17 septal area. The enrichment of cell suspensions in cholinergic neurons was assessed by analyzing the purified fraction and its complement by acetylcholine esterase (AChE) histochemistry, LNGFr and gamma-aminobutyric (GABA) immunohistochemistry. A highly enriched population of viable cholinergic neurons was isolated. The cholinergic fetal cell suspension was then transplanted into the neuron depleted parenchyma of an adult host and the cholinergic expression of the transplanted tissue was compared with the cholinergic expression of transplants obtained from non-purified suspensions. After 40 days survival, grafts obtained from purified transplants were darkly stained by AChE histochemistry, with nearly all the neurons being LNGFr immunoreactive. More than half the grafted neurons were also ChAT positive, while only a very few were GABA positive. By contrast, grafts obtained from non purified cell suspensions contained very few LNGFr and ChAT immunoreactive neurons but a majority of GABA positive neurons. These results indicate that LNGFr positive neurons can be selected from fetal septal cell suspensions, and the majority of the selected neurons mature into cholinergic neurons when transplanted into an adult parenchyma.

Supported by a grant from INSERM.

232.8

BEHAVIORAL EFFECTS OF AF64A STRIATAL LESIONS AND REVERSAL OF LEARNING DEFICITS BY NEURAL TRANSPLANTS.

M. Giordano*, M. Sánchez-Alavez, R. Salado-Castillo and R. Prado-Alcalá. Centro de Neurobiología-UNAM, Departamento de Fisiología, Facultad de Medicina-UNAM, México D:F.

Ethylcholine mustard aziridinium ion (AF64A) is an irreversible ligand for high affinity choline transport system, a cholinotoxin and an inhibitor of choline acetyltransferase. Intrastriatal and i.c.v. injections of AF64A have been shown to disrupt the acquisition and retention of learning tasks. Neural transplants can reverse behavioral abnormalities and establish anatomical connections with the host brain. In the present study, the effects of striatal AF64A lesions and fetal striatal grafts on a passive avoidance task and on spontaneous locomotor behavior were evaluated. Male Wistar rats (250-300 g) were habituated to activity chambers and their spontaneous activity was recorded for 15 min before and after each experimental manipulation. Animals received bilateral infusions of AF64A (11 nmols/µl, 0.2 µl/min) into the striatum. One week after the lesion, group 1 received bilateral solid fetal striatal transplants, group 2 received bilateral dissociated fetal striatal transplants, group 3 received bilateral solid cerebellar transplants, group 4 received only bilateral vehicle infusions, group 5 was a naive group. Six weeks after the transplant, animals were trained and tested (30 min and 24 h later) in a passive avoidance task. After the lesion 6/54 animals showed self-injurious behavior and were excluded. The results showed that locomotor activity was at its highest one week after AF64A lesions and for most groups it returned to baseline levels six weeks after the transplant. In the passive avoidance task, sham and cerebellar transplanted groups were significantly different from naive animals in their retention scores 24 hours after training ($t=2-13$, $t=2.25$ respectively, $p<0.05$). There were no significant differences between naive and striatal transplant groups. These results show that striatal transplants can reverse the retention impairment induced by the cholinergic lesion, and that this effect appears to be selective.

Support provided by UNAM-DGAPA to R. Prado-Alcalá.

232.9

TRANSPLANTATION OF HUMAN TERATOCARCINOMA NEURONAL (hNT) CELLS INTO ISCHEMIC RATS PRODUCES RECOVERY OF MOTOR FUNCTION AND PASSIVE AVOIDANCE BEHAVIOR.

P.R. Sanberg* and C.V. Borlongan. Div. Neurol. Surgery, Depts. Surgery, Neurology, Psychiatry, and Pharmacology, Univ. South Florida Coll. Med., Tampa, FL 33612.

Transplantation of fetal striatal tissue into ischemic rats introduced to occlusion of the middle cerebral artery (MCAo) has been shown by Nishino and colleagues (1993) to reverse profound deficits in passive avoidance learning and memory. The present study was designed to replicate this and to determine if transplantation of human hNT cells into ischemic rats can produce recovery of function. Eight-week old rats initially received MCAo ischemic lesions or sham lesions. One month following surgery, animals were tested for asymmetric motor behavior (EBST) and passive avoidance behavior, then assigned to one of the following groups: Normal animals, Sham-surgery/with medium infusion, Stroke-surgery/with medium infusion, Sham-surgery/with striatal cell transplant, Stroke-surgery/with striatal cell transplant, Stroke-surgery/with cerebellar cell transplant, Stroke-surgery/with fresh hNT cell transplant and adjunct Cyclosporine (CsA) treatment, Stroke-surgery/with cyropreserved hNT cell transplant and CsA treatment, Stroke-surgery/with fresh hNT cell transplant and no CsA treatment, Stroke-surgery/with CsA treatment only. At 1, 2, and 3 months post-transplant, animals were evaluated again on the behavioral tests. After completion of these tests, approximately half of the animals were sacrificed for histological immunohistochemistry. At 1 month post-transplantation there was significant recovery in both EBST and passive avoidance task in all three groups given hNT cells or fetal striatal tissue. This behavioral recovery continued for all three months post-transplant, except in the hNT fresh group without CsA which showed a significant decreasing trend of behavioral recovery for two and three months post-transplant. Control animals showed no behavioral recovery. Histological analysis will be reported by Trojanowski and colleagues. Transplantation of hNT cells into the infarcted striatum of rats introduced to transient focal ischemia has been demonstrated to ameliorate motor and cognitive deficits associated with such ischemia. The observed recovery following transplantation of hNT cells paralleled that of the transplantation of fetal striatal cells which has been the primary graft source for a number of neurodegenerative disorders such as Huntington's disease and recently with cerebral ischemia.

(This study was supported by Layton Bioscience, Inc., Atherthon, CA.)

232.10

XENOTRANSPLANTED CHOLINERGIC NEURONS INTO ANIMAL MODELS OF COGNITIVE DYSFUNCTION

C. Le Blanc[1] L. Burns[1]* P. Borghesani[1] T. Deacon[1,3] J. Dinsmore[2] O. Isacson[1]. Neuroregeneration Laboratory, McLean Hospital, Belmont, MA 02178 and Program in Neuroscience, Harvard Medical School[1], Diacrin, Inc., Charlestown, MA 02129[2] Boston University, Boston, MA 02115[3]

Recent clinical observations and specific use of immunotoxins selective for the forebrain cholinergic system support the notion of cholinergic synaptic function in cognitive processes such as those disrupted in Alzheimer's disease. We have produced cholinergic dysfunction in rats either by lesions of the nucleus basalis using excitotoxins or by infusions of saporin conjugated antibodies to the rat p75NGF-receptor into the lateral ventricle. IgG saporin lesions were found to deplete 75-98% of cholinergic neurons in the septum diagonal band region and basal forebrain. We compared cholinergic neuron production from different regions of pig fetal brains. Medial ganglionic eminence, fronto-medial telencephalon, and fronto-basal telencephalon were selectively dissected from fetal pigs (E30-E35) and cell suspensions from these tissues were transplanted into the nucleus basalis of immune suppressed lesioned animals. After 2-3 months survival, histological analysis with a second p75NGF-receptor antibody and a 70kd neurofilament antibody, both reactive with pig but not rat neurons, demonstrated cholinergic cells from all three graft types. Both p75NGFr- and NF70-immunoreactive axons could be traced long distances within the host brain. Behavioral recovery by porcine grafts is currently being tested in a Morris water maze. This study demonstrates the capacity of transplanted xenogeneic cholinergic neurons to survive and replace neurons previously lost in an animal model of a cholinergic neurodegenerative process.

(Supported by funds from Diacrin Inc. and McLean Hospital.)

232.11

RESTORATION OF MNEMONIC FUNCTIONS WITH TRANSPLANTS OF FETAL ENTORHINAL CORTEX. L.A. Rothblat[1,2*], T.C. Gleason[1], R.M. Torrisi[1], S.M. Matzke[1], N. Vnek[1], and J.M. Rosenstein[2]. [1]Dept. of Psychology; [2]Dept. of Anatomy & Cell Biology, The George Washington Univ., Washington, DC 20052.

It has been shown that bilateral transections of the angular bundle (AB) impairs retention, but not acquisition of visual object discriminations (Vnek et al., J. Neurosci., 1995). This lesion (1) removes entorhinal cortex (EC) inputs to the hippocampus and (2) results in degeneration of layer II EC cells. In this study we wanted to see if the memory impairment that results from AB transections can be reversed with fetal transplants. Seventeen rats received bilateral knife cuts intended to partially transect the perforant pathway. Seven to 10 days later, 7 of the lesioned rats received embryonic EC grafts placed bilaterally into the dentate gyrus (DGT). Ten rats had lesions with no transplants (AB) and 6 served as normal controls (NC). After two weeks, rats began behavioral testing on a spatial alternation task and then were trained on a series of object discriminations. For the discrimination task, animals learned 3 problems simultaneously. Following a 3 week interval, they were tested for retention (RT1), then tested again two weeks later (RT2).

While DGT and AB rats were impaired on the spatial task, rats in all 3 groups learned the object discriminations normally. On retention, only the AB group was significantly impaired. DGT scores were somewhat elevated on RT1, but were normal on RT2. These results suggest that fetal EC grafts placed directly into the dentate gyrus can ameliorate some of the deficits that result from EC-hippocampal disconnection. The EC transplants could improve nonspatial mnemonic function either by restoring perforant path inputs to the hippocampus (Woodhams et al., Neurosci., 1992) or by providing trophic factors that prevent axotomy-induced degeneration in entorhinal cortex (Peterson et al., J. Neurosci., 1994). *Supported by Alzheimer's Association Grant PRG-94-172.*

232.12

PHENOTYPIC EXPRESSION OF HUMAN FETAL CEREBELLAR CELLS FOLLOWING TRANSPLANTATION INTO NUDE MOUSE CEREBELLUM. L. L. Pundt*, E. A. Jörn, J. A. Conrad, W. C. Low. Depts. of Neurosurgery, and Physiology, and Program in Neuroscience, Lions Research Bldg., University of Minnesota Medical School, Minneapolis, MN, 55455.

Previous rodent studies have demonstrated the capacity of grafted cerebellar cells to organize into the trilaminar cell layers typical of the normal cerebellum. Utilizing Purkinje cell deficient hosts, transplanted Purkinje cells originating from fetal cerebellum of wild-type donors have been shown to migrate from the graft site to the location of the missing neurons, and form synaptic contacts. In addition, it has been demonstrated that fetal grafts of normal cerebellar tissue transplanted into the Purkinje cell degeneration (PCD) mutant mouse results in an improvement of motor behaviors in open field and balance rod tests. These studies indicate the potential therapeutic use of neural transplantation in patients with cerebellar ataxia. In the present study, human fetal cerebellar tissue (8.5 weeks post conception; n=2) was dissociated and transplanted into the cerebellum of normal nude mice (n=10). Six months following transplantation, histological analysis revealed donor cells within the host cerebellum of nine animals. Calbindin staining revealed the presence of donor Purkinje cells which were organized in discrete cellular layers within the transplant neuropil, and in most cases, demonstrated dendritic processes orientated in the same direction (in the "molecular layer"). Human neurofilament immunostaining revealed donor fibers within the transplant neuropil. Human astrocytes were located within the transplant region and adjacent host tissue. These results demonstrate the capacity of human fetal cerebellar cell suspension to develop into Purkinje cells following transplantation into the rodent cerebellum. Supported by PHS grant RO1-NS-24464 and the Bobby Allison Ataxia Center.

232.13

NEURONAL RESCUE BY EMBRYONIC NEURAL CELL GRAFTS IN LURCHER MUTANT MICE, J. A. Heckroth*, Department of Anatomy and Neurobiology, St. Louis Univ. Sch. of Med., St. Louis, MO 63104.

Lurcher mutant mice ten days of age received embryonic day 12 cerebellar cell suspension grafts in the cerebellar hemisphere. The donor embryos were obtained from a transgenic mouse line (provided by Dr. J. G. Sutcliffe of the Scripps Research Institute) which carries a neuron specific enolase promoter-LacZ construct. Host mice received grafts of between 500,000 and 1,000,000 live cells as determined by Trypan blue staining of the cell suspensions. The cells were injected at 2-4 sites in the right cerebellar hemisphere. Host mice were euthanized at 90 days of age, and the brains processed for: 1) visualization of beta-galactosidase activity, 2) immunohistochemical localization of 28K calcium binding protein (antiserum provided by Dr. Anthony Norman, UC Riverside), or 3) Nissl staining. Olivary neuron numbers were estimated in the right and left olivary complex of ten graft hosts and five 90 day old lurcher mice. Analysis of variance indicates no left-right asymmetry in lurcher mice and shows that the right olivary complex of the graft hosts is identical in cell number to ungrafted lurcher mice. The left inferior olivary complex of the graft hosts, however, contains statistically more neurons than the right, and more neurons than ungrafted lurcher mice. When analyzed by olivary subdivision only the caudal medial accessory olive and dorsal accessory olive exhibit significant neuronal rescue. Given that the grafted neurons undergo a period of differentiation after grafting which may delay their potential therapeutic effects for 5-10 days and considering that olivary degeneration has already begun at the time of grafting, it appears that the grafts are highly efficient in their rescue of olivary neurons. These results prove that neural cell grafts can function to reduce transneuronal degeneration in a progressive neurodegenerative disease. Research supported by NINDS grant #NS33969

232.14

AUTORADIOGRAPHIC STUDIES OF AMINO ACID RECEPTOR BINDING IN CEREBELLAR GRAFTS. K. Stasi, A. Mitsacos, E.D. Kouvelas and L.C. Triarhou*. Dept. of Physiology, Univ. of Patras Med. Sch., 265 00 Patras, Greece and Dept. of Pathology and Lab. Medicine, Indiana Univ. Sch. of Med., Indianapolis, IN 46202

We used quantitative autoradiography of [^{3}H]CNQX (200 nM), [^{3}H]muscimol (13 nM) and [^{3}H]flunitrazepam (10 nM) binding to study the distribution of non-NMDA and $GABA_A$ receptors in the cerebellum of *pcd* mutant mice with unilateral cerebellar grafts. Non-specific binding was determined by incubation with 1 mM Glu, 200 µM GABA or 1 µM clonazepam, respectively. Saturation parameters were defined in wild-type and mutant cerebella. In mutants, non-NMDA receptors were reduced by 35% in molecular layer (ML), by 36% in granular layer and by 33% in deep cerebellar nuclei (DCN). [^{3}H]CNQX binding sites were visualized at higher density in grafts that had migrated to the cerebellar cortex (11 and 23 pmole/mg prot. at 23 and 37 days after grafting), and at lower density in grafts arrested intraparenchymally (8 and 12 pmole/mg prot.). The reduction of non-NMDA receptors in *pcd* ML supports their localization on Purkinje dendrites. The pattern of expression of non-NMDA receptors in cortical vs. parenchymal grafts suggests a possible regulation of their levels by transacting elements from host parallel fibers. $GABA_A$ binding levels in the grafts for both ligands used were similar to normal ML. Binding was increased in DCN of mutants: the increase in [^{3}H]muscimol binding over normal was 271% in the non-grafted and 198% in the grafted side; for [^{3}H]flunitrazepam, the increase was 134% in the non-grafted and 112% in the transplanted side over normal. Such increases in the *pcd* DCN reflect denervation-induced supersensitivity subsequent to loss of Purkinje axon terminals. The relatively smaller increase of $GABA_A$ receptors in the transplanted side compared to the contralateral side indicates a partial normalizing effect of the grafts on postsynaptic supersensitivity in the reinnervated DCN, possibly mediated through the synapses established by donor Purkinje cells. (Supported in part by USPHS R29-NS29283 and EU Biomedicine Programme Contract No. BMH1-CT94-1378).

232.15

IMPLANTATION OF ENCAPSULATED CNTF-PRODUCING CELLS PREVENTS THE BEHAVIORAL DEFICITS AND STRIATAL DEGENERATION IN A RODENT MODEL OF HUNTINGTON'S DISEASE, D.E. Emerich*, M.D. Lindner, S.R. Winn, E-Y Chen[1], B.R. Frydel, J.H. Kordower[1], CytoTherapeutics, Inc. 2 Richmond Square Providence, RI 02906 and Research Center for Brain Repair and Department of Neurological Sciences, Rush Presbyterian Medical Center, Chicago Illinois 60612[1].

Delivery of neurotrophic molecules to the central nervous system has gained considerable attention as a potential treatment strategy for neurological disorders. In the present study, a DHFR-based expression vector containing the human ciliary neurotrophic factor (hCNTF) was transfected into a baby hamster kidney fibroblast cell line (BHK). Using a polymeric device, encapsulated BHK-control cells and those secreting hCNTF (BHK-hCNTF) were transplanted unilaterally into the rat lateral ventricle. Twelve days later, the same animals received unilateral injections of quinolinic acid (QA, 225 nmol) into the ipsilateral striatum. Following surgery, animals were behaviorally tested for apomorphine-induced rotation behavior and for skilled forelimb function using the staircase test. Rats receiving BHK-hCNTF cells rotated significantly less than animals receiving BHK-control cells. No behavioral effects of hCNTF were observed on the staircase test. Nissl-stained sections demonstrated that BHK-hCNTF cells significantly reduced (approximately 50%) the extent of striatal damage produced by QA. Quantitative analysis of striatal neurons further demonstrated that both ChAT and GAD-immunoreactive neurons were protected by BHK-hCNTF implants. In contrast, a similar loss of NADPH-diaphorase-positive cells was observed in the striatum of both implant groups. Analysis of retrieved capsules revealed numerous viable and mitotically active BHK cells that continued to secrete hCNTF. These results support the concepts that implants of polymer-encapsulated hCNTF-releasing cells can be used to protect striatal neurons from excitotoxic damage and that this strategy may ultimately prove relevant for the treatment of Huntington's disease.

232.16

STRIATAL GRAFT GROWTH DEPENDS ON STRIATAL LESION SIZE IN A RAT MODEL OF HUNTINGTON'S DISEASE C. Watts*, E Torres and SB Dunnett. MRC Cambridge Centre for Brain Repair. University of Cambridge. Cambrige CB2 2PY. UK.

The effect of lesion size on the growth and development of intrastriatal striatal transplants was evaluated in the rat model of Huntington's disease.

Thirty female Sprague Dawley rats weighing between 180 - 200g were randomly allocated to 4 groups. 3 groups received unilateral striatal lesions involving stereotaxic injection of 2 x 0.5ul of either 20nM, 40nM,or 90nM quinolinic acid (quin) into the striatum (n=8 per group). The 4th group remained unlesioned (n=6). Seven days after lesioning, 6 rats from each lesion group and all 6 unlesioned rats received a cell suspension graft of 2.3million cells in a volume of 2 ul. The cells were obtained from the whole striatal anlage of E16 (CRL 15mm) rat embryos. Ten weeks after grafting the rats were perfused with 10% formalin and 60 micrometer frozen sections were processed for Tyrosine Hydroxylase (TH) and Dopamine and Cyclic Adenosine 3:5 monophosphate Regulated Phosphoprotein of 32 kdaltons (DARPP-32) immunoreactivity.

Lesions of increasing severity were obtained with increasing concentrations of quin. Graft survival was good in all the lesioned rats. Striatum-like tissue, as defined by DARPP-32, was present in all grafts in localised patches (P-zones). TH staining within the grafts was confined to these P-zones which received TH positive fiber ingrowth from the host neuropil. In contrast, graft survival in intact animals was poor with no P-zones present and little TH fibre ingrowth within the parts of the grafts localised within the host striatum.

These results suggest that grafts will grow and develop areas of stratal tissue in both partial and complete lesions but growth of striatum-like tissue in the intact striatum is inhibited by the intact striatal neuropil. Supported by the Wellcome Trust and MRC.

232.17

THE EFFECT OF INTERVAL BETWEEN LESION AND TRANSPLANTATION ON STRIATAL GRAFT SURVIVAL AND INTEGRATION IN THE QUINOLINIC ACID-LESIONED NEOSTRIATUM OF RATS. S.Thian, C.Watts, E.M.Torres, B.J.Everitt, W.F.Blakemore and S.B.Dunnett (SPON: European Brain and Behaviour Society). MRC Cambridge Centre for Brain Repair, Forvie Site, Robinson Way, Cambridge CB2 2PY, UK.

In order to examine factors influencing neuronal graft survival and integration in an animal model of Huntington's disease, excitotoxic lesions were produced at specific timepoints prior to transplantation. Quinolinic acid (45nmol in 0.5µl) was slowly injected into the neostriatum of five groups of rats, 6 hours, 24 hours, 2 days, 7 days and 6 weeks prior to the transplantation of cell suspensions prepared from E14 rat striatal primordium. The grafts were placed in the same site as the lesion. Survival and integration were assessed histologically after 6 weeks, with immunocytochemical and enzyme stains. The grafts survived well in all groups, even in that with excitotoxin injection only 6 hours prior to transplantation. However the grafts differed in their extent of growth, which was least in the 1 day and 2 day groups. There was also a difference in the shape of the grafts, which was more cylindrical at the 6 hour interval, and more spherical at all longer intervals. The proportion of striatal patch to non-patch compartments within the graft did not differ significantly between groups. Tyrosine hydoxylase immunohistochemistry revealed somewhat greater fibre ingrowth into the non-patch as well as patch areas of grafts implanted at shorter intervals after lesioning. This may point to an interaction between a declining plasticity of host nigrostriatal terminals following loss of their intrinsic target, and the trophic properties of patch and non-patch neuronal compartments within the striatal graft.

Supported by the Wellcome Trust and the Medical Research Council.

232.18

BILATERAL INTRASTRIATAL TRANSPLANTATION OF RAT FETAL STRIATAL TISSUE INTO THE 3-NITROPROPIONIC ACID RAT MODEL OF HUNTINGTON'S DISEASE. C.V. Borlongan, T.K. Koutouzis, S.G. Poulos, S. Saporta, R.A.Hauser, D.W. Cahill*, T.B. Freeman and P.R. Sanberg. Div. Neurol. Surgery, Depts. Surgery, Neurology, Psychiatry, and Pharmacology, Univ. South Florida Coll. Med., Tampa, FL 33612.

The promising results in clinical neural transplantation for Parkinson's disease coupled with the demonstration of positive effects of fetal neural transplantation in ameliorating neurobehavioral deficits in animal models of Huntington's disease (HD) have prompted several researchers to propose clinical trials for HD. Accordingly, the implications of the evidence accumulated from HD animal models should be closely evaluated. To date, excitotoxic (e.g. kainic, ibotenic or quinolinic acids) animal models of HD have reproduced only the hyperactive stage of HD. Recently, we showed that chronic low dose injection of 3-nitropropionic acid (3-NP, a mitochondrial toxin) in mature rats leads to hypoactivity. Most, if not all, fetal tissue transplantation in animal models of HD have been carried out using the excitotoxic-induced hyperactive model of HD. In the present study, we used the 3-NP-induced hypoactive model of HD to demonstrate whether fetal tissue transplantation can ameliorate the behavioral deficits associated with the advanced stage of HD. Twelve-week old Sprague-Dawley rats were introduced to the 3-NP dosing regimen (10 mg/kg, i.p., once every 4 days for 28 consecutive days). At the end of this injection period, the rats were tested for general spontaneous locomotor activity using the Digiscan locomotor apparatus. All rats displayed significant hypoactivity compared to their pre-3-NP injection locomotor activity. Randomly selected rats then received bilateral intrastriatal solid grafts of fetal striatal (lateral ganglionic eminence) tissues from embryonic day 14 rat fetuses. Approximately 1/3 of each striatal tissue in hibernation medium was infused into each lesioned host striatum (AP: +1.2, ML: ±2.6, DV: -4.7). In control 3-NP-induced hypoactive rats, medium alone was infused intrastriatally. A 3-month posttransplant maturation period was allowed prior to locomotor activity test. Animals receiving fetal striatal grafts exhibited a significant increase in locomotor activity compared to their or to controls' post-3-NP injection activity or to the controls' posttranplant activity. Histological characterization of host-graft interaction will be reported. This observation supports the use of fetal striatal transplants to correct the akinetic stage of HD. To the best of our knowledge, this is the first study that has directly investigated the effects of fetal striatal transplantation in a hypoactive model of HD.

STAINING, TRACING, AND IMAGING TECHNIQUES II

233.1

DOES ULTRASOUND IRRADIATION AMPLIFY IMMUNOHISTOCHEMICAL LOCALIZATION ? N. Haykal-Coates, K.M. Crofton* and S. Barone Jr. Neurotoxicology Division , National Health & Environmental Effects Research Laboratory, U.S. Environmental Protection Agency, Research Triangle Park, NC 27711

Ultrasound irradiation has been used to enhance electron microscopic procedures (Robb *et al.*, 1991), and in amplifying immunohistochemistry (Podkletnova *et al.*, 1993). Since ultrasound irradiation is still regarded as a novel tool and is suggested as a method for amplifying immunohistochemical staining, we tested its usefulness and reproducibility with different antisera on sections from perfusion fixed adult rat brains. Immunohistochemistry was performed on 40 µm free-floating sagittal sections collected using a freezing-stage microtome. The sections were incubated for 30 minutes at room temperature on a shaker in 10% normal goat serum to block the nonspecific binding sites of the proteins. The sections were then transferred to polystyrene scintillation vials with one of 3 polyclonal antisera: TH (Tyrosine Hydroxylase), PKC γ diluted 1:10,000, or glial fibrillary acidic protein (GFAP) diluted 1:40,000. Using a conventional ultrasound bath, the sections were briefly exposed to 30 seconds of irradiation then incubated for 30 min or 18 hrs at 4°C in primary antisera. Immunohistochemical staining was carried out using routine immunoperoxidase techniques. Ultrasound irradiation increased TH immunoreactivity (IR) considerably at both time points but the longer incubation had the greatest increase in cellular and fiber staining. PKC-IR at short incubation times was poor regardless of ultrasound treatment. Ultrasound treatment increased PKC-IR only at the longer incubation time. Ultrasound treatment improved GFAP-IR only at the earlier incubation time, with no significant difference in IR at the longer incubation time. There was no significant increase in the temperature of the incubation medium and no increase in the background staining. Our results show that ultrasound irradiation could be used as an option to improve immunohistochemical techniques while reducing the incubation time in primary antisera and increasing the dilution of primary antisera. This method may have great utility; however, its usefulness in non-neural tissues and with different antisera must be determined empirically.

233.2

USE OF PEROXIDASE SUBSTRATE VIP™ FOR MULTIPLE STAINING IN LIGHT MICROSCOPY. J.L. Lanciego, P.H. Goede, F.G. Wouterlood, and M.P. Witter. (SPON: European Neuroscience Association). Research Institute Neurosciences Amsterdam. Department of Anatomy and Embryology. Vrije Universiteit. 7 van der Boechorststraat, 1081 BT Amsterdam. The Netherlands.

Study of the distribution of a fiber input in a particular brain area and the anatomical relationships in this area with both projection- and interneurons requires a triple-staining that allows the unequivocal distinction of each of the three involved components in one and the same histological section. We investigated in this respect the properties of a recently introduced peroxidase substrate, VIP (Vector Labs; forms a purple precipitate) in combination with two traditional substrates, standard diaminobenzidine (DAB; brown precipitate) and nickel-enhanced DAB (black). In rats, the tracers biotinylated dextran amine (BDA, anterograde tracer) and fluorogold (FG, retrograde tracer) were injected in the perirhinal cortex and hippocampus, respectively. Transported BDA was detected with an avidin-biotin-peroxidase complex (Veenman et al., 1992) whereas the transported FG was detected via PAP method (antiserum kindly provided by Dr. H. Chang, Dept. Anat. Neurobiol. Univ. of Tennessee). Tracing with BDA and FG was combined with immunocytochemistry with antibodies against the calcium-binding proteins parvalbumin or calbindin. We report on various combinations and staining sequences. Best results were obtained with a staining sequence that comprises first a BDA stain with DAB-Ni, second a FG protocol with DAB and then parvalbumin- or calbinding immunocytochemistry with VIP. The sequence in which chromogens were applied appeared critical. The first stain should be done using DAB-Ni, the second using DAB and the third using VIP. Color separation is excellent, and the sensitivity is good. Sections are mounted on slides and dried. The only specific caution to be exerted is to dehydrate in alcohol rapidily before coverslipping in order to prevent fading of the VIP reaction product. For dehydratation, toluene can be used as an alternative to ethanol.

233.3

ANTIBODY-BASED TECHNIQUES ARE SUPERIOR TO ROUTINE HISTOCHEMICAL METHODS FOR THE EVALUATION OF MYELIN. K.M. Weidenheim* and W.D. Lyman. Department of Pathology, Albert Einstein College of Medicine, Bronx, NY 10461.

The evaluation of myelin in biopsy and autopsy CNS tissue is limited because of formalin fixation, paraffin embedding and hematoxylin-based or copper-thiocyanate dyes. However, because immunohistochemical methods employing antibodies that recognize myelin-specific proteins have become available, we have evaluated the relative utility of immunologic techniques using antibodies which recognize myelin basic protein (MBP), proteolipid protein (PLP) and myelin associated glycoprotein (MAG) for the evaluation of myelin in sections of 17 formalin-fixed human spinal cords obtained at autopsy from patients without significant neurologic disease. These results were compared to those obtained using the Luxol fast blue technique (LFB). Staining intensity was evaluated by light microscopy and graded as +3 (uniform staining in all funiculi); +2 (mild variability in staining), +1 (marked variability in staining intensity), and 0 (no staining). Methods for MBP and PLP revealed uniform, +3 staining of myelinated tracts in 16/17 cases. The LFB technique revealed variability in myelin staining with 11/17 cases staining +2 and 6/17, +3. MAG immunohistochemistry produced inconsistent results. These results suggest that visualization of MBP and PLP myelin epitopes provide a sensitive and specific method for assessing myelin integrity in autopsy material obtained and fixed by widely available methods.

(Supported in part by USPHS grants MH 47667 and MH 46815)

233.4

AN ANTISERUM TO CALRETININ FIXED IN THE CALCIUM-FREE FORM REVEALS CEREBELLAR GRANULE CELL IMMUNOREACTIVITY. L. Winsky* Lab. of Clinical Science, NIMH, Bethesda, MD 20892.

Results of a previous report (Winsky and Kuznicki, 1996, J. Neurochem.) indicated that commonly used antibodies to calretinin (CR), calbindin D28k and parvalbumin preferentially recognize calcium bound proteins. The present studies extend these earlier findings in an attempt to produce an antiserum to CR that is suitable for immunohistochemistry and which preferentially recognizes the calcium free conformation of CR. Recombinant CR was cross-linked with formalin following incubation with low calcium buffer and injected into a rabbit for the production of a polyclonal antiserum. Tests for immunoreactivity were made of pre-immune serum and sera obtained at various times after injection of the CR solutions. These tests included the examination of CR immunoreactivity on brain sections of rats and guinea pigs, on western blots and on dot blots containing serial dilutions of unfixed or formalin fixed recombinant CR. Results revealed similar overall patterns of immunoreactivity to that seen with a previous CR antiserum in most brain regions. However, granule cells of the cerebellum, which contain CR mRNA but have previously only shown low immunoreactivity where intensely immunoreactive in some lobules. No cross-reacting proteins were seen on western blots of cerebellum but the new antiserum preferentially recognized CR cross-linked in a calcium free form on dot blots. These preliminary results suggest that it may be possible to develop calcium binding protein antisera for studying the calcium binding status of these proteins in cells. (Supported by NIMH DIRP)

233.5

IMMUNOHISTOCHEMICAL LOCALIZATION OF THE MULTICATALYTIC PROTEINASE (PROTEASOME) IN THE RAT CENTRAL NERVOUS SYSTEM. [1]E. Mengual*, [2]P. Arizti, [3]J. Rodrigo, [1]J.M. Giménez-Amaya and [2]J.G. Castaño. [1]Departamento de Morfología and [2]Departamento de Bioquímica, Facultad de Medicina, U.A.M., 28029 Madrid and [3]Departamento de Neuroanatomía Comparada, Instituto Cajal, C.S.I.C., 28002 Madrid, Spain.

The multicatalytic proteinase (MCP, proteasome or prosome) is a 20S complex which plays a major role in non-lysosomal pathways of intracellular protein degradation. In this study, a polyclonal antibody against rat liver MCP was used to investigate the distribution of MCP in the central nervous system (CNS) of the rat and its subcellular localization within the neurons. As expected, MCP-immunoreactivity (MCP-ir) is ubiquitously distributed in the rat CNS, but its distribution is not homogeneous. At the subcellular level, MCP-ir is primarily localized in the nuclei of neurons and glial cells. The most intensely stained neurons were the pyramidal cortical neurons of layer 5 and the motor neurons of the ventral horn in the spinal cord. These cells displayed an intense nuclear and cytoplasmatic MCP-ir and clearly stained processes. Additionally, some populations of large neurons in the mesencephalon and brainstem also displayed a moderate MCP-ir in their perikarya. The vast majority of neurons in the remaining structures did not show a strong cytoplasmatic MCP-ir, but their nuclei displayed an intense MCP-ir. The ubiquitous distribution of MCP in the rat CNS and its subcellular localization are discussed in relation to: (1) the brain distribution of calpain, the other major non-lysosomal cellular protease, that has been implicated in neuronal vulnerability to ischemia; and, (2) the possible role of MCP in the degradation of key transcription factors which are essential in many neuronal responses (e.g.: in short-term and long-term memory processes).
Supported by FIS 93/0337, SAF 94-0685 and Fundación Ramón Areces.

233.6

ISOFORMS OF DYSTROPHIN PROTEIN Dp71 IN RAT BRAIN: IDENTIFICATION, CELLULAR AND SUBCELLULAR LOCALIZATION, V. Alemán*, D. Martínez, O. Chávez, B. Osorio, D. Mornet and A. Rendón. Dept. of Physiology, CINVESTAV, México, D.F. 07300. Mexico.

Dp71 a C-terminals isoform of dystrophin has been identified as the major DMD gene product in CNS. It has been reported the existence of four different isoforms of Dp71 originated by alternative splicing for exons 71 and for 78 (Human Mol. Gen. 4, 1475-1483, 1995). MW of Dp71 isoforms ranged from 71-75 KD. Here we analyzed by Western blot (Wb) the Dp71 isoforms expression in extracts from whole brain, hippocampus and cultured hippocampal neurons (HN) and brain astroblasts. Three distincts monoclonal antibodies were used: Dys2 and H5A3 detect isoforms retaining the C-terminal sequence of dystrophin and 5F3 the Dp71 isoform containing the alternative 31 amino acid C-terminus. Using Dys2 antibody a single isoform of Dp71 was detected in cultured neurons. Immunofluorescent observations revealed neurite and cytoplasmic staining which appears as a fine reticulum of filaments. Comparative Wb analysis with H5A3 detects a full length dystrophin in whole brain, HN and glial cells. Furthermore, in brain extracts two Dp71 isoforms were detected, which migrate as a doublet 71-73 KD. In neurons but not in glia a single reactive band was observed. Immunofluorescent analysis of neurons showed a similar stain than Dys2. Wb of neurons and astroblasts were shown to contain a single Dp71 band revealed by 5F3 antibody, which appears to label the endoplasmic reticulum in both type of cells.

233.7

IMMUNOHISTOCHEMICAL LOCALIZATION OF LIPOCORTIN 1. M-Z Zhang* and JA McKanna. Dept. of Cell Biology, Vanderbilt University School of Medicine, Nashville, TN 37232.

Lipocortin 1 (LC1) has received considerable attention as a substrate for protein kinases, as a Ca^{2+}- and phosphatidylserine- binding protein, and as a mediator of glucocorticoid anti-inflammatory effects. However, progress in this field has been confused by contradictory immunohistochemical localizations; e.g., according to various reports, LC1-ir localizes in rat brain exclusively to either neurons or astrocytes or microglia. Hypothesizing that the contradictory data may arise from unusual properties of the antigen, we developed a novel brain slice model to test hundreds of fixation and staining variables. The results show that several commonly used immunohistochemical procedures have profound but unexpected effects on LC1-ir. As defined by (a) sensitivity to pre-absorption of 1^0 sera with LC1 purified from either human placenta or recombinant yeast and (b) staining with 1^0 antibodies affinity purified with immobilized recombinant LC1, "true" LC1-ir was readily apparent in microglia of brains fixed with acidified aldehydes and embedded in paraffin. However, LC1-ir was eliminated either by fixation with neutral/alkaline aldehydes, or by freezing prior to strong acid fixation, or by staining prior to partial de/rehydration. We suggest that the sensitivity of LC1 epitopes to [proton] and [water] activities may reflect molecular properties important to LC1's roles *in vivo*, and also suggest the similar sensitivities may influence immunolocalization of other proteins with phospholipid affinity. Regardless of methodological variables tested to date, true LC1-ir is never detected in neurons or astrocytes. The specific expression of LC1 in microglia supports the hypothesis that the anti-inflammatory and immuno-suppressive properties of LC1 may play important roles as these cells respond to damage and disease in the mammalian central nervous system.
Supported by DOD Medical FEL program and NS-32660HD15052.

233.8

SECRETION AND RETENTION OF CHROMOGRANIN A IN FIBROBLASTS: A BIOCHEMICAL AND EM STUDY. J.-H. Tao-Cheng*, A. Iacangelo, and L. E. Eiden. Sec. Molec. Neurosci., Lab. of Cell Biology, NIMH & Lab. of Neurobiology, NINDS, Bethesda, MD 20892.

Chromogranin A (CGA), an acidic glycoprotein expressed in neuronal and endocrine cells, is a prohormone for pancreastatin and other bioactive peptides, and has also been suggested to participate in the formation of secretory granules.To investigate the latter properties of CGA, this protein was expressed in CV-1 fibroblasts using the vaccinia virus/bacteriophage T7 hybrid system. Secreted and retained CGA was measured by Western blot analysis. The subcellular localization of CGA was examined via EM immunocytochemistry (ICC) with an anti-CGA (anti-WE-14) antibody.

The majority of CGA produced by CV-1 cells over a 2-24 hr period after initiation of expression was secreted into the medium. A portion of the CGA immunoreactivity was retained in the cells. EM ICC revealed copious staining for CGA within structures consistent with constitutive vesicles and endoplasmic reticulum. Compared to mock-transfected or untransfected cells, CGA-expressing CV-1 cells formed many more secretory granule-like structures, with the appearance of both immature and mature granules. Only the 'immature' (less electron dense) ones were markedly stained for the WE-14 CGA epitope. This pattern is consistent with WE-14 staining in bovine adrenal chromaffin cells where WE-14 was exclusively in granules and preferentially in immature granules.

Expression of CGA in CV-1 fibroblasts results in its sorting to both a constitutive secretory pathway and, to a lesser extent, to secretory granule-like structures which are absent in untransfected cells. These findings suggest that CGA is involved in secretory granule formation and that additional factor(s) are required for efficient formation of secretory granules in these non-neuroendocrine cells. (NIH intramural funding)

233.9

USE AND INTERPRETATION OF DiI IN ELECTRON MICROSCOPY. L.L. Bruce*, M.A. Christensen, M. Khachab, and B. Fritzsch. Dept. Biomedical Sciences, Creighton Univ., Omaha, NE 68178.

The fluorescent neuronal tracer, DiI, will diffuse through aldehyde-fixed tissue, and can then be photoconverted and processed for analysis with electron microscopy. We obtained optimal results using the parameters below. Furthermore, transneuronally and directly labeled DiI profiles can be easily and clearly differentiated with electron microscopy. *EM fixative*: 4% paraformaldehyde, 0.25% glutaraldehyde, 4% dextrose, and 0.005% calcium chloride in 0.05 M phosphate buffer. Keep tissue in this solution during diffusion. *DiI application*: Dissolve crystals of DiI in a drop of dimethylformamide. Immerse a nylon filter strip in this solution, air dry. Place strip in a cut through the application site. *Time vs. temperature*: DiI will diffuse approximately 3 mm in 2 days at 37°C, or 3 mm in 12 days at 25°C. Similar labeling patterns are achieved under both conditions. *Photoconversion of DiI*: After sectioning tissue, soak section to be photoconverted in DAB solution (1 mg/ml DAB in 0.1 M phosphate buffer, pH 8.3) for at least 1 hr. Place in fluorescent light, using 20X or 10X objectives for 15-40 min, until axons are lightly but definitively labeled. *Direct vs. transneuronal labeling*: Spiral ganglion cells labeled directly by applying DiI to a severed eighth nerve contained very dense labeling throughout the cytoplasm and along all membranes, but not within the mitochondrial matrix. Spiral ganglia labeled transneuronally by applying DiI to a cut olivocochlear efferent bundle contained labeling in some endoplasmic reticulum membranes, but never in the plasma membranes, mitochondrial membranes, Golgi apparatus, or in the cytoplasm. This differential labeling, together with the methods described above, make DiI easy to use in amniote embryos and neonates yielding a quality superior to biotin-based electron-microscopic tracing techniques.

Supported by NIH grant DC00215-09.

233.10

QUANTIFYING IMMUNOGOLD LABELLED NEUROTRANSMITTERS AND RECEPTORS BY ELECTRON MICROSCOPY AND IMAGE ANALYSIS. F.M.S. Haug*, V. Desai[1], P.O. Nergaard[2] and O.P. Ottersen. Dep. anat., Inst. bas. med. sci., Univ. of Oslo, Norway, Soft Imaging Software GmbH[1], Münster. Germany and Norsk Philips AS[2], Oslo, Norway.

We have optimised an image analysis system for rapid evaluation of immunogold labelled ultrathin sections. Video images are seamlessly fused into high-resolution images of 1280 x 1024 pixels and analysed on the monitor. Relative average concentrations of glutaraldehyde fixed neuroactive amino acids in different types of tissue structures are estimated as relative average numbers of particles per unit area. Profiles are outlined and classified interactively. Gold particles are detected by thresholding and classified by size for double or triple labelled sections.

Relative average densities of receptors or transporters in membranes are estimated as relative numbers of particles per unit length. Binding to a membrane antigen is suggested for particles within a distance of the membrane that depends on the antibody-gold particle complex, the epitope's position in the membrane (usually unknown) and other factors. The centres of 15 nm particles coupled to membrane antigens via secondary and primary antibodies should theoretically be within 23.5 nm of the epitope. An adequate electron optical magnification is carefully calibrated. Membrane segments are drawn and classified. Particle-to-membrane distances are measured interactively. Alternatively, thresholded particles are superimposed on a "map" that holds each pixel's distance from the membrane. The value corresponding to a particle's centre of gravity is then obtained automatically, but the accuracy of the latter method remains to evaluate. Number of particles per unit length is calculated directly, or after subtracting background particle density.

Particle densities are tabulated per profile, or per membrane segment, and exported to external statistical and graphical software for further analysis.

Sponsored by the Norwegian Research Council and EU Biomed (PL 950851).

233.11

A RAPID METHOD FOR COMBINED CONFOCAL MICROSCOPIC AND EM VISUALIZATION OF BIOCYTIN- OR NEUROBIOTIN-LABELED NEURONS. X.J. Sun*[1], L.P. Tolbert[2], J.G. Hildebrand[2], and I.A. Meinertzhagen[1]. [1]Life Sciences Centre, Dalhousie University, Halifax, NS, B3H 4J1, Canada; [2]ARL Division of Neurobiology, University of Arizona, Tucson, AZ 85721, USA.

The combination of intracellular recording and staining is still one of the most powerful and heavily used techniques for correlating the morphology and physiology of neurons. The recent widespread application of laser scanning confocal microscopy (LSCM) offers a particularly useful tool in neuroanatomy because of its capacity to reconstruct rapidly the complex shapes of neurons in three dimensions (3D). Light microscopic investigation of a neuron, however, often raises the question of whether synaptic connections exist at a particular site on the neuron's surface, for which electron microscopy (EM) is needed. The combination of intracellular staining with LSCM and EM is difficult, however, because of incompatibility of the staining protocols for the two types of microscopy. We have reported previously an immuno-gold method for rendering intracellularly injected neurons simultaneously fluorescent and electon-dense (Sun et al., J. Histochem. Cytochem. 43:329-335, 1995); but this method is sufficiently time-consuming to be worthwhile pursuing on only a small number of preparations. Here, we report a technique that quickly and easily converts a fluorescent label into a more stable and electron-dense stain, a method less flexible but more suitable for routine use. With this technique, a Neurobiotin or biocytin-labeled neuron can be imaged with LSCM after reacting with fluorophore-conjugated avidin. Then the fluorescent label is converted to an electron-dense material which will permit long-term storage or EM study. This single-step conversion uses the commercially available ABC kit (Vector Labs) with DAB as a substrate for the peroxidase. We find the current method to be more sensitive and allowing greater penetration for EM staining compared with our previous immuno-gold method, and faster and less labor-intensive than the photooxidation procedure in common use. At the same time, it retains the opportunity to examine interesting areas of the stained neuron and localize these easily for thin-sectioning prior to EM study, by using the 3-D information obtained with LSCM. Supported by award from Univ. of Arizona, Center for Insect Science, to XJS, and grants to I.A.M. (NIH EY-03592, NSERC A 0065), to LPT and JGH (NIH NS-28495) and to JGH (NIH AI-23253).

233.12

MAPPING REGIONAL BOUNDARIES AND MODELING THE COMPRESSION OF ULTRASTRUCTURAL THIN SECTIONS THROUGH THE USE OF VIDEO IMAGERY J.P. Pierce* and T.A. Milner, Dept. of Neurology and Neuroscience, Cornell Univ. Med. Coll., NYC, NY 10021

The increasing availability of video cameras that are used in conjunction with both light and electron microscopes has allowed the development of precise mapping procedures that can aid the electron microscopist. By capturing a light level *enface* image of a blockface, and comparing the dimensions of this image to a captured low magnification electron microscopic image of a thin section from the block, the extent of compression produced by sectioning can be determined, and then used to compress a map of tissue boundaries and locations, generating a template that can be overlayed on an image of any section from that block. Specifically, *enface* blockface images are captured after thin sectioning (using a x4 light level objective and NIH Image 1.5 software), and thin section images are captured (using x18 mag. on the electron microscope and the MCID package). The scale of the two images is then matched (by comparing the length of the images, and making adjustments with zeroxes), and the extent of compression determined (by comparing the widths of the images). A map of the blockface, with boundaries and locations marked, is then traced into Image using a drawing tablet, and saved as a Pict file. The Pict file is imported into Adobe Illustrator as a template and autotraced. The lines are then linked, and the Free Distort function is applied, to model the precise extent of compression, along a ruler. Finally, the compressed map is printed, and the scale again adjusted, so that it can serve as an overlay for an image of any section from that block, that needs to be examined. This approach has been useful both in studies that involve the random sampling of specific subregions, and the localization of specific labeled elements that can be seen at the light level. Additionally, determining the extent of compression provides feedback on the quality of sectioning, and allows for the correction of density measurements taken at the ultrastructural level. Supported by DA08259 and MH42834.

233.13

FAST METHOD FOR SCREENING CONNEXIN-IDENTITY USING RT-PCR ASSAYS. M. Urban, R. Rozental, F.-C. Chiu*, M. Kremer, R. Dermietzel and D.C. Spray. Dept. of Neurosci., A. Einstein Coll. Med., Bronx, NY 10461; Inst. Anat., U. Regensburg, Germany; Inst. Biophys., Fed. Univ. Rio de Janeiro, Brazil.

Gap junctions are large aggregates of transmembrane channels, composed of connexin proteins (Cx), that interconnect neighboring cells. Cloning of connexin cDNAs has revealed at least twelve distinct isoforms in mammalian tissues. Since the same Cx-type can be expressed in different tissues and more than one Cx-type can be expressed by the same cell, identification of which Cx-type is expressed has become quite laborious. The Cx family exhibits both conserved (extracellular and transmembrane regions) and divergent (cytoplasmic domains) regions. Based on their cDNA sequences, Cxs have been divided in Groups I (β; Cx26→Cx32) and II (α; Cx33→ Cx50). We have now used "Universal Primers" (*sense primer" B"*, degenerate oligo corresponding to a region of the 1st extracellular domain; *antisense primer "C"*, degenerate oligo complementary to the 2nd extracellular domain) for RT- PCR assays to amplify unknown DNA sequences starting from total RNA; Cxs belonging to Groups I and II present band sizes of 360-380 and 420-520 bp, respectively. Subsequently, we perform second generation of PCR products to identify which Cxs have been amplified within a certain group by using specific restriction endonuclease enzymes. This method thus provides a simple and rapid procedure for identifying Cx-types expressed in a variety of tissues.

233.14

QUANTIFICATION OF CYCLIC AMP IN SINGLE DEVELOPING GLIAL CELLS, A.P. Wiemelt, M.J. Engleka, and F.A. McMorris*. The Wistar Institute, 3601 Spruce Street, Philadelphia, PA 19104.

Previous findings in our lab have demonstrated that either the application of cyclic AMP (cAMP) analogs, or the stimulated increase of endogenous cAMP levels cause an acceleration in the rate of differentiation of glial precursor cells into mature oligodendrocytes. We seek to determine what changes in endogenous cAMP levels occur during the normal course of oligodendrocyte development, and what role such changes have in regulation of development. We have used a novel fixation procedure employing the aldehyde acrolein to cross-link cAMP to proteins and fix cells in a single step. We have generated polyclonal antibodies to cAMP by injecting rabbits with cAMP linked to carrier proteins by acrolein fixation. The resulting antibodies recognize acrolein-fixed cAMP-protein conjugates at dilutions of 32,000 and greater in ELISA assays, and indirect FITC immunofluorescence microscopy shows elevated cAMP levels in C6 glioma cells in response to isoproterenol (IPTN) treatment. Competition studies indicate that this reactivity is specific for cAMP, when other nucleotides were tested. cGMP did exhibit some cross reactivity, but this did not interfere in cell staining. Primary glial cultures show increased cAMP following activation of adenylcyclase with forskolin (FSK) and cholera toxin (CTX). Double staining with antibodies that recognize different stages of oligodendrocyte development and cAMP antibodies indicate that cAMP is inducible during a critical developmental window, when cells express the O4 antigen, as the cells are committing to a fully differentiated phenotype. Early A2B5 expressing (immature) or late O1 expressing (mature) cells do not induce cAMP in response to FSK/CTX. Supported by 5T32CA09171, NS32394, NS32122, NS26119.

233.15

GFP-SYNAPTOBREVIN CHIMERIC PROTEIN AS A MARKER FOR SYNAPTIC VESICLES IN DEVELOPING NEURONS . X-h. Wang* and M-m. Poo, Dept. Biol., UCSD, La Jolla, CA 92093.

Several cDNA clones coding for synaptobrevin II (SybII) were obtained from brain cDNA library of *Xenopus Laevis*. The *Xenopus* protein (XsybII) shows 89.5% identical with the amino acid sequence of rat SybII and 78.1% identical with rat SybI. A chimeric gene (GFP-XsybII) coding green fluorescence protein (GFP) and XsybII was constructed. Synthesized cRNA of the fusion protein was obtained by *in vitro* transcription and microinjected into one of the blastomeres of early *Xenopus* embryos. Expression of the chimeric protein was shown by the green fluorescence in cultured nerve and muscle cells prepared from injected embryos. In neurons, a large green spot was usually observed in the soma and small punctuate fluorescence spots of varying sizes were observed in the neurite, growth cone, and presynaptic terminals. Immunostaining with synpatophysin antibodies showed that these small green spots colocalize with synaptophysin staining, suggesting that they represent clusters of synaptic vesicles. Electrophysiological recordings of spontaneous and evoked synaptic currents showed that expression of GFP-XsybII in these neurons does not affect the synaptic activity. Thus GFP-XsybII may be useful as a marker to study the biogenesis, trafficking and recycling of synaptic vesicles in developing neurons.
Supported by NIH grant NS 31923.

234.1

DISTINGUISHING NEURONS FROM GLIA IN REPLICAS OF HIPPOCAMPUS AND SPINAL CORD: REASSIGNMENT OF CELLS WITH GAP JUNCTIONS

J. E. Rash*, T. Yasumura, H. S. Duffy, R. K. Dillman, B. Bilhartz, and F. E. Dudek. Dept. of Anatomy and Neurobiology, Colorado State Univ., Ft. Collins, CO 80523

In previous studies of gap junctions between neurons in adult rat spinal cord, we used confocal "grid-mapped" freeze-fracture to develop affirmative criteria for distinguishing neurons from glia. Criteria developed in that study are now applied to analyses of the dentate gyrus and CA1-3 regions of adult and developing rat hippocampus. Defining criteria for neurons are described elsewhere (Rash, et al., Proc. Nat. Acad. Sci., 1996). In hippocampus and spinal cord, oligodendrocytes are exclusively identified by: 1) their continuity with myelin; 2) presence of tight junctions in the outer layers of myelin; 3) presence of abundant gap junctions on their somata and dendrites, one type having irregular arrays of connexons (i.e., heterologous gap junctions to astrocytes), and a less-abundant type having regular arrays of connexons (i.e., homologous gap junctions, primarily at nodes of Ranvier and in the internodal myelin); 4) a single-strand "necklace" of IMPs surrounding E-face images of heterologous gap junctions; 5) "moth-eaten appearance" of somatic and dendritic plasma membranes where they contact myelin; 6) numerous grooves and furrows in their somatic and dendritic plasma membranes, representing the impressions of glial and neuronal processes; and less definitively, 7) by their small nuclei and thin rim of cytoplasm that is devoid of the widely-spaced (i.e., ¼-½μm) parallel stacks of Nissl substance that are found only in neurons. Astrocytes and ciliated ependymal cells are identified by square arrays in their plasma membranes and GFAP filaments in their cytoplasms. By these criteria, previous freeze-fracture images purported to represent neurons with gap junctions are now reclassified as oligodendrocytes, or less commonly, as astrocytes or ependymal cells.

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234.2

EXPRESSION OF SYNAPTIC PROTEINS IN PRIMARY CULTURED ASTROCYTES.

R. P. Sanzgiri*, L.-E. Trudeau and P. G. Haydon. Lab. of Cellular Signaling, Department of Zoology and Genetics, Iowa State University, Ames, IA 50011.

Astrocytes, a subtype of glial cells, have been shown to release the neurotransmitter, glutamate, which can cause a subsequent excitation of neurons (Parpura *et. al.* (1993), *Nature*, 369, 744-747). A possible mechanism of release is vesicular exocytosis. Consistent with this idea, it was found that a subset of neuronal synaptic proteins including synaptobrevin and syntaxin but not SNAP-25, are expressed in astrocytes (Parpura *et. al.* (1996), *FEBS Lett.*, 77, 489-492). Given that astrocytes can release neurotransmitter yet only express a sub-set of synaptic proteins, they may act as a convenient cellular expression system for studying synaptic protein interactions and their functional roles.

Towards this end, we have demonstrated that primary astrocyte cultures are effective expression systems by performing direct nuclear microinjections. By coinjecting a fluorescent dye, fluoruby, with either CMV or RSV promoter driven plasmids, we determined that 80% of microinjected cells express the desired protein. Expression is detected as early as three hours after microinjection. Microinjection of two constructs results in reliable co-expression. Overexpressed proteins appear to be correctly localized as synaptotagmin showed a punctate staining pattern consistent with its expression in intracellular organelles while synapsin and SNAP-25 are more diffusely expressed. Preliminary experiments suggest that overexpression of SNAP-25 a together with synaptotagmin reduces the accumulation of synaptotagmin within perinuclear golgilike structures, compatible with a role of SNAP-25 a in regulating the export of synaptic proteins to the synapse.

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234.3

INGROWTH OF ACTIVATED ASTROCYTES INTO GELATIN SPONGE AFTER STAB INJURY OF ADULT RAT BRAIN.

P.R. Perillan, C.C. Park, M. Gorospe, J.M. Simard*. Division of Neurological Surgery, University of Maryland School of Medicine, 22 South Greene Street, Baltimore, MD 21201.

The response of the central nervous system to traumatic injury involves activation of astrocytes, with proliferation, hypertrophy and an increase in expression of glial fibrillary acidic protein (GFAP). Western blot analysis and *in vitro* recovery of activated astrocytes from implanted gelatin sponge were used to assess ingrowth of these cells during the gliotic response to injury. Adult rat brains were subjected to a stab injury followed by implantation of pieces of gelatin sponge. After 8 d *in vivo*, the sponge pieces were harvested and cultured. When the sponge pieces were left in the culture dishes, cell counts performed at 8 d revealed a homogeneous population of cells that became confluent after 3 wk *in vitro*. More than 90% of cells were identified as astrocytes based on their immunohistochemical profile, including positive staining for GFAP, S-100 and vimentin, and western blot analysis for GFAP, as well as negative staining for α-actin and MAC-1. By comparison, when sponge pieces were removed from the culture dishes at 24 hr, cell counts performed at 8 d revealed no cells. Also, culture for 8 d of pieces of gelatin sponge that had been implanted for less than 3 d, of pieces of previously injured brain, or of pieces of freshly isolated but otherwise uninjured brain, invariably failed to yield reproducible cellular outgrowth. We conclude that astrocytes recovered in primary culture originated from the gelatin sponge, rather than from adherent tissue, that migration of cells into the sponge pieces required >3 d *in vivo*, and that migration of cells out of the sponge pieces required >24 hr *in vitro*.

Funding: National Heart, Lung and Blood Institute (HL42646 and HL51932)

234.4

IDENTIFICATION AND CHARACTERIZATION OF NINJURIN, AN ADHESION MOLECULE EXPRESSED IN SCHWANN CELLS AFTER NERVE INJURY

T. Araki* and J. Milbrandt Department of Pathology, Washington University Medical School, MO 63110

After injury to a peripheral nerve, the axons and myelin sheath distal to the site of injury degenerate by a process known as Wallerian degeneration. During this process Schwann cells undergo a number of phenotypic changes, including alterations in patterns of gene expression, that are important for axonal regeneration. Using differential screening strategies, we identified a novel protein, termed *ninjurin*(Nerve Injury Induced protein), that is up-regulated after axotomy in Schwann cells in the distal nerve segment which contains the degenerate axons. Ninjurin is expressed in embryos and adult animals in a number of tissues including liver, thymus, bone, and kidney. Interestingly, it is expressed predominantly in epithelial cells in each of these tissues. Sequence analysis revealed that ninjurin is a 16 kD protein that is predicted to contain two membrane spanning domains, but has no homology to other characterized proteins. Immunohistochemical and cell surface labeling demonstrated that ninjurin is located on the cell surface. Aggregation assays using transfected cells expressing ninjurin indicated that ninjurin functions as an adhesion molecule, although it does not have any previously identified adhesion motifs. Our data suggest that ninjurin may play an important role in nerve regeneration as well as in the formation and function of other tissues.

This work was supported by a grant from the Ronald McDonald Children's Charities. J.M. is an Established Investigator of the American Heart Association.

234.5

RAT SPINAL CORD ASTROCYTE RESPONSES TO LYSOPHOSPHATIDIC ACID

T. Manning* and H. Sontheimer. Neurobiology Research Center and Dept. of Physiology and Biophysics, University of Alabama at Birmingham, Birmingham, AL 35294

Lysophosphatidic acid (LPA) is a biological signaling molecule that is produced by activated platelets and is bound to serum albumin. It can have a wide variety of effects in different cell types. LPA stimulates stress fiber formation and DNA synthesis in fibroblasts, promotes platelet aggregation, and inhibits gap junction coupling in hepatocytes. During pathological situations where the blood brain barrier is disrupted, the brain is exposed to blood constituents including LPA. We examined the effects of LPA on primary cultures of rat spinal cord astrocytes using fluorescent Ca^{2+} imaging, proliferation assays, and time-lapse video microscopy. At nanomolar concentrations of LPA, astrocytes responded with long trains of $[Ca^{2+}]_i$ oscillations that were dependent on release from intracellular stores. Low micromolar concentrations of LPA also produced oscillations, but often with a sustained rise that was dependent on $[Ca^{2+}]_o$. The response to LPA showed little desensitization and transients could be observed for periods of up to 45 minutes. ^{3}H-thymidine incorporation was monitored to examine mitogenic effects of LPA. Treatment with nanomolar concentrations of LPA increased DNA synthesis by ~90% as compared to untreated controls. It has previously been shown that in serum free media, flat, protoplasmic astrocytes round up and adopt a more stellate morphology following increases in intracellular cAMP. This process can be initiated by the application of the cAMP analogue dibutyryl cAMP or the adenylate cyclase activator forskolin. The presence of LPA in defined medium inhibited the morphological changes induced by these compounds. Time-lapse video microscopy showed that the stellation could be completely reverted if LPA was added after the change in morphology. It remains to be seen whether LPA can affect some of the known astrocytic functions such as buffering of K^+ and clearance of neurotransmitters.

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234.6

THE MICROTUBULE ARRAY OF THE OLIGODENDROCYTE IN VITRO

K.F. Lunn*, P.W. Baas, and I.D. Duncan. Departments of Medical Sciences and Anatomy, University of Wisconsin, Madison, WI 53706.

Glial cell cultures were prepared from the spinal cords of 10 day old rats, and the microtubule (MT) arrays of oligodendrocytes (OLs) were studied by electron microscopy (EM), immunofluorescent antibody labeling and confocal microscopy after 4 days *in vitro*. EM and confocal microscopy showed that MTs form a network throughout the OL cell body with no discrete organizing centers detected. A hooking protocol was used to show that the MTs which form parallel arrays in OL processes have a mixed polarity orientation, but the majority (70-80%) of MTs have plus ends distal to the cell body. Labeling with an antibody to tyrosinated tubulin showed that recently polymerized tubulin was present throughout the OL cytoskeleton, and extended to the ends of the processes. An antibody to acetylated tubulin showed that this more stable isoform was not present at the distal tips of the OL processes. The sensitivity of OL MTs to the MT-depolymerizing drug nocodazole was assessed by quantifying the total MT polymer in OLs after exposure to drug for times ranging from 15 minutes to 6 hours. Total fluorescence of cells labeled with an anti-β tubulin antibody was used as a measurement of MT polymer mass, and in OLs this showed 2 distinct phases of loss in nocodazole, indicating the existence of both drug-labile and drug-stable polymer in these cells. A separate analysis of OL cell bodies and processes showed that while both types of polymer are present in both regions, relatively more of the total MT polymer in the processes consists of the drug-labile form. (Supported by NIH grant NS32361)

234.7

ASTROCYTES PROMOTE PROCESS OUTGROWTH BY ADULT HUMAN OLIGODENDROCYTES THROUGH INTERACTIONS OF bFGF AND ASTROCYTE MATRIX. Oh Y.S., & Yong, V.W.* Montreal Neurological Institute, Department of Neurology and Neurosurgery, McGill University, Montreal, Canada H3A 2B4.

An early event in myelin formation is process outgrowth by oligodendrocytes (OLs). We demonstrate that process outgrowth by adult human OLs is enhanced in co-culture with human astrocytes. To investigate the mechanism by which astrocytes promote oligodendroglial process outgrowth, we exposed adult human OLs to growth factors known to be produced by astrocytes. Of a variety tested, only bFGF exerted significant promoting effects on process extension by OLs; in concordance, a neutralizing antibody to bFGF retarded the ability of astrocytes to promote oligodendroglial process outgrowth. The effects of bFGF was less potent that that of live astrocytes, and no synergistic effects of bFGF with other soluble growth factors were observed. Further investigations on the role of astrocytes revealed that the astrocyte extracellular matrix (ECM) could also promote process extension. Significantly, the co-treatment of OLs with bFGF and astrocyte ECM reproduced the potent effects of live astrocytes. To identify the astrocyte ECM involved in process extension, purified ECM molecules known to be produced by astrocytes (laminin, vitronectin, fibronectin, HSPG & CSPG) were tested. None of these by themselves promoted process outgrowth by OLs, but laminin and fibronectin augmented the effects of bFGF. We conclude that bFGF and astrocyte matrix are important factors that enhance process extension by adult human OLs.

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234.8

SELECTIVE ATTACHMENT OF ASTROCYTES TO VARIOUS BASEMENT MEMBRANE AND EXTRACELLULAR MATRIX PROTEINS. L. Kam[1], R. Bizios[1], W. Shain[2], J.N. Turner[1,2*], [1]Rensselaer Polytechnic Institute, Troy, NY 12180, [2]Wadsworth Center and School of Public Health, Albany NY 12201-0509

Selective attachment of astrocytes to substrates in vitro may improve methods of cell preparation and biocompatibility of prosthetic devices designed for brain insertion. Fluorescently labeled cells were used to characterize astrocyte attachment and identify portions of proteins that mediate attachment. Astrocytes were prepared from 3-day old rat-pup cortices and maintained in DMEM containing 5% FBS and antibiotics. After 14 days in culture, cells were labeled with Hoechst-33342 (2 μg/mL; overnight incubation at 37°C), enzymatically dissociated, pelleted, and resuspended ($1x10^5$ cells/mL). Collagens I and IV, laminin, and vitronectin (10-0.04 μg/mL) were adsorbed to 96-well microfluor plates (Dynatech; overnight incubations at 4°C), blocked with 1% bovine serum albumin (BSA; 3 hr at 37°C), and washed 2X with HEPES-buffered Hanks' saline (HH). Cell suspensions (100 μL) were placed in each well and incubated for 2 hr–a period of time sufficient for maximum attachment. Wells were aspirated and washed 2X with HH and cells lysed (200 μL of HH containing 2.5% triton). The fluorescence signal of each well was read using a plate reader on a Perkin-Elmer fluorescence spectrophotometer. Cell attachment to substrates was presented as % corrected maximal binding (fluorescence signal from untreated polystyrene wells containing cells - signal from wells containing no cells). Treatment of blank wells with BSA blocked cell attachment. Cells attached to substrates in a concentration-dependent fashion. Maximum binding and apparent affinity were determined using Michaelis-Menton kinetics. Maximal attachment was different for each of the proteins, 0.95 (collagen IV), 0.80 (collagen I), 0.3 (laminin) and 0.16 (vitronectin). Cells showed highest affinity to collagen IV (0.05 μg/mL) and lower affinity to collagen I (0.44 μg/mL). Cells bound with intermediate affinity to vitronectin (0.31 μg/mL) and very low affinity to laminin (2.28 μg/mL). Assessment of attachment to collagen IV is being made with hydrolytic peptides of native collagen and synthetic peptides. (This work is partially supported by NIH RR-10957.)

234.9

MIGRATION OF SCHWANN CELLS ON THE POLYESTER FIBERS IN VITRO AND THE EFFECT OF LAMININ X.Y.Shen* and J.Yang, Department of Anatomy, Shanghai Medical University, Shanghai 200032, P.R.China

It has been reported about the purification and migration of the Schwann Cell (SC) in vitro. However, what will happen if the SC co-culture with the artificial materials, the Polyester fiber (PF). Sciatic nerves from 30 mice aged P 3-7 were cut into small pieces (1mm) and cultured on the collagen-coated round coverslip with the PF (diameter 10㎜). Living observations under reversed phase contrast microscopy in the 2,4,6, 8 and 10 d.i.v., And Sudan black staining, S-100 protein immunocytochemical labelling and electron microscopies (SEM/TEM) were also made. Results showed that the cultured SC migrated along the PF in a good compatibility. The SC showed in typical fusiform shape and connected together one by one and their process always made plexus wrapping onto the PF. No any myelin-like structures were ever seen. The experimental group in which Laminin was added to the medium showed its obvious effect in promoting migration as well as proliferation of the SC in comparing to the control ones.

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234.10

IN VIVO EVIDENCE SUPPORTING A ROLE FOR MYELIN-ASSOCIATED GLYCOPROTEIN AS A CELL RECOGNITION-ADHESION MOLECULE C.Li[1], A.Peterson[2], J.Roder*[3], [1]Dept. Physiol., Univ. Toronto, Canada. [2]Neurosciences, Montreal General Hospital, Canada. [3]Dept. Med. Genet., Univ. Toronto, Canada.

MAG (myelin-associated glycoprotein) has been postulated to play an important role in initiating myelination *in vivo* based on several lines of *in vitro* evidence: 1) MAG-containing liposomes bind specifically to neurons; 2) MAG is the first myelin protein to be detected upon myelinogenesis; 3) MAG belongs to the immunoglobulin supergene family; 4) MAG is primarily found in the periaxonal region of a myelinated fiber; and 5) up or down regulation of MAG resulted in accelerated or diminished myelination, respectively. We tested this hypothesis by targeted disruption of the *mag* locus in mice and showed previously that the mutant mice had no gross perturbations of myelination as judged by X-ray diffraction, immmunohistochemistry, Western blotting and light microscopy. Ultrastructural studies by electron microscopy, however, revealed subtle impairment of periaxonal organization, indicating a role for MAG in the normal formation and maintenance of periaxonal structures. Here we show that MAG mutants exhibit an increase in the proportion of unmyelinated axons and a reciprocal decrease in myelinated axons in optic nerves. Aberrant forms of myelination, such as redundant myelin, multiple myelin and myelination of multiple axons are also occasionally encountered. In addition, axons of MAG null optic nerves appear less densely packed. Taken together, these lines of evidence support a role for MAG as an axon-glia cell recognition and adhesion molecule. Compromised cell recognition and adhesion in the absence of MAG might underlie the abnormalities observed.

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234.11

DYSTROGLYCAN IS A DUAL RECEPTOR FOR AGRIN AND LAMININ-2 IN SCHWANN CELL MEMBRANE. K. Matsumura[1*], H. Yamada[1], A. J. Denzer[2], M. A. Ruegg[2] and T. Shimizu[1] [1]Dept. of Neurology & Neuroscience, Teikyo Univ. Sch. of Med., Tokyo 173, Japan and [2]Dept. of Pharmacology, Biozentrum, Univ. of Basel, CH-4056 Basel, Switzerland

We have shown previously that α-dystroglycan with a molecular mass of 120 kDa is a Schwann cell receptor of laminin-2, the endoneurial isoform of laminin comprised of the α2, β1 and γ1 chains. Here we show that Schwann cell α-dystroglycan is also a receptor of agrin, an acetylcholine receptor-aggregating molecule having partial homology to laminin α chains in the C-terminus. Immunochemical analysis demonstrates that the peripheral nerve isoform of agrin is a 400 kDa component of the endoneurial basal lamina and is co-localized with α-dystroglycan surrounding the outermost layer of myelin sheath of peripheral nerve fibers. Blot overlay analysis demonstrates that both endogenous peripheral nerve agrin and laminin-2 bind to Schwann cell α-dystroglycan. Recombinant C-terminal fragment of the peripheral nerve isoform of agrin also binds to Schwann cell α-dystroglycan, confirming that the binding site for Schwann cell α-dystroglycan resides in the C-terminus of agrin molecule. The binding of recombinant agrin C-terminal fragment to Schwann cell α-dystroglycan competes with that of laminin-2. These results indicate that α-dystroglycan is a dual receptor for agrin and laminin-2 in the Schwann cell membrane.

234.12

ANALYSIS OF L1/MAG DOUBLE KNOCKOUT MICE, C. A. Haney*[1,2], C. Li[3], X. Yin[2], P. Soriano[4], J. Roder[3], and B.D. Trapp[1,2], [1]Department of Neuroscience, Case Western Reserve University, Cleveland, OH 44106 [2]Department of Neuroscience, Cleveland Clinic Foundation, Cleveland, OH 44195, [3]Mt Sinai Hospital, Neurobiology, Toronto, Canada. [4]Fred Hutchinson Cancer Research Center, Seattle, WA 98194

Molecules of the Immunoglobulin (IgG) gene superfamily mediate cell-cell interactions in the developing nervous system. The myelin-associated glycoprotein (MAG), is an IgG superfamily member which is expressed exclusively by myelinating cells and enriched in the adaxonal membrane of the myelin internode. MAG knockout mice are myelinated but have compromised periaxonal spaces. L1, another member of the IgG superfamily, is expressed during initial stages of Schwann cell ensheathment of axons and by Schwann cells of unmyelinated fibers. To test if L1 could be compensating for MAG during myelin formation in the MAG knockout animals, we generated L1/MAG knockout mice. Affected male animals (MAG -/-, L1 -/y) are runted and display delayed muscle mass development of the hind limbs. Morphological analysis of 28 day sciatic nerve demonstrated comparable numbers of myelinated axons in both knockout and control animals. These observations fail to support a compensatory role for L1 in MAG knockout mice, and raise the possibility that growth of long axons or formation of neuromuscular junctions is compromised. Currently, we are looking at younger mice to identify initial changes in myelination, axonal growth or muscle innervation that could account for the runted phenotype.

This work was supported by NIH grant NS 29818 and the MRC.

234.13

REVERSIBLE CHANGES IN BASAL LAMINA SUBJACENT TO THE SUPRAOPTIC NUCLEUS.

J.B. Bobak* N. Hawrylak and A.K. Salm. Departments of Anatomy and Physical Therapy, West Virginia University, Morgantown, WV, 26505

Previous studies have suggested that astrocytes associated with the supraoptic nucleus participate in the plasticity of this system. We recently demonstrated reversible morphological changes in astrocytes forming the glial limitans subjacent to this nucleus. In the present study we investigate potential changes in basal lamina associated with these astrocytes within, and ventral to, the glial limitans. The supraoptic nucleus was stimulated in adult male rats by replacing drinking water with 2% saline for 2 (n=5) or 9 (n=5) days. Control (n=5) animals received free access to tap water. A rehydrated group (n=5) was given 2% saline for 9 days, then returned to tap water for 9 days. Animals were sacrificed and their brain tissue was processed for electron microscopy. Electron micrograph montages of astrocytes (3,887X) in the glial limitans were constructed and the total volume fraction of basal lamina was estimated by means of an unbiased stereological point counting method. Group differences were assessed by an ANOVA. A post-hoc Tukey's t-test showed a significant decrease in the volume fraction of basal lamina by 28% from control levels with 2-days of dehydration. A 31% decrease was observed with 9-days of dehydration when compared with control animals. Upon rehydration, the volume fraction of basal lamina reversed to control levels. From these data we conclude that the basal lamina reversibly changes in association with dehydration. Since basement membranes, of which the basal lamina is a component, can act as barriers to the movement of cells and macromolecules, we suggest these changes contribute to an alteration in the barrier function of the glial limitans. Supported by NSF-9109827.

234.14

GROWTH AND FATE OF NEONATAL PSA-NCAM+ RAT PRECURSOR CELLS, T. Ben Hur[1], G. Rougon and M. Dubois-Dalcq*. Institut Pasteur, Paris and Institut de Biologie du developpement, Marseille, France

Oligodendrocytes, the myelin forming cells of the CNS, are derived from neural stem cells in the developing brain. Oligodendrocyte differentiation from O-2A progenitor cells has been well characterized, yet it is unclear how early precursors become committed to the oligodendrocyte lineage (OL). Pre-O-2A progenitor cells are small round cells that express the embryonic, polysialylated form of neural cell adhesion molecule (PSA-NCAM, Greenspan et-al, JNR 41:540, 1995). The aim of this study was to characterize OL pre-progenitors and to identify factors controlling their fate. OL cells were shaked off from primary cultures of 1 day old rat cerebral hemispheres, immunopanned with PSA-NCAM antibody and grown on poly-ornithine coated dishes. Addition of bFGF to defined medium induced proliferation of PSA-NCAM+ cells which formed clusters of round and unipolar cells. BrdU incorporation increased from 4% at 1 day to 18% at day 4 in the presence of bFGF while in its absence, extensive cell death occurred. After 1 week in bFGF, the clusters of PSA-NCAM+ cells were surrounded by multipolar OL cells stained by O4 antibody. Such differentiation of PSA-NCAM+ cells was blocked by growing them on uncoated dishes where cell clusters progressively evolved into spheres. After 1 week, clusters of PSA-NCAM+/O4- cells were dissociated in single cells which grew new clusters in response to bFGF. The effects of bFGF on growth and survival of PSA-NCAM+ cells was dependent on the presence of thyroid hormone in defined medium. Therefore these factors permit sustained growth of OL pre-progenitors on non adhesive substrate. We are currently studying whether these PSA-NCAM+ cells still have the potential to differentiate along other neural lineages.

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GENE STRUCTURE AND FUNCTION: PROMOTER ANALYSIS

235.1

NEURON SPECIFIC SILENCERS AND REGION SPECIFIC ENHANCERS DETERMINE GABAERGIC NEURON SPECIFIC EXPRESSION OF THE MOUSE GAD67 GENE. G. Szabó[1]* Z. Katarova[1], E. Mugnaini[2], G. Sekerkova[1], E. Körtvély[1], U. Zsolt[1], J. Mann[3], R. Greenspan[4], A. Aszódi[5] and Zs. Bősze[5]. [1]Lab. of Mol. Neurogenetics, BRC Inst. of Biochem. P.O.Box. 521, H-6701 Szeged, Hungary; [2]Inst. of Neurosci., Northwestern Univ., Chicago IL 60611; [3]Div. of Biol., Beckman Res. Inst., City of Hope Med. Cent. Duatre, CA 91010; [4]Dept. of Biology, NYU, New York, NY 10003; [5]Agricult. Biotech. Center, H-2101 Gödöllő Hungary.

Glutamic acid decarboxylase (GAD), the GABA synthetic enzyme is localized in 20-30% of all CNS neurons found in all regions of the brain.

To study the mechanisms underlying the transcriptional control and the region-specific expression of the gene encoding the 67 kDa form of GAD, we have characterized the promoter and the 5'-upstream regulatory region of the mouse gene. Within 400 bp of the flanking region, three tandemly arranged promoters of two different classes were identified. The most proximal promoter (P1) with multiple transcription start sites contains several GC boxes and is devoid of TATA and CAAT boxes, similar to the promoters of housekeeping genes. It is embedded in a CpG island, which spans P1, the fist exon, and the first intron. Distally to the CpG island, two additional closely spaced tissue specific like promoters with TATA and CAAT boxes were found. In the multiple promoter region, we have identified several putative transcription factor binding sites and neuronal specific regulatory elements including egr-1, NFκB, and the neuronal restrictive silencer element (NRSE).

As revealed by using transgenic mice, neuron-specific and cell type-specific expression of GAD67 is mediated through the combination of silencers identified in the basic promoter region and/or the first intron, and upstream region-specific enhancers.

Supported by grants T016971 and T020638 from the Hungarian Scientific Research Fund (OTKA).

235.2

REGULATION OF SODIUM CHANNEL GENE EXPRESSION BY TRANSCRIPTIONAL SILENCING. B.J.L. Eggen*, J. Tapia-Ramirez and G. Mandel. Department of Neurobiology and Behavior, State Univ. of New York at Stony Brook, Stony Brook, NY 11794-5230.

In the central nervous system (CNS), fast electrical signaling is often mediated through sodium-based action potentials. A predominant Na channel in rat brain is the type II CNS-specific sodium channel. The presence of a 28 bp DNA element in the promoter region of the type II gene is likely required for restricting the expression of this gene to the CNS. The transcription factor (REST[1]/NRSF[2]) that binds to this silencer element in the type II gene and mediates its neuron-specific expression has been identified. Cotransfection assays of PC12 cells with type II reporter genes and a recombinant REST cDNA results in silencing of the type II promoter. The domains of REST responsible for the transcriptional silencing of the type II promoter were studied using deletion mutants and GAL4 DNA binding domain fusion genes introduced into PC12 cells with type II reporter genes. The results from these studies suggest that specific domains in REST can function as autonomous silencer domains. This may have implications for future studies directed at perturbing expression of genes in vivo.

1) Chong J.A., Tapia-Ramírez J., Kim S., Toledo-Aral J.J., Zheng Y., Boutros M.C., Altschuller Y.M., Frohman M.A., Kraner S.D. and Mandel G. (1995) Cell 80, 949-957.
2) Schoenherr C.J. and Anderson D.J. (1995) Science 267, 1360-1363.

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235.3

SILENCING THE GAP-43 GENE. J.R. Weber and J.H.P. Skene*. Dept. of Neurobiology, Duke Univ., Durham, NC 27710.

Expression of the gene for the growth cone protein GAP-43 is highly restricted to the developing or regenerating nervous system, and its 5' flanking region can confer a substantial degree of neural specific expression to a reporter gene. In order to identify tissue specific regulatory elements, we have used mutational analysis of the most neuronal specific subdivision of the 5' flanking region, a 386 bp promoter located distally to the coding region. We showed previously that this region contains several positive acting elements used in neurons, including two CCAAT boxes, a TATA box, an AP-1 site, and two apparently novel transcription factor binding sites, Cx1 and Cx2. However, these positive elements cannot account for the high degree of neuronal specificity displayed by this promoter. Rather, we now show that a novel 29 bp negative element located in the transcription start region is primarily responsible for controlling tissue specificity. Transferring this silencer element to heterologous promoters blocks expression in non-neuronal cells. Conversely, mutations in this element permit strong expression of the 386 bp GAP-43 promoter in non-neuronal cells. Considering that silencer elements have been found in several other neuron specific genes, the control of tissue specificity by negative elements may turn out to be a common theme in neuronal differentiation. This mechanism offers the advantage of being able to use many widely expressed, non-tissue specific transcriptional activators to modulate and fine tune the activity of neuronal genes, yet still keep expression confined to the nervous system. Supported by NIH grant EY07397.

235.4

FUNCTIONAL ANALYSIS OF THE 5'-UPSTREAM PROMOTER REGION OF THE MURINE TRYPTOPHAN HYDROXYLASE GENE USING THE IMMORTALIZED PINEAL CELL LINE FROM TRANSGENIC MICE. S.V.Yim[1], J.H.Chung[1]*, S.J.Park[1], S.O.Huh[2], J.H.Son[2] and T.H. Joh[2]. [1]Department of Pharmacology, KyungHee University Medical College, Seoul, Korea. [2]Cornell University Medical College at The W.M. Burke Medical Research Institute, White Plains, NY, 10605.

Tryptophan hydroxylase (TPH) catalyzes the initial step of serotonin and melatonin biosynthetic pathway in neuroendocrine cells of the pineal gland. TPH gene expression is differentially regulated in the pineal gland during circadian rhythm. Previously, we have identified putative cis-acting motifs such as CRE, CTF/NF1, SP1, AP2, AP1 and OCT in the first 1 kb of the mouse TPH upstream region. We also established the immortalized neuroendocrine pineal cell lines, PGT-811 and PGT-β, by targeted tumorigenesis using the TPH promoter. PGT-β cells express the pineal marker enzymes, TPH and N-acetyltransferase. The pharmacological study demonstrated the utility of PGT-β cells as *in vitro* model system for the investigation of TPH gene expression by circadian regulation (Son *et al.* Mol. Brain Res. in press). To localize important cis-acting elements for the cell type-specific and/or inducible TPH expression in the pineal gland, a series of plasmids containing different lengths of the TPH upstream region fused to the luciferase as a reporter gene were constructed. The lipofection method reproducibly provided high transfection efficiency. In conjunction with the gel mobility shift assay, the DNA sequence analysis of further upstream promoter region revealed both positive and negative cis-acting elements in the 5' proximal region of the murine TPH promoter, which can be crucial to the cell type-specific and/or inducible regulation of TPH expression in the pineal gland. Supported by KyungHee University Foundation.

235.5

NEURAL SPECIFIC EXPRESSION OF THE PNMT GENE.

M.J. Evinger*, E. Mathew, J. Egan‡, Y.Ughrin†, W. Rosenberg, and Y-S.E. Lee. Depts. Pediatrics, Pharmacology‡ and Neurobiology†, SUNY, Stony Brook, NY, 11794.

Expression of the PNMT gene is restricted to cells of neural lineage. Previously, we identified two sequences in the 5' upstream region of the rat PNMT gene which repress expression of PNMT promoter-reporter constructs transfected into non-neuronal cell lines and which share sequence similarity with neural-specific elements in the Type II Na^+ channel and SCG-10 genes.

We sought to resolve whether neural-specific silencing of the PNMT gene is mediated in the same manner as that characterized for the Na^+(II) and SCG-10 genes. Electrophoretic mobility shift assays resolve two complexes of L6 nuclear proteins binding ^{32}P- oligonucleotides representing the PNMT Mid (-603 bp) and Proximal (-557 bp) putative silencers. The faster complex shares the migration pattern observed with Na^+(II) and SCG-10 RE-1 oligos. Excess Na^+(II) or SCG-10 oligo competes with binding to the lower band produced with PNMT Mid and Proximal oligos. The upper band is competed only by PNMT oligos. Antisera to REST silencer protein (from G. Mandel) supershifts material from the lower PNMT and from the Na^+ (II) oligo bands.

Cotransfection of plasmids expressing either wild type or dominant negative REST (G. Mandel) into L6, PC12, BE-2C, and primary chromaffin cells produces elevated expression of PNMT promoter constructs. In contrast, co-transfection of Na^+(II) RE-1-CAT with wild-type REST suppresses CAT expression in PC12s while dominant negative REST produces elevates CAT expression in L6 cells. Thus, structural and functional properties of the PNMT Mid and Proximal silencers differ from those in the Na^+(II) and SCG-10 genes and indicate a distinctive silencing mechanism is responsible for neural-specific expression of the PNMT gene. (Sponsored by NIH grant GM 46588 to MJE.)

235.6

CHARACTERIZATION OF THE CELL-SPECIFIC SILENCER REGION OF THE DOPAMINE β-HYDROXYLASE GENE.

H.-S. Kim* and K.-S. Kim Dept of Neurology, University of Tennessee, Memphis, TN38163.

Previous transient expression assays identified a cell type-specific silencer domain which resides between -486 bp and -262 bp from the transcription start site of the human DBH gene. This upstream domain encompasses a nucleotide fragment (termed DNRE) with a significant homology to the neuron-specific silencer motif found in several neuronal genes, suggesting that the DNRE is responsible for the cell specific silencer function of that domain. The DNRE exhibited repressive activity when placed in front of the homologous DBH or heterologous thymidine kinase promoter. However, site-directed mutation of the DNRE did not significantly diminish the repressive activity of the silencer domain, indicating that additional cis-regulatory motifs may reside within that silencer region, and coordinately regulate cell-specific silencer function. To address this possibility, that silencer domain was further analyzed by DNase I footprint analysis using nuclear proteins prepared from DBH-expressing SK-N-BE(2)C and DBH-nonexpressing HeLa cell lines. Three potential cis-regulatory elements, residing at -254 to 267 bp (domain I), at -295 to -316 bp (domain II), and at -324 to -348 bp (domain III) appeared to interact with nuclear extracts. While domain II was protected by both nuclear extracts, domain I and III were protected only by HeLa extracts in a cell-specific manner. In support of this, the electrophoretic mobility shift assay showed a similar pattern of cell-specific DNA-binding activities. Taken together, these results support our hypothesis that multiple cis-regulatory elements are involved in the cell type-specific silencer function of the upstream region between -486 bp and -262 bp of the human DBH gene.

NIH grant MH48866 and Gerwin Neurodegenerative Grant to KSK

235.7

DIFFERENTIAL EFFECT OF THE NEURON-SPECIFIC SILENCING FACTOR (REST) ON THE TRANSCRIPTIONAL ACTIVITY OF THE TYROSINE HYDROXYLASE (TH) AND DOPAMINE β-HYDROXYLASE (DBH) PROMOTERS.

C.Y. Yang* and K.-S. Kim Dept of Neurology, University of Tennessee, Memphis, TN38163.

The human DBH gene contains a negative regulatory element (termed DNRE) in the 5' upstream sequence. The DNRE shows a sequence homology and shares binding affinity with the neuron-specific silencer motifs identified in type II sodium channel, SCG10 and synapsin I genes. Recent isolation of the cognate transcription factor which binds to these silencer motifs, termed RE1 silencing transcription factor (REST; Chong et al., Cell (1995) 80, 949-957) or neural restrictive silencing factor (NRSF; Schoenherr and Anderson, Science (1995) 267, 1360-1363) made it possible to test whether the same factor can regulate the activity of the DNRE and/or DBH promoter. Here, we report that neither the 2.6 kb DBH upstream sequence nor the DNRE is subject to repression by REST using a cotransfection assay. In contrast, the promoter activity of the tyrosine hydroxylase (TH) upstream sequence was suppressed by REST in a dose-responsive manner. Strikingly, the dominant negative form (p73) of REST also suppressed the TH promoter activity with a similar efficiency. Taken together, these results dispel the possibility that REST may regulate the silencer activity of the DNRE, but are consistent with the hypothesis that REST may suppress the promoter function of certain genes (e.g., TH) via protein-protein interactions.

NIH grant MH48866 and Gerwin Neurodegenerative Grant to KSK

235.8

MULTIPLE FACTORS INTERACT WITH *CIS*-REGULATORY ELEMENTS OF THE PROXIMAL PROMOTER IN A CELL-SPECIFIC MANNER AND REGULATE TRANSCRIPTION OF THE DOPAMINE β-HYDROXYLASE GENE.

H Seo* and K.-S. Kim Dept of Neurology and Anatomy & Neurobiology, University of Tennessee, Memphis, TN38163.

A cAMP response element (CRE) residing at -181 to -174 bp from the transcription start site of the human dopamine β-hydroxylase (DBH) gene appears to be essential for DBH transcription. Potential *cis*-regulatory motifs such as AP1 and YY1 occur proximal to and overlap this CRE, endowing the area with a composite promoter structure. Using the DBH-expressing human neuroblastoma SK-N-BE(2)C and DBH-negative HeLa cell lines as model systems, we here report that this CRE/YY1/AP1 area interacts with multiple nuclear proteins including CRE-binding protein (CREB) and transcription factor YY1 in a cell-specific manner. In support of the notion that multiple proteins bind to the CRE/YY1/AP1 area, DNase I footprinting analysis demonstrated that nuclear extracts protected an extended region (from -186 bp to -150 bp), relative to that protected by purified CREB (from -186 bp to -171 bp). Site-directed mutational analysis revealed differential roles of potential *cis*-regulatory motifs in regulation of DBH transcription. Furthermore, three additional DNA-binding sites were identified by DNase I footprint analysis in the upstream 260 bp promoter region of the human DBH gene, of which two sites were cell-specific. These results support a model whereby multiple proteins bind to the 5' proximal area in a cell-specific manner and coordinately regulate the cell type-specific transcriptional activation of the DBH gene.

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235.9

CRE IS INVOLVED IN THE TRANSCRIPTIONAL UPREGULATION OF HUMAN DOPAMINE β-HYDROXYLASE GENE BY TPA.

H. Ishiguro*, K. Yamada, N. Ichino, T. Nagatsu Institute for Comprehensive Medical Science, Fujita Health University, Toyoake, Aichi 470-11, Japan.

Protein kinase A (PKA) and protein kinase C (PKC) upregulate the transcriptional level of human dopamine β-hydroxylase (DBH) gene in neuroblastoma SK-N-SH cells. Although functional CRE of human DBH gene (TGACGTCC) has 1-nucleotide change of consensus CRE sequence (TGACGTCA), PKA upregulates the transcription of the gene. Previous studies showed that YY-1 binding to the promoter was important to the induction of human DBH gene transcription by the treatment of TPA. To identify the TPA response region (TRR) in human DBH gene promoter, deletion mutant constructs from -604bp to -171bp were prepared and these luciferase reporter genes (Luc) were transfected to SK-N-SH cells. TRR resided in the upstream of CRE between -223bp and -175bp of human DBH gene, and there were no similar sequences in other known TRE sequences. When the sequences of TRRs (-223bp to -199bp or -206bp to -181bp) was placed in the 5'-position of the tk promoter (tkLuc), there were no transcriptional induction by the addition of TPA. To analyze the mechanism of TRRs, CRE sequences were inserted between TRRs and tk promoter in these reporter genes. Region from -206bp to -181bp in TRRs enhanced the transcriptional level of this reporter gene by the addition of TPA. These data define the TRR, residing at -206bp to -181bp upstream of the transcription initiation site, as an essential DNA region for the induction of DBH gene transcription by TPA. (Supported by MESC Japan)

235.10

ANALYSIS OF CELL TYPE SPECIFIC EXPRESSION BY THE RAT TYROSINE HYDROXYLASE PROMOTER USING TWO DIFFERENT TRANSGENIC APPROACHES.

K.L. O'Malley*, J.P. Merlie†, R.D. Todd‡, and J. Liu. Dept. of Anat. & Neurobiol., Dept. of Mol. Biol. & Pharm†., Depts. of Psychiatry & Gen.‡, Washington Univ., Sch. of Med., St. Louis, MO 63110

As the rate-limiting enzyme in the biosynthetic pathway of catecholamines, tyrosine hydroxylase (TH) is regulated both spatially and temporally in its pattern of gene expression. In order to study cell type specific gene expression, we examined different lengths of the rat TH promoter directing the *LacZ* gene in founder transgenic animals without establishing transgenic lines. In contrast to the usual practice of establishing inbred lines, this approach allowed us to economically analyze a large number of animals and constructs in a relatively short period of time. Fragments encompassing 6.0 kb, 3.4 kb, 2.5 kb and 0.8 kb of the rat TH promoter were used for this analysis. Descriptively as well as statistically, our results showed that cell type specific enhancer elements for adrenergic/noradrenergic cells in the brain stem and locus coeruleus and dopaminergic cells in midbrain, hypothalamic and olfactory periglomeruli were all located within 6.0 kb of the promoter sequence. Cell type specific elements for peripheral tissue such as adrenal chromaffin cells were located within 2.5 kb of the promoter sequence. In contrast to studies by others, we also found that a mesencephalic cell type specific element was located within 2.5 kb of the TH promoter sequence, which seemed to be negatively regulated by the addition of upstream sequences. We have also established independent transgenic lines using the 6.0 kb construct. Comparison of the variance in cell type specific gene expression between the "kill the founder" approach (n=9) and an analysis of gene expression within one transgenic line (n=6) showed no significant differences. These studies demonstrate the feasibility and reliability of using founder transgenic animals for promoter analysis. (Supported in part by NIH 45330)

235.11

INDUCIBLE cAMP EARLY REPRESSOR (ICER) CAN MODULATE TYROSINE HYDROXYLASE (TH) GENE EXPRESSION AFTER STIMULATION OF cAMP SYNTHESIS. C. Tinti*, B. Conti, J.F. Cubells, K.-S. Kim, H. Baker and T.H. Joh. Cornell Univ. Med. Coll. at Burke Med. Res. Inst., White Plains, NY., 10605.

The forskolin-induced activation of the cAMP pathway regulates transcription of TH, the rate-limiting catecholamine biosynthetic enzyme, through a CRE sequence located in the proximal 5' promoter of the gene. To identify specific nuclear factors that modify TH transciption through the TH-CRE, gel mobility shifts were performed with nuclear extracts derived from untreated or forskolin-treated PC12 cells. Retardation patterns, supershift analysis, as well as Western- and Northern blots showed induction by forskolin of a small protein corresponding to inducible cAMP early repressor (ICER). ICER, a strong transcriptional repressor, belongs to the family of CRE binding proteins and is derived by alternative promoter usage in the CREM gene. Transient transfection experiments in PC12 cells combining a previously-characterized TH2400CAT plasmid with the expression vector CMV-ICER-Ib, showed that ICER can repress transcription driven by the proximal 2.4 kb of the rat TH promoter. ICER also repressed protein kinase A-stimulated transcription of the TH2400CAT construct. Finally reserpine, which profoundly stimulates TH transcription, induced ICER in the rat adrenal gland. These results suggest that ICER can modulate cAMP-stimulated transcription of the TH gene and may, therefore, mediate rapid reversal of increased TH transcription following elevations in cAMP. (Supported by NIH grant MH24285)

235.12

TRIPLEX FORMING OLIGONUCLEOTIDE TARGETED TO THE AP-1 SITE OF THE RAT TYROSINE HYDROXYLASE PROMOTER. N.A. Papanicolaou, B. Nankova and E.L. Sabban*. Dept. of Biochemistry and Molecular Biology, New York Medical College, Valhalla, N.Y. 10595.

The rat tyrosine hydroxylase (TH) gene is regulated by a variety of pharmacological and physiological signals. Sequences between -205 and -182 contain an AP-1-like motif which regulates the rat TH gene expression in tissue culture cells in response to NGF and phorbol esters. Moreover, nuclear extracts isolated from the adrenal medulla of rats subjected to immobilization and cold stress, exhibit increased binding of fos/jun complexes to the AP-1 site of the rat TH promoter. Triplex forming oligonucleotides (TFOs) may be used to modulate gene transcription in a sequence specific manner. In an effort to specifically inhibit transcriptional activators acting at the AP-1 site, a 22 nucleotide TFO (Thap-1) was synthesized. Thap-1 (1-100μM) was found to bind to two rat promoter fragments (32 bp and 128 bp) containing the AP-1 site and to reduce their electrophoretic mobility. Competition experiments showed that Thap-1 binds specifically the AP-1-containing oligonucleotides and prevents the interaction of fos/jun complexes in nuclear extracts from the adrenal medulla of immobilized rats. These results suggest that TFOs may be useful in modulating transcriptional activation at the AP-1 site of the TH promoter (Supported by NIH grant NS28869).

235.13

NEURON-SPECIFIC EXPRESSION IN TRANSFECTED RETINAL ORGAN CULTURES USING THE CR16 PROMOTER. W.H. Ashman, Y.-C. Li, A.P. Young, J.N. Masters*. Department of Cell Biology, Neurobiology and Anatomy and the Neurobiotechnology Center, The Ohio State University, Columbus, OH 43210

CR16 is a novel glucocorticoid-regulated gene expressed in subpopulations of rat brain neurons. In order to characterize the cis-acting DNA elements mediating the neuron-specific expression, we have determined the structure of the CR16 gene and assayed promoter function in HeLa cells and retinal organ cultures. Primer extension and RACE cloning suggest that a single promoter is responsible for transcription initiation in hippocampal neurons. The CR16 promoter sequence lacks TATA and CAAT boxes, but is contained in a possible GC island with multiple SP-1, AP-2 and E-box elements. Cell type specific expression was determined by electroporation of chicken E12 retina with β-gal reporter constructs followed by primary culture and immunocytochemistry as described (Li et al., J. Mol. Neurosci. 6:169 1995). Reporter constructs containing the RSV or SV40 promoters were relatively non-selective in terms of neuronal vs glial expression (65% neurons, 35% glia) while the glutamine synthetase promoter was relatively glial selective (20% neurons, 80% glia). The CR16 promoter from -492 to +71 (relative to the transcription initiation site) is predominantly expressed in neurons (95% neuron) and this specificity is maintained in deletion clones containing as little as 101 bp of upstream sequence. Future studies will further localize and identify the neural-specific enhancer and/or glial suppressor elements. The identification of these elements may be useful in preparing neuron-targeting vectors. Supported by NIH AG09425 (JNM) and EY05063 (APY).

235.14

TRANSCRIPTIONAL REGULATION OF THE NEURONAL GLUCOSE TRANSPORTER (GLUT 3). R.A. Rajakumar, R.K. Menon, K. D. Ryan*, and S.U. Devaskar. Department of Pediatrics, University of Pittsburgh, Magee-Womens Research Institute, Pittsburgh, PA 15213.

The glucose transporter, Glut 3, is targeted specifically to the axonal processes of mature neurons. Glut 3 expression is developmentally regulated with low amounts being noted in the fetal and early postnatal brain and peak amounts being observed at postnatal day 21 in both the rat and mouse. To examine the role of transcription in the developmental regulation of Glut 3 we undertook the present investigation. Employing a mouse Glut 3 genomic clone containing -1884 bp 5'-to the translation start site (J. Takeda & Bell; Genebank no. U11844), 5'-deletional constructs were made by PCR and subcloned into a promotorless, enhancerless luciferase vector. Following transient transfection of cultured immortalized embryonic fetal rat neural cells which exhibit a neuronal phenotype (Eves et al., PNAS 1992) and N2A mouse neuroblastoma cells (ATCC), the luciferase activity (reporter) was assayed and standardized to β-galactosidase activity (n=3 each). The results of these studies focused the Glut 3 promoter activity to -149 to -124 bp of the 5'-sequence. Subsequent competitive gel shift and super shift assays have demonstrated Sp1, the ubiquitous nuclear protein to bind this domain. The Sp1- Glut 3 DNA binding activity increases from the 1d to the 21d postnatal age. We conclude: 1) the GC rich region of a CAAT box domain forms the cis-element and Sp1 a trans-activating factor regulating neuronal transcription of mouse Glut 3; 2) the Sp1 DNA-binding activity increases with development. [Supported by NIH-HD-33997]

235.15

EXPRESSION PATTERNS OF MURINE ALDOLASE C (ZEBRIN II) TRANSGENES. S. M. Maricich, E. Walther, M. Dichgans, S. Dziennis, E. Deneris*, S. Zackson, R. Hawkes and K. Herrup. Case Western Reserve Univ., Cleveland, and University of Calgary.

Although many genes with compartmentalized patterns of expression in cerebellum have been identified, the specific *cis*-acting elements responsible for these expression patterns have remained elusive. Aldolase C (zebrin II), expressed in Purkinje cells, is one such gene product. To study its regulation, we designed three different reporter-gene constructs containing bacterial ß-galactosidase (*lacZ*). Multiple independent transgenic lines were created using each of the constructs. Expression was CNS-specific in all lines which showed reporter activity. Those with *lacZ* in the final exon, exon 9, (MAC Z9) showed glial expression throughout the brain. A deletion construct lacking all structural gene sequence between the second and ninth exons (MACZ2Δ9) showed more restricted and sporadic expression. Finally, a construct with the reporter in exon 2 (MACZ2) showed no expression. None of the constructs reproduced the pattern of expression of the endogenous gene. Copy number analysis, Northern blots of transgene expression, and in situ hybridization using specific probes for aldolase A and aldolase C were also performed. We propose a model where elements 5' to those in our constructs as well as the spacing of various *cis*-acting elements, some present within the body of the gene, are important for achieving normal patterns of aldolase C expression.

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235.16

TISSUE-SPECIFIC, 5' TRANSCRIPTIONAL CONTROL OF THE MOUSE ACETYLCHOLINESTERASE GENE. E. Atanasova*, S. Chiappa, and S. Brimijoin. Mayo Clinic, Rochester, MN 55905.

A previously uncharacterized portion of the gene for mouse acetylcholinesterase (AChE), extending from position -1267 to -493 in the 5' untranslated region, was obtained by RAGE-PCR (Rapid Amplification of Genomic Ends). The RAGE-DNA co-hybridized with coding-sequence cDNA in a mouse genomic library and was 61.5% identical to the human AChE promoter region. Analysis revealed a CCAAT box and consensus sequences for transcription factors including SP1, ATF/CREB, and AP2, as well as clustered MyoD and NFkB sites. To investigate possible promoter activity, RAGE-DNA linked to the open reading frame of a luciferase reporter-gene was transfected into neural (N1E.115 mouse neuroblastoma) and skeletal muscle cells (L6P rat myoblastoma). At 48 hr, lysate luciferase was compared with activity produced by two other reporter-constructs: 1) with the recognized minimal promoter and 2) with a 1.2 kb DNA extending from the 5' end of the RAGE sequence to position -75. In L6P, the 1.2 kb construct and the minimal promoter were, respectively 10 and 7 times as effective as the RAGE-construct. In N1E.115, the 1.2 kb construct was 6 times as effective as the RAGE-construct ($p < 0.001$ by Mann Whitney Test), but the RAGE DNA was just as effective as the minimal promoter. These data suggest that the RAGE sequence might play a biological role in neural tissue. Further evidence for this idea was derived by applying 5' RACE-PCR (Rapid Amplification of cDNA Ends) to mouse brain cDNA. Thus, DNA products cloned into TA vector and transformed into INVaF' cells yielded colonies hybridizing with both RAGE-DNA and cDNA from the AChE coding sequence. (Supported by NS28646).

235.17

MOLECULAR COMPONENTS OF DEVELOPMENTALLY REGULATED FAR-DISTANT REPRESSION OF THE ENKEPHALIN GENE, A. Dobi, M. Palkovits, A. Eitel, M. Mahan, F. Lim, M. Ring, C.G. Palkovits, and D.v. Agoston*, MCN, Lab. Dev. Neurobiol.,NICHD, NIH, Bethesda, MD 20892

The peptide neurotransmitter enkephalin is primarily expressed in neurons of the caudate nucleus, whereas the thalamus and the cortex contain 10 to 100-fold less neurons that express the ENK gene. We previously identified an AT-rich region [(ATT)19 repeat] (at -2450 bp) which specifically interacts with the proximal promoter region in a development- and brain region-specific manner through a complex of DNA binding proteins. The DNA-protein complex binds with high affinity to cationic lipids. Competitive mobility shift assays with various mutants of the ATT motif indicate that the protein complex requires a specific DNA structure characterized by AT rich sequences. The protein complex binds to the DNA in an all-or-nothing fashion suggesting cooperative binding between proteins or protein subunits. Based on UV crosslinking, the complex is composed of four proteins of MW ~200 kD, ~ 90 kD, and 43 kD. The 43 kD protein is a novel protein characterized by very high proline content. Decoying the binding of the endogenous protein complex to the ATT repeat in developing primary cortical neurons resulted in a 3-5-fold increase in ENK mRNA expression further supporting the hypothesized repressor role for this element. Also, distamycin, which specifically binds to the minor groove and thereby perturbs minor groove-protein interactions, induced a several-fold increase in ENKmRNA in primary cortical neuronal cultures. Our results suggest that the major regulatory mechanism in the mammalian brain is likely selective gene repression.

235.18

CONTRIBUTIONS OF MULTIPLE CIS-ACTING ELEMENTS TO BASAL CELL-SPECIFIC AND SECOND MESSENGER-STIMULATED TRANSCRIPTION OF THE VIP GENE. S. H. Hahm and L. E. Eiden*. Sec. Molec. Neurosci., Lab. of Cell Biol., NIMH, NIH, Bethesda, MD 20892.

A construct containing five kb of 5' flanking sequence, and extending through the second exon of the VIP gene, fused to a sequence encoding luciferase, was used to demonstrate that an upstream region (-5.2-2.5 kb) previously shown to be important for cell-specific regulation of the VIP gene (PNAS 85:9547, 1988) is absolutely required for cell-specific expression in EP and SK-H-SH neuroblastoma cells. The proximal VIP CRE (cyclic AMP response element) is also required for full cell-specific constitutive expression in the context of the 5.2 kb VIP-luc construct. Cyclic AMP (forskolin) responsiveness absolutely requires the CRE sequence. Contrary to previous reports using heterologous promoters to assay the activity of the VIP-CRE, responsiveness to phorbol esters is independent of the CRE and dependent on the presence of a region upstream of the CRE containing a consensus AP-1 site.

Multiple cis-acting sequences of the VIP gene act combinatorially to affect full basal and stimulated expression of VIP. The VIP-CRE functions only as a cyclic AMP response element, and not as a TPA-response element (TRE), in the context of the VIP gene. An additional TRE exists in the VIP gene, probably at the TGACTCA sequence approximately 2.3 kb upstream of the start of transcription of the VIP gene.

235.19

CHARACTERIZATION OF THE PROMOTER ELEMENTS AND TRANS-ACTING FACTORS INVOLVED IN THE REGULATION OF FGF-2 EXPRESSION BY GROWTH FACTORS AND SECOND MESSENGERS IN HUMAN ASTROCYTES. J. Moffett, E. Mordechai, E Kratz, A. Schwartz*, M.K. Stachowiak. Barrow Neurological Institute, Phoenix AZ 85013.

Mitogenic FGF-2 is involved in the transition of astrocytes from the quiescent to the reactive, proliferating state. We utilized cultures of human astrocytes to examine whether soluble growth factors and second messenger systems may also control expression of FGF-2. Astrocytes express the 18 kDa and high molecular weight (HMW; 21/22, 24 kDa) FGF-2 isoforms. Treatment of serum-free cultures with cytokine Il-1β increases the intracellular content of predominantly the 24 kDa FGF-2 isoform. Growth factors EGF and PDGF increased predominantly the levels of the 18 kDa FGF-2 isoform. Incubation with 18 kDa recombinant FGF-2 increased the content of the 18 kDa as well as the HMW FGF-2 isoforms. The induction of FGF-2 was accompanied by an increase in FGF-2 mRNA levels. Transfection of FGF-2 promoter-luciferase constructs showed that Il-1β and growth factors stimulate transcription of the FGF-2 gene by acting through a -550/-512 bp promoter region. Forskolin or PMA increase FGF-2 mRNA and FGF-2 promoter activity from the promoter region -650/-555 indicating that adenylate cyclase or PKC signaling pathways are not involved in FGF-2 gene stimulation by growth factors. In vitro DNA-protein binding experiments indicate that stimulation of FGF-2 gene results from increased binding of trans-activating factors to the growth factor-responsive and cAMP/PKC promoter regions, respectively. DNase I footprinting of the -650/-512 region revealed several sequences within this region that may bind trans-acting factors involved in FGF-2 regulation. UV-crosslinking identified at least 3 proteins that may be involved in trans-activation of the FGF-2 promoter. The data indicates that the FGF-2 promoter is regulated by trans-acting factors binding to novel cis-èlements in the FGF-2 promoter (supported by NSF, ADCRC, Univ. of Arizona Biomed. Res. Prog.).

235.20

REGULATION OF THE CHICKEN OVALBUMIN UPSTREAM PROMOTER TRANSCRIPTION FACTOR (COUP-TF I) PROMOTER. K. Brubaker, H. O. Nornes*, and T. Neuman Department of Anatomy and Neurobiology, Colorado State University, Fort Collins, CO 80523.

The gene for COUP-TF I codes for a transcription factor that belongs to the Steroid/Thyroid nuclear hormone receptor superfamily. Receptors in this family transduce hormone signals involved in development and cellular processes through molecules such as the retinoids and thyroxin. One function of the COUP-TF subfamily is to repress the receptor function of other superfamily members. In this way the responsivity of cells to hormone signals is inhibited. Identification of the regulatory elements of the COUP-TF I promoter may allow for the expression to be blocked, thereby enhancing cellular response to hormone signals.

The promoter region for COUP-TF I was sequenced and the start site identified. Transgenic mice containing the reporter gene (CAT) under the control of the COUP-TF I 4kb regulatory region were generated. Comparison of COUP-TF I and reporter gene expression using *in situ* hybridization demonstrate that the 4kb regulatory region is sufficient for proper expression in the developing and adult CNS. Transient CAT assays were performed to characterize regulatory elements contained within the promoter region using PCC7, 3T3, and C33A cell lines.

This research was funded by the Spinal Cord Society.

EXCITATORY AMINO ACID RECEPTORS: STRUCTURE, FUNCTION, AND EXPRESSION—FUNCTIONAL PROPERTIES

236.1

CHARACTERIZATION OF THE PH SENSITIVITY OF NMDA RECEPTOR GLYCINE SITE LIGAND BINDING AND DEVELOPMENT OF A MOLECULAR MODEL IMPLICATING NR1 SUBUNIT HISTIDINE RESIDUES B.M. Baron*, B.W. Siegel, R. Vaz, and B.L. Harrison, Hoechst Marion Roussel, Cincinnati, OH, 45215

The effect of pH on NMDA receptor glycine site ligand affinity was studied using the radiolabeled antagonist [^{3}H]MDL 105,519. The pH optimum for binding to rat brain membranes was pH 6.0. Similar results were obtained using stably expressed NMDA NR1 subunits indicating that the molecular substrate is the NR1 protein. In brain membranes, acidification increased the affinity of the radioligand without changing the total number of binding sites. The K_i values were determined at pH 6.0 and 7.4 for a panel of unlabeled agonists and antagonists. Three distinct effects were noted which were correlated with ligand structure. Agonists possess a charged nitrogen atom and showed a 5-fold reduction in affinity at the more acidic pH. Antagonists possessing a distally-located carboxylic acid moiety (e.g. MDL 105,519 and related structures) exhibited a 3-5-fold increase in affinity at the acidic pH. Antagonists lacking this side chain (e.g. ACEA 1021 and amide derivatives of MDL 105,519) were unaffected by these pH changes.

We hypothesized that the effect of pH on ligand affinity results from coulombic interactions with charged histidine residues ($pK_a = 6.1$) in the binding pocket. A molecular model of the putative glycine recognition site on the NMDA NR1 subunit was generated using the crystal structure of the homologous bacterial protein LAOBP with a loop-search technique to map non-homologous regions. Docking MDL 105,519 into the NR1 model using coordinates defined by LAOBP-bound lysine, revealed a potential ionic interaction with two histidine residues. As brain ischemia is associated with acidosis, the combination of decreased agonist affinity and increased antagonist affinity may make glycine antagonism a distinctly efficacious approach or provide selectivity for the ischemic zone. Future, work should be directed to testing the hypotheses concerning the amino acids responsible for these effects.

236.2

ASPARAGINE$_{616}$ IN THE NR1 SUBUNIT CONTROLS SPERMINE BLOCK, SPERMINE POTENTIATION, AND pH SENSITIVITY. M.F. Burgess*, J. Powers and S.F. Traynelis Dept. of Pharmacology, Emory University, Atlanta, GA 30322 and Dept. of Chemistry, Kennesaw State College, Marietta, GA 30061

Our previous studies have demonstrated that inclusion of exon5 in the NR1 subunit of the NMDA receptor leads to a voltage-independent relief of the tonic inhibition exerted by physiological pH. The polyamine spermine appears to mimic the effects of exon5 by reducing pH inhibition for NR1 splice variants that lack exon5. To further explore the structural basis of spermine and pH effects on NMDA function, we have used site-directed mutagenesis to investigate the role of residues that are suggested to line the channel pore, because these residues may control other pH-independent effects of spermine. We found that a single amino acid (Asn$_{616}$) controls voltage-dependent block of the channel by 100 and 1000 μM spermine. Substitution of arginine for the asparagine removes spermine-induced outward rectification of recombinant NR1(±exon5)/NR2 receptor responses in the *Xenopus* oocytes (n=35 oocytes). Interestingly, this mutation also decreased both the pH sensitivity (n=50) and spermine (100 μM) relief of tonic proton block (n=6). We conclude that either the Asn$_{616}$ contributes to a complex interaction that involves exon5, spermine, and the pH-sensor, or Asn$_{616}$ controls the gating of the channel in a pH-sensitive fashion.

Supported by the John Merck Fund, NIH-NINDS, and the American Epilepsy Society

236.3

PERMEATION PROPERTIES OF WILD TYPE (NR1/NR2A) AND MUTANT (NR1-E603K/NR2A) NMDA CHANNELS J.Kupper, R. Schneggenburger, J.Neyton and P. Ascher* Laboratoire de Neurobiologie, CNRS URA 1857, Ecole Normale Supérieure, 75005 Paris, France.

In the primary sequence of NMDA channels composed of the NR1 and NR2A subunits the glutamate (E) in position 603 of the NR1 subunit is the only negative charge near the Q/R/N site. We have compared the permeation properties of wild type NR1/NR2A channels to those of mutant NR1 E603K/NR2A channels in which the glutamate is replaced with a positively charged lysine (K). The channels were expressed in *Xenopus* oocytes or HEK cells. In outside out patches with symmetrical 150mM CsCl the single channel i/V relation of NR1 E603K mutant channels shows a much more pronounced inward rectification than what is observed for wild type channels. Increasing the CsCl concentration from 150 mM to 500mM increases only marginally the single channel conductance of the wild type channel, indicating that this channel operates near apparent saturation under physiological conditions. In contrast, the same change of ionic concentrations increases the single channel conductance of the NR1 E603K mutant from ≈35pS to ≈53pS (at -80mV) and reduces the inward rectification. Reversal potential measurements of macroscopic currents in HEK cells show that the calcium permeability of the NR1-E603K/NR2A channel is reduced by ≈50 % as compared to the wild type channel. Measurements of fractional calcium currents (P_f) in the voltage range between 0mV and -60mV indicate that close to 0mV P_f has the value predicted from reversal potentials but with hyperpolarization the P_f value of the mutant channel increases up to the value of the wild type. Taken together these observations are consistent with the idea that the E603K mutation introduces a positive surface charge in or close to the inner mouth of NMDA channels. *Supported in part by a HFSP fellowship to J.K., an EC fellowship to R.S. and EC grant (BIO2-CT93-0243).*

236.4

Ca^{2+} SELECTIVITY AND PERMEATION IN NMDAR CHANNELS. L.P. Wollmuth*, T. Kuner+, D.-S. Koh, P.H. Seeburg+, and B. Sakmann. MPI für med. Forschung, Abt. Zellphysiologie, Jahnstr. 29, +Center for Mol. Biology (ZMBH), Heidelberg Univ., Im Neuenheimer Feld 282, Heidelberg, Germany.

The critical contribution of the *N*-methyl-D-aspartate receptors (NMDAR) to synaptic physiology and plasticity arises in part because its channel is more selective for Ca^{2+} than for monovalent cations. The mechanism of Ca^{2+} selectivity and transport in NMDAR channels is unknown. A GHK formalism which assumes ions permeate independently seems inappropriate: (i) the addition of 1.8 mM Ca^{2+} externally (to 120 mM NaCl) shifts the reversal potential of currents rightward as expected for a more permeant ion but reduces the current amplitude over the entire potential range indicating that Na^+ and Ca^{2+} ions do not cross the channel independently; (ii) channels with substitutions of the NR1 N-site asparagine have an attenuated P_{Ca}/P_{Cs} but surprisingly invert relative to wild type the voltage dependence of fractional Ca^{2+} currents (P_f); (iii) channels with substitutions of the NR2 N+1 site asparagine also attenuate P_{Ca}/P_{Cs} without affecting P_f, indicating along with (ii) that the magnitude and voltage dependence of fractional Ca^{2+} currents are not strictly determined by Ca^{2+} selectivity. The deviation from GHK reflects in part that Ca^{2+} permeation in NMDAR channels depends on Ca^{2+} interacting with two sites: the NR1 N-site positioned at the narrow constriction (50-60% across the transmembrane electric field) as well as a site positioned less than 10% across the field.
Supported in part by a Alexander von Humboldt fellowship to LPW.

236.5

MOLECULAR DETERMINANTS OF CA-DEPENDENT INACTIVATION IN RECOMBINANT NMDA RECEPTORS
J. Krupp*, B. Vissel†, S. Heinemann† & G. L. Westbrook, Vollum Institute, Oregon Health Sciences University, Portland OR and † Salk Institute, La Jolla CA.

Ca-dependent inactivation of the NMDA receptor regulates transmission at hippocampal synapses. We have previously shown that Ca-dependent inactivation in NR1-1a/2A heteromers is similar to hippocampal neurons, but does not occur in NR1-1a/2C heteromers. To determine the molecular basis of Ca-dependent inactivation, we first tested whether differences in Ca influx could explain the apparent subunit specificity using combined calcium-imaging and whole-cell patch-clamp measurements in HEK293 cells (Vh: -50). The intracellular solution contained 200 µM fluo-3, 100 µM EGTA and no added Ca. In NR1-1a/2A heteromers 10 µM NMDA (Cao= 2 mM) induced inward currents with prominent Ca-dependent inactivation (43.8 ± 8.2 %, n = 4) concomitant with a calcium transient (F/F0 = 1.3-5.2). Similar calcium transients were evoked in NR1-1a/2C, but there was no Ca-dependent inactivation of the whole-cell current. Thus the slightly lower Ca influx of 1a/2C receptors (Burnashev et al., J. Physiol. 1995) does not explain the subunit specificity of inactivation.

The C-terminus of NR1 has been shown to bind Ca-CaM, resulting in reduced channel activity in inside-out patches of NR1/2A receptors (Ehlers et al., Cell 1996). We tested whether Ca-dependent inactivation simply reflects CaM binding by cotransfecting NR2A with a splice variant of NR1 (NR1-4a) that lacks the C-terminal inserts containing a PKC site and one of the CaM sites. We also constructed two deletion mutants (NR1-stop838 and NR1-stop863), terminating 5 and 30 aa after the putative TMiv. Ca-dependent inactivation was retained with NR1-4a (54.3 ± 13.0%, n = 7) as well as NR1-stop863 (32.8 ± 18.0%, n = 5), but was absent with NR1-stop838 (5.5 ± 27.1 %, n = 8). Thus, Ca-dependent inactivation requires the first 30 aa after TMiv of NR1 that contains one calmodulin binding site. However, calmodulin inhibitors have no effect on Ca-dependent inactivation in native receptors, suggesting that Ca-dependent inactivation involves additional receptor domains.
Supported by NIH (MH46613), HFSP (JK & BV] and J Aron Charitable Foundation (BV).

236.6

IDENTIFICATION OF RESIDUES LINING THE EXTRACELLULAR VESTIBULE OF THE NMDA RECEPTOR CHANNEL. C. Beck, P.H. Seeburg, T. Kuner* Center for Molecular Biology (ZMBH), University of Heidelberg, and MPI für Medizinische Forschung, 69120 Heidelberg, Germany

The current model of glutamate receptor topology depicts three membrane spanning segments, M1, M3, and M4, and a membrane reentrant segment, M2. The M2 segment forms a channel-lining loop which originates and ends at the cytoplasmic side of the membrane. The selectivity filter is formed by a cluster of hydrophilic residues at the tip of the loop. The ascending, α-helical segment, and the descending, extended segment of the loop form part of the cytoplasmic channel wall. Such a structure of M2 (Kuner et al., Soc. Neurosci. Abstr. 21,85) suggests, that other domains form the external vestibule of the channel.

We used the substituted-cysteine-accessibility method (SCAM, Akabas et al., 1992 & 1994) to identify channel-lining residues within, and flanking, the membrane spanning segments (M1, M3, M4) of the NR1 subunit. NR1(mut)-NR2C channels expressed in *Xenopus* oocytes were probed from the extracellular side of the membrane with differently sized methanethiosulfonate reagents. From the irreversible current reduction in mutants P539C (preM1), L544C (M1), N632C (M3) and G797C (M4) we infer that these positions are exposed to the channel lumen. No current reduction was found in mutants W545 to V552 of the M1 segment. We propose that the region preceding M1, the M3 segment and the M4 segment of the NR1 subunit cooperate to form the outer vestibule of the NMDA receptor channel.

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236.7

NMDA RECEPTOR SUBTYPES STABLY EXPRESSED IN HUMAN KIDNEY 293 CELL-LINES. F. Besnard, S. Renard, M. Partiseti, C. Drouet-Petre, S.Z. Langer* and D.Graham, Synthelabo Recherche, 92500 Rueil Malmaison, France

NMDA receptors are composed of many different subunits subdivided in two families: NR1 (8 splice variants) and NR2 (4 related subunits A-D). They are involved in a number of important CNS functions such as motor coordination and delayed neuronal cell death. As subtype-selective drugs could help determine the composition and role of the different native NMDA subtypes we developed a novel strategy to establish stable cell lines expressing various NMDA receptor subunit combinations in order to identify such drugs. These cell lines were established using a tetracycline inducible promoter in order to protect the cells against cytotoxicity induced by NMDA receptor expression. The NR1A subunit was subcloned downstream of the tetracycline regulated promoter and after transfection and selection, clones were screened by Western-blot with a NR1 specific antibody. Several clones showed expression patterns that could be modulated from background to high levels by removing tetracycline for 24 hours. One clone was selected for surtransfection with different NR2 subunits. After a second round of selection two cell-lines expressing binary combinations of NR1A/NR2A and NR1A/NR2D were obtained and characterized. Expression of NR1/NR2 subunits in these cells upon tetracycline removal was confirmed by Western-blot analysis with subunit-selective antibodies as well as by induction of cytotoxicity. The pharmacological and electrophysiological properties of these cell-lines will be presented.

236.8

EFFECTS OF A MONOCLONAL ANTIBODY TO THE GLUTAMATE BINDING PROTEIN ON NMDA AND L-GLUTAMATE- ACTIVATED ION CHANNELS. R. E. Ragan*, G.L. Aistrup, K.N. Kumar, R.D. Schowen, E.K. Michaelis, M.L. Michaelis. Dept. of Pharmacol. & Toxicol. and Ctr. Neurobiol. & Immunol. Res., Univ.of Kansas, Lawrence, KS 66045

Excess activation of the *N*-methyl-D-Aspartate (NMDA) subtype of brain glutamate receptors has been implicated in neurodegeneration occurring as a result of massive Ca^{2+} influx. A group of proteins has recently been isolated from rat brain synaptic membranes that have binding characteristics consistent with those reported for the NMDA receptors (JBC 269:27384, 1994). This complex does not contain the NMDAR1 protein or any other glutamate receptors that have been sequenced to date. Monoclonal antibodies (mAb's) were raised against the glutamate binding protein (GBP) subunit of this complex. The studies reported here were undertaken to determine whether these mAb's inhibited the glutamate/glycine-activated ion currents detected when this protein complex was reconstituted into planar lipid bilayers and whether these same mAb's decreased NMDA-activated Ca^{2+} influx into primary hippocampal neurons. The protein complex reconstituted in lipid bilayers exhibited a glutamate/glycine activated ion current which was inhibited by the NMDA receptor blockers 2-AP5 and MK-801. Addition of 10ng/ml of the purified α-GBP mAb's inhibited nearly 100% of glutamate/glycine- activated ionic current. The same mAb's also decreased the NMDA-activated Ca^{2+} influx into primary hippocampal neurons by approximately 50%, suggesting that a portion of the NMDA-activated Ca^{2+} influx is regulated by this protein complex. This mAb should serve as an important tool for probing the physiological relevance of this group of proteins in the function of neuronal NMDA receptors. (Funded in part by PHS grants GM07775, AA04732, and by a KTEC grant to the Center for Neurobiol. & Immunol. Res.)

236.9

SUPEROXIDE INDUCED INHIBITION OF L-[^{3}H]GLUTAMATE BINDING TO NMDA RECEPTORS IN BRAIN SYNAPTIC MEMBRANES, TO A PURIFIED PROTEIN COMPLEX AND TO ONE OF ITS SUBUNITS.

X. Chen*, N. S. Ranciat, M.L. Michaelis, and E. K. Michaelis. Dept. Pharmacol. Toxicol.,Cntr. Neurobiol. & Immunol. Res., Univ. of Kansas, Lawrence, KS 66045

Oxidative stress in neurons may be triggered by hyper-activation of NMDA receptors (NMDAr's) and may be the cause of NMDA-induced neuronal degeneration. Superoxide ($O_2\bullet^-$) and nitric oxide (NO•) are rapidly formed and may modify the structures of proteins, DNA and cell membrane lipids. The NMDAr's may themselves be among the proteins modified by reactive oxygen species (ROS). This may either alter the rate of formation of ROS. In the present studies, rat brain synaptic membranes were treated with 5μM xanthine (X) and 0.02 U/ml of xanthine oxidase (XO) to generate $O_2\bullet^-$. The NMDA-sensitive L-[^{3}H]-glutamate (Glu) binding to the membranes following treatment was less than 20% of control. The X+XO treatment primarily reduced the B_{max} and had only a modest effect on the K_d. The purification of the NMDA R1/R2 complex in a functional state is difficult to achieve. Therefore, a further molecular definition of the targets of $O_2\bullet^-$ action was pursued by purifying a complex of proteins with ligand-binding characteristics similar to those of NMDAr's Treatment of this complex with X + XO produced nearly identical results to those for the synaptic membranes. Furthermore X + XO treatment decreased the B_{max} for L-[^{3}H]-Glu binding to the glutamate-binding protein (GBP) subunit of the complex: Treatment of with X, XO, or H_2O_2 (10μM) alone did not alter the binding of L-[^{3}H]-Glu to any of these preparations. The effect of $O_2\bullet^-$ formation on the function of NMDAr's in intact cells is currently being explored in cerebellar granule cells and cortical neurons in culture. (Supported by grants AG12993 & AAO4732).

236.10

SUBUNIT-SPECIFIC PROPERTIES OF HUMAN RECOMBINANT NMDA RECEPTORS. C. Deal, S.D. Hess*, M. Washburn, G. Veliçelebi and E.C. Johnson. SIBIA Neurosciences, Inc., La Jolla, CA 92037.

Recombinant receptors formed from combinations of cDNAs encoding human NMDAR1A and NMDAR2A-D expressed in *Xenopus* oocytes or HEK293 cells were studied using voltage-clamp techniques. In HEK293 cells transiently expressing hNMDAR1A/2D receptors, there was an approximate 10 mV shift in the reversal potential upon changing the bath from Ringer containing 135 mM Na^+ to 110 mM Ca^{2+}, suggesting an appreciable fractional calcium conductance for this receptor. For hNMDAR1A/2C receptors, we determined EC_{50} values of 22.6 μM for NMDA and 0.67 μM for glycine, and an IC_{50} of 1.8 μM for CGS 19755. For agonists, NMDA and glycine were most potent on hNMDAR1A/2D receptors, and can discriminate this combination from hNMDAR1A/2A, 1A/2B, or 1A/2C receptors. The glycine-site competitive antagonist 5,7-DCKA was more potent against hNMDAR1A/2A than 1A/2B or 1A/2D receptors, but CGS 19755 did not distinguish between the pairwise combinations. Ifenprodil displayed a high affinity for hNMDAR1A/2B receptors (IC_{50} of 114 nM) and low affinity for hNMDAR1A/2A, hNMDAR1A/2C and hNMDAR1A/2D receptors (39.5, 55.4 and 75.9 μM, respectively). We compared the ifenprodil sensitivities of NMDA receptors formed by injecting into oocytes transcripts encoding NMDAR1A and different ratios of 2A and 2B subunits. At a 1:1 2A:2B ratio, the resulting receptors were pharmacologically indistinguishable from hNMDAR1A/2B receptors in terms of sensitivity to NMDA, glycine, D-serine, and CGS 19755. The receptors expressed from a 10:1 2A:2B transcript ratio displayed an ifenprodil sensitivity that would be predicted for a population in which 51% was represented by $hNMDAR(1A)_2(2A)_3$ complexes. Our results underscore the need for subtype-selective compounds acting at novel sites in order to sufficiently probe the pharmacological differences between NMDA receptor subtypes formed by different subunit combinations.

236.11

FUNCTIONAL CHARACTERIZATION OF TWO ISOFORMS OF THE HUMAN GluR6 RECEPTOR AND DISTRIBUTION OF GluR6 RNA EDITING SITES. L.P. Daggett*, C. Jachec, F.-F. Lin, C. Deal, M.A. Varney, S.D. Hess, G. Veliçelebi, and E.C. Johnson. SIBIA Neurosciences Inc., La Jolla, CA 92037

Overlapping cDNA fragments isolated from a human fetal brain cDNA library were used to construct cDNAs encoding human GluR6a-IYQ and an alternatively-spliced carboxyl-terminal isoform, hGluR6b-IYQ. The deduced amino acid sequences of the hGluR6a and hGluR6b receptors contain 908 and 870 residues, respectively. PCR techniques were used to obtain four additional hGluR6a cDNAs that contained various combinations of RNA edits encoding I/V, Y/C or Q/R. The distribution and extent of editing at the three GluR6 RNA editing sites were analyzed by PCR techniques with cDNAs isolated from 12 different human brain regions. The expected hGluR6 PCR product (387 bp), which spanned the three editing sites, was identified in each of the 12 brain regions investigated. Restriction enzyme and DNA sequence analyses of the PCR fragments were performed and the composition of the editing events was determined for each region. In addition, $[Ca^{2+}]_i$ measurements were performed in HEK293 cells transiently transfected with cDNAs encoding hGluR6a-IYQ, 6a-VCQ, 6a-IYR, 6a-ICR, and 6a-VCR and hGluR6b-IYQ. Kainate-induced $[Ca^{2+}]_i$ responses (100-300 nM above basal) were observed in hGluR6a-IYQ-, hGluR6a-VCQ- and hGluR6b-IYQ-expressing cells. $[Ca^{2+}]_i$ responses to kainate were not observed in cells expressing hGluR6a containing an arginine (R) at the TM2 editing site. A 125-kDa protein was detected in membranes isolated from hGluR6a-IYQ and hGluR6a-VCQ-transfected cells and probed with anti-GluR6/7 antibody in Western blots. The immunoreactive protein was reduced to ~103 and ~120 kDa following digestion with N-glycosidase F and endoglycosidase H, respectively. In response to application of 100 μM kainate, in the presence of ConA, inward currents with a mean amplitude of 6.3 ± 3.1 nA were recorded from hGluR6a-IYQ-transfected cells held at -60 mV.

236.12

STABLE EXPRESSION AND CHARACTERIZATION OF THE RECOMBINANT HUMAN AMPA RECEPTOR SUBTYPE GLUR3-FLIP IN MAMMALIAN CELLS. S. Rao, M.A. Varney*, F.F. Lin, C. Jachec, R. Skvoretz, L.P. Daggett, C. Deal, S. Hess, G. Veliçelebi, E.C. Johnson. SIBIA Neurosciences, Inc., La Jolla, CA 92037.

A stable cell line expressing the human AMPA receptor GluR3-flip (hGluR3i) was established in HEK293 cells. Functional expression was demonstrated by glutamate-induced $[Ca^{2+}]_i$ signals in fluo-3-loaded cells, and glutamate-evoked currents in whole-cell patch-clamp recordings. The presence of hGluR3i protein was confirmed by immunoblot analysis.

The pharmacology of recombinant hGluR3i was investigated by measuring $[Ca^{2+}]_i$ signals and [^{3}H]-AMPA binding. The rank order of potency for agonists in the $[Ca^{2+}]_i$ assay, in the presence of cyclothiazide, was quisqualate > AMPA = glutamate > kainate. Kainate was not a fully efficacious agonist, as compared to glutamate. These potency results differed from those obtained with [^{3}H]-AMPA displacement curves, where the rank order of potency was kainate > AMPA > quisqualate > glutamate.

Total [^{3}H]-AMPA binding was reduced by cyclothiazide, and in some cases the Hill coefficients for the displacement curves were lower than unity. However, the rank order of potency for displacement of bound [^{3}H]-AMPA was the same as that obtained in the absence of cyclothiazide. GYKI-52466, a non-competitive AMPA-receptor antagonist, inhibited AMPA-stimulated $[Ca^{2+}]_i$ signals in the presence of cyclothiazide. However, in binding studies GYKI-52466 did not displace [^{3}H]-AMPA binding, or reverse the cyclothiazide-mediated reduction in [^{3}H]-AMPA binding. These results suggest that cyclothiazide and GYKI-52466 act at different binding sites on hGluR3i.

236.13

POLYAMINES PERMEATE & BLOCK GLUTAMATE RECEPTOR CHANNELS. R. Bähring*, D. Bowie and M.L. Mayer. LCMN, NICHD, National Institutes of Health, Bethesda, MD 20892.

Intracellular polyamines cause inward rectification of both voltage-activated K^+ and glutamate receptor channels. Voltage ramp experiments in the outside-out patch configuration on homomeric GluR6(Q) channels expressed in HEK 293 cells showed voltage-dependent block by 30 μM internal spermine ($G_{+50/-100} = 0.03$). Shifting the reversal potential, by lowering $[Na^+]_o$, was accompanied by a hyperpolarizing shift in the voltage dependence of block, indicating interactions within the pore between permeant ions and polyamines. Relief of block with extreme depolarization ($G_{+100/-100} = 0.51$) suggests spermine itself is permeant. The structurally related but larger toxin, PhTX 343, produces a similar voltage-dependent block but with no relief at positive potentials ($G_{+50/-100}$ and $G_{+100/-100}) = 0.01$). We suggest that relief with depolarization of block by spermine is due to permeation and that PhTX 343 cannot permeate because of its larger size.

Given the ability of polyamines to cross the channel we tested the action of externally applied spermine. Using voltage ramps (-100 to +150 mV) we observed strongly voltage dependent block with an unusually high affinity ($K_{App}0mV = 100$ nM) which was not confirmed in steady state experiments with different spermine concentrations ($IC_{50} = 65$ μM at both +40 and -40 mV). However, with 30 μM external spermine inducing inward currents by transiently hyperpolarizing the membrane potential resulted in a fast (τ~1 ms) onset block at +40 mV which showed slow recovery to the steady state level ($G_{trans} = 0.05$, $G_{SS} = 0.6$). Both the rate of onset and the amount of block were dependent on the membrane potential, the duration of the prepulse, and external spermine concentration. This suggests that with inward current flow spermine permeates the channel from the outside to cause block from an internal high affinity binding site when the membrane potential is reversed. In addition there is an external site of polyamine action which shows lower affinity and much weaker voltage dependence. SUPPORTED BY THE NIH.

236.14

DUAL BLOCKADE OF GLUTAMATE RECEPTORS BY EXTERNAL DIVALENT CATIONS AND INTERNAL POLYAMINES. Derek Bowie* and Mark L. Mayer, Laboratory of Cellular & Molecular Neurophysiology, NICHD, National Institutes of Health, Bethesda, MD 20892.

We have previously reported that the complex rectification of kainate and AMPA receptors expressed from cDNAs for GluR6(Q) and GluR-A_{flip} results from ion-channel block mediated by the endogenous cytoplasmic polyamines, spermine and spermidine (*Neuron*, **15**, 453-462, 1995). In the absence of internal polyamines the current-voltage relationship for both AMPA and kainate receptors exhibited weak outward rectification. In each case, voltage-dependent block by external divalent cations contributed to this rectification. With symmetrical sodium (120mM) solutions block at -60 mV by Ca^{2+} (30 μM - 30 mM) for GluR6(Q), $IC_{50} = 0.72 \pm 0.1$ mM (n=10) was stronger than block by Mg^{2+}, $IC_{50} = 1.34 \pm 0.1$ mM (n=7). For both ions block was incomplete even at high concentrations; extrapolated G_{min} values for Ca^{2+} and Mg^{2+} were 4.0 ± 0.74 % (n=10) and 9.97 ± 1.49 % (n=7) of the maximum response, respectively. AMPA receptors generated from GluR-A_{flip} exhibited weaker block and a lower affinity for Ca^{2+} ($IC_{50} = 2.56 \pm 0.38$ mM at -60 mV; $G_{min} = 26.43 \pm 2.59$, n=5). For GluR6(Q) inclusion of 30 μM spermine in the internal solution generated strong inward rectification but did not measurably alter block by external Ca^{2+} (IC_{50} at -60 mV = 0.78 ± 0.1 mM; $G_{min} = 4.2 \pm 1.7$ %, n=4). In addition, the extent of block by 30 μM internal spermine was similar in divalent-free solution and in the presence of 1mM external Ca^{2+} suggesting that block by divalent cations and by polyamines occurs through independent mechanisms. Our data suggest that physiological concentrations of intracellular polyamines and extracellular divalent cations will jointly control the amplitude of synaptic responses as well as the complex rectification properties of unedited versions of glutamate receptors. Supported by the NIH.

236.15

MODULATION OF GLUR1, GLUR2 AND GLUR3 EXPRESSED IN OOCYTES BY HYDROGEN ION AND CHEMICAL MODIFICATION. B. Yu*, C. Brechtel, S. C. King, J. Sims, S. Alagarsamy and B. N. Christensen. Dept. Physiology and Biophysics and Dept. Pharmacology, Univ. Tx. Med. Branch, Galveston, TX 77555.

Protons titrate non-NMDA receptor activity with a pK of 6.5, a value similar to that of free histidine. We have investigated the role of hydrogen ion and chemical agents that modify histidine residues on GLUR1-3 expressed in *Xenopus* oocytes in an attempt to identify the pH sensor. GLUR1-3 cRNA was injected into oocytes and responses to kainic acid recorded 1 week later. Both homomeric as well as combinations of GLUR1-3 were expressed. Membrane current was recorded using the two-electrode voltage clamp. Decreasing pH reduced the membrane current for both homomeric and heteromeric receptors. This is similar to the effect of pH on native mammalian and teleost non-NMDA receptors. Increasing pH increased the membrane current relative to the current measured at pH 7.5. This effect is not seen either on native mammalian or teleost non-NMDA receptors. The pK obtained from a titration curve ranged from 6.1 to 6.4 and the Hill coefficient obtained by using the Hill equation to fit the data was near 1.2. Similar to the effect of increasing pH, diethylpyrocarbonate (DEPC) a histidine modifying agent also increased the current in a concentration-dependent manner. This is similar to the reported action of DEPC on the native mammalian NMDA receptor but opposite the effect seen on native non-NMDA receptors in teleost retina. DEPC also potentiated the KA-induced membrane current in cultured cortical neurons. The pH dependence of the expressed receptors was altered following DEPC modification. At increased pH, the current was reduced compared to pH 7.5. In addition, the pK was shifted to the left near 5.8. These results indicate multiple pH sensors on GLURs and that DEPC can modify at least one. Supported by grant NEI-01897 from the NIH. cDNA for GLUR1-3 was a kind gift from the Salk Institute, La Jolla, CA.

236.16

MOLECULAR DETERMINANTS OF LIGAND SPECIFICITY AT FUNCTIONAL AMPA-TYPE GLUTAMATE RECEPTORS. J. S. Sessoms*, M. Sturgess, and T. A. Verdoorn. Dept. of Pharmacology, Vanderbilt University, Nashville, TN 37232-6600.

To determine the role of S1 and S2 domains in ligand-specific responses at functional AMPA-type glutamate receptors, site-directed mutagenesis and molecular modelling were used to examine amino acid residues predicted to line the binding pocket. Mutant receptors were examined functionally by two-electrode voltage-clamp in Xenopus oocytes and fast-application patch-clamp in HEK293 cells. Lysine 755, a positively charged S2 residue conserved among members of the iGluRs, KBPs, and LAOBP, has been mutated to a glutamate. The resulting mutant receptor altered the apparent affinity of glutamate as detected by dose-response experiments in Xenopus oocytes. The dose-response curve for mutant homomers did not plateau even at 1mM glutamate measured in the presence of 30μM cyclothiazide. The EC50 for glutamate was higher in oocytes coinjected with $A_{flip}755E/wtB_{flip}$ from wild-type AB{A755E: 1.38mM (95% CI .0047-396mM), ABwt: 9.12μM (4.17-19.5μM)}. In addition to a change in the apparent affinity of glutamate, the shape of macroscopic currents in oocytes injected with K755E dna were different from wild-type. K755E is currently being examined in HEK293 cells. Ongoing experiments will examine the role of charge at the K755 residue by measuring the effects of both glutamine and arginine substitutions at this residue. These studies are anticipated to reveal residues of the AMPA binding pocket which are critical for functional ligand recognition and specificity. Source of Funding: NS30945

236.17

MUTATIONAL ANALYSIS OF A SITE REGULATING MODULATION OF AMPA RECEPTOR DEACTIVATION AND DESENSITIZATION. M.W. Fleck, K.M. Partin* and M.L.Mayer. Lab Cellular & Molecular Neurophysiology, NICHD, NIH, Bethesda, MD 20892.

AMPA receptor subunits express an alternatively spliced exon, known as the flip/flop domain, located within the extracellular M2-M3 loop. Amino acid mutations within this 38-residue domain affect desensitization and allosteric modulation by cognition-enhancing drugs such as aniracetam and cyclothiazide. We had previously shown that a single residue, SER_{750} in A*flip* or ASN_{750} in A*flop*, underlies differential modulation of desensitization by cyclothiazide.

To further examine the role of residue 750 in regulating deactivation, desensitization and allosteric modulation, we constructed and tested a series of A*flip* point mutants converting SER_{750} to ASN, GLN, ALA, ASP, GLU, VAL, LEU, MET, CYS, THR and TYR. The mutated receptors were transiently expressed in transfected 293 cells, then analyzed in whole-cell and outside-out-patch configurations. Different phenotypes of these mutations included nonfunctional receptors, receptors with altered control responses, and receptors with altered kinetics of modulation. In whole-cell experiments, modulation of desensitization by 100 μM cyclothiazide followed a rank order of SER > ALA > ASN ≥ GLY > GLN ≥ MET, whereas modulation by 5 mM aniracetam followed a rank order of ASN > SER > ALA ≥ GLY ≥ GLN ≥ MET. To examine modulation of deactivation kinetics, 1 ms pulses of glutamate were applied to outside-out patches. Cyclothiazide slowed deactivation of A*flip* ($\tau_{control}$ 0.86 ± 0.08 ms; τ_{cyclo} 2.34 ± 0.19 ms) but did not affect deactivation of A*flop* ($\tau_{control}$ 0.82 ± 0.05 ms; τ_{cyclo} 0.76 ± 0.07 ms). In contrast, aniracetam slowed deactivation of A*flop* ($\tau_{control}$ 0.84 ± 0.08 ms; τ_{anir} 3.42 ± 0.32 ms) to a greater extent than A*flip* ($\tau_{control}$ 0.86 ± 0.08; ms τ_{anir} 1.75± 0.13 ms). These experiments characterize molecular determinants at position 750 that regulate control kinetics, modulation of desensitization, and modulation of deactivation. SUPPORTED BY THE NIH.

236.18

THE SENSITIVITY TO JORO SPIDER TOXIN OF RECOMBINANT AMPA RECEPTORS. Olimpia Meucci*, Dongjun Ren and Richard J. Miller Dept. of Pharm.Phys..Sci., Univ. of Chicago, Chicago 60637 IL.

We showed that AMPA receptors in cells of the O-2A lineage may contain variable amounts of the edited GluRB subunit (GluRB(R)) and that the subunit composition of the receptor is developmentally regulated. The inhibition of kainate-induced Ca^{2+} entry and Na^+ currents by joro spider toxin (JSTx) was found to be lower when the cells expressed high levels of GluRB(R). To further investigate the role of this subunit on the properties of AMPA receptor we have studied the toxin sensitivity and Ca^{2+} permeability of recombinant receptors in HEK 293 cells. The cells were transiently transfected with different ratios of cDNAs for GluRA and GluRB(R). Fura 2-based video imaging and simultaneous recording of Ca^{2+} entry and Na^+ currents were used to measure kainate-induced responses in the presence of cyclothiazide. The expression of the different subunits was assessed by Western blot analysis and immuno cytochemistry. Imaging studies showed that 85% of recombinat homomeric GluRA receptors responsive to kainate were inhibited 42±3% by JSTx. Cotransfection of GluRA+ GluRB(R) subunits in a 10:1 ratio did not change the toxin sensitivity significantly. However, reducing the ratio GluRA/GluRB(R) to 4:1 or to 1:1 caused a reduction in the number of responsive cells which were inhibited by JSTx (55-60%). Only 40% of cells were inhibited by the toxin when the ratio of transfection was 1:4. We also noted a difference in the magnitude of the $[Ca^{2+}]i$ rise evoked by kainate in the different conditions, the highest response being noted in cells cotransfected with the two subunits at a ratio 4:1 (465±93 nM) and the lowest one at a ratio 1:4 (234±39 nM). Homomeric GluRA receptors showed a fast Ca^{2+} response (280±56 nM). Homomeric GluRB(R) receptors were generally impermeable to Ca^{2+} and only 1 out of 30 cells showed a low Ca^{2+} response (205 nM). These results show that the toxin sensitivity and the Ca^{2+} influx of heteromeric recombinant AMPA receptors depends on the relative amount of GluRB(R). (DA-02121, MH40165, NS33502 and DA-02575 NIH grants; OM is supported by Ministero della Sanita', borsa AIDS 1995-96).

236.19

FUNCTIONAL EXPRESSION OF GluR1 AND GluR2 AMPA RECEPTOR SUBUNITS IN PC12 CELLS USING ADENOVIRAL VECTORS. K. Tsuzuki[1], M. Sudo[1], H. Okado[2], A. Miwa[2] and S. Ozawa[1]*. [1]Dept. of Physiol., Sch. of Med., Gunma Univ., Maebashi 371, [2]Dept. of Neurobiol., Tokyo Metropolitan Inst. Neurosci., Fuchu 183, Japan

Adenoviral vectors are promising means for in vivo transfer of therapeutic genes into the central nervous system. To examine the possibility of expression of functional receptor channels by adenoviral vectors, we have constructed recombinant adenoviruses encoding rat AMPA receptor subunit genes, GluR1 (AxCAR1) and GluR2 (AxCAR2X) with modified chicken β-actin promoter and cytomegarovirus immediate-early enhancer. PC12 cells, intrinsically lacking the expression of AMPA receptor channels, were infected with these adenoviruses. Expression of transferred genes in PC12 cells was confirmed by reverse transcription followed by polymerase chain reaction (RT-PCR) and immunoblotting. AxCAR1 infected cells were intensely stained by anti-GluR1 antibodies (Chemicon). Expression of AMPA receptor channels was analyzed electrophysiologically using the whole-cell patch-clamp technique. In infected cells, current responses were induced by iontophoretic application of kainate. These current responses were completely blocked by 2.5 μM 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX). Inwardly rectifying current-voltage relations of responses to kainate in these cells indicated the assembly of functional homomeric channels. On the other hand, in AxCAR1 / AxCAR2X doubly infected cells, formation of heteromeric GluR1 / R2 channels was indicated by the outwardly rectifying current-voltage relations. This work was supported by the Human Frontier Science Program and the Ministry of Education, Science, Sports and Culture of Japan.

237.1

χ-1 and χ-2, MEMBERS OF A NOVEL CLASS OF THE GLUTAMATE RECEPTOR SUPERFAMILY. K.A. Sevarino*, A.M. Ciabarra, and M.S. Forcina. Division of Molecular Psychiatry, Departments of Pharmacology and Psychiatry, Yale University School of Medicine, 34 Park Street, New Haven, CT 06508.

Ionotropic glutamate receptors mediate the majority of rapid excitatory neurotransmission in the CNS. We previously identified a novel member of the rat glutamate receptor superfamily, χ-1, that has 27% homology to NMDA receptor subunits (Ciabarra et al., J. Neurosci. *15:* 6498). Co-expression of χ-1 with other NMDA receptor subunits strongly inhibits agonist-induced currents. Ligand binding studies were performed in HEK-293 cells transiently transfected with NMDA R1 and 2A in combination with χ-1. Binding of [^{3}H]-CGP39653, a glutamate site antagonist, was upregulated nearly two fold, and binding of the glycine site antagonist [^{3}H]-DCKA was increased several fold, by co-expression of χ-1 with NMDA R1/2A hetero-oligomers. These data support a post-translational mechanism for χ-1 attenuation of NMDA receptor currents, but attempts to demonstrate co-assembly of χ-1 with NMDA subunits using C-terminally directed antisera have been unsuccessful. We have now isolated and cloned to near completion χ-2, a second member of the χ class with structural similarity to χ-1. Northern blot studies reveal that χ-2 mRNA is enriched in the dorsal and ventral striatum, in contrast to χ-1. Isolation of the full length clone, and *in situ* studies, are in progress. *These studies were supported by the Scottish Rite Schizophrenia Research Program, the Theodore and Vada Stanley Research Foundation, and the Abraham Ribicoff Research Facilities, Connecticut Mental Health Center. Special thanks to the DNA Sequencing Facility, Pfizer Central Research, Groton, CT for nucleic acid sequencing.*

237.2

DIFFERENTIAL SURFACE EXPRESSION AND REGULATION OF NR1 AND NR2B SUBUNITS IN CULTURED HIPPOCAMPAL NEURONS

R.A. Hall* and T.R. Soderling, Vollum Institute, Oregon Health Sciences University, Portland, OR 97201.

The surface expression of the NR1 and NR2B subunits of the NMDA-type glutamate receptor was studied in cultured hippocampal neurons by exposing intact cultures to extracellular treatments (either the protease chymotrypsin or the membrane-impermeant cross-linking reagent BS^3) which modify surface-exposed proteins and shift their mobility on SDS-PAGE gels. It was found that less than half of the total NR1 subunits are located in the plasma membrane, whereas almost all of the NR2B subunits in the hippocampal neurons are located at the cell surface. These data suggest that NR2 subunits are critical for the targeting of NMDA receptors to the neuronal plasma membrane, an idea which finds support in recent studies on the surface expression of NMDA receptor subunits expressed in fibroblasts (McIlhinney et al., *Neuroscience* 70:989, 1996). We are currently studying the effects of different types of neuronal stimulation on both the surface expression and the total level of protein for the various NMDA receptor subunits (supported by NIH grant NS27037).

237.3

MOST CORTICAL NMDA RECEPTORS CONTAIN THREE SUBUNITS (NR1/NR2A/NR2B). J.H. Luo, Y.H. Wang, R.P. Yasuda and B.B. Wolfe*. Department of Pharmacology, Georgetown University School of Medicine, Washington, DC 20007

A monoclonal antibody recognizing the NR1 subunit of the NMDA receptor was developed using a fusion protein corresponding to the N-terminal amino acids 341-561 (a common region for all NR1 splice variants). Using membranes from NR1 transfected cells and from rat brain tissue, this monoclonal antibody recognized a fuzzy band at 115 kDa in immunoblots. No crossreactivity with any NR2 subunit was seen. The NR1 subunit was selectively immunoprecipitated under conditions where the subunits have been solubilized under denaturing conditions. On the other hand, if the receptors are initially solubilized using non-denaturing conditions this antibody immunoprecipitated other NMDA receptor subunits indicating an interaction *in vivo*. Using the monoclonal antibody and polyclonal antibodies against the NR2A and NR2B subunits, immunoprecipitation and immunoblot experiments using rat cortical membranes were performed to determine the subunit composition of cortical NMDA receptors. We found: 1) The dominant complex comprising the NMDA receptor in rat cortex contains three subunits, NR1/NR2A/NR2B; 2) A small fraction of NMDA receptors is composed of only two subunits, NR1/NR2A or NR1/NR2B; 3) No complexes exist that are composed of NR2A/NR2B without NR1; and 4) A small fraction of each subunit exists as 'free' and is not associated with any of the other subunits measured. These results suggest that functional studies with recombinant receptors composed of at least three subunits may be the most physiologically meaningful. (Supported by NS28130, AG09973, and AHAF)

237.4

REGIONAL AND DEVELOPMENTAL EXPRESSION OF NMDA SUBUNIT NR2D PROTEIN IN RAT BRAIN USING A SUBUNIT-SPECIFIC ANTIBODY.

A. W. Dunah[1,2*], R. P. Yasuda[1,2] J. Luo[1], Y.-H. Wang[1], M. I. Davila-Garcia[1], M. Gbadegesin[2], S. Vicini[2,3], and B. B. Wolfe[1,2]. Department of Pharmacology[1], Interdisciplinary Program in Neuroscience[2], and Department of Physiology and Biophysics[3], Georgetown University School of Medicine, Washington, DC 20007.

A subunit-specific polyclonal antibody for the NMDA receptor subunit NR2D has been developed using peptides from the carboxyl terminus of the rat NR2D protein. The affinity purified antibody recognizes the free peptides on ELISA and a dominant ~160 kDa band on immunoblots of membrane fractions from transfected cells as well as from CNS tissues. This antibody shows no cross-reactivity with other NMDA receptor subunits. Enzymatic deglycosylation with PNGase F shows that the NR2D receptor subunit in both brain and transfected cells is N-glycosylated. Using this antibody, the relative densities of the NR2D subunit in nine areas of postnatal day seven (P7) and adult rat brains have been determined by quantitative immunoblotting. The NR2D subunit was found to be expressed at highest levels in the thalamus, midbrain, medulla and spinal cord, while intermediate levels of this receptor were seen in the cortex and hippocampus. More complete ontogenic profiles of the NR2D subunit have been determined in the telencephalon, diencephalon, and spinal cord of rats. The data showed that high levels of NR2D protein were present at embryonic stages of development, which rose to a peak at P7 and decreased but remained quantifiable during late postnatal life. These results suggest that native NMDA receptors containing the NR2D subunit may play functional roles not only in the developing brain but also in adult brain. (Supported by NS28130, AG09973 and the AHAF).

237.5

INCREASED CONTRIBUTION TO SYNAPTIC NMDA RECEPTORS OF NR2A SUBUNIT IN DEVELOPING VISUAL CORTICAL NEURONS.

G.Stocca* and S. Vicini Dept. of Physiology, Georgetown Univ. Med. Cntr. Washington DC 20007.

In situ hybridization studies showed the selective increase of mRNA for the NMDA receptor subunit 2A (NR2A) as compared to NR2B during postnatal development in rat neocortex. We investigate whether synaptic NMDA receptor underlying spontaneous excitatory synaptic currents (NMDA-sEPSCs) were sensitive to inhibition by haloperidol and ifenprodil, selective antagonists of recombinant receptors containing NR1 and NR2B subunits. Visual cortical neurons in brain slices of rats at different age (P7-9 and P13-16) were used. NMDA-sEPSCs from voltage-clamped (-60 mV) stellate and pyramidal neurons in layer IV/V were studied in Mg^{2+} free solution containing NBQX (5μM) with Kgluconate or $CsMeSO_4$ filled pipettes. 4-aminopyridine (2 mM) was added to bath perfusion to increase the low frequency of occurrence of NMDA-sEPSCs and in most cell induced paroxysmal bursts of synaptic events. Haloperidol and ifenprodil were surprisingly poorly effective in blocking both NMDA-sEPSCs and paroxysmal bursts. In the younger group only 20% of neurons investigated (n=11) showed a clear reduction of the averaged NMDA-sEPSCs with haloperidol (100 μM) while cells in the older group (n=27) were dramatically less affected. Fast applications of L-glutamate (1 mM, 4 msec) activated rapid inward currents in nucleated patches excised from small layer IV neurons. Haloperidol (50 μM) reversibly inhibited the inward current in all four patches from a P16 rat by more than 50%. These data indicate that an increasing proportion of synaptic NMDA receptors may comprise NR1/NR2A/NR2B subunit heteromers, insensitive to "NR2B selective blockers" with distinct pharmacological properties as compared to NMDA receptors in excised nucleated patches. Supported by NINDS grants NS28130 and NS01680.

237.6

PROPERTIES OF NMDA RESPONSES IN HEK293 CELLS EXPRESSING HETEROMERS OF NR1/NR2A/NR2B SUBUNITS.

JH.Li, JF.Wang, JH.Luo YH.Wang, BB.Wolfe and S.Vicini* Depts. of Physiology & Pharmacology, Georgetown Univ. Med. Cntr. Washington D.C. 20007

Individual plasmid cDNAs encoding the NR1a, NR2A and NR2B NMDA receptor subunits were transiently transfected in mammalian HEK293 cells using the Ca phosphate precipitation technique. Successful transfection was evaluated with the use of specific immunopurified antibodies raised in mice and rabbits against NMDA receptor subunits NR1, NR2A and NR2B. Confocal microscope analysis of immunostained transfected cells revealed that most cells expressing NR1 subunit also expressed NR2A and NR2B subunits. A small but consistent population of cells expressed individual NR1/NR2A or NR1/NR2B heteromers. The subcellular staining pattern for the NR2A subunit in confocal optical sections well matched that for the NR2B subunit. Rapid application of L-glutamate (1 mM, 4 msec) by a piezoelectric translator, activated whole-cell currents in small lifted transfected cells expressing recombinant NMDA receptor heteromers. Our previous results indicated that currents produced by rapid pulse application on NR1/NR2A transfected cells had faster deactivation kinetics compared to those containing NR1/NR2B subunits and were insensitive to haloperidol blockade. We now report that currents recorded from cells transfected with NR1/NR2A/NR2B cDNAs were characterized by a slow deactivation (range 170-300 ms) and they were insensitive to haloperidol (50 μM). However, a small population of NR1/NR2A/NR2B transfected cells had deactivation kinetic and haloperidol sensitivity characteristics of individual NR1/NR2A or NR1/NR2B heteromers, consistent with what was observed in immunocytochemical staining. Our results indicate that the formation of NR1/NR2A/NR2B heteromers produce receptors with kinetic dominated by the NR2B subunit and haloperidol sensitivity dominated by the NR2A subunit. Supported by NINDS grants NS28130 and NS01680.

237.7

Evidence for a significant pool of unassembled NR1 subunit of the NMDA receptor in the adult mouse forebrain.

Paul L. Chazot and F. Anne Stephenson* School of Pharmacy, University of London, 29/39 Brunswick Square, London. WC1N 1AX UK

The N-methyl-D-aspartate (NMDA) receptors of the mammalian brain are a pharmacological subclass of fast-acting excitatory glutamate receptors. There are five NMDA receptor genes, encoding the subunits, NR1 and NR2A-2D. The NR1 gene undergoes alternative splicing to yield eight different forms of the NR1 subunit, NR1-1 to -4a/b, which differ by the presence or absence of a 21 amino acid insert in the N-terminal region and the differential use of exons 21 and 22 which yield distinct C-termini. We have raised two anti-NR1 antibodies which distinguish between the different splice forms of the NR1 subunit; an anti-NR1 17-35 antibody which recognises all splice forms and an anti-NR1 911-920 antibody which recognises NR1-1a,1b,2a,2b.These antibodies and others have been used to characterise the native NMDA receptors of adult mouse forebrain. Thus, optimal solubilisation was achieved with 1% Triton X100 and 1M NaCl which extracted maximally 15% of the total NR1 subunits. Gel filtration of this soluble extract yielded a major anti-NR1 immunoreactive peak with Mr 710 000 and a minor peak with Mr 125 000 (40% of the total NR1 immunoreactivity). Purification of the NMDA receptors from the detergent extract by anti-NR1 911-920 immunoaffinity chromatography resulted in the isolation of the cognate subunit. No NR2A or NR2B subunit immunoreactivity was detectable. Similarly, NR2A or NR2B immunoreactivity was not detectable following immunoprecipitation from soluble forebrain extracts with anti-NR1 911-920 antibodies. This is in contrast to precipitation with anti-NR1 17-35 antibodies where NR1, NR2A and NR2B immunoreactivities were present in the immune pellets. Overall, these results demonstrate the presence in adult mouse forebrain of a significant pool of unassembled NR1 subunit comprising the NR1-1a, 1b, 2a, 2b splice forms i.e. those containing the C2 exon. *This work was funded by the BBSRC*

237.8

STOICHIOMETRY OF RECOMBINANT NMDA RECEPTOR CHANNELS. L. S. Premkumar* and A. Auerbach, Department of Biophysics, SUNY at Buffalo, Buffalo NY 14214.

Single channel currents were recorded using outside out patches from *Xenopus* oocytes injected with mouse cRNA for wild type and mutant NMDAR subunits. Wild type ζ_1 and ε_2 subunits have an asparagine (N) at position 598 and 589, respectively. Wild type receptors (ζN/εN) had one predominant conductance level of 86 pS in Ca^{++} and Mg^{++} free extracellular solution. Injection of oocytes with subunits having a glutamine (Q) mutation formed channels having specific conductance and kinetic fingerprints because they occupied a subconductance level with a high relative probability (p). Channels with mutations in both subunits (ζQ/εQ) had a main conductance level of 95 pS (p=0.52) and a subconductance level of 62 pS (p=0.48). Channels with a mutation only in ε subunit (ζN/εQ) had a main conductance level of 59 pS (p=0.67) and a subconductance level of 24 pS (p=0.33). Channels with a mutation only in the ζ subunit (ζQ/εN) had a main conductance level of 69 pS (p=0.15) and a subconductance level of 12 pS (p=0.85). When oocytes were injected with a ζQ/(εQ+εN) subunit combination, there were exactly three classes of current. Two correspond to ζQ/εQ and ζQ/εN receptors. The third class had a main conductance level of 85 pS (p=0.32) and a subconductance level of 38 pS (p=0.68). When oocytes were injected with a (ζQ+ζN)/εQ subunit combination, there were four common classes of current. Two correspond to ζQ/εQ and ζN/εQ receptors. The third class had a main conductance of 97 pS (p=0.2) and a subconductance of 69 pS (p=0.8), and the fourth had a main conductance of 59 pS (p=0.47) and a subconductance of 42 pS (p=0.53). These results suggest that there are exactly two ε_2 subunits and at least three ζ_1 subunits forming the NMDAR channel complex (*supported by NS-23513*)

237.9

LIKELY CO-ASSEMBLY OF THREE NR2 SUBUNITS IN FUNCTIONAL NMDA RECEPTOR CHANNELS. J.C. Brimecombe*, F.A. Boeckman and E. Aizenman. Dept. Neurobiol., Univ. Pittsburgh Sch. Med. Pittsburgh, PA 15261.

A recent study suggested that NMDA receptors most likely contain two NR1 subunits in a functional channel (Béhé et al., *Proc. Roy. Soc. Lond.* **262**:205; 1995). However, the number of NR2 subunits which co-assemble with NR1 has yet to be determined. We have utilized an expression system to develop a profile that can distinguish between various possible functional combinations of NR1 and NR2 subunits. Single-channel recordings were performed from outside-out patches excised from Chinese hamster ovary cells transfected with NR1 in combination with either NR2A, NR2B or both. NMDA (10 μM)-activated unitary currents were recorded under basal conditions or in the presence of either the reducing agent DTT (1 mM), the oxidizing substance DTNB (100 μM), or the ifenprodil analog CP 101,606-27 (CP; 1 μM). Channels obtained from NR1/NR2A-transfected cells (n=10) exhibited a large (1.7 ms) difference in mean open time between reduced and oxidized conditions, and were completely insensitive to CP. NR1/NR2B channels (n=7) showed a much smaller difference in open time between redox states (0.5 ms), while CP dramatically reduced both their open time and nPo. Of 12 patches obtained from cells transfected with NR1/NR2A/NR2B, 2 had channels with virtually identical properties to NR1/NR2A, while 4 were indistinguishable from NR1/NR2B. Interestingly, the remaining 6 patches could be grouped into two new additional categories. Four of these had channels with little redox sensitivity in their open time (like NR1/NR2B), but were totally insensitive to CP (like NR1/NR2A). The remaining two patches were also NR1/NR2B-like in their redox sensitivity, but CP decreased only their nPo without altering open time. These four distinct categories suggest that three NR2 subunits likely co-assemble with NR1 to form pentameric structures. Supported by NIH grant NS29365.

237.10

APPARENT CYTOPROTECTIVE ACTIONS OF NMDAR1(N616Q) MUTANT IN TRANSFECTED CELLS IS DUE TO HIGH RESIDUAL CALCIUM E. Aizenman*, K.A. Hartnett and F.A. Boeckman. Department of Neurobiology, University of Pittsburgh Sch. of Medicine, Pittsburgh, PA 15261.

Two recent studies (Cik et al., *E. J. Pharmacol.* **266**:R1, 1994; Anegawa et al., *J. Neurochem.* **64**:2004, 1995) reported that the cytotoxicity which ensues following the functional expression of NMDA receptors in transfected HEK-293 cells could be partially averted with the use of a NMDA receptor subunit which has decreased calcium permeability, NR1(N616Q). Liposome-transfected Chinese hamster ovary (CHO) cells perish following expression of NR1/NR2A and NR1/NR2B-containing NMDA receptors (Boeckman and Aizenman, *Soc. Neurosci. Abstr.* **21**:86, 1995), regardless of whether or not the NR1(N616Q) mutant is used. In contrast, the use of an NR1(N616R) mutant in CHO cells substantially increases cell survival by 40% and 70% when co-expressed with either NR2A or NR2B, respectively. It came to our attention that the well-established actions of extracellular calcium in decreasing NMDA single-channel conductance (Ascher and Nowak, J. Physiol. 399:247, 1988) were dramatically enhanced in recombinant channels containing the NR1(N616Q) mutation (Ruppersberg et al., Biochem. Pharmacol. 46:1877, 1993). We thus sought to test whether the high residual Ca^{2+} (4.4 mM) that results from the calcium phosphate precipitation transfection technique normally utilized with HEK-293 cells (Anegawa et al., *ibid*) could be sufficient to account for the observed discrepancies. High calcium conditions (4.5 mM) were observed to increase cell viability by 17 ± 7% (n=4) in NR1/NR2A-transfected CHO cells and by 31 ± 5% (n=4) in NR1(N616Q)/NR2A-expressing cells. These results suggest that the previously reported cytoprotective effects of the glutamine mutation may have been partly artifactual. Supported by NIH grant NS29365.

237.11

INTERACTION OF THE NMDA RECEPTOR WITH A NOVEL SYNAPSE ASSOCIATED PROTEIN, SAP102. L. F. Lau*, A. Mammen, M. D. Ehlers, S. Kindler, W. J. Chung, C. C. Garner and R. L. Huganir HHMI, Dept of Neuroscience, Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205 and The Univ. of Alabama at Birmingham, Birmingham, AL 35294.

Recently, *in vitro* studies have shown that members of the NMDA class of glutamate receptors interact with a synapse associated protein, SAP90 (PSD-95). However, evidence for the *in vivo* interaction of NMDA receptors with SAP family members is still lacking. In the present study, we demonstrate a specific interaction between SAP102, a novel synapse associated protein, and the NMDA receptor complex from the rat cortical synaptic plasma membranes using co-immunoprecipitation techniques. No association was observed between SAP102 and GluR1, a member of the AMPA class of glutamate receptors. To identify the domain on the NMDA receptor responsible for this interaction, we constructed hexahistidine fusion proteins from different regions of the NR1a and NR2 subunits of the NMDA receptor. Immunoblot overlay experiments showed that while the C-terminal domain of the NR2 subunit displayed strong binding, the NR1a intracellular C-terminal tail did not interact with SAP102. The site of interaction was more precisely located to the last 20 amino acids of the NR2 subunit. In summary, we demonstrate here for the first time an *in vivo* interaction between the native NMDA receptor complex and a synapse associated protein. These results suggest that SAP102 may play an important role in NMDA receptor clustering and immobilization at excitatory synapses. (This work was supported by HHMI and DDRC)

237.12

NMDA RECEPTORS IN CULTURED CEREBELLAR GRANULE CELLS: SUBUNIT EXPRESSION AND METABOLIC TURN-OVER. K.H. Huh* and R.J. Wenthold. Laboratory of Neurochemistry, NIDCD, NIH, Bethesda 20892

We used cultured cerebellar granule cells to characterize the expression of NMDA receptor subunits and to investigate the metabolic turnover rate of the receptors. Cultures were established from 7-8 day-old rat cerebella, and all analysis were done after 7-8 days *in vitro*. By Western blot using various subunit-specific and NR1 splice variant-specific antibodies (C1, C2, C2' and N-cassettes), we have identified the expression of NR2A, 2B subunits and of at least two different NR1 splice variants. NR2C subunits do not appear to be expressed at a detectable level at this developmental stage. Also, splice variants containing C1 and/or C2 cassettes are expressed more abundantly than those with the N-terminal cassette, although the reverse is true for adult cerebellum. Cells were pulse labeled with ^{35}S-methionine/cysteine and radioactive NR1 subunits were identified by fluorography after immunoprecipitation. N-deglycosidase F treatment of receptors obtained immediately after a 10 min pulse produced a deglycosylated form of NR1 subunits with a MW of 100-105 kDa, instead of 120 kDa for the glycosylated form. Complete deglycosylation is confirmed by tunicamycin treatment of cells before and during the pulse chase experiment. Based on the MW difference between glycosylated and non-glycosylated forms of subunits, it appears that, after 10 min of pulse, newly synthesized NR1 subunits are already present in the Golgi or post-Golgi compartments. Pulse labeling followed by immunoprecipitation of NR1 subunits at various chase periods indicates that $t_{1/2}$ of the NR1 subunit is about 3-5 hr, indicating a relatively rapid turnover of the NMDA receptor. (Supported by the NIDCD Intramural Program)

237.13

ADHESION MOLECULES & SYNAPTIC FUNCTION: EVIDENCE THAT ALTERATIONS IN NEURAL CELL ADHESION MOLECULE (NCAM) AFFECT THE BINDING AFFINITY OF AMPA RECEPTORS. K.B.Hoffman*, M. Kessler & G. Lynch, CNLM, Univ. of Calif., Irvine, CA 92717-3800.

The 180 and 140 kDa isoforms of NCAM are enriched in postsynaptic densities (Persohn et al., 1989) and synaptosomal membranes (Bahr et al., 1993). Interactions between NCAM isoforms and other extracellular components have been implicated in memory consolidation (Doyle et al., 1992), LTP stabilization (Lüthi et al., 1994), and LTP induction (Rønn et al., 1995). The strength of NCAM-mediated adhesion can be altered by removing its polysialic acid moieties with the enzyme neuraminidase. The present study examined whether such a treatment alters the binding characteristics of glutamate responsive receptors. Rat brain membranes were incubated for 15 min at 37°C with neuraminidase and then tested for binding of radiolabeled agonists to three glutamate receptor subtypes. [^{3}H]Glutamate binding to the NMDA receptor and [^{3}H]kainate binding were unaffected, but binding of [^{3}H]AMPA (50 nM) was increased by 20-25 % ($p<.0001$; $n=18$; paired t-test). Scatchard analysis (5 to 1500 nM) suggested that the binding sites most affected by the enzyme treatment were of the low-affinity variety. To determine if the neuraminidase treatment was directly affecting AMPA receptors, membranes were solubilized with 1% TX-100 and then exposed to the enzyme; no change in AMPA binding was observed. Treatment with neuraminidase at 0°C caused no change in AMPA binding. Western blot analysis revealed that the molecular weights of the 180 and 140 kDa isoforms of NCAM were decreased by ~10 % post-enzyme treatment (with and without membrane solubilization), while those of AMPA receptor subunits GluR1 and GluR2/3, and the NMDA receptor subunit NR-1, were unchanged. Such results suggest that modulations of NCAM-mediated adhesion directly affect the endogenous binding affinity of agonist to AMPA receptors, and hence further suggest that adhesion dynamics within the synaptic zone may play a role in transformations of synaptic functioning (supported by AFOSR 95-I-0304 and Cortex Pharm).

237.14

TYPES AND SUBUNITS OF GLUTAMATE RECEPTORS CAN DIFFER IN THEIR DISTRIBUTION IN NEURONS. M.E. Rubio*, #P.B. Manis, R.J. Wenthold NIDCD, NIH, Bethesda, MD 20892, #Johns Hopkins University, Baltimore, MD 21205.

In this study we addressed the fundamental question of whether or not all glutamate receptors that are expressed in a neuron are targeted to the same synaptic populations or if subsets are targeted to specific synaptic populations. Glutamate receptors are one of the more complex families of receptors which includes eight metabotropic receptors, and 17 subunits of ionot ropic receptors that form four receptor subtypes (AMPA, kainate, NMDA and delta). The metabotropic glutamate receptors (linked to G-proteins) may exert long-lasting actions in neurons. Among the ionotropic glutamate receptors, AMPA and kainate subunits gate fast ionic currents, and the NMDA receptors mediate slower currents subject to voltage dependent Mg^{++} block. The physiological function of the delta receptors is still unknown. It has been demonstrated that the physiological properties of the receptor are related to the subunits and subtypes expressed. As our model system, we chose the fusiform cell (FC) of the dorsal cochlear nucleus. This is a bipolar neuron with an apical and a basal dendritic tree which receive two different excitatory inputs. A direct auditory nerve input synapses on the basal dendrites, whereas parallel fibers of the granule cells synapse on the apical dendrites of FC. Studies indicate that the AMPA receptors of the ascending auditory synapses have the fastest constant of desensitization compared to other synapses of the CNS (Raman et al.,1994; Geiger et al.,1995), whereas parallel fibers may produce slower synaptic response. In order to study the distribution of these subunits in FC, we combined retrograde tracer labeling (horseradish peroxidase) with pre-embedding glutamate receptor immunocytochemistry using DAB as chromogen in P15 day old Sprague Dawley rats. Antibodies selective for the following subunits were used: (AMPA: GluR 1,2/3,2,4; kainate: GluR 6/7, KA2; NMDA: NR1, NR2A/B; metabotropic: mGluR1, mGluR2/3; delta 1/2). Our results show that while both populations of synapses contain multiple glutamate receptors on their postsynaptic membranes, there are critical differences. The results suggest that some receptor subunits are selectively targeted to the two synaptic populations. (Supported by NIDCD Intramural Program)

237.15

DISTRIBUTION AND MOLECULAR INTERACTIONS OF NON-NMDA GLUTAMATE RECEPTORS PRESENT IN DEVELOPING RAT CEREBELLUM AND CULTURED CEREBELLAR GRANULE CELLS. J. A. Ripellino* and J. R. Howe, Yale University, School of Medicine, New Haven, CT 06520-8066.

We characterized the cellular distribution and molecular interactions of ionotropic glutamate receptors present in the developing and adult cerebellum and in cerebellar granule cell cultures. Analysis of non-NMDA GluR subunits were performed using antibodies selective for GluR1, GluR2/3/4c, GluR4, GluR6/7 and KA2. At the light microscopic level, immunostaining was detected to varying degrees in each region of the cerebellar cortex at all ages studied. From P1 to P7, the distribution of immunoreactivity in the molecular and internal granule cell layers was similar with each antibody. However, at P10 to adult, a more distinct staining pattern for each subunit was observed. The GluR1 subunit was predominantly associated with the molecular layer with weak staining of the internal granule cell layer. Only the GluR2/3/4c antibody gave significant staining of Purkinje cells with intense immunoreactivity present in both the soma and dendrites. KA2 immunoreactivity was found associated with a population of cells distributed throughout the molecular layer and also with a layer of cells which reside at the level of the Purkinje cell soma, possibly consistent with basket cell staining. After 7 DIV, primary cultures of granule cells demonstrated varying degrees of immunoreactivity for all of the above GluR subunits.

Immunoprecipitation of detergent extracts prepared from postnatal days 1, 10, 18 and adult rat cerebella with anti-GluR6 resulted in the complete removal of KA2 subunit immunostaining on Western blots, indicating that all of the KA2 protein is associated with GluR6 as an heteromeric complex. In contrast, only a small proportion of GluR6 protein is depleted by immunoprecipitation with the anti-KA2 antibody. Similarly, GluR1 antibodies quantitatively immunoprecipitated GluR4 from cerebellar extracts, but all of GluR1 was not immunoprecipitated by anti-GluR4. These results were qualitatively the same at all ages studied. (Supported by NS 30996).

237.16

SUBUNIT STOICHIOMETRY AND PROPERTIES OF HETEROMERIC GLUR6/KA2 CHANNELS. K.E. Pemberton*, J.A. Ripellino and J.R. Howe, Yale University, New Haven, CT 06520-8066.

Two stable HEK 293 cell lines were identified that express different relative amounts of the kainate-type subunits GluR6(R) and KA2. In each line, virtually all of each subunit protein was immunoprecipitated with antibody directed against the other subunit, indicating that the stoichiometry of heteromeric GluR6/KA2 channels is not fixed. Average GluR6/KA2 stoichiometries of 3/2 and 4/1 were determined for the two cell lines from Western analysis (assuming the channels are pentamers).

Homomeric GluR6(R) and GluR6(Q) channels differ in their Ca^{2+} permeability, *I-V* behavior, and unitary conductance. KA2 contains glutamine at the site corresponding to the Q/R site in GluR6. Patch-clamp experiments were done to determine to what extent the properties of GluR6(R)/KA2 channels are intermediate between homomeric GluR6(R) and GluR6(Q) channels. In cells where the GluR6(R)/KA2 ratio was 3/2, the reversal potential of kainate-evoked currents shifted -16 $\pm$ 3 mV when Ca^{2+} replaced Na^+ as the only cation in the external solution. This value is closer is to that reported for GluR6(Q) channels (-24 mV; Köhler *et al.*, 1993) than to the value (-3 $\pm$ 1 mV) we obtained for GluR6(R) channels. In contrast to results for GluR6(Q) channels (Köhler *et al.*, 1993), *I-V* curves for both GluR6(R) and GluR6(R)/KA2 channels showed outward rectification at positive potentials. The unitary conductance of GluR6(R)/KA2 channels (3/2 cells) was 0.60 pS, whereas values for homomeric GluR6(R) and GluR6(Q) channels of 0.23 and 5.4 pS have been reported (Swanson *et al.*, 1996; Howe, 1996). The extent to which co-expressing KA2 with GluR6(R) confers a "Q-like" phenotype appears to differ for different channel properties. (Supported by NS 30996).

237.17

THE PHARMACOLOGY OF AMPA RECEPTOR AGONISTS AT HOMOMERIC, HETEROMERIC AND CHIMERIC GLUR1$_o$ AND GLUR3$_o$. T.G. Banke, A. Schousboe, and D.S. Pickering*. PharmaBiotec Research Center, The Royal Danish School of Pharmacy, Copenhagen, Denmark.

A series of AMPA analogues were evaluated for activity at homomeric, heteromeric and chimeric rat GluR1$_o$ and GluR3$_o$ receptors expressed in *Xenopus* oocytes, using the two-electrode voltage clamp technique.

EC_{50} (μM) $\pm$ S.E.M (Hill Coefficient $\pm$ S.E.M)

Agonist	GluR1	GluR3	GluR1+3	NG1/CG3†	NG3/CG1†
ACPA	1.1±0.3* (0.95±0.07)	0.10±0.02* (1.32±0.07)	0.6±0.2 (0.86±0.09)	0.55±0.08 (0.86±0.05)	0.44±0.07 (1.29±0.07)
TFA	1.8±0.5 (0.83±0.05)	0.6±0.2 (1.1±0.2)	0.6±0.2 (0.78±0.08)	0.45±0.07 (0.82±0.03)	1.52±0.53 (1.2±0.2)
L-Glu	6.4±1.1 (0.87±0.02)	1.3±0.3 (1.3±0.2)	7.1±0.4 (0.82±0.05)	5.1±0.7 (0.88±0.05)	8.3±1.3 (0.9±0.2)
AMPA	8.7±1.3* (0.8±0.1)	1.4±0.2* (1.36±0.07)	2.5±0.6 (0.90±0.05)	2.9±0.6 (0.70±0.08)	1.2±0.5 (1.32±0.13)
KA	27±3 (1.7±0.1)	31±3 (2.0±0.3)	29±2 (2.0±0.2)	20±3 (1.28±0.07)	54±2 (1.63±0.05)

*Significantly different by t-test, ($p<0.003$);† N- & C-terminal halves of GluR1$_o$ (G1) or GluR3$_o$ (G3).

The formation of heteromeric receptor complexes was demonstrated by cross-immunoprecipitation of both subunits from solubilized oocyte membranes. The AMPA analogue ACPA (2-amino-3-(3-carboxy-5-methyl-4-isoxazolyl) propionic acid) was the most potent and selective agonist tested at GluR1$_o$ and GluR3$_o$, the least potent being kainate. These experiments suggest that for activation, an interaction occurs between N-terminal and C-terminal domains of the receptor. Also, it seems that electronegative group substitutions on the isoxazole ring and decreasing the pK_a of the substituent at position 3 play a major role in determining the degree of receptor activation under steady-state conditions. Future studies will examine the effects of single amino acid mutations in these receptors, giving a more precise localization of the agonist binding site. (Supported by a research grant from the Alfred Benzon Foundation).

237.18

BINDING PROPERTIES OF RECOMBINANTLY EXPRESSED AMPA RECEPTOR SUBUNITS EXHIBIT AFFINITY PROFILES SIMILAR TO THOSE OF NATIVE AMPA RECEPTORS. Martin Hennegriff†*, Amy Arai†, Markus Kessler†, Peter Vanderklish†, Manpreet Singh Mutneja†, Gary Rogers¶, Rachael L. Neve**, and Gary Lynch†. † Ctr. for the Neurobiology of Learning & Memory, Univ. California, Irvine, CA 92717-3800. ¶Cortex Pharmaceuticals, Inc., Irvine CA 92718. **Dept. of Genetics, Harvard Medical School & McLean Hospital, Belmont, MA 02178.

Homomeric AMPA receptors were stably expressed in kidney cells from cDNAs encoding GluR1$_{flop}$, GluR2$_{flip}$, GluR2$_{flop}$, GluR3$_{flop}$, and GluR4$_{flop}$ subunits. The recombinant receptors were of the expected size and were functional as evidenced by whole-cell recording. Scatchard plots for [^{3}H]AMPA binding to GluR1, 3, and 4 receptors were linear, but those for GluR2 receptors showed a marked curvature, indicating the presence of two components. Upon solubilization of the GluR2 receptors, a major fraction of the lower affinity component was converted into the high affinity form. PCMBS increased [^{3}H]AMPA binding to GluR2$_{flip}$ but had only minor effects on the other receptors. These results suggest that several characteristic features of brain AMPA receptors reflect at least in part an influence of specific subunits such as GluR2.

[^{3}H]AMPA binding was inhibited in a non-competitive manner by two classes of drugs known to change the desensitization kinetics of the AMPA receptor. In agreement with physiological observations, the apparent affinity of cyclothiazide for GluR2$_{flip}$ was approximately ten times higher than for receptors made of flop subunits. In contrast, BDP-37, a rigid benzamide drug related to the benzoylpiperidine family, exhibited a three times higher potency for the flop vs. the flip isoform of the GluR2 subunit, suggesting that the action of centrally active drugs may vary across brain regions expressing receptors with different subunit compositions (supported by AFOSR 95-I-0304).

237.19

A Novel Excitatory Amino Acid Response in Purkinje Cells: Selective Activation by Aspartate and High Calcium Permeability. M. Yuzaki*, D. Forrest†, T. Curran and J. A. Connor‡. St. Jude Children's Res. Hosp., Memphis, TN38105, †Mount Sinai Sch. of Med., New York, NY 10029, ‡Lovelace Inst., Albuquerque, NM 87108.

An endogenous excitatory amino acid (EAA) neurotransmitter L-aspartate (Asp) is widely distributed in the mammalian central nervous system. In the cerebellum, Asp has been suggested to be a transmitter at climbing fiber-Purkinje cell synapses. However, the Asp responses of Purkinje cells have puzzled physiologists and pharmacologists for almost 20 years. In this study, we aimed at solving this enigma by taking advantage of mice with a disrupted NMDA receptor 1 gene (NR1), which lack functional NMDA receptors, to exclude the involvement of a classical NMDA receptor. Hippocampal neurons, cerebellar granule cells, and Purkinje cells were prepared from newborn wild type (NR1+/+) and homozygous mutant (NR1-/-) mice and maintained for 7-18 days *in vitro*. Asp induced a large inward current in Purkinje cells from NR1-/- mice (EC_{50} = 90 μM), in Mg^{2+}-free, glycine-, picrotoxin-, and TTX-containing solution. This current was sensitive to both NMDA and non-NMDA receptor antagonists. In contrast, no current was induced by Asp in hippocampal neurons and cerebellar granule cells from these same NR1-/- mice. Other known glutamate receptor agonists such as glutamate, homocysteate, AMPA and kainate did not evoke this response. NMDA acted only as a partial agonist and competitively inhibited the Asp-induced current with IC_{50} of 265 μM. In contrast to glutamate, Asp induced a large Ca^{2+} influx (P_{Ca}/P_{Cs} = 13.2) into Purkinje cells. These results suggest that in Purkinje cells, Asp activates a novel type of EAA receptor that could contribute to synaptic plasticity through its high Ca^{2+} permeability.

EXCITATORY AMINO ACID RECEPTORS: STRUCTURE, FUNCTION, AND EXPRESSION—REGULATION OF EXPRESSION

238.1

MOLECULAR DIVERSITY OF NMDA RECEPTORS: NR2B C-TERMINAL DOMAIN DELETION MICE C. Amico, R. Brusa, U. Krueth, G. Köhr*, P. H. Seeburg, R. Sprengel. Center for Molecular Biology (ZMBH), University of Heidelberg, 69120 Heidelberg, Germany.

The NMDA receptor channel is formed by the subunit NR1 expressed in different combinations of the four NR2 (2A, 2B, 2C, 2D) subunits. Considering the functional significance of the molecular diversity of NMDA receptor channels, we generated mutant mice with the NR2B c-terminal domain deletion by homologous recombination. The NR2B subunit occurs early in development (mRNA is already detectable at E13). The long c-terminal domain interacts with the postsynaptic density protein PSD95 and is the most prominently tyrosine-phosphorylated segment. Our proposal is that the long c-terminal segment plays a role anchoring the subunit from the membrane to the cytoplasm and the resulting deletion could alter both distribution and developmental pattern. To investigate the physiological role of this we transfected embrionic stem cells with a NR2B gene construct in which a translational stop codon is introduced immediately downstream from the M4 coding region. Tree positive clones were identified by PCR nested primers and Southern blots analyses of 210 G-418 resistant clones. Two independent clones were injected into host mouse blastocysties and germline chimeras were obtained at high rate.

238.2

IMPAIRED KINDLING DEVELOPMENT IN NMDA RECEPTOR NR2A C-TERMINAL MUTANT MICE Jianying Li*, Rolf Sprengel, Linda Butler, Rosella Brusa, Peter H. Seeburg, James O. McNamara. Department of Medicine, Duke University, Durham, NC27710. Center for Molecular Biology University of Heidelberg, D-69120 Heidelberg, Germany.

Kindling, an animal model of epilepsy, is a form of activity-determined plasticity of the mature nervous system. The activity consists of a focal electrical seizure or afterdischarge. Periodic elicitation of afterdischarges in an otherwise normal animal induces a lifelong seizure-prone state accompanied by diverse synaptic modifications. Activation of NMDA receptors is a key component of the activity, because NMDA receptor antagonists markedly inhibit development of kindling. The subunit composition of the NMDA receptors underlying kindling development, as well as the mechanism by which activation of these receptors contributes to kindling development are unknown. To begin to address these questions, we examined kindling development in mutant mice in which the carboxyterminal intracellular domain of NR2A subunit had been eliminated by gene targeting (Brusa et al, Neurosci Abst 20:726, 1994).

Wild type (WT) and mutant (MT) mice underwent stereotaxic implantation of a stimulating/recording electrode in the right amygdala. The rate of kindling development was measured by the number of stimulation-induced afterdischarges required to trigger three consecutive clonic motor seizures. The rate of kindling development was markedly impaired in MT (27.4±4.2 stimulations [n=8]) compared to WT (12.9±1.2 stimulations [n=10]) mice ($p<0.01$). These findings implicate the NR2A subunit as a key component of the NMDA receptors underlying kindling development. Current investigations are aimed at determining whether this mutation interferes with synaptic activation of NMDA receptors or somehow uncouples the receptor from intracellular signaling events. Supported by NS32334 from NINDS.

238.3

SUBUNIT-SPECIFIC REGULATION OF NMDA RECEPTOR EXPRESSION IN CULTURED RAT CEREBELLAR GRANULE CELLS. M. Villa, O. Weinmann, S. Haller, H. Mohler*, J.-M. Fritschy. Institute of Pharmacology, ETH and University of Zurich, 8057 Zurich, Switzerland.

The developmental expression of the NMDA receptor subunits NR1, NR2A and NR2B was monitored immunocytochemically in cerebellar granule cell cultures obtained from 5-day old rat pups. A gradual increase in NR1 and NR2A subunit staining was observed, while the NR2B subunit immunoreactivity was intense initially and decreased after 6-8 days *in vitro* (DIV) to reach very low levels by 14 DIV. These changes parallel the maturation of NMDA receptors *in vivo*. Further, we observed a frequent co-localization of synaptophysin-immunoreactive puncta with neurites positive for the NR2A subunit, but not for the NR2B subunit, suggesting that receptors containing the NR2A subunit are preferentially engaged in synaptic transmission. Incubation of 5 DIV-cultures with 100 μM NMDA for 24-48 h, a treatment known to induce a down-regulation of functional NMDA receptors, resulted in a selective decrease in NR2A subunit staining, most pronounced in neurites. In contrast, 24 h treatment with KN93, a specific inhibitor of the Ca^{2+}/calmodulin-dependent kinases II and IV, dramatically increased the NR2B-subunit staining, suggesting that the expression of these NMDA receptors is controlled by phosphorylation mechanisms involving these kinases. Our results indicate that the NR2A and NR2B subunits represent distinct populations of NMDA receptors in cerebellar granule cells, differing in developmental regulation and subcellular distribution. Support: Swiss National Science Foundation, grant 31-36327.92.

238.4

CHRONIC TREATMENT WITH NMDA RECEPTOR LIGANDS AND CHANNEL BLOCKERS MODULATES NR1 PROTEIN EXPRESSION T.M. Thiesen, O. Jeyifous and M.C. Curras* *Dept. of Neuroscience, University of California, Riverside.*

The NR1 subunit is required for the function of recombinant NMDA receptors, is found throughout the brain and is probably a component of all functional native NMDA receptors. In order to examine the plasticity of the NR1 subunit and to identify putative extracellular regulatory signals, we exposed ED 17-19 cortical cultures to general NMDA receptor/channel ligands and blockers. After 1-3 days of treatment, tissue from test and control groups was harvested, assayed for protein and subjected to SDS-PAGE and Western blotting. Immunodetection of NR1 protein was carried out with a polyclonal anti-NR1 primary antibody (Petralia et al, 1994; Chemicon), a goat anti-rabbit IgG conjugated with HRP (BioRad) and ECL. With progressive days in vitro (DIV) the NR1 immunoreactive band (100-120 kDa) increased in intensity: NR1 expression in cultures with 7 DIV > 5 DIV > 4 DIV. We used 7-day-old cultures for the present study. Chronic treatment with glutamate produced a dose-dependent (0.1 - 10 uM) decrease in NR1 expression suggesting a regulatory role of glutamate on NR1. In some experiments, however, low doses of glutamate increased NR1 expression. Prolonged treatment of cultures with D-APV (100 uM) produced a marked upregulation in NR1. Cultures treated with NMDA (15 - 60 uM) and D-APV showed less NR1 expression than those treated with D-APV alone suggesting that prolonged agonist binding may downregulate NR1. Mg^{2+} (1 mM) treatment provoked a dramatic upregulation of NR1 possibly due to prolonged channel blockade. Treatment with NMDA and Mg^{2+} resulted in lower levels of NR1 protein expression compared with Mg^{2+} alone. In the presence of Ca^{2+}(2 mM), Mg^{2+} failed to produce increased NR1 levels. These results suggest that expression of NR1 is plastic and may be regulated by extracellular levels of glutamate, divalent cations and drugs acting at NMDA receptors.

238.5

CHRONIC ADMINISTRATION OF A GLYCINE PARTIAL AGONIST (1-AMINOCYCLOPROPANECARBOXYLIC ACID, ACPC) ALTERS EXPRESSION OF NMDA RECEPTOR mRNAS. S. Bovetto[1], P. Skolnick and L.H. Fossom, Laboratory of Neuroscience, NIH/NIDDK, Bethesda, MD 20892

The glycine partial agonist ACPC is neuroprotective in animal models of focal, global and spinal ischemia. Converging lines of evidence indicate that different mechanisms are responsible for this neuroprotection following acute and chronic treatments. To investigate the possibility that chronic treatment affects NMDA receptor composition, the levels of ϵ1, ϵ2 and ϵ3 mRNAs were quantified by *in situ* hybridization histochemistry using ^{35}S-labeled riboprobes in 15 μm brain sections taken from NIH-Swiss mice 24 hr after chronic treatment (14 daily injections) with ACPC (200 mg/kg) or saline. Chronic ACPC increased ϵ1 subunit mRNA levels in the hippocampus (especially CA1 and CA2), dentate gyrus, cerebral cortex (frontal, parietal and occipital) and cerebellum. In contrast, chronic ACPC decreased ϵ3 subunit mRNA levels in the hippocampus (especially CA1), dentate gyrus, and frontal and occipital cortices, but did not alter levels in cerebellum. Chronic ACPC also decreased ϵ2 subunit mRNA in cerebral cortex (especially frontal and parietal), but did not alter levels in cerebellum, hippocampus or dentate gyrus. From studies using recombinant NMDA receptors, it may be hypothesized that an alteration in subunit composition of NMDA receptors (to a larger proportion of receptors containing ϵ1 relative to ϵ2 and ϵ3 subunits) within specific brain areas would decrease the affinity of glutamate and glycine, resulting in the observed neuroprotective actions of chronic ACPC.

[1]Post-doctoral Fellow supported by Elf-Aquitaine.

238.6

REGULATION OF THE NMDAR1 GENE PROMOTER BY SERUM DEPRIVATION STRESS IN PC12 CELLS. G. Bai*, M. Prenger and J.W. Kusiak. Mol. Neurobiol. Unit, Gerontology Research Center, NIA, NIH, Baltimore, MD 21224.

The expression of NMDAR1 gene is increased during neuronal development and may be differentially regulated in ischemia and epileptogenesis. We are interested in studying the role of trophic factors in regulating the NMDAR1 gene. We measured the activity of a 3 kb NMDAR1 promoter-luciferase reporter stably transfected into PC12 cells under conditions of serum deprivation and re-addition. We compared the serum effects with the effects of different trophic factors. The results showed that serum deprivation for 4 hr caused a down-regulation of the NMDAR1 promoter activity to about 33% of untreated cells and re-addition of serum for two hr increased the NMDAR1 promoter activity over serum-deprived cells by 13 fold. This serum-induced activity partially resides in the proximal 356 bp promoter. In the absence of serum, NGF, bFGF and EGF individually up-regulated the activity of serum-deprived cells by more than 8 fold. NGF and serum had an additive effect. NGF also had an additive effect with EGF on serum-deprived cells. But, the kinetic effects of serum re-addition and growth factors on the promoter were different: Serum re-addition caused a robust increase within 15 min while NGF induced a significant up-regulation only after one hr treatment. Pretreatment with actinomycin D prevented the serum- and NGF-induced increase in the luciferase activity. The NGF effect was sensitive to K-252a blockade. Our studies suggest that a lack of trophic support causes a down-regulation of NMDAR1 promoter and both serum and trophic factors like NGF can up-regulate the activity of this promoter but through different mechanisms. Supported by NIH and the Aluminum Association.

238.7

ANALYSIS OF THE NMDAR1 GENE PROMOTER IN TRANSGENIC MICE. T. Storck, R. Kolhekar, J. Jerecic, R. Sprengel and P. H. Seeburg*, Center for Molecular Biology, ZMBH, Univ. Heidelberg, and MPI für medizinische Forschung, 69120 Heidelberg, Germany.

The NMDAR1 receptor subunit is the essential subunit of N-methyl-D-aspartate receptors which play crucial roles in synaptic plasticity, memory formation and neurotoxicity. Targeted disruption of the NMDAR1 gene in mice results in neonatal death, precluding the analysis of its consequences in adult animals. In order to circumvent this problem we are employing the tetracycline (tet) system to produce tet-inducible NMDAR1-knockouts. Our first approach was aimed at expressing the tet-transactivator (tTA) under the control of the NMDAR1 promoter. Several lacZ reporter constructs containing different regions of the murine NMDAR1 gene were analyzed in transgenic mice. A DNA fragment encompassing 4kb of upstream sequence and the transcribed region including part of exon IV gave rise to an expression pattern resembling that of the endogenous NMDAR1 gene in three out of four transgenic animals, but the level of expression was low. Thus, an important enhancer element might be missing from this construct.

In order to recruit all cis-elements necessary for NMDAR1-specific expression, we introduced the tTA gene into the promoter region of the NMDAR1 gene via homologous recombination in murine embryonic stem cells. We are currently evaluating the regulatable expression from the engineered allele in differentiated stem cells.

Supported by Sonderforschungsbereich grant 317.

238.8

NMDAR-1 IS A POTENTIAL TARGET GENE FOR THE EGR FAMILY OF TRANSCRIPTION FACTORS Penny Jensen*[1], Noemi Millan[2], and Kenneth J. Mack[1,2] Departments of Physiology[1] and Neurology[2], University of Wisconsin-Madison, Madison, WI 53706

The EGR family of transcription factors has been implicated in synaptic plasticity including complex functions such as LTP and seizures. Yet no specific downstream target gene has been identified in orchestrating these events. The promoter region of the glutamate receptor NMDAR-1 contains the EGR cis-regulatory element (Bai and Kusiak, 1994) therefore we asked if NMDAR-1 is transcriptionally regulated by the EGR family of transcription factors. Since previous results from this lab have shown highly dissimilar levels of NGFI-A/ZIF268 following either a physiological (whisker stimulation) or pathological (seizure) stimulus in rat brain, levels of NMDAR-1 were examined following the same paradigms. RT-PCR investigation displays that in the somatosensory cortex there is relatively no change in NMDAR-1 mRNA expression following sensory stimulus, and a 50% (n=5) reduction subsequent to a PTZ (50 mg/kg) induced seizure. In contrast, RT-PCR, *in-situ*, and western analysis indicate that both mRNA and protein levels appear to increase following a seizure in the motor cortex. *In vitro* gel shift and supershift analysis shows that all EGR family members have the capability of binding to the promoter region of NMDAR-1 either alone or within nuclear homogenates. *In-vivo*, transfection analysis in PC12 cells using a luciferase reporter plasmid under control of the NMDAR-1 promoter (bps -1024 to 1) shows an increase in luciferase expression following addition of NGF. Two constructs containing sequences flanking but excluding the cis-regulatory element exhibit a significant decrease in expression 86% (n=6) and 91% (n=5) respectively for regions 5' (bp -1024 to -362) and 3' (bp -302 to 1) to the gsg-box. Within the missing region also resides an SP1 binding site, co-transfection analysis with SP1 demonstrates no increase in expression of the reporter plasmid. Co-transfection assays depict a biphasic relationship for both NGFI-A and Krox20, low levels increase expression, whereas high levels provoke a decrease. These data reveal that the EGR family may play an influential role in NMDAR-1 expression. Supported by NIHNS33913.

238.9

MOLECULAR ANALYSIS OF NMDA RECEPTOR 2 SUBUNIT EXPRESSION DURING DEVELOPMENT. M. Sasner* and A. Buonanno LDN, NICHD, NIH, Bethesda, MD 20892-4480

The refinement of synaptic inputs and neural plasticity during development are partly controlled by the activation of NMDA receptors (NR), which are composed of a NR1 subunit and at least one of the four different NR2 (A-D) subunits. The NR2 subunits are differentially expressed in various brain regions during development and the NR2 composition of the receptor influences the characteristics of the channel. The NR2B subunit is expressed in the forebrain throughout life. In the cerebellum, the NR2B subunit is expressed at high levels early in development, then replaced by the NR2C subunit during the second week of postnatal life. The down-regulation of the NR2B gene has been suggested to be activity-dependent, but activity is not sufficient to activate the NR2C gene. We are studying the molecular mechanisms that control the neural specificity of NR2 expression and the switch from the NR2B to NR2C subunit during development. We have identified the murine NR2B promoter region sufficient for neural specificity and a downstream region necessary for down-regulation in the cerebellum during development. Sequence motifs in these regions that are conserved with other neural genes include the neuron-restrictive silencer element (NRSE), the BSF1 element and two N boxes. We are currently mapping the elements that mediate the developmental down-regulation of NR2B transcription. In addition, we are developing transgenic lines that express reporter genes driven by the NR2C promoter that will be useful tools to study the molecular mechanisms that drive the NR2B to NR2C switch during cerebellar development.

Supported by the NIH intramural program.

238.10

ANALYSIS OF CHANGES IN GLUTAMATE RECEPTOR mRNAS IN EPILEPSY MODELS USING A REVERSE NORTHERN BLOT ASSAY. K. Borges*, J. Doherty, P.J. Conn and R. Dingledine. Dept. of Pharmacology, Emory Univ., Atlanta GA 30322.

The properties of glutamate receptors are largely determined by their subunit composition. To assess changes in the glutamate receptor subunit composition that might play a role in the development of epileptic seizures we developed a reverse Northern blot assay that permits measurement of the relative abundance of many specific mRNAs in a single, small (~20 mg) tissue sample. PolyA$^+$ RNA from epileptic and control tissue was isolated and reverse transcribed with random primers in the presence of α^{32}PdCTP to obtain a mixture of radiolabeled cDNA probes. After hybridizing each probe to cationic nylon filters containing dots of denatured plasmid DNA of glutamate receptor subunits (400 ng), cyclophilin (25 ng) and vector (200 ng), the signals of bound probe were quantified and normalized to cyclophilin. We compared these signal ratios from tissue of amygdala-kindled rats 24 hrs after the 10th stage 5 seizure (N=10) to ratios obtained from sham-operated rats (N=10). The signal for the NR2A subunit was significantly decreased by 29% and 21% in the kindled piriform cortex and the kindled "area tempestas" implicating a change in NMDA receptor properties. No changes were found for the NMDAR1, -2B and the GluR1,-2, -3 and -4c subunits, which contrasts to the reported downregulation of GluR2 protein found in piriform cortex by Western blotting (Prince et al., 1995. J. Neurochem. **64**, 462-465). These data suggest that 24 hr after the last kindled seizure only the NR2A mRNA is decreased.

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238.11

IDENTIFICATION OF TRANSCRIPTIONAL CONTROL ELEMENTS IN THE NEURON-SELECTIVE RAT GLUR2 PROMOTER. S.J. Myers*, J. Peters, and R. Dingledine, Dept. of Pharmacol., Emory Univ. Sch. of Med., Atlanta, GA 30322.

GluR2 is the key subunit controlling calcium permeability and rectification properties of native hetero-oligomeric AMPA receptors in neurons. A fall in GluR2 expression, relative to GluR1, R3, and R4, could lead to increased calcium entry into these cells and would have important consequences on synaptic physiology and neuronal health. For these reasons, we conducted a mutational deletion scan of the proximal GluR2 promoter (-175 to +372, relative to transcription initiation) to identify key promoter regions that regulate transcriptional expression of GluR2 in transfected cultured cortical neurons, primary glia, and C6 gliomas. Anti-luciferase staining of the neuronal cultures revealed that expression was restricted to cells with neuronal morphology. Two main regions of control were identified that together confer neuron-selective expression of the GluR2 gene: (1) Deletion of the domain harboring a silencer element (RE1 consensus) resulted in a 2- to 3-fold increase in GluR2 promoter-driven luciferase expression (GluR2pro-Luc) in non-neuronal cells. This same deletion construct had no effect in neurons. Further analysis showed that function of the GluR2 silencer was sensitive to as few as 1- or 6-bp mutations. When GluR2pro-Luc constructs were cotransfected with a repressor that recognizes RE1 (REST), luciferase expression was reduced in neurons in a silencer-dependent manner, suggesting that REST or a repressor similar to REST may regulate GluR2 expression. (2) Three additional contiguous, independent deletion constructs (~25 bps each) resulted in sequential and strong attenuation (35, 56, and 65% reduction) of GluR2pro-Luc expression in neurons. This 75-bp region was found to exhibit a high density of transcription factor consensus sequences, including four Sp1/Sp4 sites. By analysis of a combination of these deletion constructs, 35 to 56% of the GluR2 promoter activity mapped to these Sp1/Sp4 sites. Coexpression of Sp1 or Sp4 with WT GluR2pro-Luc increased luciferase expression 3- to 5-fold in neurons, glia, and C6 gliomas, consistent with the hypothesis that Sp1/Sp4 transcription factors are involved in GluR2 gene expression. In sum, we have identified a repressor and an activator domain in the GluR2 promoter that, in part, governs neuron-selective expression of the rat GluR2 gene. Supported by NS27452.

238.12

RNA EDITING OF GLUTAMATE RECEPTOR SUBUNIT 2 (GluR-B) AFTER MAXIMAL ELECTROSHOCK AND KAINIC ACID-INDUCED SEIZURES. **Ruo Q. Hu*, Jang-Ho J. Cha, and Andrew J. Cole, Epilepsy Research Laboratory, Neurology Service, Massachusetts General Hospital, Harvard Medical School, Fruit St., Boston, MA 02114.**

Introduction: RNA editing at a single nucleotide results in a Gln to Arg substitution in the second transmembrane domain of GluR-2. This critical residue determines the calcium permeability of AMPA-sensitive glutamate receptors. Disruption of the editing process in mice results in an epileptic phenotype that is lethal by day 20 (Brusa *et al., Science.* 270, 1995). Because seizures themselves may be epileptogenic, we hypothesize that certain epileptic conditions may be the result of acquired defects in the editing process. In the present study, we examined the editing status at the Q/R site of GluR-2 after seizures induced by both maximal electroshock (MES) stimulation and kainic acid (KA) administration.

Methods: Male Sprague Dawley rats were studied after single MES (85mA, 100Hz, 1s), 10 daily MES, or seizures induced by systemic KA (15 mg/kg i.p.). Rats were sacrificed 24 h after last MES and 72 h after KA treatment. Cortex, cerebellum and hippocampus were dissected for isolation of DNA and RNA. Following reverse transcription, PCR was carried out to amplify a portion of the GluR-2 coding sequence containing the Q/R editing site. Products were analyzed to determine the sensitivity *Bbv* I hydrolysis, which is abolished by the A to G conversion produced by editing.

Results: Rat genomic DNA was cleaved by *Bbv* I, while cDNA produced from cortex, cerebellum and hippocampus of control animals was resistant to hydrolysis. Neither acute MES, chronic MES, nor KA induced seizures resulted in the appearance of unedited cDNA as determined by this assay. These findings indicate that neither acute nor repeated seizures significantly affect the GluR-2 RNA editing process in these models of epilepsy. Support: Whitehall Foundation

238.13

CHARACTERIZATION OF AMPA RECEPTOR SUBUNIT (GluR-B) RNA EDITING IN RAT BRAIN NUCLEAR EXTRACTS. S.M. Rueter*, C.M. Burns and R.B. Emeson. Department of Pharmacology, Vanderbilt University, Nashville, TN 37232-6600.

Site-specific editing of pre-mRNA encoding the B-subunit of the AMPA subtype of glutamate receptor (GluR-B) alters a codon in the second hydrophobic region (TMII) of the receptor subunit, leading to alterations in both the Ca^{2+} permeability and electrophysiological properties of the resultant ion channel. The identification of an RNA duplex formed by exonic and intronic sequences surrounding the editing site (Q/R site), in conjunction with *in vitro* observations that this post-transcriptional modification occurs by creation of an inosine moiety in the substrate RNA, has indicated that this editing reaction is catalyzed by a double-stranded RNA-specific adenosine deaminase.

Unlike nuclear extracts prepared from HeLa cells, which edit GluR-B transcripts *in vitro* at both the Q/R site and intronic position +60, crude rat brain nuclear extracts did not efficiently edit GluR-B RNA substrates at the +60 site, although editing at the Q/R site was substantially higher than that observed in HeLa nuclear extracts (75% vs. 30%). To isolate and characterize the enzymatic activity responsible for catalyzing the modification of GluR-B RNA transcripts, we have fractionated rat brain nuclear extracts by anion-exchange, cation-exchange, and gel-filtration chromatography. Analyses of fractions eluting from the cation-exchange and gel-filtration columns revealed that editing at position +60 could now be observed to almost the same extent as at the Q/R site. In addition, enzymatic activity mediating the editing of the +60 adenosine residue was resolved from the activity responsible for Q/R site modification. Both peaks of activity contained a generic double-stranded RNA (dsRNA)-specific adenosine deaminase activity as assessed by thin-layer chromatographic analyses of inosine production from a radiolabeled dsRNA substrate. The apparent molecular weights of these distinct editing activities corresponded to those of two previously characterized adenosine deaminases that act on dsRNA (DRADA/dsRAD and RED1), suggesting that both of these activities may be responsible for mediating the post-transcriptional modification of GluR-B transcripts. Supported by NIH grant NS33323.

238.14

AMPA RECEPTOR EXPRESSION IN CULTURED RAT HIPPOCAMPAL NEURONS FOLLOWING SUBLETHAL OXYGEN-GLUCOSE DEPRIVATION. H.S. Ying*, J. Weishaupt[+], M. Grabb, L.M.T. Canzoniero, S.L. Sensi, H. Monyer[+], and D.W. Choi. Center for the Study of Nervous System Injury and Dept of Neurology, Washington Univ. School of Medicine, St. Louis, MO 63110. [+]Center for Molecular Biology (ZMBH), Univ. of Heidelberg, 69120 Heidelberg, Germany.

We used single cell RT-PCR to examine the effect of a sublethal preconditioning oxygen-glucose deprivation (OGD) insult on AMPA receptor gene expression in hippocampal neurons. DIV 18-22 dissociated rat hippocampal cultures were exposed to OGD for 30-45 min. 24-48 hrs later, neurons were randomly selected for RNA harvesting through a patch pipette. Reverse transcription and two rounds of PCR (40 and 35 cycles) were performed using hemi-nested degenerate oligonucleotide primers to detect the four major AMPA receptor subunits. PCR products were probed with subunit-specific oligonucleotides and quantitated using a phosphorimager. Average percent of total AMPA receptor abundance ± S.E.M. for 42 cells are given below:

CONDITION	GLUR-A	GLUR-B	GLUR-C	GLUR-D
CONTROL (n=19)	35.1±4.3	34.0±4.8	17.4±3.7	13.5±3.7
OGD (n=23)	26.0±3.5*	28.6±3.8	18.0±3.3	28.0±4.3*

* indicates difference from control using repeated-measures ANOVA, $P < 0.01$.

Thus sublethal OGD produced a relative increase in GluR-D expression at the expense of GluR-A expression. The decrease in GluR-B expression previously reported in hippocampal CA1 neurons *in vivo* exposed to severe ischemia was not observed here, although a non-significant trend towards reduction was noted. Since desensitization and deactivation time constants are negatively correlated with GluR-D abundance, decreased GluR-D gene expression -- if reflected in receptor composition -- might act as a protective mechanism to reduce AMPA receptor-mediated currents and consequent excitotoxic injury. Supported by NIH NINDS grant NS 32636 (DWC).

238.15

CALPAIN-MEDIATED REGULATION OF AMPA RECEPTORS IN ORGANOTYPIC HIPPOCAMPAL CULTURES. X. Bi*, G. Tocco, and M. Baudry. Neuroscience Program, University of Southern California, Los Angeles, CA 90089-2520.

Calpains, calcium-activated neutral proteases, have been implicated in a variety of physiological and pathological processes. We have recently demonstrated that in situ activation of calpains in frozen-thawed rat brains sections results in a partial proteolysis of GluR1 subunits of AMPA receptors. Further studies using antibodies directed towards peptide sequences in the N-terminal (N-Ab) and C-terminal (C-Ab) domains of the subunit indicated that the cleavage of GluR1 subunit by calpain-mediated proteolysis occurs in the C-terminal domain, resulting in the formation of a new species with a molecular weigh approximately 7 kD lighter than the native protein. The present study examined changes in GluR1 subunits after kainic acid (KA) treatment of organotypic hippocampal cultures. Hippocampal slices were processed for Western blots immediately (3 hr) or 3 hr (6 hr) after KA (50 μM) treatment. Immunoblots labeled with the C-Ab revealed a decrease in GluR1 immunoreactivty that appeared at 3 hr and was further enhanced by 6 hr. However, immunoblots labeled with the N-Ab indicated that KA-induced changes in GluR1 were due to alterations in the mobility rather than to a decreased amount of the protein. At 3 hr, GluR1 immunopositive bands shifted towards a sligtly lower molecular weight while a second band with a 7 kD lighter molecular weight was evident by 6 hr. These changes in GluR1 subunits were significantly reduced by the application of 100 μM calpeptin, a cell membrane permeable calpain inhibitor. These results indicate that 1) changes in GluR1 properties elicited by KA treatment are mediated by calpain activation, 2) at least two cleavage sites for calpain-mediated proteolysis exist in the C-terminal domain of GluR1 subunits (possibly at residues 885-886 and residues 833-834 or 837-838 respectively), and 3) the temporal specificity of the two cleavages indicates a diverse regulation of GluR1 by calpain-mediated proteolysis, suggesting that calpains participate in different stages of neurophysiological or neuropathological processes.

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238.16

GROWTH FACTOR-INDUCED TRANSCRIPTION OF GLUR-1 INCREASES FUNCTIONAL AMPA RECEPTOR DENSITY IN GLIAL PROGENITOR CELLS. L.J. Chew, M.W. Fleck*, P. Wright, S.E. Scherer, M.L. Mayer and V. Gallo. Lab. of Cell. and Mol. Neurophysiol., NICHD, NIH, Bethesda, MD 20892.

We have previously demonstrated that platelet-derived growth factor (PDGF) and basic FGF (bFGF) regulate the expression of glutamate receptor (GluR) subunits in cultured oligodendrocyte progenitor (O-2A) cells (Wright et al., Soc. Neurosci. Abstr. 1994, 20, 214.11). Untreated cortical O-2A cells express GluR1 mRNA at levels lower than that of the other AMPA receptor subunits. bFGF treatment caused a 2-3 fold increase in steady-state mRNA levels for GluR1, 2, 3 and 4. PDGF did not significantly modify the mRNA levels for any of the AMPA subunits, but selectively potentiated the effects of bFGF on GluR1 mRNA (5-6 fold increase over untreated). We analyzed if: i) the changes in GluR1 RNA levels triggered by PDGF+bFGF were due to modifications of GluR1 gene transcription; ii) changes in GluR1 gene expression also modified expression of functional AMPA receptors. Nuclear run-on assays demonstrated that PDGF+bFGF caused a 2.5 fold increase in the rate of GluR1 gene transcription, while having no effect on GluR2. Western analysis showed that PDGF+bFGF caused a 10-fold increase in GluR1 protein levels, but did not modify GluR2,3 and 4 protein levels. Functional receptor expression was assessed by rapid application of AMPA (500 μM) to cultured cells in the whole-cell recording configuration. AMPA receptor densities were quantified from peak current amplitudes relative to whole-cell capacitance (pA/pF) and were increased nearly 5-fold in cells treated with PDGF+bFGF, as compared to untreated cells. Further, AMPA receptor channels in cells treated with PDGF+bFGF were more sensitive to voltage-dependent block by intracellular polyamines, as expected from the robust and selective enhancement of GluR-1 expression. Our combined molecular and electrophysiological analyses indicate that GluR function can be regulated by changes in the rate of GluR subunit gene transcription. Supported by NIH.

238.17

CATALYTIC RNA-DIRECTED CLEAVAGE OF THE NMDA RECEPTOR SUBUNIT NR1 mRNA. L. Yen* and R. G. Kalb, Dept. of Neurology, Yale University, School of Medicine, New Haven, CT 06510

The NMDA subtype of glutamate receptor is believed to play important roles in the brain development, learning, memory, and neurotoxicity of many neurodegenerative diseases. The presence of NR1 subunit is required to form a functional NMDA receptor. We are working on a new strategy for regulating the expression of NR1 subunit that involves the selective destruction of its mRNA mediated by a small catalytic RNA. A small RNA can be designed (the external guide sequence or EGS) that will bind in a sequence-specific manner to NR1 mRNA and this complex becomes a substrate for the RNase P (an enzyme present in all cells). RNase P will cleave the substrate, efficiently and selectively eliminating the target mRNA from the cell.

RNase T1 mapping of a minigene of the NR1 mRNA has led us to the identification of a site highly sensitive to RNase digestion. This accessible site is within a region common to all isoforms of NR1 mRNA. Hybridization of a custom-designed EGS to this accessible site of the NR1 mRNA leads to the efficient and selective destruction of NR1 mRNA *in vitro*. Sequencing the cleaved NR1 mRNA fragments confirms that cleavage occurred at the expected location. Furthermore, control experiments indicates that the designed EGS interacts with NR1 mRNA in a sequence-specific manner and does not affect other glutamate receptor related genes *in vitro*.

We have engineered the EGS into a vector under the control of the U6 promoter. This constructed vector leads to high level expressions of EGS both in human embryonic kidney HEK 293 cells, and in rat neuronal-like PC12 cells. The expression of EGS is strictly confined to cell nucleus where RNase P is concentrated. A critical next step is to examine the efficacy of this EGS to direct cleavage of NR1 RNA in cells. We believe that this system could have important applications for the study of NMDA receptor-mediated development and neurotoxicity. (Supported by NIH NS 29837).

238.18

ALTERATION OF GLUR5 mRNA EDITING USING GENE TARGETING. Andreas Sailer, Geoffrey T. Swanson, D. K. Smetters+, Robert E. Petroski*, Charles F. Stevens+, Stephen O'Gorman#, and Stephen F. Heinemann. Molecular Neurobiology Laboratory, +Howard Hughes Medical Institute, #Gene Expression Laboratory. The Salk Institute for Biological Studies, La Jolla, CA 92037, USA.

Editing of mRNAs coding for subunits of the glutamate-gated ion channels at the Q/R site in the membrane domain 2 (MD2) has been shown to alter the channel properties in terms of Ca^{2+}-permeability, rectification, and single channel conductance. Analysis of total brain mRNA for the kainate receptor subunit GluR5 showed that about 40% of the mRNA transcripts are edited at the Q/R site in MD2. To address the physiological role of glutamate receptor mRNA editing in the brain, we have made mutant mice which express 100% edited GluR5 mRNA using ES-cell-mediated transgenesis. In the first step a targeting vector carrying a point mutation (CGG = R) at the Q/R site in MD2 and a neomycin resistance gene (flanked by loxP sites) located in the adjacent intron was transfected into ES cells. In a second step the resistance marker was excised by the transient expression of the Cre recombinase. Subsequently, recombined clones were injected into blastocysts to generate chimeric animals which upon breeding with wild type animals transmitted the mutant allele to the next generation.

This work was supported by grants from the SNF and DFG to AS, the NIH to GTS, CFS, SOG, and SFH, and the McKnight Foundation to SFH.

238.19

PHARMACOLOGICAL CHARACTERIZATION IN OOCYTES OF AMPA-RECEPTORS EXPRESSED FROM RAT AND HUMAN WHOLE BRAIN mRNA. D.A. Gurley[1], S.D.Donevan[2] and E.W. Harris[1]*, [1]Biology Dept., Astra Arcus USA, Rochester, NY 14602 and [2]Dept. Neurology, Univ. Utah, Salt Lake City, UT 84112.

Studies of cloned AMPA-receptors (AMPA-Rs) have identified toxins that selectively block Ca^+-permeable AMPA-R channels, and compounds that differentially potentiate AMPA-R splice-variants. These new pharmacological insights have been applied to AMPA-Rs expressed in injected oocytes injected with whole brain polyA^+mRNA. AMPA and glutamate yielded small currents that were potentiated >50-fold by 50μM cyclothiazide (CYZ); CYZ increased kainate currents only 2-3-fold, for adult rat and human mRNA, suggesting mostly "flop" variants, and ~6-fold for human fetal RNA, suggesting mostly "flip" variants. AMPA and Iodo-Willardiine were antagonized ~50% by 3mM SCN^-, which slightly potentiated kainate currents.

Although JSTX-sensitive (Ca^{++}-permeable) AMPA-Rs are sparse in adult brain, 1μM JSTX blocked 75% of adult brain mRNA AMPA-R current. The remaining current was blocked by 1mM pentobarbital.

A published study of human epileptic mRNA concluded that Ca^{++}-permeable AMPA-Rs had increased, since ~75% were Ca^+-permeable. Our results suggest that control mRNA may have a similar profile.

Supported by Astra Arcus USA

EXCITATORY AMINO ACID RECEPTORS: PHYSIOLOGY, PHARMACOLOGY, AND MODULATION—mGluR I

239.1

DIFFERENTIAL INVOLVEMENT OF GROUP II AND GROUP III mGLURS AS AUTORECEPTORS AT LATERAL AND MEDIAL PERFORANT PATH SYNAPSES. T.A. Macek*, D.G.Winder, R.W. Gereau IV, C.O. Ladd, and P.J. Conn Dept. of Pharmacology, Emory Univ. School of Med., Atlanta, GA 30322.

Previous reports have shown that group III metabotropic glutamate receptors (mGluRs) serve as autoreceptors at the dentate gyrus lateral perforant path (LPP) synapse. However, the possible roles of other mGluR subtypes at this synapse have not been examined. Furthermore, it is not known whether specific mGluR subtypes serve as auoreceptors at the medial perforant path (MPP) synapse. Utilizing whole cell patch clamp and field excitatory post-synaptic potential (fEPSP) recordings, we have examined the effects of group-selective mGluR agonists on transmission at lateral and medial perforant path synapses in adult rat hippocampal slices. Micromolar concentrations of the group III-selective mGluR agonist L-2-amino-4-phosphonobutyric acid (L-AP4) reduced transmission at the LPP but not the MPP synapse. In contrast, the group II selective agonist (2S,1'R,2'R,3'R)-2-(2,3-dicarboxycyclopropyl) glycine (DCG-IV) reduced transmission at the MPP but not the LPP synapse. The group I-selective agonist 3,5-dihydroxyphenylglycine (DHPG) had no effect on tranmission at either synapse. (2S,3S,4S)-2-methyl-2-(carboxycyclpropyl)glycine (MCCG), a group II selective antagonist blocked the inhibitory action of DCG-IV at MPP but not that of L-AP4 at the LPP. In contrast, the group III-selective antagonist α-methyl-L-AP4 (MAP4) blocked the effect of L-AP4 but not that of DCG-IV. DCG-IV had no effect on kainate evoked currents at a concentration that maximally reduced transmission at the MPP synapse. Taken together, these data suggest that group II and group III mGluRs serve as autoreceptors at MPP and LPP synapses respectively. Supported by NIH NINDS grant NS31373 (PJC).

239.2

ROLE AND MECHANISM OF ENDOGENOUSLY ACTIVATED mGluR1 IN MODULATING SYNAPTIC TRANSMISSION TO PHRENIC MOTONEURONS. X-W. Dong* & J. L. Feldman Depts. of Physiological Science & Neurobiology, UCLA, Los Angeles, CA 90095-1527

Activation of metabotropic glutamate receptors (mGluRs) by 1S,3R-ACPD exerts multiple actions on phrenic motoneurons (PMNs), including reduction of inspiratory-modulated synaptic currents and increase of cell excitability. These actions are mediated by different receptor subtypes: mGluR1 increases excitability and mGluR2/R4 depresses synaptic transmission. We determined their functional roles by examining whether these receptors are endogenously activated and investigated the underlying mechanisms. PMN activity was recorded under whole-cell patch-clamp conditions or from C4 root in the brainstem-spinal cord preparation of neonatal rat. Drugs were applied via pipettes over the PMN pool. To determine endogenous activity of mGluR1, the effect of the antagonist (RS)-1-Aminoindan-1,5-dicarboxylic acid (AIDCA; 200 μM) was examined. AIDCA significantly reduced C4 inspiratory discharges to 89±8% (n=5) of control, indicating that mGluR1 is endogenously activated, enhancing PMN inspiratory activity. The mGluR1 agonist (RS)-3,5-dihydroxyphenylglycine (DHPG) induced an inward current (I_{DHPG}) associated with a decrease of membrane conductance (G_N). The I_{DHPG} had a reversal potential (E_{rev}) of ~-100 mV, close to the estimated E_K (-95 mV). Elevating $[K^+]_o$ to 9 mM reduced I_{DHPG} E_{rev} to -73 mV. The K^+ channel blocker Ba^{2+} occluded DHPG effects by diminishing I_{DHPG} and the associated decrease of G_N. The Ba^{2+}-sensitive component of I_{DHPG} had similar I-V relationship to Ba^{2+}-induced current with E_{rev} at ~ -95 mV. Our data indicate that the major component of I_{DHPG} results from blockade of a Ba^{2+}-sensitive resting K^+ conductance. We conclude that mGluR1s are endogenously activated to enhance inspiratory drive to PMNs. Supported by NIH Grant NS 24742.

239.3

ROLE OF METABOTROPIC GLUTAMATE RECEPTORS IN ACTIVITY-DEPENDENT INHIBITION OF EXCITATORY SYNAPTIC TRANSMISSION IN LOCUS COERULEUS NEURONS. G. R. Dubé*, and K.C. Marshall, Dept. of Physiology, University of Ottawa, Ottawa, On. Canada, K1H 8M5.

Regulation of excitatory synaptic transmission in the locus coeruleus (LC) was investigated in an adult rat brain slice preparation. Evoked excitatory postsynaptic potentials (EPSPs) resulting from stimulation of LC afferents were measured intracellularly in LC neurons in the presence of bicuculline (10 μM). When two single stimulations were delivered to the afferents with a 600ms interval, the amplitude of the second [test (T)] EPSP was identical to the first [control(C)]. However, when a 300ms, 70 Hz train of stimulation was delivered 500 ms prior to T, the amplitude of the latter EPSP was consistently smaller than C ($p<0.001$, n=20). We proceeded to characterize the mechanisms involved in this activity-dependent EPSP inhibition. Perfusion of the slice with cyclothiazide (100 μM), an AMPA receptor desensitization blocker, resulted in a significant increase in amplitude of both C and T (142%±14 and 163%±16, respectively, $p<0.01$, n=5). This resulted in a significant increase in the ratio of EPSP amplitude of T over C (T/C), from 0.57±0.08 in regular perfusate to 0.67±0.08 in cyclothiazide ($p=0.002$). In a second set of experiments, the effects of mGluR antagonists were investigated. Bath applied MAP4 (500 μM), an mGluR group III antagonist, caused a significant increase in T/C from 0.60±0.06 to 0.67±0.06 (n=7, $p=0.01$). Conversely, (+)MCPG (500 μM), a broad spectrum mGluR antagonist, failed to significantly alter EPSP inhibition (0.73±0.05 vs 0.77±0.05, $p=0.3$). Finally, we are presently investigating the effects of $GABA_B$ and adenosine A_1/A_2 receptor agonists and antagonists. However, preliminary results provide little evidence that these were involved in activity-dependent inhibition of EPSPs. Hence, in our preparation, desensitization of the AMPA receptor following a train of stimulation can account for up to 25% of the inhibition of EPSP amplitude, while approximately 20 % of this inhibition in mGluR group III-dependent. The present study thus demonstrates that presynaptic mGluR (group III) activation and AMPA receptor desensitization are two mechanisms by which excitatory synaptic transmission can be reduced during high frequency activity of LC afferents. Supported by the MRC, Canada.

239.4

REGULATION OF SYNAPTIC TRANSMISSION IN OLFACTORY MITRAL CELLS THROUGH MULTIPLE METABOTROPIC GLUTAMATE RECEPTORS. N. E. Schoppa* and G. L. Westbrook, Vollum Institute, Oregon Health Sciences Univ., Portland, OR 97201.

Several mGluRs (mGluR1, 2, 7 and 8) are expressed in mitral cells of the olfactory bulb, but their specific roles in regulating excitatory synaptic transmission are unclear. We evaluated the effects of different mGluR agonists and antagonists on miniature excitatory post-synaptic currents (mEPSCs) in whole-cell patch recordings of cultured rat mitral cells. Experiments (at V_h= –60 mV) were conducted using a CsCl or KCl–based pipette solution containing 1 mM EGTA and 0 Ca^{2+}, and a bath solution containing 2 mM Ca^{2+} and TTx (1 μM). Picrotoxin (50 μM) and APV (100 μM) were added to isolate AMPA mEPSCs. The group I and II mGluR selective agonist 1S,3R-ACPD (100 μM) had two effects on mEPSCs. In 8 of 24 cells, ACPD caused a dramatic increase in the mEPSC frequency. This effect was apparently an indirect result of depolarization of surrounding mitral cells because it was blocked by Cd (100 μM), and ACPD reduced a resting K^+ current in the test mitral cell. In the majority of mitral cells, and in all recordings made in the presence of Cd, ACPD caused a reduction in the mEPSCs frequency (37±8% in 13 recordings made in Cd). The ACPD–induced increase in mEPSCs was mimicked by the Group I selective agonist DHPG (100 μM), whereas the ACPD–induced decrease was mimicked by the purported Group II selective agonist CCG-I (2 μM). The Group III selective agonist L-AP4 (100 μM) also caused a Cd-insensitive 34±4% (n=16) reduction in the mEPSCs frequency. 5 μM L-AP4 caused a similar magnitude mEPSCs inhibition, that was blocked by the Group III selective antagonist MAP4 (500 μM; n=3). These results suggest that multiple mGluRs can regulate synaptic transmission in mitral cells. Although mGluRs can inhibit calcium influx, the Cd-insensitivity of mEPSCs inhibition suggests that mGluRs can regulate neurotransmitter release by altering step(s) in the release process downstream of calcium influx. *Supported by 5T32-DK07680-05 (NES) and NS26494 (GLW).*

239.5

A ROLE FOR METABOTROPIC GLUTAMATE RECEPTORS IN PAIRED PULSE DEPRESSION IN SLICES OF RAT DENTATE GYRUS

Deirdre O'Leary & John O'Connor*. *Department of Human Anatomy & Physiology, University College, Earlsfort Terrace, Dublin 2, Ireland.*

There is now evidence that metabotropic glutamate receptors (mGluRs) modulate perforant path responses, via both pre- and post-synaptic mechanisms in the hippocampus. In the present study we have investigated the effect of a number of mGluR agonists and antagonists on paired pulse depression (PPD) in the medial perforant pathway of the rat dentate gyrus *in vitro*. Transverse hippocampal slices were prepared from male Wistar rats (50-150g) by standard methods. Field excitatory post-synaptic potentials (epsps) were evoked in the medial perforant pathway every 20s. Paired pulses were applied at inter stimulus intervals (ISIs) of 10, 50, 100 and 500ms. In control slices the second synaptic response was consistently smaller than the first at all ISIs tested (e.g. by 18.7±1.4% at 100ms ISI, n=16). The mGluR agonists, ACPD (10μM) and L-2-amino-4-phosphonobutyric acid (L-AP4; 20μM) reduced PPD at all ISIs less than 500ms (e.g. by 8.0±1.7% & 5.1±1.5% at 50ms ISI respectively; $p<0.01$; n=5; Students t test). Application of the mGluR antagonist, α-methyl carboxy-phenylglycine (MCPG; 100μM) inhibited the ACPD-induced reduction in PPD at all ISIs (n=7). The mGluR antagonists α-methyl-L-AP4 (MAP-4; 200μM) and α-methylserine-o-phosphate (MSOP; 200μM) did not block the L-AP4-induced reduction in PPD at ISIs less than 20ms. However both agents did inhibit the effects of L-AP4 at intermediate ISIs (50-500ms). These results further demonstrate the involvement of mGluRs in the modulation of PPD in the medial perforant pathway. (Supported by Forbairt, Ireland).

239.6

THE MIXED MGLUR AGONIST / ANTAGONIST, 4C3HPG, INHIBITS EVOKED BURSTING IN BASOLATERAL AMYGDALA NEURONS FROM KINDLED RATS. N. B. KEELE* AND P. SHINNICK-GALLAGHER, DEPT. OF PHARMACOLOGY, UNIV. OF TEXAS MEDICAL BRANCH, GALVESTON, TX 77555.

Whole-cell current and voltage clamp recordings in a slice preparation were obtained from single basolateral amygdala neurons of control and kindled rats. Previously, this laboratory has demonstrated that kindling-induced epileptogenesis produces bursting in response to both the mGluR agonist 1S,3R-ACPD and electrical synaptic stimulation. The purpose of this study is to define the pharmacology of mGluR agonist-induced bursting and to evaluate the effects of phenylglycine compounds on agonist-induced and synaptic-evoked bursting in kindled animals.

In kindled rats, application of either 1S,3R-ACPD (100μM; n=8), or the group I agonist DHPG (20μM; n=9), induced bursting. Neither group II nor group II agonists [L-CCG-I (10μM) and L-AP4 (100μM)] produced bursting in any neuron tested (n=4), suggesting group I receptors participate in bursting. Superfusing either MCPG (500μM; n=3) or 4C3HPG (100-300μM; n=5) inhibited agonist-induced bursting. We also tested MCPG and 4C3HPG on bursting evoked by lateral amygdala (LA) stimulation. MCPG had no discernible effect on synaptic-evoked bursts (n=3). In contrast, 4C3HPG showed anticonvulsant efficacy in neurons from kindled animals: (i) The threshold stimulus intensity for bursting (7±0.6V) produces only EPSP (4±0.2mV) in the presence of 4C3HPG (300μM; n=5). Increasing stimulus intensity evokes spiking, similar to control neurons, but not bursting. (ii) Burst threshold is increased dose-dependently ($EC_{50} \approx$ 350μM) by 4C3HPG [$F(3,16)=3.95$; $P<0.05$; ANOVA; control: 7±0.5V; 300μM 4C3HPG: 13±3V; $p<0.05$]. These data may suggest that the combined agonist/antagonist properties of 4C3HPG may be necessary to inhibit ictal-like burst activity. [Supported by NS24643].

239.7

ACPD INCREASES SPONTANEOUS IPSC FREQUENCY IN NEOCORTICAL NEURONS. Z. G. Chu, F.-M. Zhou and J. J. Hablitz*. Neurobiology Research Center, University of Alabama at Birmingham, Birmingham, AL 35294

Spontaneous (s) and miniature (m) IPSCs were recorded in pyramidal cells and GABAergic interneurons using whole-cell patch clamp techniques. All recordings were made at ~22 °C in slices of rat neocortex maintained in vitro. Excitatory postsynaptic currents were blocked with 20 μM D-APV and 10 μM CNQX. IPSCs were recorded at a holding potential of -60 mV. Under the recording conditions used, the Cl^- equilibrium potential was near 0 mV. Amplitudes of spontaneous IPSCs ranged from 5 to 500 pA. sIPSCs were reversibly blocked by bicuculline (10 μM) indicating mediation by $GABA_A$ receptors. Bath application of the metabotropic glutamate receptor (mGluR) agonist ACPD induced an increase in the frequency of sIPSCs in layer II/III pyramidal neurons which was reversible upon washing. The amplitude of AP-dependent sIPSCs was not significantly increased. Decay kinetics of sIPSCs were unaffected. mIPSCs recorded in the presence of TTX were not affected by ACPD. Recordings from layer I interneurons showed an ACPD-induced increase in sIPSC, but not mIPSC frequency. ACPD-evoked inward currents were occasionally observed in layer I but not layer II/II neurons. These results suggest that the potentiating effect of ACPD on IPSCs may be mediated by mGluR receptors on a subset of interneurons. Supported by NIH grant NS18145.

239.8

ACTIVATION OF METABOTROPIC GLUTAMATE RECEPTORS IN THE RAT NUCLEUS ACCUMBENS INCREASES LOCOMOTOR ACTIVITY. J.-H. Kim* and P. Vezina. Department of Psychiatry, The University of Chicago, Chicago, IL 60637.

The effect on locomotor activity of in vivo activation of metabotropic glutamate receptors in the nucleus accumbens (NAcc) was investigated in the rat. The selective metabotropic glutamate receptor agonist, (1S,3R)-1-amino-cyclopentane-1,3-dicarboxylate (1S,3R-ACPD), was microinjected into the NAcc via chronically implanted bilateral guide cannula and locomotor activity was measured for two hours. Different groups of rats injected with one of four doses of (1S,3R)-ACPD (0.005, 0.05, 0.5, or 2.5 nmol/0.5 μl/side) showed significant dose-dependent increases in both horizontal locomotion and rearing relative to control rats that received injections of the saline vehicle. In a manner similar to the locomotor effects produced by injection of amphetamine into the NAcc, increases in locomotor activity were observed predominantly in the initial 30 min of testing. During this period, the magnitude of the increases in horizontal locomotion and rearing produced by (1S,3R)-ACPD ranged from 15-40% and 34-73% above saline control levels, respectively. By comparison, injection into the NAcc of a 6.8 nmol(2.5 μg)/side dose of amphetamine produced increases in horizontal locomotion and rearing that were 113% and 167% above saline control levels, respectively. These findings are consistent with microdialysis data reported by others indicating that (1S,3R)-ACPD given into the NAcc increases extracellular levels of dopamine in this site and suggest a potentially important role for metabotropic glutamate receptors in the NAcc in the production of locomotor activity. Supported by a grant to P.V. from The Brain Research Foundation.

239.9

METABOTROPIC GLUTAMATE AGONIST-INDUCED ROTATION MEDIATED BY GROUP I MGLURS. J. Feeley Kearney* and R.L. Albin. Department of Neurology and Neuroscience Program, University of Michigan, and Ann Arbor VAMC GRECC, Ann Arbor, MI 48104-1687.

Metabotropic glutamate receptors (mGluRs) are a major class of excitatory amino acid receptors. Eight mGluR subtypes, coupled to a variety of effector systems, have been cloned. These receptors have been sub-classified into three groups based on amino acid sequence homology, pharmacology and effector systems.

The striatum possesses a high density of mGluR binding sites (Albin et al, 1992, Neurosci, 46: 35) and several mGluR mRNAs are expressed by basal ganglia neurons (Testa et al, 1994, J Neurosci, 14: 3005). In rats, unilateral intrastriatal administration of the mGluR agonist 1-aminocyclopentane-1S,3R-dicarboxylic acid (ACPD) results in vigorous contralateral rotation with delayed onset (Sacaan et al, 1992, J Neurochem, 59: 245). We sought to determine the mGluR subtype(s) mediating this behavioral effect.

In rats, the group I mGluR agonist 3,5-dihydroxyphenylglycine (DHPG) induces contralateral rotation in a dose-dependent manner. In addition, it has greater potency than the non-selective mGluR agonist ACPD. Selective agonists for group II ((2S,3S,4S)-a-carboxycyclopropylglycine (L-CCG-I)) and group III (L-2-amino-4-phosphonobutyric acid (L-AP4)) mGluRs do not elicit any significant level of rotation at any of the doses administered.

Contralateral rotation induced by ACPD or DHPG was attenuated by the group I mGluR antagonists 1-aminoindan-1,5-dicarboxylic acid (AIDA) and a-methyl-4-carboxyphenylglycine (MCPG) (group I > group II) in a dose-dependent manner. Antagonists at group II ((R,S)-a-methyl-4-tetrazolylphenylglycine (MTPG)) and group III ((R,S)-a-methyl-4-phosphonophenylglycine (MPPG)) mGluRs had no effect on ACPD- or DHPG-induced rotation. Additionally, preliminary data indicates identical modulation of ACPD- or DHPG-induced rotation by dopaminergic and purinergic agents. These results suggest that mGluR-induced contralateral rotation is mediated by group I mGluRs.

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239.10

METABOTROPIC GLUTAMATE RECEPTORS HAVE DIFFERENT EFFECTS IN DIFFERENT LAYERS OF CAT VISUAL CORTEX. N.W. Daw* and S.N.M. Reid. Dept. of Ophthalmology, Yale Univ. Sch. of Med., New Haven, CT 06510.

A group of gluamate receptors, metabotropic glutamate receptors (mGluRs) are present in the visual cortex (Reid et al., J. Comp. Neurol. 355:470, 1995). In this study, single neurons were recorded in cat primary visual cortex, and the effect of iontophoresis of the mGluR agonist trans-(1S,3R)-1-amino-1,3-cyclopentanedicarboxylic acid (ACPD) was observed. In nearly all cases, ACPD reduced the visual response. The effect of ACPD on spontaneous activity, however, was variable. Increases were generally seen in infragranular layers (V and VI), and decreases in supragranular layers (II and III). The reduction in the visual response was also largest in supragranular layers. In a number of cases in layers II and III, both visual response and spontaneous activity were almost totally abolished. In a number of cases in layers V and VI, spontaneous activity was increased and visual response was reduced to provide a large decrease in signal/noise ratio. Thus, metabotropic glutamate receptors have both facilitatory and depressive effects in visual cortex, the effect depends on the layer of the cell recorded, and the effect on the visual response and spontaneous activity are sometimes opposite to each other. Supported by RO1 EY 00053.

CATECHOLAMINES: NOREPINEPHRINE

240.1

DENDRITIC INTERACTIONS IN PERICOERULEAR REGIONS CONTRIBUTE THE SYNCHRONOUS ACTIVITY IN ADULT RAT LOCUS COERULEUS. M. ISHIMATSU and J. T. WILLIAMS* Vollum Institute, Oregon Health Sciences University, Portland, OR 97201-3098

The synchronous activity was studied in the locus coeruleus (LC) of the adult rat using an intracellular and extracellular recordings from horizontal brain slices. In tetraethylammonium chloride (TEA, 10 mM), BaCl2 (1 mM) and tetrodotoxin (1 μM) containing krebs solution oscillations in membrane potential and spontaneous field potentials were synchronous throughout the nucleus. This activity was not dependent on synaptic transmission, but was abolished by carbenoxolone (100 μM) or intracellular acidification. These results suggest synchronous activity is due to the electrotonic coupling.

LC cells were labeled with the water soluble dye (Cy5) using microelectrodes and the slices were prepared for catecholamine fluorescence. The cell body region of the LC was indicated as catecholamine fluorescent area. Confocal microscopic studies showed Cy5 filled dendrites that extended in the rostral and caudal directions beyond the cell body region. Other slices were sectioned both at the rostral and the caudal poles of the LC in order to isolate cell body region. In sectioned slices synchronous activity was abolished or remarkably reduced. In addition, [Met^5]-enkephalin (10 μM) induced current reversed the polarity at -116+/-4 mV (n=7) in these sectioned slices which was less negative than observed in normal slices (-140 mV). The results suggest the dendritic interactions in pericoerulear regions are the site at which electrotonic coupling occurs in adult rat LC. (Supported by NIH DA08163.)

240.2

ANALYSIS OF DISCHARGE CHARACTERISTICS OF LOCUS COERULEUS NEURONS DURING ACTIVATION BY DIVERSE NEUROCHEMICAL INPUTS. M.E. Page* and E.D. Abercrombie. Center for Molecular & Behavioral Neuroscience, Rutgers University. Newark, NJ 07102

Noradrenergic locus coeruleus (LC) neurons are activated by a wide variety of stimuli; from non-noxious sensory stimuli which elicit orienting responses, to intense stressors that can lead to depletion of norepinephrine in terminal fields. Activation of the LC occurs via multiple, neurochemically diverse afferents. Selective activation of distinct inputs to the LC may serve to confer response specificity as a function of the nature of the activating stimulus. For example, activation by an excitatory amino acid may result in a different profile of firing activity and biochemical responses of LC neurons as compared to activation by corticotropin-releasing factor (CRF). The present studies examined the effects of CRF and the α_2 antagonist, idazoxan on the discharge rate and pattern of LC neurons. Extracellular levels of norepinephrine (NE) also were monitored with in vivo microdialysis techniques. CRF administered locally in the LC (30 ng/60 nl) by pressure ejection resulted in a 105% increase in LC discharge rate and was associated with a 24% increase in the percentage of spikes occurring in burst-like mode. Activation of the LC by CRF was accompanied by a significant increase (70%) in extracellular NE efflux. Intravenous administration of idazoxan (1μg/kg) resulted in a similar magnitude of increase (94%) in LC discharge rate. This activation was associated with a slight increase in burst-like firing of LC neurons (from 3% to 9%). Similarly, a 10μM dose of idazoxan administered directly onto LC neurons increased firing rate by 34%, whereas no change in the percent of spikes occurring in bursts was detected with this level of activation. Studies are underway to determine if differences in the amount of NE released at the nerve terminal result from differing patterns of activation of LC neurons. Work supported by PHS MH10969 (mcp) and a Charles and Joanna Busch research grant (eda).

240.3

RELATIONSHIP BETWEEN LOCUS COERULEUS NEURONAL ACTIVITY AND NOREPINEPHRINE RELEASE IN NEOCORTEX. C.W. Berridge [*1], E.A. Abercrombie[2], R.T. Kuczenski[3], and S.L. Foote[3]. [1]Univ. Wisconsin, Madison, WI 53706; [2]Rutgers University, Newark, NJ 07102; [3]UCSD, La Jolla, CA 92093.

A major lacuna in understanding the neurobiology of the locus coeruleus (LC)-norepinephrine (NE) system is a lack of information concerning the relationship between LC discharge activity and rates of NE release. We have begun to examine this relationship utilizing a probe that combines electrophysiological recordings of LC neurons and local infusions of drugs to increase/decrease LC activity in halothane-anesthetized rat. In vivo microdialysis is used to assess NE release in the ipsilateral frontal cortex. The day following dialysis probe implant, animals are anesthetized and the recording/infusion assembly positioned with the electrode 200-300 μm below the dorsal border of LC and the infusion needle 200-500 μm lateral to LC. 90-120 min later, collection of 15-min baseline dialysis samples (approx. 2.5 pg/20 μl) and LC neuronal recordings are initiated. Initial observations are: 150nl infusions of the cholinergic agonist, bethenachol (0.5-2.0 ng/nl), increased LC discharge rates to a maximum of 100-1000% above basal levels (approx. 1.5Hz), recovering to baseline within 10-20 min. LC activation 60-200% above basal rates elicited approximately linear increases in NE in the first of two 5-min samples. Increases in LC activity beyond 200% did not elicit further NE increases. In the second 5-min sample, NE concentrations were proportional to the level of LC activation, except at extreme LC activation (800-1000%). This suggests that brief depletion of releasable NE does not contribute to the asymptotic NE response observed with moderate LC activation. Conversely, complete suppression of LC activity by local infusion of clonidine (150 nl; 0.1-1.0ng/nl) decreased NE levels to about 15%-30% of basal levels (approaching the 0.3-0.5 pg limit of detection). During the return of LC activity from clonidine-induced suppression, NE returned to basal levels at a faster rate than did LC activity, occasionally exceeding preinfusion levels. Supported by NSF (CWB).

240.4

PREFRONTAL CORTEX ACTIVATION POTENTLY EXCITES LOCUS COERULEUS (LC) NEURONS IN ANESTHETIZED RATS. E. Jodo[1] and G. Aston-Jones*. Dept. of Psychiatry, Med Coll PA & Hahnemann Univ, Phila., PA 19102; [1]Dept. of Physiology, Fukushima Medical College, Fukushima, Japan.

We found that the medial prefrontal cortex (mPFC) projects to rostral ventromedial peri-LC zone densely populated with LC dendrites (Zhu & Aston-Jones, this meeting). To examine the possible influence of the mPFC on LC activity, electrical or chemical stimulation was applied to either the infralimbic/prelimbic area (PL) or the dorsolaterally adjacent anterior cingulate/rostromedial agranular field (Cg1/Fr2 of Paxinos & Watson) while recording impulse activity of individual LC neurons in anesthetized rats. Most electrical stimulation experiments were conducted in rats (N=32) with 6-hydroxydopamine-induced lesions of the ascending dorsal bundle (DB) of noradrenergic LC fibers to eliminate the possible influence of antidromically activated LC collaterals. Single pulse stimulation (1 mA, 0.3-0.5 msec) synaptically activated 16/56 LC cells for the PL region, and 12/15 LC neurons for the Cg1/Fr2 region. The median onset latency was 32 msec for PL stimulation, and 40 msec for Cg1/Fr2 stimulation. Train stimulation (20 Hz) synaptically activated more LC cells, 41/50 for the PL and 11/12 for the Cg1/Fr2. Weak inhibitory responses were found for 9/56 LC neurons with PL stimulation; no purely inhibitory responses were detected with Cg1/Fr2 stimulation. Chemical stimulation of the mPFC with L-glutamate (10-100 mM) or D,L-homocysteic acid (10 mM) typically produced phasic excitation, sometimes followed by long-lasting oscillatory activity (15/68 LC cells with PL stimulation, and 15/26 LC neurons with Fr2 stimulation). No LC cells exhibited purely inhibitory responses with chemical stimulation of any mPFC site. Lidocaine microinjection (2%, 180 or 300 nl) into the mPFC reduced LC firing rates in 7/19 cells for the PL, and 6/10 neurons for the Fr2; in no case did lidocaine in any the mPFC site activate an LC neuron. Though most of these experiments were conducted in halothane anesthetized rats, similar results were obtained in pentobarbital anesthetized rats. These results indicate that activation of the mPFC potently excites LC neurons. Supported by PHS grants NS 24698 and DA 06214.

240.5

THE MEDIAL PREFRONTAL CORTEX PROMINENTLY INNERVATES A PERI-LOCUS COERULEUS (LC) DENDRITIC ZONE IN RAT. Y. Zhu* and G. Aston-Jones, Dept. Psychiatry, Med Coll PA & Hahnemann Univ, Philadelphia, PA 19102.

We previously found that injections of retrograde tracer into the LC that incorporated peri-LC areas often yielded retrogradely labeled neurons in the medial prefrontal cortex (mPFC), whereas injections restricted to the LC nucleus proper did not produce labeled cortical cells. Here, we examined whether the mPFC innervates the rostral ventromedial peri-LC (pLCrvm) region that contains numerous dendrites of LC neurons. Focal injections of cholera toxin b subunit (CTb) into the pLCrvm produced numerous retrogradely labeled neurons throughout the mPFC. Topography was apparent in these connections: injections centered in the dorsal pLCrvm (focused between the LC and the IVth ventricle) produced neurons most numerously in the infralimbic/prelimbic and anterior cingulate cortices, whereas more ventral pLCrvm injections also produced marked labeling in the dorsolaterally adjacent rostromedial agranular field (rostral FR2 of Paxinos & Watson). Large injections into the pLCrvm also produced many labeled neurons in the orbital cortex. Injections of phaseolus vulgaris leucoagglutinin (PHA-L), biotinylated dextran amine (BDA) or wheat germ agglutinin-conjugated horseradish peroxidase into these mPFC sites confirmed these connections by yielding prominent anterogradely labeled fibers in the pLCrvm. Notably, these cortical injections also confirmed the near absence of innervation to the LC nucleus proper. Physiological experiments in a companion study (Jodo and Aston-Jones, this meeting) reveal that electrical or chemical activation of these mPFC areas potently excite LC neurons, whereas lidocaine inactivation of these areas decreases LC activity. These results indicate that projections to the pLCrvm may contact distal LC dendrites and have strong functional effects on LC neurons; ultrastructural studies are needed to confirm this hypothesis. The potent influence of the mPFC on LC activity is compatible with the complex discharge properties that we observe in LC neurons in animals performing cognitive behavioral tasks (see Rajkowski et al., Kubiak et al., and Ivanova et al., this meeting). Supported by PHS grants NS 24698 and MH 55309.

240.6

ELECTRICAL STIMULATION OF THE LOCUS COERULEUS (LC): EFFECT OF FREQUENCY AND PATTERN OF DISCHARGE ON EXTRACELLULAR NOREPINEPHRINE (NE) RELEASE IN THE PREFRONTAL CORTEX (PFC). S. M. Florin-Lechner*, J.P. Druhan, G. Aston-Jones and R.J. Valentino, Dept. Psychiatry, Medical College of Pennsylvania and Hahnemann University, Philadelphia, PA 19102.

Evidence suggests that stimuli that activate the brain noradrenergic nucleus locus coeruleus (LC) will facilitate cortical processing of stimulus-related information by targets via synaptically-released norepinephrine (NE). The present study was designed to examine the relationship between frequency and pattern of LC activation and NE release in the medial prefrontal cortex (PFC), a target of LC projections. The LC was electrically stimulated (700 μA, 0.2 ms pulses) at varying frequencies for 20 min and extracellular NE levels were measured in the medial PFC of halothane-anesthetized rats using *in vivo* microdialysis. Tonic electrical stimulation of the LC increased cortical NE levels in a frequency-dependent manner, with 3 Hz stimulation having little effect and 5 and 10 Hz stimulation increasing NE levels by 48 ± 4 % and 66 ± 22%, respectively. The LC was also phasically stimulated with bursts of pulses every 4 s designed to deliver the same number of stimuli in a 20 min period as delivered by tonic stimulation at 3 Hz (12 pulses at 24 Hz) or 10 Hz (40 pulses at 80 Hz). Norepinephrine levels were significantly higher with 24 Hz burst stimulation compared to 3 Hz tonic stimulation. In contrast, 80 Hz burst stimulation was no more effective than 10 Hz stimulation. The present results indicate that both frequency and pattern of LC discharge are determinants of NE terminal release. Additionally, bursts of LC activity may be more effective in increasing terminal NE release than tonic increases in activity. Supported by the Human Frontiers Science Program (RG-16-93) and PHS Grants MH40008, MH00840 and DA06214.

240.7

ALTERATIONS IN pH MAY MODULATE SUBTHRESHOLD RHYTHMIC OSCILLATIONS (SROS) AND SPONTANEOUS DISCHARGE IN LOCUS COERULEUS (LC) NEURONS. A.Y. Ivanov* and G. Aston-Jones, Dept. Psychiatry, Med. Coll. PA. & Hahnemann Univ., Philadelphia, PA 19102.

SROs are a prominent feature of LC neurons. We found that these oscillations (best observed in TTX plus Ba++) depend upon low-voltage activated Ca++ currents (Soc. Neurosci Abstr., 1995, p. 633). Here, we report that $[Na+]_o$ and pH are also important determinants of SROs and spontaneous discharge in LC cells. Intracellular recordings were obtained from LC neurons in slices using sharp microelectrodes. Superfusion with low $[Na+]_o$ (25-50 mM) suppressed spontaneous discharge, hyper-polarized membrane potential and eliminated SROs (n=7 neurons). TTX also eliminated spontaneous discharge but did not hyperpolarize or eliminate SROs, nor did it prevent these effects of low Na+. We investigated whether TTX-independent actions of low Na+ may be due to changes in Na+/H+ exchanger activity with altered pH in LC neurons. Sodium propionate (10 or 25 mM; to decrease intracellular pH) suppressed spontaneous discharge and hyperpolarized LC neurons (n=5); Na+ propionate treatment also suppressed SROs and HVA (high-voltage activated) Ca++ spikes in TTX (1 μM) plus Ba++ (2 mM; n=3). This indicates that superfusion with low Na+ (which may decrease Na+/H+ exchanger activity) produces changes in LC neurons similar to intracellular acidification. In addition, NH_4Cl (10 mM; to increase intracellular pH) increased spontaneous discharge and the frequency of SROs observed in TTX plus Ba++. The amplitude and frequency of SROs and HVA Ca++ spikes (in TTX plus Ba++) were also increased in 60 mM $NaHCO_3$ (pH adjusted to 7.3; to increase intracellular pH; n=3). However, the same solution with pH 7.9 (intracellular alkalization also expected; n=4) hyperpolarized LC neurons and blocked spontaneous discharge and SROs. The hyperpolarization evoked in LC neurons by raising extracellular pH was substantially suppressed in the presence of TTX plus Ba++ but HVA Ca++ spikes and SROs were not restored (n=4). These data indicate that SROs and spontaneous discharge in LC neurons may be modulated by Na+/H+ exchanger activity and pH. Supported by PHS grants NS 24698 and DA 06214.

240.8

OPIATE WITHDRAWAL-INDUCED HYPERACTIVITY IN LOCUS COERULEUS (LC): MEDIATION BY SUBREGIONS OF THE ROSTRAL VENTRAL MEDULLA H. Hirata*, and G. Aston-Jones, Dept. Psychiatry, Hahnemann Univ. , Philadelphia, PA 19102.

LC neuronal hyperactivity during opiate withdrawal (OW) is thought to underlie many withdrawal symptoms. Previous work indicates that this LC hyperactivity is in large part mediated by excitatory amino acid inputs to the LC from the nucleus paragigantocellularis lateralis (PGi) in the rostroventral medulla. We have previously found that neurons projecting to the LC are widely scattered throughout the PGi region. The purpose of this study was to identify subregions of the PGi that are responsible for OW-induced LC hyperactivity. Extracelluar recordings were obtained from individual LC neurons in halothane-anesthetized rats that were chronically pretreated with morphine (osmotic minipump; ~ 34 mg/kg/day s.c., 6 days). OW precipitated by intravenous naloxone HCl (NLX; 0.1 mg/kg) produced strong activation in all LC neurons recorded (~4-fold increase in firing rate). When the discharge rate stabilized (~3-5 min after NLX), lidocaine (2%; 250-300 nl) was microinfused over 1-2 min into a PGi region from a glass micropipette (~20 μm tip) using pneumatic pressure (Picospritzer). Of 10 cells examined, 4 exhibited ~20-40% reduction of OW-induced activity after lidocaine infusion into the PGi. Preliminary histological results indicate that PGi areas where lidocaine attenuated OW-induced hyperactivity in LC are more rostral than those where lidocaine yielded little or no change in LC hyperactivity. We have also previously reported that lidocaine in the PGi can attenuate LC response to footshock stimulation (Chiang and Aston-Jones, Neuroscience, 1993). Here, we found in 2 cases that lidocaine microinfused into caudal PGi sites attenuated footshock responses of LC neurons but did not appreciably decrease OW-induced hyperactivity in LC cells. The present results indicate that specific subregions of the PGi may be responsible for OW-induced vs. footshock-induced activation of LC neurons. Supported by PHS grants DA 06214 and NS 24698.

240.9

NEURONAL RESPONSE TO NOREPINEPHRINE IS DETERMINED BY CELL'S LOCATION RELATIVE TO BARREL COLUMN WITHIN RAT SOMATOSENSORY CORTEX. D.T. Fleischer* and B.D. Waterhouse. Dept. of Neurobiology & Anatomy, MCP/Hahnemann University, Philadelphia, PA 19102-1102.

Norepinephrine (NE)-containing axons from locus coeruleus innervate multiple sensory circuits throughout the mammalian CNS. It has been proposed that this widespread projection system facilitates sensory signal transmission during arousal and vigilance. Previous studies from this laboratory and elsewhere have examined the effects of NE on extracellularly recorded response properties of cells in primary sensory circuits. The goal of the present study was to categorize NE's effects on intrinsic membrane properties of electrophysiologically and morphologically identified pyramidal neurons in layer II/III of the barrel field of rat somatosensory cortex. Intracellular recordings were obtained from neurons in cortical tissue slices maintained *in vitro*. Intrinsic membrane properties of individual cells were measured under current clamp conditions using glass microelectrodes filled with 2M K Ac plus 1% neurobiotin. NE was bath applied at doses up to 10μM. After each experiment recorded cells were labeled with neurobiotin for morphologic identification. The tissue was then stained using cytochrome oxidase histochemistry to confirm each cell's location within a barrel column. Based upon previously established criteria all recorded layer II/III neurons were identified as regular spiking with pyramidal morphology. In the presence of NE at 1-10μM, neurons which were located at the periphery of a barrel column showed an increase in input resistance and decrease in rheobase, whereas cells located within the center of a barrel column showed a decrease in input resistance and an increase in rheobase. Thus, NE appears capable of eliciting distinctly different changes in neuronal excitability depending upon the cell's location with respect to the center or periphery of a barrel column. These findings raise the possibility that the manner in which NE modulates neuronal excitability is correlated with the location of a cell relative to well defined, functionally-related cytoarchitectonic landmarks. Finally, our data support an emerging concept that endogenously released NE can selectively modulate specific neural components of sensory cortical networks.

This work was supported by NINDS NS32461 to B.D.W.

240.10

EFFECT OF INCREASING TONIC LEVELS OF NOREPINEPHRINE ON GLUTAMATE EVOKED DISCHARGE OF SINGLE NEURONS IN LAYERS II/III AND V OF RAT BARREL FIELD CORTEX. D. M. Devilbiss and B. D. Waterhouse* Dept. Neurobiology and Anatomy, Medical College of Pennsylvania and Hahnemann University, Philadelphia, PA 19102

Recent microdialysis studies support the widely accepted view that forebrain levels of norepinephrine (NE) fluctuate as tonic output from the nucleus locus coeruleus changes. Previous studies from our laboratory and elsewhere have employed a variety of strategies to assess the potential impact of synaptically released NE on the responsiveness of target neurons to synaptic inputs. The goal of the present study was twofold: (1) to assess the effects of different tonic levels of NE on glutamate (GLU) evoked discharge of cortical neurons and (2) to clarify the results of earlier studies which showed, that depending on the neuron recorded and the dose of NE, both facilitation and suppression of stimulus evoked discharge could be observed. To this end we utilized an in vitro cortical tissue slice preparation to identify possible laminar differences, pre- versus postsynaptic effects, and iontophoretic dose dependence of NE's modulatory influence on GLU evoked discharge in rat barrel field somatosensory cortical neurons. Extracellular responses to iontophoretically applied GLU were recorded from neurons in layers II/III and V of the barrel field cortex before, during and after incremental microiontophoretic doses of NE (1-50nA). For each neuron recorded peri-event histograms were quantatively analyzed in order to determine iontophoretic dose response relationships. Our results showed that for neurons in both layers II/III and V there were two possible modes of interaction between NE and GLU evoked discharge: (1) suppression of GLU evoked response at all doses of NE tested and (2) an "inverted U" dose response relationship, such that GLU evoked responses were progressively facilitated to an optimum as NE levels were increased and then progressively suppressed as NE levels were increased further. We also noted that in the same cell minimal responses to GLU were modulated (ie. facilitated or suppressed) by NE more than maximal responses. A similar set of findings were observed in aCSF containing low Ca^{2+} and high Mg^{2+}. This latter result suggests that neither the facilitating nor the suppressant effects of NE on GLU evoked discharge rely upon presynaptic mechanisms. In summary, the demonstration that NE can either facilitate or suppress cortical neuron responsiveness emphasizes the intrinsic heterogeneity of cortical neurons with respect to noradrenergic modulatory actions. More importantly, these results suggest that under ideal conditions modulatory interactions between NE and GLU can be optimized. Finally, we believe it is particularly significant that our finding of an "inverted U" dose response relationship between NE and GLU evoked discharge parallels the results of Aston-Jonesand colleagues who have described an "inverted U" relationship between tonic rates of locus coeruleus neuron discharge and primate performance on a sustained attention task. Supported by NINDS NS32461 and DA05117 to BDW.

240.11

INNERVATION OF THE NUCLEUS ACCUMBENS SHELL AND VENTRAL TEGMENTAL AREA BY DOPAMINE BETA-HYDROXYLASE-POSITIVE FIBERS: POSSIBLE SITES FOR NOREPINEPHRINE-DOPAMINE INTERACTIONS. A. Saleem*, Y. Zhu, J. Druhan and G. Aston-Jones, Dept. Psychiatry, Med Coll PA & Hahnemann Univ., Phila, PA 19102.

Several lines of evidence indicate that norepinephrine (NE) brain systems may influence functions associated with nucleus accumbens dopamine (DA). However, the site(s) for such interactions have not been identified. We undertook anatomical studies to determine whether NE fibers innervate the nucleus accumbens or the ventral tegmental area (VTA) which contains DA neurons that innervate the accumbens. Sections of rat VTA and nucleus accumbens were stained with an antibody to dopamine beta-hydroxylase (DBH) to identify NE-containing fibers. Using dual immunohistochemistry, the VTA sections were also stained for tyrosine hydroxylase (TH) to identify DA neurons and processes, and the sections through the nucleus accumbens were also stained with an antibody against substance P to identify accumbal boundaries. In the VTA, we found moderate densities of DBH+ fibers in dorsocaudal regions that also contained DA somata and dendrites. Numerous DBH+ fibers were also present in the medial shell of the nucleus accumbens. Large injections of Fluoro-Gold into the shell of the accumbens produced retrogradely labeled NE neurons in the locus coeruleus; more focal injections with other tracers are needed to confirm this result. Injections of the anterograde tracers Phaseolus vulgaris leucoagglutinin (PHA-L) or biotinylated dextran amine (BDA) into the LC produced anterogradely labeled fibers in the same VTA regions as contained DBH+ fibers; source(s) of DBH in the accumbens are less certain. These findings indicate that both the VTA and nucleus accumbens shell are possible sites for NE-DA interactions. Such interactions could be important for regulation of motivated behaviors and in drug abuse. Supported by PHS grants DA 06214 and DA 10088.

240.12

DISTRIBUTION OF DOPAMINE-β-HYDROXYLASE (DBH)-LIKE IMMUNOREACTIVE FIBERS WITHIN THE NUCLEUS ACCUMBENS SHELL. A.E. Kelley[1], T.R. Stratford[1], S.L. Foote*[2], and C.W. Berridge[3]. [1]Psychiatry Dept., [3]Psychology Dept., Univ. Wisconsin, Madison, WI 53706, [2]Psychiatry Dept., Univ. California, San Diego, La Jolla, CA 92093.

The nucleus accumbens can be divided into distinct subfields, delineated on the basis of afferent and efferent projections, as well as on histochemical markers. Much attention has been focused on the functions of the "shell" subregion, which has reciprocal relationships with a variety of limbic regions and brainstem autonomic structures, and has been suggested to participate in the modulation of stress- and arousal-related processes. The locus coeruleus-noradrenergic system has also been implicated in stress and arousal. Previous tracing studies suggest that the locus coeruleus projects to the accumbens shell. To better understand the anatomical substrate through which LC could influence activity of the accumbens shell, the extent and distribution of noradrenergic innervation within this structure was examined. Sections of rat brain from the rostral accumbens through the bed nucleus of the stria terminalis were processed for DBH immunoreactivity, to visualize noradrenergic axons. In some animals, alternate sections were processed for immunohistochemical localization of substance-P, in order to delineate core, shell, and pallidal compartments. Moderate to dense DBH-like immunoreactivity (DBHir) was found in the approximately posterior one-third of the shell, particularly in the septal pole and ventral shell. The innervation of the septal pole was contiguous with a dense innervation of the bed nucleus of the stria terminalis. Very few fibers were observed in the rostral accumbens, core, or caudate-putamen. Many DBHir fibers were of small caliber with numerous varicosities, features indicative of terminal fields. These observations suggest noradrenergic systems might modulate processes associated with stress, behavioral state, or reinforcement via actions within the accumbens shell. Supported by NIDA DA04788 and Univ. of Wisconsin.

240.13

Localization of β-Adrenergic Receptor Responses and Tyrosine Hydroxylase Positive Fibers in the Deep Cerebellar Nuclei of F344 Rats. Thomas J. Gould[1*], Cathy E. Adams[2] & Paula C. Bickford[1, 3]. [3]Dept. of Vet. Affairs Med. Ctr., Denver CO; [2]Dept of Psychiatry; [1]Dept of Pharm. Univ. Colorado, Health Sciences Center, Denver, Colorado 80262.

Norepinephrine (NE) is a neurotransmitter that also acts as a neuromodulator. At levels subthreshold for producing depression of spontaneous firing, NE can facilitate excitatory or inhibitory neurotransmission. Whereas a significant body of work has examined cerebellar cortex and noradrenergic function (Bloom, 1979), little work has examined NE pharmacological function in the deep cerebellar nuclei (DCN). The presence of norepinephrine (NE) and NE activated cells in the deep cerebellar nuclei (DCN) of male F344 rats was investigated using electrophysiology during iontophoresis of the β-adrenergic agonist isoproterenol (ISO) and using immunohistochemistry. During extracellular electrophysiology, GABA was iontophoretically applied to the cell and ISO was then co-applied to in an attempt to modulate the GABAergic inhibition of cell firing in the DCN. Immunohistochemistry was used to detect tyrosine hydroxylase (TH) positive fibers in the DCN. Isoproterenol modulated GABAergic inhibition in 51% of the DCN tested. In addition, TH-positive fibers that appeared to make contact with DCN cells were found. Thus, this study demonstrated that functional NE receptors exist in the DCN and NE appears to be present in fibers in the DCN. This work was supported by USPHS grant AG04418, grant AG05686-01 and the VAMRS.

240.14

FISCHER AND LEWIS RATS EXHIBIT DIFFERENTIAL BEHAVIORAL AND NORADRENERGIC RESPONSES TO STRESS AND AMPHETAMINE CHALLENGE. L.A. Rosario* and E.D. Abercrombie, Center for Molecular and Behavioral Neuroscience, Rutgers University, Newark, NJ 07102.

Fischer (F344) and Lewis (LEW) rats show differential behavioral and physiological responses to stress, i.e., F344 rats are hyper-responsive while LEW rats are hypo-responsive in their locomotor activity (LA), ACTH, and corticosterone responses to stressful stimuli. The locus coeruleus (LC), which may underly differences in behavioral reactivity, plays a role in stress and arousal. This study investigates the locomotor and noradrenergic responses to stress or amphetamine (AMPH) in these strains and examines the LC as a substrate underlying differential behavioral reactivity. LA was measured in the home cage during the light/dark cycle, in response to tail pinch stress (TP), AMPH (1 mg/kg or 5 mg/kg) challenge and novelty stress. LA increases with the onset of the dark phase in F344 and LEW rats ($p<0.0001$); this increase is greater in F344 rats ($p<0.006$). Similarly, the initial LA in response to novelty is greater in F344 rats ($p<0.0003$). TP-induced LA is not different between the groups. Administration of AMPH increases LA in both strains. Low dose AMPH (1.0 mg/kg) induces greater LA in F344 rats ($p<0.0011$) whereas at the higher dose (AMPH 5.0 mg/kg) there is no statistical difference between the groups. *In vivo* microdialysis was used to measure hippocampal NE and DOPAC efflux in F344 and LEW rats in response to TP or AMPH (1.0 mg/kg). Extracellular NE levels are an indicator of the evoked responsiveness of the LC-NE system whereas DOPAC efflux serves as an indirect index of tyrosine hydroxylation. TP increases NE release in both strains ($p<0.03$); this increase is greater in the F344 rats ($p<0.0006$). Similarly, AMPH-induced NE release is greater in F344 rats ($p<0.002$). TP or AMPH injection increases DOPAC efflux only in the F344 rats ($p<0.0003$). In summary, LA in F344 rats is greater than LEW rats during arousal and/or mild stress. Also, F344 rats exhibit a potentiated noradrenergic response to TP stress and AMPH challenge compared to LEW rats. Increased hippocampal DOPAC efflux in F344 rats suggest differences in tyrosine hydroxylation may contribute to the differential noradrenergic profile observed in these rats. Supported by DA08086 and a Charles and Joanna Busch Research Grant.

240.15

AN ELECTROPHYSIOLOGIC APPROACH TO THE STUDY OF THYROID HORMONE (TH) ACTION IN BRAIN: MEASUREMENTS OF UNIT ACTIVITY IN BRAIN SLICES. M.B. Dratman,* D. W. Pfaff, and L.-M. Kow, Laboratory of Neurobiology and Behavior, Rockefeller University, New York, NY 10021

Previous studies (Rozanov and Dratman, Neuroscience, IN PRESS) have demonstrated that triiodothyronine (T_3) is strongly localized in locus coeruleus and its projection sites, including molecular and pyramidal cell layers (PC) of hippocampus (Hi). To search for functional correlates of the morphologic observations in Hi, unit activities (basal firing rates and responses to test substances) recorded from PC-CA1 of euthyroid (EU) or hypothyroid (HYPO) rats were measured during perfusion of brain slices with ACSF ± norepinephrine (NE). TH, NE+TH or their diluents. TH-induced effects were small and, as observed against the dynamic background of unit activity, were subtle. These preliminary results were graded and subjected to analysis by the ordered chi-square test for trend. Firing rate changes after NE, judged as +4 to -2, were significantly greater ($p=.0002$) in EU (median=4) then in HYPO (median=1). Surprisingly, the effects of T_3 and thyroxine (T_4) were different; T_3 increased and T_4 decreased both basal and NE-induced firing rates ($p=.005$ and .002 respectively). Dampened PC-x CA1 unit activity after NE in HYPO, may reflect down-regulation of adrenergic receptors during low TH states. While opposite effects of T_3 and T_4 were unexpected, they conform with known homeostatic adjustments of TH metabolism in brain (Dratman, et al, Am. J. Physiol., E185-193, 1983). The rapid time course of response to TH makes it unlikely that genomic mechanisms account for its effects on basal and NE-induced firing rates. Instead, both functional and morphologic observations are consistent with mediation through synaptic (adrenergic?) membrane receptors.
Support from MH45252 and Pat Kind Fund for Thyroid Research

240.16

EFFECTS OF 2 WEEK AND 8 WEEK HIGH SALT DIET ON NOREPINEPHRINE CONTENT IN DISCRETE BRAIN NUCLEI OF BORDERLINE HYPERTENSIVE RATS (BHR). I.P. Edgemon, R. Finkel, G.-P. Zheng, N.E. Dobson, and J.E. Lawler*. Department of Psychology, University of Tennessee, Knoxville, TN 37996-0900.

The BHR has a mixed genetic history for developing hypertension when exposed to environmental stressors. Previous research in our laboratory has established that chronic stress alone or combined with salt or exercise affects norepinephrine (NE) content of hypothalamic and brainstem nuclei associated with cardiovascular regulation in BHR. While varying durations of high salt diet have been shown to alter blood pressure levels in animals with genetic histories for hypertension, less is known about the effects of salt alone on the NE content of discrete brain centers. The purpose of this study was to investigate the effects of varying durations of high salt diet on NE content of brainstem (A2, A1, C1, LC) and hypothalamic (PH, Arc, DMH, LH, VMH, PVH, Ant, SO) nuclei of BHR and their normotensive parent strain, Wistar-Kyoto rats (WKY). Male BHR and WKY remained on either normal (1%) or high (8%) salt chow for 2 or 8 weeks such that the diets were completed at 12 weeks of age. Following sacrifice, the brains were removed and sliced, and nuclei were extracted using the Palkovits technique. NE content was later determined using liquid chromatography and electrochemical detection. Results indicated that: 1) both high salt diets elevated NE content in many brainstem and hypothalamic nuclei of BHR and WKY; 2) the distribution of nuclei affected within rat strains was different based on the brain region and diet duration; and 3) 2 week high salt diets produced elevations, while 8 week diets produced reductions, in NE content of BHR nuclei compared to WKY. Data are consistent with the hypothesis that BHR lack sufficient central noradrenergic inhibition to counteract the hypertensive effects of a chronic high salt diet.
(AHA-TN Affiliate)

240.17

α_2 ADRENERGIC RECEPTORS (ARS) REDUCE AND β ARS POTENTIATE SYNAPTIC TRANSMISSION BETWEEN THE ENTORHINAL CORTEX AND THE RAT BASOLATERAL AMYGDALA. E. Pralong, M. Kiraly*, P.J. Magistretti and B. Ferry. Laboratoire de recherche neurologique, CHUV et Institut de Physiologie, Université de Lausanne, Suisse.

Horizontal rat brain slices preserving the synaptic connections betwen the basolateral amygdala (BLA), the external capsule (eca) and the entorhinal cortex (EC) were used to record synaptic responses in 156 (BLA) neurones to either EC, eca or direct BLA stimulations. Comparison of the time-to-peak latencies for the fast excitatory postsynaptic potentials (EPSPs) evoked by stimulation of the EC, eca or BLA (13.3, 7.9 or 7.2 ms respectively) suggests that the EC-BLA connections are mostly mono or oligosynaptic. Individual components of the post-synaptic potentials evoked by direct BLA stimulation were pharmacologically isolated using CNQX, d-AP5 and bicuculline methiodide. NA reduced the fast EPSPs by ~40 % and the slow EPSP by ~50 %. A similar effect was obtained with the α_2 agonist UK 14,304. In contrast, the β agonist isoproterenol increased the fast EPSP by ~40 % and the slow EPSP by ~20 %. Accordingly, the effect of NA on the EPSPs was blocked only by the α_2 antagonist yohimbine. NA was ineffective on the isolated IPSPs, the fast and slow IPSPs being reduced by ~5 and ~10 % respectively. These results suggest NA action on the excitatory transmission can be dissected into a predominant α_2 AR-mediated inhibition and a β AR-mediated excitation.

Supported by the FNRS 31-040545.94

240.18

REGULATION OF [^{3}H]NOREPINEPHRINE ([^{3}H]NE) RELEASE FROM GUINEA PIG HIPPOCAMPAL SLICES BY SIGMA$_2$ (σ_2) RECEPTORS. J.K. Weatherspoon*, T.I. Netoff, G.M. Gonzalez-Alvear and L.L. Werling. Dept. of Pharmacology, The George Washington University Medical Center, Washington, DC 20037.

We have shown that σ receptors regulate NMDA-stimulated [^{3}H]NE release from slices of rat hippocampus (HIPP) (Gonzalez-Alvear and Werling, Brain Res. 673: 61-69, 1995). Although a significant portion of regulation of release appeared to be via a σ_2 receptor, we had no σ_2-selective drugs with which to confirm this hypothesis. In the current study, we tested σ regulation of NE release in slices of guinea pig HIPP, a tissue in which a larger percentage of inhibition appeared to be via σ_2 receptors. We employed the σ_2 ligand BIMU-8, whose binding profile had been determined, but whose agonist/antagonist status at σ_2 receptors had not been identified. We tested the σ agonists (+)pentazocine (500 nM - 5 μM) and BD737 (0.1 nM - 100 nM), both alone and in the presence of the σ_1-selective antagonist Dup734 (100 nM), or BIMU-8 (100 nM). (+)Pentazocine (500 nM - 5 μM) alone inhibited NMDA-stimulated release. When the σ_1 component of (+)pentazocine inhibition was blocked by inclusion of DuP734, BIMU-8 fully reversed the remaining inhibition produced by (+)pentazocine. BIMU-8 alone had no effect on basal or NMDA-stimulated release. The inhibition mediated by BD737 was not significantly reversed by Dup734, suggesting the inhibition mediated by BD737 in HIPP includes another component. BIMU-8 did not reverse inhibition of release by low concentrations of BD737 (chosen to selectively activate σ_1 receptors). We are currently testing BIMU-8 against higher concentrations of BD737 that should also activate σ_2 receptors. Our results suggest that (1) BIMU-8 is an antagonist at σ_2 receptors and that (2) σ_2 receptors contribute to regulation of NE release in HIPP. Supported by NIDA.

SEROTONIN: TRYPTOPHAN HYDROXYLASE

241.1

CELLULAR LOCALIZATION OF TRYPTOPHAN HYDROXYLASE mRNA IN POSTMORTEM HUMAN BRAINSTEM AND PINEAL GLAND. M.C. Austin*, S.M. O'Donnell, J.L. Rhodes. Department of Psychiatry, University of Pittsburgh School of Medicine, Pittsburgh, PA 15213.

Tryptophan hydroxylase (TPH) is the rate-limiting enzyme involved in the biosynthesis of serotonin. Recent studies have documented that the human TPH gene exhibits an unusual splicing complexity in the 5'-untranslated region which produces four distinct mRNA transcripts (Boularand et al. 1995). Although TPH mRNA has been visualized in raphe neurons in the rodent and monkey brain (Kim et al. 1991; Pecins-Thompson et al. 1995), there have been no reports documenting the cellular localization of TPH mRNA in postmortem human brain. The present study was designed to characterize the cellular distribution of TPH mRNA in the human brainstem and to compare the level of mRNA expression to that in the pineal gland. Postmortem human brain tissue (N=5) was obtained at autopsy, dissected and frozen. All cases had negative toxicological results and no known neurologic or psychiatric disorders. A 345 bp TPH cDNA fragment was amplified from human pineal RNA using oligo primers and RT-PCR. The cDNA fragment was then inserted into a pDIRECT vector (Clontech). Coronal tissue sections (20μm) of the brainstem and pineal were thaw-mounted on gelatin-coated slides and processed for in situ hybridization using ^{35}S-labeled cRNA probes. Abundant hybridization signal corresponding to TPH mRNA was localized to neurons in the median and dorsal raphe as well as in the oral pontine and supralemniscal nuclei. Hybridized sections of the human pineal gland revealed very high levels of TPH mRNA in pinealocytes. It was evident from both the film and emulsion autoradiograms that TPH mRNA levels were much higher in the pineal tissue compared to the raphe, an observation which has been previously documented in rodents. These findings reveal the cellular localization of TPH mRNA in the human pineal gland and in serotonergic neurons of the brainstem. Understanding the nature of TPH gene expression in the human brain may provide new insights into the pathophysiological mechanisms underlying serotonergic dysfunction in neuropsychiatric disorders. (Supported by the Stanley Foundation).

241.2

INCREASED LEVELS OF AROMATIC-L-AMINO ACID DECARBOXYLASE PROTEIN AND mRNA IN HUMAN CARCINOID TUMORS. J.A. Gilbert,* L.M. Frederick, and M.M. Ames, Div. of Oncology Res., Mayo Clinic & Foundation, Rochester, MN 55905

The carcinoid tumor is an uncommon neuroendocrine malignancy whose primary characteristic is excessive production of serotonin (5-HT), synthesized by conversion of tryptophan (trp) to 5-OH-tryptophan (5-OH-trp) by trp hydroxylase (TPH) and decarboxylation of 5-OH-trp to 5-HT by aromatic-L-amino acid decarboxylase (AAAD). As a preliminary step to developing mechanism-based agents selective against carcinoid tumors, we characterized these enzymes in patient sets of carcinoid hepatic metastases and surrounding normal liver. Carcinoid AAAD, while similar in substrate affinity to AAAD from normal liver, had a 50-fold higher maximal activity. Western immunoblot analyses of the same matched sets indicated that the AAAD polypeptide content of carcinoid tumor was >20-fold that of normal liver. Preliminary Western analyses of patient sets of primary ileal carcinoid tumors and adjacent normal ileum samples indicated a similarly high ratio of carcinoid:normal AAAD protein. Northern blot analyses were performed on total RNA or mRNA isolated from matching carcinoid hepatic metastases and normal liver samples. Blots were sequentially probed with a synthetic 40-mer complementary to the mRNA coding region of human AAAD and a commercially available, 1.0-kb human glyceraldehyde-3-phosphate dehydrogenase cDNA fragment. A higher level of AAAD mRNA was detected in carcinoid tumor than in normal liver. These results suggested that increased levels of carcinoid AAAD protein existed in both primary and metastatic lesions, and that increased levels of carcinoid AAAD mRNA, as determined in matched sets of hepatic carcinoid and normal samples, may be a primary contributing factor. Supported in part by grant CA 58450, NCI, DHHS.

241.3

AMINO TERMINAL PHOSPHORYLATION OF TRYPTOPHAN HYDROXYLASE S.C. Kumer*, S.M. Mockus, and K.E. Vrana. Dept. Of Physiology and Pharmacology, Bowman Gray Sch. of Med., Medical Center Blvd., Winston-Salem, NC 27157-1083.

Tryptophan hydroxylase (TPH) catalyzes the rate-limiting and committed step in serotonin biosynthesis. Within this enzyme, two distinct domains have been hypothesized to exist: an amino terminal regulatory domain and a carboxyl terminal catalytic domain. In the present experiments, the functional boundary between the putative domains was defined utilizing deletion mutagenesis. A full-length cDNA clone for rabbit TPH was engineered for expression in bacteria. Amino terminal deletions were constructed using polymerase chain reaction (PCR). Five amino terminal deletions were created: NΔ50, NΔ60, NΔ90, NΔ106, and NΔ116 (referring to the number of amino acids deleted from the amino terminus). Enzymatic activity was determined for each mutant following expression in bacteria. Specific activities for NΔ50, NΔ60, NΔ90, and NΔ106 were comparable to the wild type recombinant TPH enzyme. Deletion of 116 amino acids, however, abolished activity. As a corollary, the ability to phosphorylate TPH using cAMP-dependent protein kinase (PKA) was also examined. Deletion of the first 60 amino terminal residues abolished the ability of the enzyme to serve as a substrate for PKA, yet the unmodified and the NΔ50 constructs maintained phosphorylation. In conclusion, we believe that the first 106 amino acids compose a regulatory domain which is modified at a consensus sequence for phosphorylation by PKA between amino acids 50 and 60. Moreover, this postulated regulatory-catalytic domain boundary is analogous to the similar domain structure in the related enzyme tyrosine hydroxylase. *This work was supported by T32DA07246 (to S.C.K.) and GM38931 (to K.E.V.).*

241.4

SALT-INDUCED ACTIVATION OF MIDBRAIN SEROTONERGIC NEURONS: ASSESSMENT OF cFOS, TPH, 5HT, AND 5HIAA CONTENTS BY QUANTITATIVE RADIOIMMUNOCYTOCHEMISTRY, IMMUNOBLOT, AND HPLC. S.D. Doughman, J.A. Saydoff*, J.Spangenberg, and M.S. Brownfield. Dept of Comp Biosci, Univ of Wisconsin, Madison, WI 53706 and INCSTAR Corp, Stillwater, MN 55082.

Brain serotonin (5HT) stimulates the secretion of vasopressin (VP) and oxytocin (OT) and plays a role in the osmoregulation of these neurohormones in conscious rats. These studies were designed to determine the location of salt activated 5HT neurons. Rats were given 2% sodium chloride to drink for 2 days as an osmotic stimulus. Control rats drank tap water. Single and dual label immunocytochemical (ICC) and radioimmunocytochemical (RICC) studies were done on brains fixed by perfusion with 4% paraformaldehyde. RICC studies employed antisera against tryptophan hydroxylase (TPH, rate limiting enzyme in the synthesis of 5HT), and 5HT and 5HIAA (serotonin metabolite). Serial cryostat sections were incubated with appropriately diluted antibodies, washed and incubated with 125-I labeled recombinant protein A/G (Pierce), washed and exposed to 3H-Ultrafilm. Immunoblots (using chemiluminescent detection) and high performance liquid chromatography were done on extracts of unfixed brains. Labeled section and immunoblot images on film were quantified by computer densitometry (MCID).

Routine ICC revealed salt-induction of cFOS immunoreactivity in VP and OT cells and in the dorsal (DR) and median (MR) raphe nuclei. Hindbrain 5HT cell groups were not labeled. Dual label ICC studies verified that most cFOS labeled cells were 5HT positive. RICC studies showed significant reductions of TPH and 5HT and an increase in 5HIAA content in the DR and MR, but no change in raphe pontis (RP). Quantitative immunoblots for TPH and HPLC for 5HT and 5HIAA showed similar changes in the midbrain and no change in pontine or medullary hindbrain content of these substances.

These studies show that salt loading induces a selective activation of midbrain 5HT nuclei, but does not affect pontine or medullary serotonergic sites. These studies also show the utility of these approaches for establishing sites of activation of the serotonergic system. Supported by American Heart Association Grant No 94016290 to MSB.

241.5

CHANGES IN TRYPTOPHAN HYDROXYLASE (TPH) mRNA EXPRESSION IN THE DORSAL RAPHE NUCLEUS FOLLOWING LESION OF THE DORSOLATERAL HYPOTHALAMUS (DLH) IN THE RAT. V. Ljubic-Thibal, A.J. Morin*, M. Diksic and E. Hamel. Montreal Neurological Institute, McGill University, Montréal, QC, Canada H3A 2B4.

Recent retrograde tracing studies with Cholera toxin β subunit coupled to immunocytochemistry of TPH, the synthesizing enzyme for serotonin (5-HT), suggest that the major serotonergic innervation of the DLH originates from neurons in the lateral aspect of the ipsilateral dorsal raphe nucleus (DRN)(Soc. Neurosc. Abst. 21, #540.5, 1995). As well, 5,7-dihydroxytryptamine (5,7-DHT) lesion of the DLH induces significant increases in TPH levels in 5-HT neurons of the DRN with a similar lateral location (Ljubic-Thibal et al., Brain Res. 1996, in press). In the present study, we investigated whether the local compensatory changes in TPH activity and content, apparently limited to the DLH projecting neurons, also involve changes at the TPH gene transcriptional level. We examined TPH mRNA expression in the rat brainstem raphe nuclei 5 days after a unilateral stereotaxic 5,7-DHT lesion (3µg in 0.25 µl) of the DLH (Bregma,-3.0; 1.0 lateral; 8.1 from brain surface). Rat brains were perfusion-fixed, cryostat-cut (10 µm) and processed for cellular *in situ* hybridization using a sense or antisense 800 bp ^{35}S-labelled TPH cRNA probe (cDNA fragment generously provided by Drs. KS Kim and TH Joh). Sham (injected with 0.25 µl of vehicle) and control brain sections were run in parallel. The distribution of TPH-expressing neurons in the DRN was similar between lesioned, sham and control rats. However, cells expressing very high levels of TPH mRNA, determined by silver grain density, were only detected in the lesioned rats and primarily so, in the lateral aspects of the ipsilateral DRN. On average, the number of cells expressing high levels of TPH mRNAs was 6.95 ± 0.55 on the ipsilateral side as compared to 1.41 ± 0.18 (d.f.=8, $p < 0.001$), on the midline or contralateral side. These results show that DLH lesion results in changes in TPH mRNA levels in a small population of 5-HT neurons in the ipsilateral DRN, most likely corresponding to those projecting to the DLH. They suggest that, following a local damage in the DLH, there is a feedback regulation of the TPH gene transcription in order to provide sufficient amount of TPH protein for the *de novo* synthesis of 5-HT. Supported by the NIH and the HSF of Québec.

241.6

EXPRESSION AND DELETION MUTAGENESIS OF TRYPTOPHAN HYDROXYLASE FUSION PROTEINS: ROLE OF HISTIDINE RESIDUES IN ENZYME FUNCTION. C.M. D'Sa, R.A. Arthur, Jr., and D.M. Kuhn*. Dept. Psychiatry & Beh. Neurosci., Wayne State Univ. Sch. Of Med., Detroit, MI 48201

Tryptophan hydroxylase (TPH) is the initial and rate limiting enzyme in the synthesis of the neurotransmitter serotonin. Complimentary DNAs encoding the full length enzyme and deletion mutants comprising the catalytic core of TPH (amino acids 99-444) were cloned and expressed in *E. coli* as GST-fusion proteins. Both full length TPH and the catalytic core were expressed as inactive apoenzymes which were converted to holoenzymes by the addition of ferrous iron It is not known which amino acid residues in TPH are involved in the coordination of iron to determine enzyme stability and catalytic activity. The TPH sequence contains a highly conserved His-X4-His motif within the catalytic core, representing a putative iron binding site. Treatment of purified GST-TPH fusion proteins with diethyl pyrocarbonate (DEPC), a highly specific histidine carbethoxylating reagent, significantly reduced TPH activity in a concentration- and time-dependent manner. The effect of DEPC was prevented by free histidine but not by lysine or tyrosine. DEPC inhibition of TPH was reversed by hydroxylamine. Putative iron binding sites in the catalytic core were studied for their role in catalysis by site-directed mutagenesis. Conserved His residues 272 and 277 were mutated to lysine residues as were the non-conserved His residues 258 and 343. Mutations of His 272 or 277 produced dramatic effects on TPH activity whereas mutations of the latter histidine residues did not. Taken together, these data indicate that histidine residues 272 and 277 form an important iron coordinating center within the catalytic core of TPH and are essential for the expression of catalytic activity.

SEROTONIN: GENERAL

242.1

IDENTIFICATION AND QUANTITATION OF SEROTONIN IN THE CROP OF THE COCKROACH *PERIPLANETA AMERICANA* BY HPLC WITH ELECTROCHEMICAL DETECTION. Z.A. Abdullah, S.L.Myracle, T.D.Kimbrough, P. Archer* and N. Narasimhachari. Dept. of Biology, Virginia Commonwealth University, Richmond, VA 23284.

Some previous reports have indicated no serotonin (5-HT) in the gut of the cockroach. In this study crop tissues of the cockroach *Periplaneta americana* were examined for serotonin by high performance liquid chromatography with electrochemical detection (HPLC-ECD). The cockroaches were either sham treated or treated with phenelzine sulphate (20ul; 5mg. phenelzine sulfate in 100 ml of saline) for 3 days. The denervated and untreated specimens were harvested, homogenized with 250 ul of mobile phase. After centrifugation the supernate was filtered and the clear filtrate (5 ul) was injected into hplc system. A Supelco C18,3um column with a Waters guard column was used. Phosphate buffer (0.01M pH 4.0 or pH 3.16) with 10.0% acetonitrile was the mobile phase and the flow rate was 1.2 ml/min. The oxidation potential was set at 0.9 volts. The serotonin level in the sham treated crop was 10.8 ng/mg and in the phenelzine treated one was 44.5ng/mg. We also found N-accetyl serotonin in the crop 0.81 ng/mg tissue. The homogenates were extracted into ethylacetate at pH 9.0, and the extracts evaporated under nitrogen, the residue redissolved in mobile phase and injected into hplc. Both 5-HT and N-acetyl 5-HT were identified in the extracts, thus confirming their identification.

242.2

MONOCLONAL ANTIBODIES TO BUFOTENINE Naokuni Takeda *

Department of Biotechnology, Research and Development center, COSMO Research Institute, Satte, Saitama, 340-01, Japan

There are no chemical indicators that can be used to diagnose the psychiatric abnormalities. We suggested that the presence and levels of bufotenine might be useful and important markers of some kind of psychiatric disorders (Neuro-Report 6: 2378-2380, 1995). Bufotenine (5-hydroxy-N, N-dimethylserotonin) is a dimethylated derivative of serotonin via N-methylserotonin. For the detection of bufotenine by the immunochemical methnod, monoclonal antibodies to bufotenine were made. Among three kind of hapten antigens, formylated antigen conjugating to BSA showed higher immunoreactivity than the others. By using spleen cells of immuno-Mice (Balve/c:female), cell fusions and clonings were performed repeatedly. The specificity of antisera was tested by the ELISA method with competition experiments. Among 14 strains, ten antibodies showed large cross-reactivity with both serotonin and N-methylserotonin and two antibodies with N-methyl-serotonin. However, we can get two kind of antibodies to bufotenine which is possible for the sample analysis with small cross-reactivity.

242.3

SEROTONERGIC AND DOPAMINERGIC OPTIC NERVE PATHWAYS IN RAT AND GOLDFISH. M. Urbina and L. Lima*. Lab. Neurochemistry, CBB, IVIC, Apdo. 21827, Caracas 1020-A

Serotonin (5HT)-amacrine cells occur in the fish retina, but not in rodents, the latter possess a raphe-retinal pathway non-demonstrated for the fish. The aim of this work was to correlate the concentration of 5HT, dopamine (DA) and their metabolites (ng/mg prot) determined by HPLC-ED in the retina and the optic nerve of rats and goldfish. The concentration of DA, homovanillic acid (HVA) and 3,4-dihydroxyphenylacetic acid (DOPAC) were 3.8, 1.2 and 1.8 for rat, and 5.3, 0.5 and 0.8 for goldfish retina. For 5HT and 5-hydroxyindoleacetic acid (5HIAA) the values were 0.3 and 0.09 in the rat, and 1.2 and 0.5 in the goldfish. DA, HVA and DOPAC were 0.4, 0.9 and 0.3 in the optic nerve of rat. In the goldfish DA was 0.3 5HT and 5HIAA levels were 0.4 and 0.3 in rat and 0.2 and 0.08 in goldfish. The ratio 5HT in retina/5HT in optic nerve were 0.7 and 6.2 in rat and goldfish, reflecting the in situ neuronal production for the goldfish. For DA, produced in both retinas, these ratios were 10.0 and 21.3. The effect of 5,7-dihydroxytryptamine icv or io has been evaluated. Rat ganglion cells contain DA, which might be its source in the optic nerve.

242.4

DIURNAL VARIATION IN CEREBRAL SPINAL FLUID (CSF) SEROTONIN CONCENTRATIONS IN HEALTHY HUMANS. P. D. Kirwin, C. J. McDougle*, G. M. Anderson, G. R. Heninger, T. D. Geracioti, P. B. Chappell, J. F. Leckman, L. H. Price. Clinical Neuroscience Research Unit, Yale Univ. Sch. of Med., New Haven, CT 06519.

The role of serotonin (5-HT) in the pathogenesis and treatment of depression continues to be the subject of extensive research. Previous studies examining central 5-HT functioning measured CSF levels of 5-hydroxyindoleacetic acid (5-HIAA) by using single or multiple lumbar punctures. Recently several groups have demonstrated the feasibility of continuous CSF sampling via an indwelling lumbar catheter. **Methods:** Four healthy female volunteers, aged 21-34 years, underwent continuous CSF sampling. CSF was collected at a constant rate of 1 ml every 10 minutes over a 30-hour period, with levels of tryptophan (TRP) and 5-HIAA, measured every hour. Plasma was also obtained hourly for TRP determination. **Results:** The results of this study indicate a trend toward significant diurnal variation in CSF 5-HIAA levels. CSF TRP and plasma TRP levels showed variation over time, but failed to show diurnal fluctuation. **Conclusion:** Continuous CSF sampling is safe and feasible in humans, and may prove useful for studies of central neurochemicals in neuropsychiatric illness.

Funding Source: National Institute of Mental Health

242.5

DISTRIBUTION OF 5-HT TERMINALS AND ASCENDING PROJECTIONS OF THE DORSAL AND MEDIAN RAPHE NUCLEI IN THE HAMSTER. LP Morin* and E Meyer-Bernstein. Dept. Psychiatry, HSC, Stony Brook Univ., NY 11794.

5-HT neurons in the dorsal (DR) and median (MR) raphe differentially innervate nuclei of the circadian rhythm system (J. Neursci. 16, '96). However, other targets of the DR and MR have not been described in the hamster. Therefore, we used iontophoretic injections of *Phaseolus vulgaris* leucoagglutinin to trace the ascending DR and MR projections. Those patterns are compared to the normal distribution of 5-HT fibers and terminals, also not previously described in the hamster.

The DR projects in a moderately robust fashion to most areas. Regions which receive little, if any, innervation include several layers of the hippocampus, olfactory tubercle, medial habenula, arcuate n., suprachiasmatic n., medial geniculate (ventral), reticular n., oculomotor n. and medial terminal n. MR projections are not found in many areas including much of the basal ganglia and ventral telencephalon, bed nucleus of the stria terminalis, arcuate n., supraoptic n., ventromedial n., medial habenula, non-midline thalamic nuclei, most of the pretectum, the accessory optic n. and outer layers of the superior colliculus. Normal 5-HT innervation is generally quite robust, although there is great variation in detail. E.g., cortical layer 1, unlike the inner layers, is very densely innervated. The pattern of 5-HT innervation is more similar to the distribution of DR than MR efferents. However, because the raphe nuclei have non-5-HT efferents as well, it is not presently possible to determine the extent to which either the MR, DR or both contribute to normal 5-HT innervation.

Supported by NS22168 to LPM.

242.6

MORPHINE-INDUCED INCREASE IN EXTRACELLULAR SEROTONIN (5-HT) RELEASE IS ATTENUATED DURING PROLONGED TREATMENT. R. Tao*, Z. Ma, S.B. Auerbach. Nelson Biol. Lab., Rutgers Univ., Piscataway, NJ 08855.

The aim of this study was to determine if tolerance to morphine-induced increases in 5-HT release develop during prolonged treatment. 5-HT was measured by *in vivo* dialysis in the dorsal raphe nucleus (DRN) of freely behaving rats. After subcutaneous implantation of 2 morphine pellets (75 mg size), 5-HT was increased by 46.0±5.6% (n=5; $P<0.05$) above levels in the DRN of rats implanted with placebo pellets. Morphine pellets also induced analgesia as measured by a 11.7±3.1 sec increase (n=5; $P<0.05$) in hot plate latencies. In contrast, with 2 pellets in place for 7 days, extracellular 5-HT in both treatment groups were no longer significantly different: morphine, 4.5±1.0 pg (n=6); placebo, 5.0±1.5 pg (n=6). Furthermore, implantation of 2 more morphine pellets at this time produced no significant increase in extracellular 5-HT (9.4±7.1%, n=6; $P>0.05$) or hot plate latencies (0.8±0.4 sec, n=6; $P>0.05$). As a further test, rats were exposed to morphine or placebo pellets for 14 days. One day after removal of these pellets, challenge with morphine (20 mg/kg, sc) produced no significant increase in DRN 5-HT in the morphine treatment group (4.4±11.9%, n=6), compared with a significant increase in placebo rats (55.5±16.4%, n=4). These results suggest that tolerance to the 5-HT-enhancing and analgesic effects of morphine develop in parallel. Supported by NIMH grant #51080.

242.7

IMPAIRED DEVELOPMENTAL ELIMINATION OF CALLOSAL AND CLAUSTRAL PROJECTIONS TO CORTICAL AREAS 17 AND 18 IN CATS NEONATALLY DEPLETED OF SEROTONIN. K. Turlejski*, R. Djavadian. Nencki Institute of Experimental Biology, 3 Pasteur st., 02-093 Warsaw, Poland.

Some projections to areas 17 and 18 in the newborn cat are broad and are later limited by the process of axon elimination. We studied the influence of serotonin depletion on this process. Three experimental and one normal kitten were injected on the first two postnatal days with desipramine (0.02 mg/g) and then with 5,7-dihydroxytryptamine (5,7-DHT, 0.075 mg/g). At the age of three months several injections of Fast Blue were done into the cortical areas 17 and 18 of one hemisphere in each cat under Rompun and ketamine anesthesia. After one week survival the animals were killed with an overdose of pentobarbital and perfused with 4% paraformaldehyde. The brains were freeze-cut for 40 μm. Immunolabelling of spare sections for serotonin showed that serotonergic axons were scarce in the areas 17 and 18 of the lesioned animals. In the normal kitten callosally projecting neurons were limited to the transient zone of areas 17/18 (representation of the vertical meridian) and the part of area 18 representing the visual field 10-15 deg from the vertical meridian. In the 5,7-DHT injected kittens far periphery of the area 17 was also void of callosal projection, but the retrogradely labelled neurons were found in its part representing 5-10 deg from the vertical meridian. Callosally projecting neurons were also found in almost all of the area 18. Another structure that showed a significantly changed pattern of cortical projections was claustrum. In the ipsilateral claustrum of the 5,7-DHT injected cats the number of the labelled neurons was increased by 30-50%, while in the contralateral claustrum the labelled neurons were 2-3 times more numerous. These results show that depletion of serotonin disturbs the developmental process of partial elimination of excessive projections that finally shapes the cortical connections.

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242.8

HYPOTHALAMIC SEROTONERGIC ACTIVITY AND BRAIN TEMPERATURE ACROSS THE SLEEP-WAKE CYCLE. L. Imeri*, C. Gemma, M.G. De Simoni°, M.R. Opp§ and M. Mancia. Istituto di Fisiologia Umana II, Università degli Studi, 20133 Milano, Italy; °Ist. Mario Negri, 20157 Milano, Italy; §Dep. of Psychiatry & Behavioral Sciences, UTMB, Galveston TX 77555-0428, USA.

Recordings of serotonergic raphe cells discharge rates or of 5-HT release indicate that the activity of these cells varies in phase with the sleep-wake cycle. Sleep and waking are associated with changes in several physiological functions, including EEG activity, brain temperature, and locomotion. The aim of the present study was to clarify which of these parameters better correlates with serotonergic activity.

To this purpose, voltammetric recordings of 5-HT metabolism in the Medial Preoptic Area and polygraphic recordings of fronto-parietal EEG, nuchal EMG activity and of brain cortical temperature (T_{cort}) were simultaneously performed in freely moving rats. Correlations between 5-HT metabolism and the variables under investigation (sleep-wake activity, power in the EEG delta band, T_{cort} and EMG activity) were estimated.

Univariate analyses of variance revealed that each variable was statistically correlated with 5-HT metabolism. When the variables were entered into the model simultaneously, both partial correlation and stepwise multiple regression analyses indicated that the highest correlation exists between 5-HT metabolism and T_{cort}.

Since T_{cort} could be regarded as a parameter representing the integral of different physiological variables, the data in this study are in agreement with the hypothesis that serotonergic activity is reflective of the general behavioral state of the organisms (Jacobs, B.L. and Azmitia, E.C., *Physiol. Rev.*, 1992, 72:165-229).

242.9

LEVELS OF 5-HT IN THE HIPPOCAMPAL FORMATION OF THE RAT FOLLOWING ELECTRICAL STIMULATION OF THE DORSAL OR MEDIAN RAPHE NUCLEI D.J. Mokler*, D. LaRiviere, S. Sinclair, E. Housner, D. W. Johnson, J. Bronzino[1], and P. Morgane. Depts. Pharmacol. & Physiol., University of New England, Biddeford, Me. and (1) Trinity College, Hartford, Ct..

Sprague-Dawley rats were surgically implanted with stainless steel bipolar electrodes into either the dorsal raphe nucleus (DRN) or the median raphe nucleus (MRN); and guide cannula into the dorsal hippocampus (DH) or the ventral hippocampus (VH), respectively. Probes were inserted into the hippocampus on the day of the experiment without anesthesia and perfused with artificial CSF containing 1 μM sertraline. Following three hours for stabilization, electrical stimulation (ES) was given for twenty minutes (200μA, .1-.5 msec, 10-20Hz). Twenty min samples were collected over an additional two hours. At the completion of the experiments electrode and probe placements were verified histologically. Basal levels of 5-HT were 2 fmoles/sample in the DRN-DH and 6 fmol/sample in the MRN-VH in the presence of sertraline. ES of the dorsal raphe nucleus at 20 Hz resulted in an increased release in 5-HT in a stimulus-bound manner. ES of the MRN at 0.5 msec, however, led to a decrease in 5-HT release. Changes in 5-HT release following ES may affect 5-HT terminal autoreceptor activation, thus decreasing release following MRN stimulation. ES may also enhance synaptic release following DRN stimulation. (Supported, in part, by grant HD 22539.)

242.10

INCREASED DAILY TURNOVER OF NORADRENALINE AND SEROTONIN IN VENTRAL MEDIAL HYPOTHALAMUS (VMH) OF OBESE VERSUS LEAN ZUCKER RATS ASSESSED BY IN VIVO MICRODIALYSIS. S. Luo*, J. Luo, S. Hodge and A. H. Cincotta. Ergo Sci. Dev. Corp., Charlestown, MA 02129.

Noradrenergic activation of the VMH is known to increase feeding, hepatic glucose output, and adipose lipolysis while inducing obesity in mammals. Moreover, serotonin agonists have been shown to potentiate noradrenergic release within the hypothalamus. Recently, we showed that improvements in body composition and insulin sensitivity are strongly associated with decreases in 24 hr VMH turnover of noradrenaline and serotonin in naturally obese Syrian hamsters. The present study employed *in vivo* microdialysis to investigate the temporal changes of monoamine profile in the VMH of freely moving lean and obese Zucker rats. Rats (9 weeks old) held on 12 hr daily photoperiods were cannulated at the right VMH. Dialysate samples were collected every hour for 24 hr through the microdialysis probe (perfused with Ringer's solution at 0.12 µl/min), and analyzed by HPLC with electrochemical detection. Blood samples were obtained from the tail vein before microdialysis to measure blood glucose, insulin and free fatty acid (FFA) levels. Plasma insulin and FFA levels in obese rats were dramatically higher than in lean rats (407 ± 28 vs 49 ± 8 µU/ml and 2.1 ± 0.4 vs 0.6 ± 0.1 mmol/L respectively, $p<0.01$), while plasma glucose levels were also higher than in lean rats (198 ± 8 vs 169 ± 5 mg/dl, $p<0.01$). Extracellular levels of metabolites of serotonin (5-hydroxyindolacetic acid) and norepinephrine (3-Methoxy-4-hydroxy-phenylglycol) within the VMH were 40% higher ($p<0.05$) in the obese relative to lean counterparts throughout a day. These findings, along with other available evidence, suggest that increased noradrenergic and serotonergic activity within the VMH of obese Zucker rats is associated with and may contribute to the development of hyperinsulinemia and obesity.

Ergo Sci. Dev. Corp.

242.11

SEROTONERGIC AND CHOLINERGIC MODULATION OF VISUALLY-IDENTIFIED INTERNEURONS IN SLICES OF IMMATURE RAT SENSORIMOTOR CORTEX. Foehring, R.C., van Brederode, J.F.M., and Spain, W.J.*, Dept. of Anatomy & Neurobiology, Univ. of Tenn., Memphis, TN 38163 and Dept. of Neurology, VA Medical Center, Seattle, WA 98108.

Very little is known concerning the effects of modulatory neurotransmitters on neocortical interneurons. We therefore tested the effects of serotonin (5HT: 30-60 μM) and the cholinergic agonist carbachol (10 μM) by recording from visually-identified interneurons in layers I-III using infrared video-microscopy in brain slices (300 μM) from rats aged P9-P21. Whole-cell recordings were made in current-clamp mode at 30ºC using a K-methylsulphate internal (0.1mM EGTA, 0.5% Biocytin). Cell type was confirmed in all cases by histological analysis after recording. Neurons accepted for study had resting potentials (RPs) of at least -60 mV, fired repetitively in response to long current injections and had overshooting action potentials. Of the first 16 cells tested, 13 were confirmed by histology to be interneurons. 11 of these were in layer I and 2 in layer II. In addition, we recorded from 3 pyramidal cells. Seven of 13 interneurons were hyperpolarized (2-9 mV) and the remaining 6 were depolarized (4-15 mV) by 5HT. Variable changes in input resistance accompanied these membrane potential changes. In the pyramidal neurons, 5HT hyperpolarized 1 cell and depolarized 2 cells, and all 3 cells fired more rapidly in response to long current injections (at original RP). This increased firing was accompanied by decreased sAHPs and the induction of an afterdepolarization (ADP) after repetitive firing. Carbachol caused a depolarization (2- 15 mV) in 4/4 interneurons tested and in 3/4 cells, an ADP followed repetitive firing in carbachol. We have not found a clear correlation between morphological cell type and response to 5HT or carbachol. Supported by NINDS grant NS33579 (R.C.F.) and a VA Merit award (W.J.S.).

242.12

COLD INCREASES SYMPATHETIC ACTIVITY AND SEROTONIN IN SPINAL CORD MICRODIALYSATES. A.M. Passerin* and W.N. Henley. Dpt. of Biol. Sci., Ohio U., Athens, OH 45701.

We have previously reported increases in serotonin (5HT) metabolism in spinal cord following 2, 8, 24, 48, and 96hr of cold exposure (3°C). Additionally, increased synthesis of 5HT was found after 24 hr at 3°C. To further investigate the importance of 5HT for cold-induced sympathoexcitation, microdialysates were obtained from spinal cords of urethane-anesthetized, temperature controlled, adult, male Sprague-Dawley rats (L1 or L2; intermediolateral region; CSF, 1.2μl/min). Dialysates were analyzed for [5HT] using HPLC-EC. Blood pressure and HR were also measured. [5HT] was stable 2.5hr. after probes were implanted; recovery averaged 28.1%. An ice pack was placed on the caudal half of the rat's body. When rectal temperature fell below 33.5°C, increases ($p<0.05$) in [5HT] in dialysates were noted. These cold-induced increases in 5HT were accompanied by significant ($p<0.05$) increases and decreases in MAP and HR, respectively; cold effects were reversible. In a second experiment the activity of sympathetic nerves innervating brown adipose tissue was recorded during application of a similar cold stimulus. It was found that when rectal temperature was dropped below 33.5°C, nerve activity increased in a reversible manner. Together, these data suggest that 5HT release may be an important mechanism associated with cold-induced sympathoexcitation. Supported by the Ohio U. College of Osteopath. Med.

242.13

CENTRAL SEROTONIN METABOLISM CORRELATES WITH AUTONOMIC CONTROL AND THYROID STATUS. W.N. Henley*, P.I.Harness, T.J.Koehnle, and L.L. Bellush. Dpts. of Biol. Sci. and Psychol., Ohio U., Athens, OH 45701.

Adult male, Sprague-Dawley rats were placed on either 0.02% methimazole/water (HYPO; n=8) or were maintained on tap water. After 2 wks, HYPO and 8 euthyroid rats (EUTH; n=8) had placebo implants while 3 additional groups of euthyroid rats had either 2.5, 5.0, or 7.5mg triiodothyronine (T_3) pellets implanted (s.c.; n=8/group). Overnight urine collections were obtained 5d post-implant and rats were killed by decapitation 7d post-implant. Neurochemical analysis (HPLC-EC) indicated significant ($p<0.05$) increases in serotonin metabolism ([5HIAA], 5HIAA/5HT, or both) in brainstem and spinal cord regions (T1-T5, T6-T10, T11-L2) from hypothyroid rats. Decreased 5HT metabolism was observed in all 3 spinal cord regions in rats with T_3 implants when compared to EUTH or HYPO. Similar significant increases in urinary norepinephrine excretion with HYPO and decreases with T_3 implants were also seen. Statistical analysis indicated that a significant correlation existed between central 5HT metabolism and urinary NE excretion with changes in thyroid status. Preliminary findings indicate that selective brain stem replacement of T_3 in HYPO may elicit cardiovascular responses predicted by the neurochemical changes reported here. Supported by the Ohio U. Coll. of Osteopath. Med.

242.14

IN VIVO ELECTROCHEMICAL MEASUREMENTS OF EXTRACELLULAR SEROTONIN CLEARANCE IN RAT DORSAL HIPPOCAMPUS. L.C. Daws*[1], G.M. Toney[1], G.A. Gerhardt[2] and A. Frazer[1] [1]Dept. of Pharmacology, University of Texas, HSC, San Antonio, TX 78284. [2]Dept. of Psychiatry, University of Colorado, HSC, Denver CO, 80262.

The regulation of 5-HT transporter function has been studied by several techniques. However, few have used rapid (5-10 Hz) *in vivo* electrochemical detection techniques to measure uptake and clearance of exogenously applied serotonin (5-HT). Male Sprague-Dawley rats were anaesthetized with chloralose/urethane and a Nafion-coated, carbon fiber electrode, attached to a multibarrel micropipette, was positioned in the CA1 region or dentate gyrus (DG) of the hippocampus. Serotonin (10 to 75 nl, 2 to 15 pmol), the selective 5-HT reuptake inhibitor fluvoxamine (FLUV), the selective norepinephrine reuptake inhibitor desipramine (DMI), or vehicle, were pressure ejected (50 to 200 nl, 2.5 to 200 pmol), 150-200 μm from the recording electrode. Serotonin signals were reproducible and dependent on the volume delivered. Vehicle injection produced no detectable effect alone and did not alter the 5-HT signal. FLUV had no effect at doses lower than 10 pmol, but increased the total time course of the 5-HT signal by 90 ± 18 % (n=7 rats) at 25 pmol, and by 200 ± 40 % (n=6) at 55 pmol. Surprisingly, DMI produced comparable dose-dependent increases in the time course of the 5-HT signal. Responses were similar in the CA1 region and DG. By contrast, in the corpus callosum, a fiber tract devoid of transporter, neither drug altered the 5-HT response. In rats treated 3-4 weeks previously with 6-hydroxydopamine (6-OHDA, 200 μg i.c.v. with citalopram pretreatment), FLUV produced similar results to those observed in intact rats; however, DMI was without effect. Further, for a given amount of applied 5-HT, the signal amplitude was increased 2-fold in 6-OHDA treated rats. Taken together, the data support the hypothesis that both serotonergic and noradrenergic systems are involved in regulating the clearance of exogenously applied 5-HT. (Supported by funds from USPHS (MH29094), NSF (BIR913392) the VA and The Upjohn Co.).

242.15

MORPHOLOGICAL STUDIES OF MICE OVEREXPRESSING THE SEROTONERGIC GROWTH FACTOR S-100β. P.M. Whitaker-Azmitia, R. Gerlai, M. Wingate and A. Borella. Dept. of Psychiatry, SUNY, Stony Brook, New York, 11794-8101.

S-100β is a neurotrophic factor released by astroglial cells in response to stimulation of the serotonergic 5-HT1a receptor. Once released, S-100β acts as a serotonergic and cortical trophic factor. Thus, animals overexpressing this trophic factor would be expected to show significant changes in the development of cortex and the serotonergic system. Moreover, since the gene for S-100β is within the oligate region for Down's Syndrome (DS), and high levels of S-100β are found in DS patients, animals overexpressing S-100β may serve as a model for DS.

Mice overexpressing S-100β, a gift from Dr. John Roder, Mt. Sinai Research Institute in Toronto, Canada, were produced by insertion of a human genomic fragment containing the three exons and the transcription control elements of the S-100β gene. The resultant animals express S-100β levels approximately 5X that of control animals and have learning deficits.

Control CD1 and transgenic mice were perfused at one year and immunochemically stained for the dendritic marker MAP-2. Tissue was analyzed densitometrically and morphologically using the Optima system. One year old transgenic animals showed a statistically significant 38% increase in thickness of cortical layers 1-4, a 23% decrease in layer 5 and no change in layer 6. In hippocampus, thickness of the granule cell layer of the dentate gyrus showed a 25% increase with no change in the molecular layer.

Finally, transgenic animals showed pronounced somatic staining of MAP-2 in hippocampus, while normals showed MAP-2 staining confined to dendrites, as expected. (Funding through National Institute on Aging).

242.16

DIFFERENTIAL VULNERABILITY TO AGING IN THE RAT SEROTONERGIC SYSTEM. A. Nishimura[1,2]*, S. Ueda[3], Y. Takeuchi[2], T. Sawada[2], M. Kawata[1]. [1]Dept. of Anatomy and Neurobiol., and [2]Dept. of Pediatrics, Kyoto Pref. Univ. of Med., Kyoto 602, Japan., and [3]Dept. of Histology and Neurobiol., Dokkyo Univ. Sch. of Med., Tochigi 321-02, Japan.

During the process of aging, the structure and the distributional pattern of many chemically identified neuronal systems in the central nervous system are altered. Morphological aberrations of serotonergic (5-HT) fibers have also been reported previously (Van Luijtelaar et al., 1992, Nishimura et al., 1995). The present study examines immunohistochemically the distributional changes associated with aging of the 5-HT neuron system. Wistar rats ranging from 3 months old (young adult) to 3 years old (aged) were used. In the aged rat brain, abnormal morphology of the 5-HT processes was noted. Aberrant-shaped 5-HT fibers observed in the aged rat were classified into two groups: One was thin fibers with a large number of varicosities outgrowing like bunches of grapes, and the other was thick fibers with smooth-surfaced varicosities distorting and clustering like a ball of worsted. The former type of fibers was observed in the regions projecting from the dorsal raphe nucleus, whereas the latter existed in the restricted areas projecting from the median raphe nucleus. These findings suggest that the vulnerability to aging in these two types of aberrant 5-HT fibers may be different.

242.17

ELECTROCONVULSIVE SHOCK INCREASES INTRANEURONAL STAINING OF THE SEROTONERGIC GROWTH FACTOR, S-100β. J. Bell 3rd *, M. Halasz, A. Borella, E. Azmitia and P.M. Whitaker-Azmitia Dept. Psychiatry, SUNY, Stony Brook, New York, 11794-8101.

Work from our lab has shown that serotonergic 5-HT1a receptor agonists can release S-100β and that once released S-100β can act as a trophic molecule both for serotonergic and cortical neurons. Once released, the molecule influences phosphorylation states of neuronal proteins such as MAP-2 and GAP-43.

Electroconvulsive shock (ECS) in animals has been shown to release high amounts of serotonin and animals lesioned with the serotonergic neurotoxin, 5,7-DHT, do not show the usual neurochemical changes associated with ECS. Thus, serotonin appears to be involved in the response to ECS. The current study was therefor undertaken to determine if serotonin-released S-100β might be increased by ECS.

Adult male Sprague-Dawley rats were treated with unmodified ECS every other day for ten days. On Day 11, control and treated rats were perfused and immunocytochemically stained for S-100β and the dendritic marker MAP-2. S-100β immunostaining is generally confined to astroglial cells of the adult rat brain, however the ECS animals showed high levels of intraneuronal staining. As well, there was an increase in MAP-2 staining principally due to increased dendritic size, rather than number.

Our results suggest that ECS may have its therapeutic effects throught serotonergic mediated release of the trophic factor, S-100β.

(Funding through National Institute on Aging).

SEROTONIN: BEHAVIOR

243.1

IDENTIFICATION OF NOVEL GENES REGULATING BRAIN SEROTONERGIC HYPERINNERVATION BY DIFFERENTIAL DISPLAY RT-PCR IN MURINE ANOREXIA (*ANX*) MUTANT. C.H. PENG, B. CONTI, J.W. JAHNG, T.H. JOH, S.J. KIM* AND J.H. SON. Cornell University Medical College at The W.M.Burke Medical Research Institute, White Plains NY, 10605.

The central serotonergic system modulates many important physiological functions including appetite, sleep, motor activity, endocrine secretion, sex activity, learning and memory. The developmental and functional alterations in serotonergic systems have been implicated in human neuropsychiatric disorders. The serotonergic system matures postnatally and the target innervation density seems to depend on unknown factors present in the mature target tissues. Previous work from this laboratory has demonstrated extensive serotonergic hyperinnervation in target fields such as hippocampus, cortex and cerebellum, but no serotonergic hyperinnervation in nontarget fields, in the brain of an *anorexia (anx)* mutant (Son et al. *Mol. Brain Res.*, 25, 129 '94). In order to identify novel mRNAs which are specifically regulated by or contribute to the serotonergic hyperinnervation in the brain of *anx* mutant, PCR differential display was employed. Total RNAs from both hippocampus and cortex of either *anx* mutant or normal littermate were isolated and used for RT-PCR at postnatal day 16. By comparing the mRNAs from both *anx* and normal littermate brains, a total of 25 differentially displayed bands between 150 to 400 bp were identified. Of these, 5 clones were selected based upon their brain region-specific expression pattern determined by *in situ* hybridization. Each differentially displayed band is being further examined in order to elucidate the molecular nature of serotonergic hyperinnervation in the brain of *anx* mutant.

(Supported in part by NIH grant MH24285)

243.2

S 15535-3, A 5-HT$_{1A}$ RECEPTOR PARTIAL AGONIST, INCREASES RATES OF PUNISHED RESPONDING IN RATS: COMPARISON WITH CHLORDIAZEPOXIDE, IPSAPIRONE AND WAY 100635. R. Samanin#, C. Bonvicini#, M. J. Millan°, E. Mocaer°, M. T. Tacconi#* and L. Cervo#. #Istituto di Ricerche Farmacologiche "M. Negri", Milano, Italy, °Institut de Recherches Internationales Servier, France.

The effect of S 15535-3, a highly selective agonist and antagonist (weak partial agonist) at pre- and post-synaptic 5-HT$_{1A}$ receptors respectively, was compared with that of chlordiazepoxide, ipsapirone and the 5-HT$_{1A}$ receptor antagonist WAY 100635 in a Geller-Seifter conflict test. Administered orally 1 h before testing , S-15535-3 at 10 and 30 mg/kg selectively increased punished responses. The same effect was found with 3-30 mg/kg chlordiazepoxide administered orally 1 h before testing. Administered subcutaneously 30 minutes before testing, S-15535-3 significantly increased punished responses and reduced unpunished responding at 3 (but not 0.3 and 1) mg/kg. Ipsapirone administered subcutaneously at 5 and 10 mg/kg 30 minutes before testing significantly increased punished responses. The higher dose of ipsapirone reduced unpunished responding. At subcutaneous doses ranging from 0.1 to 1 mg/kg administered 1 h before testing WAY-100635 had no effect on punished and unpunished responses. The results have shown that S 15535-3 has benzodiazepine-like effects in a conflict test, whereas WAY-100635 causes no effects in this test. The results suggest that S 15535-3 may have favourable effects in the treatment of anxiety disorders.

The study was supported by Servier, Courbevoie, France.

243.3

ANTI-AGGRESSIVE EFFECTS OF S-15535, A NOVEL AND SELECTIVE ANTAGONIST AT POSTSYNAPTIC 5-HT$_{1A}$ RECEPTORS AND AGONIST AT 5-HT$_{1A}$ AUTORECEPTORS. E. Mocaer[1], M. Lesourd[1], S.F. de Boer[2], B. Bohus[2]* and J.M. Koolhaas[2]. [1]Institut de Recherches Internationales Servier, 6 place des Pléiades, 92400 Courbevoie, France; [2]Department of Animal Physiology, University of Groningen, P.O. Box 14, 9750 AA Haren, The Netherlands.

The novel benzodioxopiperazine S-15535 potently acts as a competitive antagonist at postsynaptic 5-HT$_{1A}$ receptors and as an agonist at 5-HT$_{1A}$ autoreceptors. This unique pharmacological profile makes S-15535 a useful tool for assessing presynaptic and/or postsynaptic mediation of 5-HT$_{1A}$-induced responses. Recently, we convincingly demonstrated that selective 5-HT$_{1A}$-receptor agonists specifically suppresses offensive aggression. It is not known, however, whether this anti-aggressive effect is pre- or postsynaptically mediated. Therefore, the present experiments investigated the effects of S-15535 (0.5, 1, 2.5 and 10 mg/kg), given either alone or in combination with the selective 5-HT$_{1A}$ receptor antagonist WAY 100635 (0.1 mg/kg) on offensive and defensive aggression in wild-type rats using a resident-intruder paradigm. Commencing at 45 min (WAY-100635) and 30 min (S-15535) after SC injection of drugs or vehicle, behavior of drugged resident rats (offensive aggression test) or drugged intruder rats (defensive aggression test) was examined during a 10 min social encounter. S-15535 exerted a potent dose-dependent decrease in offensive aggressive behavior in resident rats (ED_{50}=1.34 mg/kg), which was completely antagonized by WAY-100635. Other non-social behavioral elements, i.e., exploration, grooming and inactivity, as well as defensive aggression were not significantly affected by S-15535. No anti-aggressive effects were seen after WAY-100635 treatment alone. The data shows that S-15535, similar to other selective 5-HT$_{1A}$ agonists i.e., alnespirone, effectively and specifically suppresses offensive aggression.Given the pharmacological properties of S-15535, these data provide evidence that the anti-aggressive effects of 5HT$_{1A}$ receptor agonists are mediated via their action on 5-HT$_{1A}$ autoreceptors.

243.4

ANTI-AGGRESSIVE EFFECTS OF ALNESPIRONE (S-20499), A POTENT 5-HT$_{1A}$ RECEPTOR AGONIST, IN WILD-TYPE RATS. M. Lesourd[1], S.F. de Boer*[2] and J.M. Koolhaas[2]. [1]Institut de Recherches Internationales Servier, 6 place des Pléiades, 92400 Courbevoie, France; [2]Department of Animal Physiology, University of Groningen, P.O. Box 14, 9750 AA Haren, The Netherlands

Alnespirone (S-20499) is a novel selective and potent 5-HT$_{1A}$ receptor agonist. Since the 5-HT$_{1A}$ receptor is known to play an important role in anxiety, S-20499 has reliably been reported to possess potent anxiolytic activity in a variety of animal behavioral models of anxiety. Besides the involvement in anxiety, 5-HT$_{1A}$-receptors are reported to be involved in aggressive behavior as well. Therefore, the present experiments investigated the effects of S-20499 (1, 5, 10 mg/kg) on offensive and defensive aggression in wild-type rats using a resident-intruder paradigm, in comparison to 8-OHDPAT (0.05, 0.1, 0.25, 1.0 mg/kg), eltoprazine (1 mg/kg) and ipsapirone (5 mg/kg). Thirty min after SC injection of drugs or vehicle, behavior of drugged resident rats (offensive aggresion test) or drugged intruder rats (defensive aggression test) was examined during a 10 min. social encounter. S-20499, like 8-OHDPAT, eltoprazine and ipsapirone, exerted a potent dose-dependent decrease in offensive aggressive behavior in resident rats. Other non-social behavioral elements, i.e., exploration, grooming and inactivity, were not significantly affected by S-20499. In contrast, the anti-aggressive profile of eltoprazine and especially ipsapirone and 8-OHDPAT was paralleled by a strong increase in behavioral inactivity. Pretreatment with the selective 5HT$_{1A}$ receptor antagonist WAY 100635 (0.1mg/kg) completely antagonized the anti-aggressive effects of S-20499 as well as the behavioral effects of 8-OHDPAT, eltoprazine and ipsapirone. In the defensive aggression test, S-20499 did not significantly change any behavioral element of the intruder rats. The data indicate that S-20499 effectively and specifically suppresses offensive aggression without interfering with adequate defensive aggressive behavior. Furthermore, these data provide convincing evidence for the involvement of 5HT$_{1A}$ receptors alone in the modulation of aggressive behavior.

243.5

B-20991, A NOVEL SELECTIVE $5HT_{1A}$ RECEPTOR AGONIST WITH A POTENTIAL ANXIOLYTIC PROFILE. Beneytez, M.E.[1], López Rodríguez[2], M.L. Morcillo[2], M.J. Rosado[2], M., Orensanz[3], L.,Fuentes, J.A.[1] and Manzanares, J*[1]. [1]Department of Pharmacology, School of Pharmacy, [2]Department of Organic Chemistry , School of Chemistry, Complutense University, and [3]Department of Research, Ramon y Cajal Hospital, Madrid 28040, Spain

The purpose of this study was to characterize the effects of 2-[4-(o-Methoxyphenyl)piperazin-1-ylmethyl]-1,3-dioxope rhydroimidazo[1,5-α]pyridine (B-20991) on several biochemical and behavioral assays. Rat brain membranes were used to carry out binding affinity receptor studies for the following receptors: $5HT_{1A}$, $5HT_2$, D_2 and α_1-adrenoceptor.The pharmacological activity of B-20991 was estimated performing the following assays after s.c. administration: 1) hypothermia in mice, 2) 5HT syndrome (flat body posture, FBP; lower lip retraction, LLR) in rat, 3) 5HT and DA neuronal activity in mice hypothalamus by HPLC-EC, 4) open-field locomotor activity (LA) in mice. The anxiolytic activity of B-20991 was assessed by using the social interaction (SI) and light/dark box (LDB) tests.

Results of binding studies showed that B-20991 binds with high affinity to the $5HT_{1A}$ receptor (K_i =31.7±1.7 nM) and low affinity (K_i> 1000) to $5HT_2$,D_2 and α_1-adrenergic receptors. B-20991 caused a dose- and time-related decrease in rectal temperature, increased FBP and LLR, decreased 5HIAA/5HT ratio in hypothalamus, did not alter LA and increased SI and LDB scores.

Taken together, these results suggest that B-20991 is a selective $5HT_{1A}$ receptor agonist with anxiolytic activity.

243.6

EQUIVALENT EFFECTS OF STIMULATION OR BLOCKADE OF 5-HT1A RECEPTORS UPON AMPHETAMINE-INDUCED LOSS OF AUDITORY GATING. **K.E. Stevens*, R.G. Johnson, J. Luthman and G.M. Rose. Dept. Psychiatry, Pharmacology, and Neuroscience, Univ. Colorado Health Sci. Ctr; VA Medical Center, Denver, CO and the Karolinska Institute, Stockholm, Sweden.**

Deficient sensory filtering or "gating", which is a hallmark of schizophrenia, can be demonstrated as an inability to inhibit the response to the second of a pair of identical click stimuli given at a 0.5 sec interval. This deficit can be mimicked in rats by administration of the psychotomimetic, amphetamine. Clinical success with the atypical neuroleptic clozapine, which also improves gating in schizophrenics, has focused attention on the serotonergic system. The 5-HT1A receptor is of specific interest since it is localized almost exclusively in limbic structures, certain of which have been implicated in schizophrenia and the concomitant sensory filtering deficit. Administration of a 5-HT1A antagonist, (-)UH-301 (5,2,0.5 mg/kg, s.c.), produced a normalization of amphetamine-induced loss of gating while a lower dose (0.1 mg/kg, s.c.) was not effective. (-)UH-301 alone had no significant effect upon gating. Interestingly, administration of a 5-HT1A agonist, 8-OH-DPAT (0.1 mg/kg, s.c.) also normalized amphetamine-induced loss of gating. Again, a low dose (0.05 mg/kg, s.c.) was not effective. These data suggest an important function for the 5-HT1A receptor in the modulation of auditory gating and, further, suggest that both pre- and post-synaptic receptors are involved. The present studies may have implications for the use of serotonergic compounds in the clinical treatment of schizophrenia. Supported by NIMH R29 MH51931-02.

243.7

DOI, A 5-HT2 AGONIST, NORMALIZES AUDITORY GATING IN ISOLATION-REARED RATS. **R.G. Johnson[1]*, K.E. Stevens[2,3] and G.M. Rose[1,3]. Depts. of [1]Pharmacology and [2]Psychiatry, UCHSC and [3]Medical Research, VAMC, Denver, CO 80220**

Auditory gating is a form of sensory processing which is impaired in schizophrenia. We have previously shown that rats which are reared from weaning in social isolation also show gating deficits. In such rats, as with schizophrenics, gating is not improved after administration of the neuroleptic, haloperidol. However, gating in schizophrenics is normalized by clozapine, an atypical neuroleptic with actions at serotonin receptors. The goal of the present study was to determine whether DOI (2,5,-dimethoxy-4-iodoamphetamine), a selective agonist for $5\text{-}HT_2$ receptors, would restore gating in isolation-reared rats. The animals were prepared for recording by implanting a skull screw at vertex (-4.0 from bregma, on midline). After recovery, auditory evoked potentials were recorded in response to paired clicks (87 dB, 0.5 sec between clicks). Isolation-reared rats consistently showed impaired gating in this paradigm. However, administration of DOI (2.5 mg/kg, i.p.) normalized gating for a period lasting 2-3 hours. These data suggest a role for the $5\text{-}HT_2$ receptor in the modulation of auditory gating, and may have implications for future therapeutic interventions for schizophrenia. Supported by MH-44212 and the VAMRS.

273.8

INVOLVEMENT OF SEROTONERGIC NEURON SYSTEM IN THE RAT AGGRESSIVE BEHAVIOR (MURICIDE). S.Ueda*, M.Aikawa, A.Nishimura, K.Yoshimoto. Dept. of Histology and Neurobiology, Dokkyo Univ. Sch. of Med., Tochigi 321-02, Japan, Dep. of Pediatrics and Legal Medicine, Kyoto Pref. Univ. of Med., Kyoto 602, Japan.

Transplantation of fetal serotonergic neurons into the hypothalamus restored mouse killing behavior (muricide) in the rat with raphe lesion induced by serotonergic neurotoxin 5,7-dihydroxytryptamine. Immunohistochemical and neurochemical analyses demonstrate that recovery of serotonergic innervation in the lateral hypothalamic area by the graft is brought about the inhibition of muricide. The grafted serotonergic neurons are strongly related to the inhibitory action on the muricide. Transplanted serotonergic neuron system can result in a reinnervation of the host, and leads to a reestablishment of the aggressive behavior.

243.9

ESTROGEN MODULATES $5\text{-}HT_{1A}$ RECEPTORS. A. Jackson*, L. Sinclair-Worley, M. Caldarola-Pastuszka, H. Conrad-Webb, M. Rudick, and L. Uphouse. Department of Biology, Texas Woman's University, Denton, TX. 76204.

Treatment of ovariectomized rats with 25 μg of estradiol benzoate (EB) reduces the ability of the $5\text{-}HT_{1A}$ agonist, 8-OH-DPAT, to inhibit lordosis behavior. The effect of EB requires 3-4 days to develop and results in a reduced potency of the $5\text{-}HT_{1A}$ agonist. It is not known if estrogen's modulation occurs on a shorter time scale. Ovariectomized rats were injected with 2.5, 7.5, or 25 μg EB (s.c.) followed 48 hrs later with 500 μg progesterone. Four to six hrs later, rats were infused bilaterally into the ventromedial nucleus of the hypothalamus (VMN) with 8-OH-DPAT (50 to 200 ng). The effects of 8-OH-DPAT varied with the dose of EB. The mechanisms responsible for estrogen's attenuation of the effects of 8-OH-DPAT are not known. Studies are in progress to examine the effects of estrogen on $5\text{-}HT_{1A}$ receptor mRNA. Supported by NIH RO1 HD28419 and NIGMS 08256.

243.10

8-OH-DPAT DESENSITIZATION OF $5\text{-}HT_{1A}$ RECEPTORS. S. Maingano, J. Hines*, and L. Uphouse. Department of Biology, Texas Woman's University, Denton, TX. 76204.

Several investigators have suggested that agonist-induced modulation of somatodendritic $5\text{-}HT_{1A}$ receptors occurs more rapidly and/or with less agonist activation that is evident for postsynaptaic $5\text{-}HT_{1A}$ receptors. To further evaluate the dose-dependency of the agonist-induced modulation, we pretreated ovariectomized rats (60 to 90 days) with varying doses of the $5\text{-}HT_{1A}$ receptor agonist, 8-OH-DPAT (0.15, 0.25, or 0.5 mg/kg; s.c.). A second 8-OH-DPAT injection (0.15, 0.25, or 0.5 mg/kg; s.c.) was given 24 hrs later; the effects of the drug on flat body posture and hypothermia (evidence of postsynaptaic $5\text{-}HT_{1A}$ receptor activation) and hyperphagia (evidence of somatodendritic $5\text{-}HT_{1A}$ receptor activation) were monitored for 30 min after injection. For all the parameters examined, prior treatment attenuated the response to the second treatment. Relative to the dose required to attenuate hypothermia and flat body posture, a lower dose of 8-OH-DPAT was required to reduce drug-induced hyperphagia. These data are consistent with previous suggestions that a higher dose of the $5\text{-}HT_{1A}$ agonist is required to modulate postsynaptic than presynaptaic $5\text{-}HT_{1A}$ receptor function. Supported by NIH RO1 HD 28419

243.11

MEDIOBASAL HYPOTHALAMIC SEROTONIN DURING THE RAT ESTROUS CYCLE AND FOLLOWING GONADAL HORMONES. S. Maswood*, B. Truitt, M. Caldarola-Pastuszka, and L. Uphouse. Department of Biology, Texas Woman's University, Denton, TX. 76204 and Department of Psycholoigy, University of Colorado, Boulder, Co. 80309.

Serotonin inhibits lordosis behavior by activating 5-HT_{1A} receptors while a facilitatory role for 5-HT_2 receptors has been postulated. Tissue concentrations of serotonin fluctuate in response to estrogen and progesterone and during the female rat estrous cycle, but the degree to which variations in tissue levels reflect changes in synaptic concentration of the neuotransmitter are still unclear. A more direct examination of extracellular changes in the biogenic amine is possible with microdialysis procedures. In the following studies, extracellular serotonin in the mediobasal hypothalamus was examined in diestrous, proestrous, and estrous rats and in hormonally-primed, ovariectomized rats. Microdialysate serotonin was significantly lower during proestrus and estrus than during diestrus. Since estrogen increased serotonin in the microdialysate, the relatively low concentration of the neurotransmitter on proestrus and estrus may reflect a progesterone-mediated attenuation of the effects of estrogen.
Supported by NIH RO1 HD28419 and TARP 003646003

243.12

THE 5-HT_{1A} ANTAGONIST S-(-)UH 301 BY ITSELF INDUCES THE HEAD-TWITCH RESPONSE (HTR) AND POTENTIATES THE EFFECTS OF COCAINE AND SERTRALINE ON THE 5-HTP-INDUCED HTR IN MICE. N.A. Darmani*, Dept. of Pharmacol., KCOM, Kirksville, MO 63501.

5-HT_{1A} receptor antagonists appear to increase the basal firing rate of raphe neurons by antagonizing the inhibitory endogenous serotonin tone operating on the somatodendritic 5-HT_{1A} autoreceptors. This effect should enhance the concentration of 5-HT in serotonergic terminal fields which may then activate other postsynaptic 5-HT receptors. However, microdialysis studies show that generally 5-HT_{1A} antagonists do not increase the basal 5-HT release but potentiate the ability of serotonin reuptake blockers to increase the neuronal serotonin output. In this study we determined whether antagonism of the endogenous serotonin tone can potentiate the activity of other postsynaptic serotonin receptors. We utilized the head-twitch response (HTR) in mice as a model of postsynaptic 5-HT_{2A} receptor function. The selective 5-HT_{1A} antagonist S-(-)UH 301 produced the HTR in normal but not in reserpinized animals. The 5-HT_{2A} antagonist, SR 46349B, completely prevented S-(-)UH 301-induced HTR. Pretreatment with S-(-)UH 301 potentiated 5-HTP-induced HTR both in normal and in reserpinized mice. The 5-HT_{1A} selective agonist, 8-OH DPAT, significantly but partially inhibited 5-HTP-induced HTR. S-(-) UH 301 pretreatment not only antagonized but also broke through the inhibitory effect of 8-OH DPAT on 5-HTP-induced HTR. The selective (sertraline) and nonselective (cocaine) serotonin reuptake blockers potentiated the ability of 5-HTP to induce the HTR. Pretreatment with S-(-)UH 301 enhanced the potentiating effect of serotonin reuptake blockers on the 5-HTP-induced HTR. These results suggest that an endogenous 5-HT tone via the discussed mechanism controls the terminal field synaptic activity of serotonergic neurons. This study was supported by NIDA grant DA 07627.

243.13

5-HT_3 RECEPTORS IN THE VMN AND FEMALE LORDOSIS BEHAVIOR. N. Maswood* and L. Uphouse. Department of Biology, Texas Woman's University, Denton, TX. 76204.

Within the ventromedial nucleus of the hypothalamus (VMN), serotonin exerts a dual role in the control of female lordosis behavior. Most emphasis has been placed on the 5-HT_{1A} and 5-HT_2 receptors, which inhibit and facilitate the behavior, respectively. In the current experiment, a potential role for the 5-HT_3 receptor was examined. Ovariectomized rats, hormonally primed with 25 µg estradiol benzoate and 500 µg progesterone, received VMN infusions with 100 ng, 250 ng or 500 ng of the 5-HT_3 receptor antagonist, tropisetron. Lordosis behavior was inhibited within 10 min of the 500 ng infusion. The 5-HT_3 receptor agonist, m-chlorophenylbiguanide (mCPBG), dose-dependently protected against tropisetron. The 5-HT_3 receptor agonist, alone, had no effect on the lordosis to mount (L/M) ratio, but it slightly reduced lordosis quality. These observations extend prior evidence for the complexity of serotonin's regulation of lordosis behavior. To our knowledge, this is the first evidence for a functional role of VMN 5-HT_3 receptors in the control of lordosis behavior.
Supported by NIH RO1 HD28419

243.14

LORDOSIS BEHAVIOR AFTER CO-INFUSION OF KETANSERIN AND DOI INTO THE VMN. A.Wolf* and L. Uphouse. Department of Biology, Texas Woman's University, Denton, TX. 76204.

We have reported that the 5-HT_2 antagonist, ketanserin, inhibited lordosis behavior after infusion into the VMN; similarly, the 5-HT_2 agonist, DOI, facilitated lordosis behavior. The present studies were designed to evaluate the ability of (a) ketanserin to reduce the 5-HT_2 agonist-induced facilitation or (b) DOI to attenuate the inhibitory effects of the 5-HT_2 antagonist. Ovariectomized rats with VMN cannulae were injected s.c. with 0.5 µg estradiol benzoate followed 48 hrs later with 500 µg progesterone. Four to six hrs later, rats were infused with DOI (2000 or 3000 ng) and/or ketanserin (3000 ng). Rats with preinfusion lordosis to mount (L/M) ratios > 0.5 were inhibited by ketanserin; co-infusion with DOI completely prevented the inhibition. L/M ratios of rats with preinfusion L/M ratios ≤ 0.5 were facilitated following the DOI infusion. Although co-infusion with ketanserin failed to prevent the facilitation, prior treatment with ketanserin attenuated the response to DOI. These findings lend further support to the hypothesis that 5-HT_2 receptors in the VMN facilitate lordosis behavior.
Supported by NIH RO1 HD28419 and NIGMS 08256.

243.15

ESTROGEN REDUCES 8-OH-DPAT'S LORDOSIS-INHIBITING POTENCY IN THE VMN. T. Price, A. Trevino, A. Wolf, M. Caldarola-Pastuszka, and L. Uphouse*. Department of Biology, Texas Woman's University, Denton, TX. 76204.

Serotonin (5-HT) inhibits lordosis behavior by acting on 5-HT_{1A} receptors; the ventromedial nucleus of the hypothalamus (VMN) was identified as an effective site for the inhibition. However, when ovariectomized rats received two weeks of treatment with estradiol benzoate (EB), infusion with 200 ng 8-OH-DPAT inhibited lordosis behavior on the first, but not the second week of treatment. In the present experiment, ovariectomized rats, with VMN cannulae, were injected s.c. with 25 µg of EB. Seven days later, rats received a second injection of EB followed 48 hrs later with 500 µg progesterone (s.c.). A second group of rats received a single EB treatment followed 48 hrs later by progesterone. Four to six hrs after progesterone, rats were pretested for sexual behavior and were infused into the VMN with 200 ng, 1000 ng, or 2000 ng 8-OH-DPAT. Lordosis behavior of rats receiving a single EB treatment was rapidly inhibited following all doses of 8-OH-DPAT. However, in animals that had received two EB treatments, the potency of the drug was reduced.
Supported by NIH ROI HD28419 and NIGMS 08256.

243.16

5-HT1B KNOCK-OUT MICE DISPLAY IMPULSIVE BEHAVIOR IN A FOOD REWARD CHOICE TASK. [1]D. Brunner, [2]M. A. Hofer*, [3]R. Hen and [4]M.-C. Buhot. [1]Unit 50-NYSPI, [2]Unit 40-NYSPI , [3]Center for Neurobiology & Behavior, Columbia University, New York NY 10032, [4]Lab. Neurosci. Comportementales & Cognitives, CNRS-URA 339, Univ. Bordeaux I, Talence, France.

Impulsiveness has been associated with aggression, addiction and OCD, psychopathologies which are likely be linked to a dysfunctioning of the central serotonergic system. We studied impulsiveness in knock-out mice lacking the 5-HT1B receptor because previous studies demonstrated that these mice displayed a reduced latency to attack in an aggression test and acquired self-administration of cocaine faster than wild-type mice.

Since impulsiveness can be described as a lack of self-control of waiting capacities, we first examined possible learning and time perception deficiencies in these mutant mice in an operant learning. Both wild-type and mutant mice were able to learn and showed no difference in timing accuracy, with a shorter latency to respond in mutants.

Our second experiment involved a 2-arm maze with a choice between a small reward or a larger one. Again, both genotypes discriminated equally. A behavior consisting in switching to the alternative arm within the consumption time of the large reward decreased progressively as training advanced in wild type mice, but was significantly higher and persistent in mutants.

The shorter response latencies found in the operant experiment suggest that switching within a reward may not be due to a decreased motivation but to a lowered threshold for responding. Our results may be relevant for the understanding of impulsiveness in childhood hyperactivity, which results in an inability to wait, and support the involvement of the serotonergic system in behavioral inhibition.

Supported by NIMH, NYS Psychiatric Institute, NIDAR01DA09862, NATO, CNRS and a Ludwig Schaefer award.

243.17

ON THE MECHANISM OF ACTION OF THE PUTATIVE ANTIPSYCHOTIC MDL 100,907 IN RELATION TO MK-801 INDUCED PERTURBATIONS IN MICE. P.Martin*[1], N.W. Waters[1], C.J. Schmidt[2], A. Carlsson[1] and M.L. Carlsson[1] Dept. of Pharmacology[1], Gothenburg University, Sweden and Hoechst Marion Roussel Inc.[2], Cincinnati, Ohio.

MK-801 is an un-competitive N-methyl-D-aspartate (NMDA) receptor antagonist which induces a behavioral syndrome in mice that includes hyperlocomotion (HLMA). The potent and selective 5-HT2a-receptor antagonist MDL 100,907 efficiently counteracted the MK-801 (0.3 mg/kg ip.) induced HLMA (cf. Abstract 809.7, 1995), a dose-dependent effect that was abolished when endogenous serotonin stores were depleted by pretreatment with para-chlorophenylalanine (pCPA).

pCPA pretreatment by itself reduced the MK-801 induced HLMA to 75% of MK-801 treated controls. This reduction of HLMA was not affected by replenishment of 5-HT with (+)-5-HTP (2.5-10-40 mg/kg). However, during concomitant treatment with MDL 100,907 (0.1 mg/kg), (+)-5-HTP given in the same doses elicited a marked, dose-dependent reduction of the MK-801 induced HLMA in pCPA treated animals.

These results suggest that MK-801 induced HLMA is accompanied by an activation of, but is not fully dependent upon, CNS serotonergic systems. MDL 100,907, by blocking the 5-HT2a-receptors unveils the anti-hyperlocomotor effects of (an)other serotonin receptor(s).

243.18

FUNCTIONAL SUPERSENSITIVITY OF 5-HT_{2A} AND 5-HT_{2C} RECEPTORS MEDIATING ACTH SECRETION FOLLOWING 5-HT DEPLETION PRODUCED BY PCPA BUT NOT PCA TREATMENT IN RATS. G. Hahn, P. Mazzola-Pomietto* C. S. Aulakh, Christine Wichems, and D. L. Murphy. Lab. of Clinical Science, National Institute of Mental Health, Bethesda, MD 20892-1264.

Systemic administration of both the phenylisopropylamine hallucinogen 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI) and m-chlorophenylpiperazine (m-CPP) produce dose-related increases in plasma adrenocorticotropic hormone (ACTH) concentrations, most likely by stimulation of 5-HT_{2A} and 5-HT_{2C} receptors, respectively. In the present study, we investigated the effects of 5-HT depletion produced either by inhibition of 5-HT synthesis following intraperitoneal administration of parachlorophenylalanine (PCPA, 150 mg/kg x3 days) or by degeneration of 5-HT nerve terminals following subcutaneous parachloroamphetamine (PCA, 10 mg/kg x2 days) treatment to male Wistar rats. In the PCPA study, separate groups of animals were challenged with various doses of either DOI or m-CPP 72 hrs after the last dose of PCPA. In the PCA study, separate groups of animals were challenged with various doses of DOI or m-CPP 9 days after the last dose of PCA. The animals were sacrificed by decapitation 30 min after m-CPP or DOI administration. Depletion of 5-HT produced by PCPA treatment accentuated both DOI-induced and m-CPP-induced increases in ACTH concentrations. On the other hand, depletion of 5-HT produced by PCA treatment did not modify either DOI-induced or m-CPP-induced ACTH secretion. These findings demonstrate functional supersensitivity of both 5-HT_{2A} and 5-HT_{2C} receptors mediating ACTH secretion following inhibition of 5-HT synthesis but not following degeneration of nerve terminals.

243.19

REGULATION OF 5-HT_2 RECEPTORS BY CHRONIC DOM, DOI, RITANSERIN OR DOM PLUS RITANSERIN: BEHAVIORAL EVIDENCE. K. R. Bonson*, and D. L. Murphy. 10/3D41, Laboratory of Clinical Science, National Institute of Mental Health, Bethesda, MD 20892-1264.

Regulation of the 5-HT_2 receptor is unusual in that chronic exposure to 5 HT_2 agonists as well as 5-HT_2 antagonists reduces receptor numbers. The present study used changes in behavioral responses of rats to a challenge dose of the 5-HT_2 agonist, DOM (2.5 mg/kg sc), as an index of 5-HT_2 receptor sensitivity after chronic exposure to 5-HT_2 agonists, an antagonist or their combination. In treatment groups receiving either DOM or DOI (2.5 mg/kg, sc) for 1-4 d, the head shake response induced on Day 1 was reduced. When rats in these two groups received a challenge dose of DOM 24 or 48 hrs after final agonist injection, the head shake response was reduced compared to saline-treated rats. In contrast, DOM or DOI (2.5 mg/kg) administered by osmotic minipump did not induce head shakes on Day 1, and the head shake response to DOM challenge after 4 d DOM or DOI was equal to that of saline-treated rats 24 hrs after the minipumps were removed. Although 14 d exposure to both ritanserin (5 mg/kg, sc) and ritanserin (5 mg/kg) + DOM (2.5 mg/kg, sc) reduced the head shake responses to DOM challenge compared to DMSO-treated rats 24 hrs after final injection, ritanserin itself induced head shake responses as well as hyperthermia after 10 d administration. No drug treatment altered the 0.5°C increase in temperature upon DOM challenge. Changes in ACTH, corticosterone and prolactin responses to DOM challenge varied between treatment groups. These data offer evidence that certain 5-HT_2 agonist-induced behavioral responses can be altered by chronic exposure to 5-HT_2 agonists, dependent on route of administration, and that the treatment effects of chronic agonist exposure are not blocked or potentiated by concurrent administration with an antagonist.

243.20

ATTENUATION OF FOOD INTAKE SUPPRESSANT EFFECT OF THE 5-HT_3 ANTAGONIST MDL-72222 BY DEXAMETHASONE PRE-TREATMENT IN RATS. C. S. Aulakh, P. Mazzola-Pomietto, N. Garrick*, and D. L. Murphy. Lab. of Clinical Science, National Institute of Mental Health, Bethesda, MD 20892-1264.

We have recently demonstrated that systemic administration of the 5-HT_3 receptor antagonist MDL-72222 but not ondansetron produced dose-related decreases in food intake and locomotor activity in rats (Psychopharmacology 121:488-493, 1995). Unlike ondansetron, MDL-72222 has been shown to inhibit 5-HT uptake into guinea pig hippocampal synaptosomes (Drug Dev. Res. 23:27-33, 1991) as well as interact with GABA-A receptors (Alcoholism 18:410-414, 1994). In an attempt to understand the mechanism of its anorectic effect, we studied the effects of various serotonergic, noradrenergic, cholinergic, opiate and GABAergic receptor antagonists on MDL-72222 (10 mg/kg)-induced suppression of food intake in male Wistar rats. Pretreatment with metergoline (0.01-1.0 mg/kg), mesulergine (1.0 mg/kg), mianserin (1.0 mg/kg), ritanserin (1.0 mg/kg), spiperone (0.1 mg/kg), ondansetron (2.0 mg/kg), propranolol (20 mg/kg), scopolamine (0.1-1.0 mg/kg), naloxone (4.0 mg/kg), phenoxybenzamine (1.0 mg/kg), picrotoxin (1.0 mg/kg) or muscimol (1.0 mg/kg) did not affect MDL-72222-induced suppression of food intake. On the other hand, pretreatment with dexamethasone (0.1-1.0 mg/kg) significantly attenuated MDL-72222-induced suppression of food intake but not locomotor activity. Daily administration of MDL-72222 (10 mg/kg/day) for 9 days failed to produce tolerance to its suppressant effect on food intake. These findings suggest that glucocorticoids may be involved in mediating the food intake suppressant effect of MDL-72222.

SEROTONIN: UPTAKE AND RELEASE

244.1

ORAL ADMINISTRATION OF FLUVOXAMINE INDUCES FOS-IR IN THE MALE RAT BRAIN. J.G.Veening, L.M.Coolen, W.J.P.M.Spooren, J.Mos, E.Ronken and B.Olivier*. Dept. of Anat.& Embr., Fac.Med Sci., Univ. of Nijmegen, the Netherlands; *Dept. of CNS Pharmacology, Solvay Duphar B.V., Weesp, the Netherlands. (SPON: European Neuroscience Association).

Fluvoxamine is a specific 5-HT reuptake inhibitor with antidepressant effects. To detect the brain areas possibly involved in these effects, the distribution of Fos-IR neurons was studied in the male rat brain after acute and chronic oral administration.

90 min. after acute administration (30 mg/kg) increased numbers of activated neurons were observed in *the nucleus of the solitary tract, medial part (NTSm); the lateral parabrachial nucleus, external part (PBle); the bed nucleus of the stria terminalis, dorsolateral part (BSTdl); and the central amygdaloid nucleus, lateral part (ACl).* After chronic administration (14 days, 30 mg/kg/day) the location of the Fos-IR neurons did not appear to be different, but the numbers were much lower, and no longer significantly different from control values. Using double immuno-stained sections, no Fos-IR could be observed in the 5-HT-IR neurons of the raphe nuclei, but a clear topographical relationship was observed with the GAL-IR fibers in the NTSm and PBle, and with the VIP- and CRH-IR fibers in the BSTdl and ACl.

Treatment by a 5-HT_{1A} -receptor-antagonist (WAY 100,635) or a 5-HT_3 antagonist (ondansetron) before acute oral administration of fluvoxamine did not prevent the induction of Fos-IR in the four brain areas, mentioned above.

We hypothesize that the Fos-IR induced in these areas, as an acute effect of Fluvoxamine, may be the result of an emesis-like activation of the NTSm, probably of peripheral origin.

(Funding sources: Solvay Duphar B.V.; University of Nijmegen).

244.2

DEXFENFLURAMINE PRODUCES DOSE-DEPENDENT BIOCHEMICAL AND FUNCTIONAL IMPAIRMENT OF BRAIN 5-HT SYSTEMS

W. Pinto*, F. Garcia, Q. Li, A. Vicentic, L.D. Van de Kar, W. A. Wolf and G. Battaglia Neuroscience Grad. Prog. and Dept. of Pharmacology, Loyola University of Chicago, Stritch School of Medicine, Maywood, IL 60153.

This study investigated the biochemical and functional consequences of short-term, repeated dexfenfluramine (d-FEN) treatment on brain 5-HT nerve terminals. Adult, male Sprague-Dawley rats (300-350g) were treated with either saline or d-FEN (0.5, 2.0 and 12.0 mg/kg, s.c, b.i.d, 4 days). Based on our previous studies with d,l-fenfluramine, the doses chosen represent a low, moderate and a maximally effective dose. 14 days post-treatment, rats were challenged with a single dose of the pre-synaptic 5-HT releaser, d-fen (5.0 mg/kg, i.p.), 15 minutes prior to decapitation. Pre-treatment with low-dose d-FEN (0.5 mg/kg) did not alter 5-HT levels in frontal cortex (FCX) or 5-HT uptake sites in FCX and hypothalamus (HYPO). Consequently, no functional alterations were observed in plasma ACTH or oxytocin responses to a d-FEN challenge. In contrast, pre-treatment with a moderate dose of d-FEN (2.0 mg/kg) significantly reduced cortical 5-HT levels (-22%) as well as 5-HT uptake sites in FCX (-30%) and HYPO (-26%). Consistent with these biochemical reductions, significant functional deficits were observed in the d-FEN challenge induced elevation of plasma ACTH (-42%) and oxytocin (-49%). The maximally effective dose of d-FEN (12.0 mg/kg) produced significantly greater reductions in cortical 5-HT (-77%) and 5-HT uptake sites in FCX (-87%) and HYPO (-59%) with correspondingly greater functional reductions in oxytocin (-80%) and ACTH (-73%) responses to a d-FEN challenge. Together, these data indicate that short-term, repeated dexfenfluramine treatment produces dose-dependent, biochemical and functional impairment of brain 5-HT nerve terminals, which is persistent for at least 2 weeks post-treatment. (Supported by Potts Foundation)

244.3

COMBINED ADMINISTRATION ENHANCES NEUROTOXICITY OF PHENTERMINE AND FENFLURAMINE ON 5-HT NEURONS: AN AUTORADIOGRAPHIC ANALYSIS. R. Lew* and L.S. Seiden, Depart of Pharmacol & Physiol Sciences, Univ of Chicago, 947 E58th St Chicago IL 60637.

Using 3H-Citalopram autoradiography, we present further evidence demonstrating combined adminstration enhances the neurotoxicity of phentermine (Phen) and (±) fenfluramine (Fen) on 5-HT neurons. Recently, we reported combined Phen/Fen administration causes significantly greater depletion of serotonin (5-HT) than either drug alone. In the present study, 3H-Citalopram autoradiography was used to measure 5-HT transporters after Phen/Fen treatment . Male Holtzmann Sprague-Dawley rats (275-300 g) were injected i.p on a 4 x 1 hr regimen with either saline, Phen (20 mg/kg), Lo-Fen (3.125 mg/kg), Hi-Fen (12.5 mg/kg) or Phen/Fen (20 mg/kg/3.125 mg/kg). One week after cessation of treatment, rat brains were perfused and processed for autoradiography. On the day of experiment, brain sections (20 um) were incubated in incubation buffer (50mM Tris HCl pH 7.4, 22 ^{o}C, 120 mM NaCl) containing 3H-Citalopram (2nM) for 60 min. Non-specific binding was defined by 0.5 uM fluoxetine. After incubation, sections were washed in cold incubation buffer, rinsed in ddH2O and air dried. Sections were apposed to Biomax film in 4-5 wks before photoprocessing autoradiograms. In preliminary studies, fluoxetine>RTI-121> nisoxetine in competing with 3H-Citalopram binding, indicating binding was to the 5-HT transporter. In autoradiography studies, combined Phen/Fen reduced 3H-Citalopram binding in cingulate, frontal, frontal-parietal and occipital-temporal cortex, nucleus accumbens, olfactory tubercle, striatum, hippocampus and ventromedial hypothalamus. The reduction in binding by Phen/Fen was greater than that observed with either Phen or Lo-Fen. The present study suggests that caution should be exercised in the prescription of Phen and Fen. (This work was supported by research grant DA00085; L.S.S is supported by RSA MH-105 62)

244.4

SUPEROXIDE-MEDIATED OXIDATION OF SEROTONIN: POTENTIAL ROLE IN NEURODEGENERATIVE DISEASES. M.Z. Wrona and G. Dryhurst*. Department of Chemistry and Biochemistry, University of Oklahoma, Norman, OK 73019.

There is a general consensus that oxygen free radicals contribute to degeneration observed in many neurodegenerative disorders, including Alzheimer's (AD) and Parkinson's (PD) diseases, transient cerebral ischemia, and alcohol and amphetamine abuse. There is also a widely accepted assumption that their deleterious effects are caused by damage to proteins, nucleic acids and lipids. However, many other biomolecules, including neurotransmitters, may be targets for free radicals and these types of reactions may lead to formation of endogenous neurotoxin(s). Recent studies from this laboratory have demonstrated that the serotonergic transmitter 5-hydroxytryptamine (5-HT) is an easily oxidized compound. It is also well known that degeneration of serotonergic pathways contributes to the overall neuropathology observed in AD, cerebral ischemia and methamphetamine abuse. Recently, we demonstrated that 5-HT is readily oxidized at physiological pH by hydroxyl radicals ($HO^{\bullet}$), generated by Fenton chemistry, to give, initially, a mixture of 2,5-, 4,5- and 5,6-dihydroxytryptamine (DHT). Two of these compounds 2,5- and 5,6-DHT, are unique products of the $HO^{\bullet}$-mediated oxidation, whereas 4,5-DHT is also formed in electrochemical or enzyme-mediated oxidations. The neurotoxic properties of 5,6-DHT are well established while those of 2,5- and 4,5-DHT are under current investigation. In the present study, it has been established that 5-HT is very rapidly oxidized by superoxide radicals ($O_2^{-\bullet}$), generated by the xanthine-xanthine oxidize system. Two products of this reaction, 4,5-DHT and 5,5'-dihydroxy-4,4'-bitryptamine) (DBT), could be detected by HPLC with electrochemical detection. Yields of both products increase rapidly with enzyme concentration and incubation times shorter than 9 min., reaching after that steady levels. DTPA has no effect on the overall reaction, whereas catalase inhibits formation of DBT with only a small decrease in 4,5-DHT formation. The effects of glutathione and other antioxidants on the rate and mechanism of $O_2^{-\bullet}$- mediated oxidation of 5-HT as well as the mechanism of this reaction and its potential biological consequences will be discussed.

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244.5

EFFECTS OF DEXFENFLURAMINE ON THE RELEASE OF SEROTONIN AND DOPAMINE IN THE RAT STRIATUM. A. Balcioglu* and R.J. Wurtman, Massachusetts Institute of Technology, Brain and Cognitive Sciences, Cambridge MA 02139

D-fenfluramine (DF), when given to animals in doses that produce brain levels more than 10-fold greater than those occurring in obese patients taking therapeutic doses, can cause a prolonged but reversible decrease in brain serotonin (5HT) unassociated with behavioral changes. Since this decrease reportedly is enhanced by L-dopa but not by 5HTP, it might be mediated by dopamine (DA) release occurring at these high brain levels.

We examined the dose relationships of DF's release of brain 5HT and DA, using in vivo microdialysis. Rats given 0.5, 1.0 or 2.5 mg/kg of DF i.p. exhibited significant increases in ECF 5HT by 20-40 minutes after DF administration; peak increases were 123%, 150% and 433%, respectively. Small increases in ECF DA occurred 80-100 minutes after the 0.5 mg/kg dose, and 80-100 or 60-80 min after the other doses. Peak increases are 115%, 118% and 150%, respectively.

Methiothepin, a serotonin 5HT receptor antagonist, largely blocked the increase in ECF DA caused by DF.

These data are compatible with the hypothesis that DF increases DA release via a trans-synaptic action mediated by 5HT.

244.6

NEUROTOXICITY OF METHAMPHETAMINE: DEGENERATION VERSUS DEPLETION, A STUDY USING SEROTONIN TRANSPORTER AS A MARKER.

S. B. Bledsoe* and F. C. Zhou. Indiana University, School of Medicine, Department of Anatomy, Indianapolis, IN 46202.

It is not conclusive whether the neurotoxicity of hallucinogenic drugs of the amphetamine analogue, e.g. methamphetamine (METH), causes depletion or degeneration of serotonin (5-HT) in the brain. This vital difference is not distinguishable since detection markers are subject to the 5-HT level. In this study, using immunocytochemistry against serotonin transporter, we report that METH caused definitive damage to 5-HT fibers at the dosage of 12.5-50mg/kg i.p., within hours. Immunocytochemical 5-HTT staining detected that 5-HTT-immunostained (5-HTT-im) fibers became vesicular along the axons within minutes to hours after METH injection, not normally detectable by anti-5-HT antibodies. The blebs enlarged from 2 to 20μm and eventually perforated when exceeding 15μm-20μm. The perforation of the blebs caused fragmentation which in turn resulted in degeneration of 5-HT fibers. Reduction of 5-HTT-im and 5-HT-im fibers in cortical and hippocampal regions was observed within hours. This rapid damage of 5-HT fibers preceded the normal course of degeneration which takes place over several days. Four days after METH injection, the 5-HTT-im fibers were greatly reduced in many regions of the brain, particularly in frontal, parietal, and occipital cortices, hippocampus, striatum, and thalamus. The sparse thick 5-HTT-im and 5-HT-im fibers that were scattered in the temporal cortex, septal nucleus, nucleus accumbens, hypothalamus and brainstem are more resistant to the METH insult. The damage of 5-HTT-im and 5-HT-im cell bodies in the raphe, if it exists is largely unnoticeable with the current method. In summary, we reported that there are two phases of 5-HT fiber damage by METH: the initial physical damage--vesiculation and perforation; second, the degeneration due to fragmentation and lack of further supply of nutrients and vital proteins occurres within days.

(NIH R24HD30508)

244.7

FENFLURAMINE-INDUCED SEROTONIN NEUROTOXICITY IN BABOON BRAIN DETECTED WITH PET. **U. Scheffel, Z. Szabo, W. Mathews, P. Finley, R. Dannals, H. Ravert, B. Callahan, J. Yuan, and G. Ricaurte*. Departments of Radiology and Neurology, Johns Hopkins Medical Institutions, Baltimore, Maryland 21205.**

Fenfluramine is a widely prescribed appetite suppressant that has the potential to damage brain serotonin (5-HT) neurons in animals. While fenfluramine's 5-HT neurotoxic effects in rodents and nonhuman primates are well documented, fenfluramine's neurotoxic potential in humans has not been investigated, largely because of difficulties inherent in evaluating 5-HT neuronal integrity in the living brain. The present studies sought to determine whether PET imaging with the newly developed 5-HT transporter ligand, [^{11}C](+)McN-5652, could be used to detect fenfluramine-induced 5-HT neurotoxicity in the brain of living primates. A 22.3 kg male baboon (*Papio anubis*) underwent 6 PET studies: 3 before and 3 after treatment with fenfluramine (5 mg/kg, s.c., twice daily for 4 days). The 5 mg/kg dose is known to produce a profound loss of 5-HT axon markers, and is approximately two times higher than a dose of fenfluramine reported to produce small and inconsistent weight loss in baboons. In each PET study, 25-32 mCi of [^{11}C](+)McN 5652 (the active enantiomer) and [^{11}C](-)McN 5652 (the inactive enantiomer) were injected, followed by dynamic PET acquisition over 115 min. PET studies 18, 45 and 81 days after fenfluramine revealed marked reductions in regional brain specific binding of [^{11}C](+)McN-5652. Findings with PET corresponded well with post-mortem results indicative of 5-HT neurotoxicity. These results suggest that PET imaging with [^{11}C]McN-5652 will be useful for evaluating the 5-HT neurotoxic potential of fenfluramine and related drugs in living humans. [Support: DA19487, DA06275 and DA06309]

244.8

SELECTIVE INCREMENTS OF 5-HT RELEASE IN THE MEDIAN AND DORSAL RAPHE NUCLEI BY MILD STRESS A. Adell*, J.M. Casanovas, F. Artigas. Dept. of Neurochemistry, C.S.I.C. Barcelona, Spain.

The serotonergic system participates in the neurobiological adaptations to aversive stimuli. Severe stressful situations increment the turnover of 5-HT and its release in forebrain structures. Less attention has been paid to the response of 5-HT neurons to mild aversive situations. Using microdialysis in conscious rats, we have examined the effects of mild stress on 5-HT release in the dorsal and median raphe nuclei (DRN and MRN, respectively) and in forebrain areas with selective innervation from both (striatum, frontal cortex, hippocampus). The release of 5-HT in the MRN doubled that in the DRN (45 ± 5 vs 22 ± 2 fmol/20-min in presence of 1 μM citalopram, probe length 1.5 mm) and both were markedly superior to that in various forebrain areas (8-13 fmol/fraction). A saline injection increased 5-HT release only in the DRN and MRN (160 % and 210 % of basal, respectively). Also, 5-HT release in the MRN increased to 195 % of basal in rats handled for 30 sec and to 140 % in rats subjected to a mild stress procedure (noise, water spill) within their cages for 5 min. These procedures attenuated or reversed the spontaneous decline of 5-HIAA output in the MRN but not in forebrain. Rats subjected to forced swimming for 5 min experienced a moderate increment of 5-HT release in the midbrain which was accompanied by a reduction in medial prefrontal cortex and hippocampus. These results indicate that the release of 5-HT in the DRN and MRN is very sensitive to mild aversive situations. Given the presence of somatodendritic 5-HT$_{1A}$ autoreceptors, the enhanced 5-HT release in these midbrain nuclei may be a physiological mechanism by which 5-HT neurons could attenuate unwanted increments of activity during stressful situations. *Supported by FIS 95/266*

244.9

ESTROGEN INCREASES SEROTONIN TRANSPORTER (SERT) BINDING SITES AND SERT mRNA EXPRESSION IN FEMALE RAT BRAIN. J.K. McQueen, H. Wilson and G. Fink*. MRC Brain Metabolism Unit, University Department of Pharmacology, 1 George Square, Edinburgh, UK.

In the rat, estrogen stimulates a massive increase in the expression of 5-HT_{2A} receptor mRNA in the dorsal raphe nucleus[1], and significant increases in the density of 5-HT_{2A} receptors in several forebrain areas[2]. The key regulator of serotonergic transmission in brain is the reuptake of extracellular 5-HT by the 5-HT transporter (SERT). We have investigated the possible effects of estradiol-17β on the expression of SERT mRNA and the density of SERT binding sites in female rat brain. Adult female rats were ovariectomized under halothane anaesthesia between 09.00 and 12.00 h on di-estrus, and immediately injected s.c. with either 10 μg estradiol benzoate (EB) in 0.1 ml arachis oil (n = 7) or with oil alone (n = 7). The rats were killed between 16.30 and 18.00 h on the following day and the brains removed. SERT mRNA levels were determined by *in situ* hybridization using a 45 base oligonucleotide probe labelled at the 3' end with ^{35}S-γ-ATP and SERT binding sites were measured by quantitative autoradiography using ^{3}H-paroxetine as ligand. EB increased (~50%) the numbers of cells expressing SERT mRNA in the dorsal raphe nucleus and the density of SERT binding sites in lateral septum (90%), basolateral amygdala (20%), ventromedial nucleus of hypothalamus (250%) and ventral thalamus (250%). Sites in periaqueductal central gray were decreased (by 15%). These results show that estrogen has potent effects on the serotonin transporter as well as 5-HT_{2A} receptors, suggesting that effects of estrogen on mood and mental state may be mediated through both of these central serotonergic mechanisms.

1 Sumner, B E H and Fink, G (1993) *Mol. Cell. Neurosci.* **3**, 83-92

2 Sumner, B E H and Fink, G (1995) *J. Steroid Biochem. Mol. Biol.* **54**, 15-20

244.10

PRESYNAPTIC CONSEQUENCES OF 5,7-DIHYDROXYTRYPTAMINE IN THE AGING HIPPOCAMPUS. A. Dugar*, C. Patanow, M.L. Billingsley and J.M. Lakoski. Depts. of Pharmacology & Anesthesia, The Pennsylvania State University College of Medicine, Hershey, PA 17033.

The hippocampus is vulnerable to selective damage with aging; however, few studies have examined age-related cellular physiological changes in this region following neurotoxic injury. Following administration of the neurotoxin 5,7-dihydroxytryptamine (5,7-DHT) into the fimbria-fornix/cingulum bundle (FF/CB) to denervate serotonergic afferents to the dorsal hippocampus, age-dependent alterations in hippocampal serotonergic function were investigated using electrophysiological and immunohistochemical techniques. Female Fischer 344 rats were anesthetized with sodium pentobarbital, pretreated with nomifensine (15 mg/kg, i.p.) and bilaterally injected with 5,7-DHT or vehicle (0.1% ascorbic saline) into the FF/CB (2 μl total).

Extracellular *in vivo* recordings of hippocampal CA3 subfield pyramidal neurons in chloral hydrate anesthetized rats (3 and 18 mo) were performed three weeks post-lesion. Microiontophoretic applications of 5-HT, 8-OH-DPAT and WAY 100,135 were used to assess serotonergic neuronal function. The rate of recovery was significantly increased following application of 5-HT in the 18 mo 5,7-DHT group compared to the 18 mo Vehicle and 3 mo 5,7-DHT groups (3.3 and 2.6 fold, respectively). Immunohistochemical analysis of the hippocampus was performed using rabbit polyclonal antibodies directed against the presynaptically localized protein, SNAP-25. SNAP-25 expression did not decline in intact aged animals (3, 18, 21 and 29 mo) or in 18 mo animals following 5,7-DHT or vehicle treatment (7 and 21 days post-lesion).

The physiological data revealed the development of increased presynaptic sensitivity to 5-HT in aged animals following neurotoxic lesion, suggesting 5-HT neurotransmission is differentially altered in young versus old animals. These data provide evidence for an age-dependent decline in presynaptic function of the CA3 pyramidal neurons, which may underlie injury-induced responses in the aging hippocampus. *Pub. No. 56A supported by PO1 AG 10514 (JML).*

244.11

BEHAVIORAL EVIDENCE OF SEROTONERGIC TRANSMISSION IN THE SUBSTANTIA NIGRA PARS COMPACTA OF FREELY MOVING RATS. **J.L. Góngora-Alfaro*, F. Heredia-López, F. Alvarez-Cervera and J.L. Bata-García, Centro de Investigaciones Regionales, Universidad Autónoma de Yucatán, Mérida, Yuc., México, 97000.**

The substantia nigra pars compacta (SNc) receives serotonergic innervation from the raphe nuclei. Here we investigated the behavioral effect of duloxetine, a serotonin (5-HT) reuptake inhibitor, microinjected into the SNc. Drugs were applied through a cannula aimed at the SNc of the right cerebral hemisphere of male rats. Each rat received two consecutive injections 60 min apart. Circling was counted with an automated rotometer at 5 min intervals. PBS (0.5 μl) had no effect. Duloxetine elicited a modest contralateral circling: 2 μg, 18.4 ± 5.5 turns/60 min; 4 μg, 19.8 ± 5.2 turns/60 min. 5-HT applied alone induced a weak effect. However, an intense contralateral circling developed when 5-HT (8 μg) was co-injected with duloxetine. The potentiating effect of duloxetine was dose-dependent: 2 μg, 53.6 ± 9.1 turns 60/min (n=7); 4 μg, 110.4 ± 16.8 turns/60 min (n=8). It is concluded that 5-HT spontaneously released at the SNc modulates motor behavior. (Supported by grant 1831-M9211 from CONACyT, México.)

244.12

LACK OF EFFECT OF SEROTONIN LESIONS ON COCAINE-INDUCED SENSITIZATION. R. T. Windh*, M. Martinez, G. Slean and K.A. Cunningham. Dept. Pharmacol. and Toxicol., Univ. Texas Med. Branch, Galveston, TX 77555.

The locomotor-activating effects of cocaine (COC) have been largely attributed to an increase in striatal synaptic dopamine (DA) subsequent to blockade of DA reuptake. Serotonin (5-HT) can modulate DA neurotransmission by interacting with both DA cell bodies and terminals, and it is generally reported that 5-HT exerts a negative net control on amphetamine-induced locomotor activity (LMA). The role for 5-HT in COC-induced LMA, or in the progressive increase in COC-induced activity known as sensitization, is not known. On the basis of these earlier studies, one might predict that 5-HT lesions would enhance COC sensitization; the present studies were undertaken to test this hypothesis. Parallel studies were run in which male Sprague-Dawley rats were treated with one of two 5-HT neurotoxins: *dl*-fenfluramine (FEN; 12 mg/kg sc, bid, 4 days) or 5,7-dihydroxytryptamine (DHT; 8 μg base/2 μl aCSF with 0.1% ascorbic acid, pH 7, injected into the dorsal raphe nucleus under pentobarbital anesthesia) or their vehicles, and then treated with a sensitizing regimen of cocaine (15 mg/kg ip, bid, 7 days) or saline (SAL; 1 ml/kg) in their home cages 4 (FEN study) or 7 days (DHT study) following neurotoxin treatment. LMA was assessed during a 45 min challenge with COC (15 mg/kg ip) 72 hrs after the last sensitizing injection. LMA in response to acute COC administration was not altered in either FEN- or DHT-lesioned rats compared to vehicle-treated controls. Two-way ANOVA of the LMA in response to the COC challenge indicated no main effect of lesion treatment (p=.31, .1 for FEN and DHT studies, respectively), a significant main effect of repeated COC treatment indicating sensitization (p=.005, .02) and no interaction between the variables (p=.42, .26). Therefore COC treatment produced sensitization in each study, and neither FEN nor DHT altered the LMA response to acute COC or the expression of COC sensitization as assessed by this assay. Initial immunohistochemical analysis indicated substantial 5-HT loss in lesioned animals from each study; more quantitative assessments are currently underway. Supported by DA 06511 and DA 05637.

244.13

IN VIVO CRITERIA TO DIFFERENTIATE MONOAMINE UPTAKE INHIBITORS (MARIs) FROM SEROTONIN RELEASING DRUGS: SIBUTRAMINE IS A MARI. C. Gundlah, K.F. Martin[1], D.J. Heal[1], J. Schjott, S.B. Auerbach*. Nelson Biol. Lab., Rutgers Univ., Piscataway, NJ 08855 & [1]Knoll Pharmaceuticals, Nottingham G.B.

Monoamine reuptake inhibitors (MARIs) and releasers increase extracellular serotonin (5-HT), and it can be difficult to differentiate these two mechanisms in vivo. The MARIs, fluoxetine, paroxetine and sibutramine (1-10 mg/kg i.p.) dose-dependently increased 5-HT levels in rat hypothalamus by 2-3 fold. By contrast, the 5-HT releaser fenfluramine (10 mg/kg i.p.), also increased dialysate 5-HT but to a much greater extent (12-fold). The effects of the MARIs, but not fenfluramine, were attenuated by administration of 8-OH-DPAT. All drugs increased extracellular 5-HT when infused into the hypothalamus via the dialysis probe and the effect of the MARIs was attenuated by subsequent systemic administration. In contrast, systemic fenfluramine did not attenuate the increase produced by prior local infusion of fenfluramine into the hypothalamus. Therefore, MARIs can be distinguished from 5-HT releasers by (1) magnitude of their effect, (2) susceptibility to inhibition of neuron firing and (3) blockade of local MARI action by systemic MARI administration, which was not the case for fenfluramine. The data also demonstrate that sibutramine has the pharmacological characteristics of a monoamine reuptake inhibitor and is not a 5-HT releaser. Supported by NIMH grant #51080 and Knoll Pharmaceuticals.

244.14

ELEVATIONS IN BODY TEMPERATURE THAT ARE INDUCED BY D-FENFLURAMINE (D-FEN) EXPOSURE CORRELATE WITH DECREASING CONCENTRATIONS OF SEROTONIN (5-HT).

C. W. Stewart, P. Clausing, J. F. Bowyer and W. Slikker, Jr.* Division of Neurotoxicology, National Center for Toxicological Research/FDA, Jefferson, AR 72079.

D-Fen induces elevation of core body temperatures in a warm dosing environment (> 25°C). Other reports demonstrate that d-fen exposure produces a dose-dependant decrease in 5-HT. In this experiment, adult male Sprague-Dawley rats were dosed with either 5 or 10 mg/kg d-fen in a 22 and 28°C dosing environment. All animals were sacrificed 7 days after d-fen exposure and 5-HT concentrations were measured by HPLC in the frontal cortex, hippocampus and striatum. In both dosing environments, there was a dose-dependant decrease in 5-HT concentrations. A negative correlation was observed between peak body temperatures of individual animals and 5-HT concentrations for both 5 and 10 mg/kg d-fen. To determine if the pharmacokinetics of d-fen were altered by the warm environment (28°C), plasma samples were taken (1, 2, 2.5, 3, 4 and 8 hrs) from another group of animals that were dosed with 5 mg/kg d-fen in a 22 and 28°C environment. Analysis of the means at each time-point revealed that pharmacokinetics of d-fen and d-norfenfluramine were not altered by the warm environment. These data indicate that as peak body temperatures increase, 5-HT concentrations decrease and that these effects induced by d-fen are not the result of pharmacokinetic alterations.

244.15

INTERACTION BETWEEN THE FORCED SWIMMING TEST AND FLUOXETINE TREATMENT ON EXTRACELLULAR 5-HT IN THE RAT. **L.G. Kirby* and J. Lucki. Departments of Psychiatry and Pharmacology, Institute of Neurological Sciences, University of Pennsylvania, Philadelphia, PA 19104.**

This study examined extracellular levels of 5-hydroxytryptamine (5-HT) during the forced swimming test (FST), a behavioral test that is sensitive to the effects of antidepressant drugs. Cannulae were implanted under surgical anesthesia into either the striatum or lateral septum. One week later, dialysis probes were lowered through the cannulae and dialysis samples collected throughout two sessions of the FST: 1) a 15 min pretest swim, and 2) a 5 min test swim 24 hours later. The antidepressant fluoxetine (20 mg/kg, s.c.), a 5-HT selective uptake inhibitor, or saline was administered 23.5, 5, and 1 hour prior to the test swim. In the striatum, the pretest swim increased 5-HT by 80-90% in both treatment groups. On the second day, the test swim had no effect on 5-HT in saline-treated rats. Fluoxetine-treated rats showed higher basal levels of 5-HT on the second day. In contrast to saline treatment, the test swim decreased striatal 5-HT by 23%. In the lateral septum, the pretest swim produced a 40-50% decrease in 5-HT. On the second day, septal 5-HT did not change following the test swim in saline-treated rats. Fluoxetine-treated rats showed higher basal levels of 5-HT on the second day. In contrast to saline treatment, the test swim decreased septal 5-HT by 38%. 5-HIAA levels were decreased in both brain regions and treatment groups by 15-50% after each swim exposure, although fluoxetine decreased basal 5-HIAA levels on the second day in each region. Therefore, fluoxetine treatment prevented adaptation of the regional response of extracellular 5-HT ordinarily produced in the FST. Fluoxetine reversed the response to the initial swim in striatum and restored the response to the initial swim in lateral septum. It is possible that the ability of antidepressant drugs to alter the neurochemical adaptation to repeated swim might underlie their behavioral effects in the FST, a commonly used animal model of depression. Supported by USPHS grants MH 36262 and MH 17168.

244.16

PHARMACOLOGICAL PROFILE OF THE MIXED 5HT-RE-UPTAKE INHIBITOR/5HT1A-AGONIST EMD 68843

G.D. Bartoszyk*, A. Barber, H. Böttcher, H.E. Greiner, J. Leibrock, J.M. Martínez, and C.A. Seyfried. Merck KGaA Pharm. Res., CNS-Research, 64271 Darmstadt, Germany.

EMD 68843 (5-{4-[4-(5-cyano-3-indolyl)-butyl]-1-piperazinyl} -benzofuran-2-carboxamide, HCl) exhibited the following binding profile *in vitro* (receptor and IC_{50}-values, resp.): $5HT_{1A}$ 0.5 nM, σ 17 nM; affinity to $5HT_{1D}$, $5HT_{2A/C}$, $5HT_4$, $D_{1/2/3/4}$, BZD/GABA, $\alpha_{1/2}$, $H_{1/2}$, $M_{1/2}$, opioid, NMDA, AMPA, kainate, glycine was ≥ 100 nM. Reuptake was inhibited *in vitro* with IC_{50}-values of 0.2 nM for 5HT, 60 nM for NE, and 90 nM for DA and *in vivo* for 5HT with ED_{50}-values of 0.7 mg/kg s.c. or 3.8 mg/kg p.o.. Accumulation of 5HTP was inhibited by 40% in rat striatum with 10 mg/kg p.o. whereas DOPA levels were not affected. In behavioral animal models, EMD 68843 inhibited ultrasonic vocalization in rats with ED_{50}-values of 3.8 mg/kg s.c. and 12.8 mg/kg p.o. with a long duration of action; the effect was totally counteracted by the $5HT_{1A}$ antagonist WAY 100635. After repeated treatment, EMD 68843 reduced immobility in the behavioral despair test and the tail suspension test in mice with doses of 30 to 55 mg/kg p.o.. The results characterize EMD 68843 as a drug with mixed antidepressant/anxiolytic activity.

244.17

PRESYNAPTIC $5\text{-}HT_{1D}$ RECEPTORS INHIBIT GLUTAMATE RELEASE IN HUMAN CEREBRAL CORTEX.

G. Maura, M. Marcoli, M. Tortarolo and M. Raiteri*. Institute of Pharmacology and Pharmacognosy, University of Genoa, Genoa, Italy

Activation of 5-HT receptors has been reported to control excitatory amino acid transmission in rat central nervous system. In the present study the effects of 5-HT receptor activation on the release of endogenous glutamate from human cerebral cortex synaptosomes have been assessed. Synaptosomes were prepared from fresh cerebral cortex samples obtained from patients undergoing neurosurgery. Depolarization with high K^+ (15 mM) increased endogenous glutamate release from superfused synaptosomes; the depolarization-evoked overflow of glutamate was almost totally calcium-dependent. Serotonin or agonists selective for 5-HT receptor subtypes (receptor selectivity in parentheses) were found to inhibit endogenous glutamate overflow. The rank order of potency of agonists was: 5-HT ≥ sumatriptan ($5\text{-}HT_{1D}/5\text{-}HT_{1B}$) > 8-OH-DPAT ($5\text{-}HT_{1A}$) >> phenylbiguanide ($5\text{-}HT_3$). The inhibitory effect of 5-HT (0.1 μM) was insensitive to (+)WAY 100135 ($5\text{-}HT_{1A}$ antagonist), spiperone ($5\text{-}HT_{1A}/5\text{-}HT_2$ antagonist) or ICS-205930 ($5\text{-}HT_3/5\text{-}HT_4$ antagonist), at concentrations up to 1 μM. Metergoline, a $5\text{-}HT_1/5\text{-}HT_2$ receptor antagonist which has been reported to behave as a partial agonist or as a full agonist at $5\text{-}HT_{1D}$ receptors, inhibited the endogenous glutamate overflow. Metergoline and 5-HT were almost equieffective as inhibitors of glutamate overflow. In preliminary experiments the $5\text{-}HT_1/5\text{-}HT_2$ receptor antagonist methiothepin prevented the effects of both 5-HT and metergoline.

It is suggested that glutamatergic axon terminals in the human cerebral cortex possess 5-HT receptors that mediate inhibition of glutamate release; the receptors resemble the $5\text{-}HT_{1D}$ subtype.

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SEROTONIN TRANSPORTERS

245.1

REGIONAL VARIATION OF EXPRESSION OF SEROTONIN TRANSPORTER MRNA IN DORSAL AND MEDIAN RAPHE NUCLEI.

M. Rattray*[1], J. Lee[1], G.Michael[2], C.Bendotti[3]& J.V. Priestley[1,2]. [1]UMDS Biochem. & Mol. Biol., [2]UMDS Physiology, Guy's Hospital, London SE1 9RT, U.K. [3] Mario Negri Institute, Via Eritrea 62, 20157 Milan, Italy

We have previously noted that a number of drugs including fenfluramine, MDMA and PCPA down-regulate serotonin transporter (SERT) mRNA levels. Neurons in the ventromedial subdivision of the dorsal raphe nucleus (DRv) are more sensitive than neurons in the dorsomedial subdivision (DRd). The basis for this is unknown, but may be due to regional variation in expression of proteins involved in serotonin neurotransmission.

We have used radioactive *in situ* hybridization (ISH) using liquid emulsion autoradiography and image analysis techniques to determine the levels of expression of the mRNAs for SERT, tryptophan hydroxylase (TpH) and l-aromatic amino acid decarboxylase (AADC) in subdivisions of the dorsal raphe and in median raphe (MR). In addition we have combined SERT ISH with serotonin immunocytochemistry. SERT mRNA is expressed by all serotonin positive cells, but the levels of SERT mRNA and serotonin display a wide variation between individual neurons. The mean cellular expression level of SERT mRNA is highest in DRv, with neurones in the lateral wings of the dorsal raphe (DRl) having the lowest levels. SERT mRNA levels in MR and DRd were intermediate. TpH and AADC mRNA levels also showed regional variation (TpH mRNA levels in DRv ≥ MR >> DRl = DRd; AADC mRNA levels in MR ≥ DRv >> DRl = DRd). Conversely, the levels of serotonin immunoreactivity were lowest in DRv cells and highest in DRl cells. These results support the hypothesis that serotonin neurones have subtle differences in chemical phenotype, according to their location. This may account for variation in their drug response. Supported by MRC

245.2

IMIPRAMINE CAN PROTECT SEROTONIN UPTAKE INHIBITION INDUCED BY CITALOPRAM,

C. Sur, H. Betz* and P. Schloss. Dept. of Neurochemistry, Max-Planck Institute for Brain Research, Frankfurt am Main, Germany.

Tricyclic antidepressants and selective serotonin uptake inhibitors are widely used for the treatment of depression. To gain further insight into the interactions between imipramine, citalopram and serotonin (5-HT) at the rat serotonin transporter, SERT1, we have analyzed the effects of these drugs on 5-HT transport into a stable cell line expressing SERT1. Previously, we have shown that imipramine binds to two different sites on this molecule; a high affinity sodium-dependent site and a low affinity site that also binds citalopram. Competition experiments revealed that both citalopram and imipramine competitively inhibit 5-HT uptake. Whereas the Ki value for citalopram was similar to its Kd value, the inhibitory potency of imipramine was more than one order of magnitude lower than its Kd value as determined in direct ligand binding. However, binding of imipramine to its high affinity site allosterically displaced citalopram. Consequently, in the presence of sodium ions, low concentrations of imipramine partially protected 5-HT transport from citalopram inhibition. These results confirm the existence of two distinct binding sites for imipramine on SERT1 and suggest that they may exert different effects on 5-HT uptake depending on which site is occupied.

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245.3

REGULATION OF THE SEROTONIN TRANSPORTER.
L. M. Hall* and G.M. Anderson. The Child Study Center, Yale University School of Medicine, New Haven, CT 06510.

The regulation of the serotonin transporter (SERT) has been examined by a number of groups. A consistent finding has been the short-term down-regulation of transport rates (Vmax) following protein kinase C (PKC) activation. There are also reports that rapid regulation can occur after receptor stimulation, protein kinase G (PKG) activation, or following exposure to nitric oxide (NO). We now report that ADP does not reduce platelet 5-HT uptake through receptor stimulation, but rather via a non-competitive effect at the transporter; similar effects are seen in platelet and synaptic vesicles. A reported stimulatory effect of histamine on platelet 5-HT uptake was not been observed in a series of studies examining a range (0.1-1.0 uM) of histamine concs and exposure times. We also have not observed the stimulatory effects reported for NO and PKG activation on 5-HT transport by RBL cells or brain slices when studying the platelet SERT. Data from studies on the effects of protein phosphatase inhibitors, alone or in combination with PKC-activators, on synaptic vesicle and platelet uptake will also be presented. We will attempt to reconcile the varied results obtained using platelets, cell-lines, synaptosomes, and brain slices.
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245.4

Chronic cocaine binge administration fails to alter serotonin and norepinephrine transporter mRNA levels from behaviorally sensitized rats. S.A. Burchett* and M. J. Bannon. Depts. of Psychiatry and Behavioral Neurosciences and Pharmacology, Wayne State University, Detroit, MI 48201.

Cocaine binds with high affinity to the dopamine, serotonin and norepinephrine transporters (DAT, SERT and NET, respectively). While the effects of cocaine administration on DAT expression have been investigated intensively, few studies have investigated changes in SERT and NET expression. Furthermore, both the dopamine and serotonin systems may be involved in the progressive development of the well-described phenomenon termed sensitization. In the present study, the effects of chronic cocaine in a binge paradigm of administration on DAT, SERT and NET transporter mRNA levels were compared in the brains of sensitized rats. Male Fisher rats were injected 3 times (spaced 1 hr apart) with 15 mg/kg cocaine for 14 days. Differences in the behavioral scores between day 1 and day 14 indicate that the cocaine treated rats had become sensitized during the treatment regimen. Quantitative in situ hybridization using antisense cRNA probes was utilized to map the distribution and abundance at the cellular level of the DAT, NET and SERT mRNAs. Although significant regional heterogeneity of DAT and SERT mRNA abundance was evident, chronic cocaine administration did not consistently alter the abundance of the SERT and NET mRNAs in the regions examined, suggesting that the SERT and NET in the rat do not exhibit compensatory changes in response to cocaine. In addition, correlational analysis indicates that SERT and NET mRNA levels do not underlie cocaine-induced sensitization. Supported by NIH grant DA 06470.

245.5

REGULATION OF SEROTONIN TRANSPORTER EXPRESSION BY CAMP AND INTERFERON IN VARIOUS TISSUES AND IN BEWO CELLS. O. Morikawa*, T. Deai, N. Murakami, N. Sakai and N. Saito, Laboratory of Molecular Pharmacology, Biosignal Research Center, Kobe University, Kobe 657, Japan

The serotonin transporter (SET) is known to be the main target of the antidepressants. We have reported that we cloned the promoter region of SET from mouse genomic library and the promoter activity of SET was enhanced by the treatment of dibutylyl cyclic AMP (Dib-cAMP) (Sakai et al. 1995). To elucidate the involvement of SET in the pathogenesis of the affective disorders, it is of interest to examine the mechanism of the transcriptional regulation of SET expression.

In the present study, we investigated the effect of Dib-cAMP, IFN-α and IFN-γ on the transcriptional level of SET mRNA in Bewo cells which constitutively express SET, using Northern blot analysis. Significant increase in level of SET mRNA was observed by the treatment of Dib-cAMP for 24 hrs. The result was in agreement with the previous findings Dib-cAMP enhanced the promoter activity of SET assessed by CAT assay. The treatment of IFN-α and IFN-γ produced slight increase in level of SET mRNA. It is suggested that the cAMP dependent process may regulate the SET expression and may be involved in the pathogenesis of the affective disorders. We also investigated the change in the level of SET mRNA in mouse brain stem, adrenal and bone marrow by the intraperitoneal administration of Dib-cAMP, IFN-α and IFN-γ.

245.6

EFFECT OF CHRONIC CITALOPRAM TREATMENT ON THE SEROTONIN TRANSPORTER, J. Lawrence, H.J. Olverman, J.S. Kelly* and [1]S.P. Butcher, Department of Pharmacology and [1]Fujisawa Institute of Neuroscience, University of Edinburgh, 1 George Square, Edinburgh, EH8 9JZ.

This study was undertaken to investigate the effects of chronic treatment with citalopram, a selective serotonin uptake inhibitor used widely in the treatment of depression, on the distribution and density of serotonin transporter (SERT) sites in rat brain. Citalopram (10mg/kg s.c.) was administered twice daily for 21days. The density of SERT sites was measured by immunohistochemical techniques, using antibodies raised against selected sequences of SERT, conjugated to keyhole limpet haemocyanin. The rats were perfused through the aorta with 4% paraformaldehyde, 0.05% glutaraldehyde. Brains were removed and cryoprotected in 30% sucrose overnight and then sectioned at 30μm. Sections were preblocked in PBS containing 4% normal goat serum for two hours then incubated with antibody (1:1000 dilution) overnight. SERT immunoreactivity was detected with the Vectastain ABC Elite kit. Chronic administration of citalopram produced a dramatic reduction in the density of terminal field staining in the striatum with a smaller reduction seen in the hippocampus. These data support the findings of Piñeyro et al (1994) where chronic paroxetine treatment was seen to reduce ^{3}H-5HT uptake and ^{3}H-paroxetine binding in rat hippocampal and cortical membranes. Similarly Lesch et al (1993) have demonstrated a significant reduction in SERT mRNA levels in rat midbrain, after chronic antidepressant treatment, as measured by Northern blot analysis.

Lesch, K.P. et al (1993) Mol.Brain.Res., 17, 31-35.
Piñeyro, G. et al (1994) J.Neurosci., 14.5, 3036-3047.

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245.7

INTERACTION BETWEEN WAY 100635 A SELECTIVE 5-HT1A RECEPTOR ANTAGONIST AND LOW FLUOXETINE DOSE ON SEROTONIN LEVELS AT NERVE TERMINALS: AN IN VIVO MICRODIALYSIS STUDY. I. Malagié*, C. Jacquot and A.M. Gardier. Lab. Neuropharmacol. JE DRED 92-372, Fac. Pharm Univ. Paris-Sud, F92296 Chatenay-Malabry, France.

In the rat, a single administration of fluoxetine (Flx), a selective serotonin reuptake inhibitor (SSRI) at low doses, i.e., comparable to those used therapeutically, increases extracellular serotonin (5-hydroxytryptamine, 5-HT) levels in the vicinity of cell bodies and dendrites of serotoninergic neurons of the median and dorsal raphe nuclei. In these areas, high densities of 5-HT$_{1A}$ autoreceptors and of 5-HT transporters have been described. This effect is more marked than that observed in regions rich in 5-HT nerve endings (frontal cortex FC, ventral hippocampus vHi) (Malagié et al., *Eur. J. Pharmacol.*, 286 (1995) 213). This could be explained by activation of somatodendritic 5-HT$_{1A}$ autoreceptors by endogenous 5-HT in the raphe nuclei, thus leading to decreases in the firing rate of serotoninergic neurons, thereby limiting the frontocortical effects of the antidepressant. To test this hypothesis, we studied the changes in extracellular 5-HT levels in the FC and vHi, induced by the combined administration of a highly selective 5-HT$_{1A}$ receptor antagonist, WAY 100635 (0.1 mg/kg, i.v.), and Flx (1 mg/kg, i.p.) to conscious rats. In the 2 brain areas studied, no change in extracellular 5-HT concentrations was observed following Flx administration over the 210 min post-injection period. However, in animals co-administered with WAY 100635 + Flx, the maximal increase in 5-HT levels in FC dialysates was to 215% of the respective basal value (100%), while no change was found in hippocampal 5-HT. We also found that the major active metabolite of Flx, norfluoxetine (NFlx), concentrated in the frontocortical tissue but not in the hippocampal one, thus achieving a mean ± S.E.M. [NFlx]-to-[Flx] concentration ratios of 2.1 ± 0.16 and 1.29 ± 0.08 n=7, respectively, as measured when the effect on extracellular 5-HT was maximal in FC , i.e., 120 min after Flx administration alone or combined with WAY 100635. Our data underline the role of somatodendritic 5-HT$_{1A}$ receptor blockade in revealing the neurochemical effects of clinically relevant SSRI doses.

245.8

MOLECULAR CLONING OF THE MURINE SEROTONIN TRANSPORTER GENE AND IDENTIFICATION OF BRAIN TRANSCRIPTION INITIATION SITES. A.L.Bauman*, N. Flattem, M.D. Malone, J.D. Fritz, R.D. Blakely, Dept. of Pharmacology and Ctr. Mol. Neuroscience, Vanderbilt University Sch. of Medicine, Nashville, TN 37232.

The serotonin (5HT) transporter (SERT) is responsible for termination of synaptic transmission at central and peripheral serotonergic synapses. SERT gene expression in the CNS is restricted to midbrain and brainstem serotonergic neurons and regulated by cAMP, antidepressants, and growth factors. The mechanisms of cell-specific and activity-mediated SERT gene expression are not understood, nor are the consequences of compromised SERT expression. To pursue these questions, we have isolated a murine SERT (mSERT) genomic clone and have used 5'RACE to identify transcription start sites in mouse brain. Oligonucleotides complementary to mSERT cDNA sequences were used to identify an amplicon from mouse genomic DNA. The identity of the amplicon was verified by Southern blotting and direct sequencing. The amplicon primers were subsequently used to obtain a genomic clone from a murine ES 129 P1 library. Verification and mapping of the mSERT genomic clone was accomplished by Southern analysis using mSERT cDNA and oligonucleotide probes, as well as through direct sequencing. The mSERT genomic clone spans more than 20Kb and, like the human SERT gene, is comprised of multiple exons. 5'RACE reactions performed on mouse brain RNA identified 560 bp of 5' noncoding sequence upstream of the mSERT initiator methionine. The sequence has been mapped on the P1 clone, significantly upstream of previously reported initiation sites (Genbank U26452), and indicate at least one distal noncoding exon likely to contribute to CNS SERT mRNAs. (Supported by NIH grant DA07390)

245.9

VOLTAGE-DEPENDENCE OF 5-HT-TRANSPORT-ASSOCIATED CURRENT. A. Galli, C. I. Petersen, M. deBlaquiere, S. Ramsey, R. D. Blakely* and L. J. DeFelice. Dept. of Pharmacology, Vanderbilt Univ., Nashville, TN 37232.

Serotonin (5-HT) transporters (SERTs) are the targets for antidepressants and drugs such as cocaine. We have microinjected cRNA encoding the Drosophila serotonin transporter (dSERT) into Xenopus oocytes to determine 5-HT uptake characteristics and the ion permeation of the 5-HT transporter under voltage-clamp conditions. Several recent reports indicate that co-transporters generate large currents that are incompatible with a fixed stoichiometry. Recently, channel behavior has been described in mammalian 5-HT transporters. Here we report the possibility that the channel mode of conduction is also able to provoke a non-stoichiometric-coupled 5-HT associated current. In these experiments the correlation of [^{3}H]5-HT uptake with net charge movement in same voltage clamped oocytes shows the average quantity of charge translocated per 5-HT molecule varies with membrane potential and increases at negative voltages (150 e at -120 mV versus 15 e at 0 mV). By addressing the effect of voltage on [^{3}H]5-HT uptake and associated currents, we can examine mechanistic aspects of 5-HT uptake at the single transporter level. Supported by NIH DA 07390, NS 34075-01, NARSAD.

245.10

ACUTE ADMINISTRATION OF RESERPINE MODULATES SEROTONIN TRANSPORTER GENE EXPRESSION IN A TIME DEPENDENT MANNER IN THE RAT BRAIN. Q. Xiao, A. Pawlyk and S. M. Tejani-Butt*. Department of Psychiatry, University of Pennsylvania School of Medicine, Philadelphia, PA 19104.

Despite recent advances in determining central serotonin function, the basic aspects by which serotonin neurotransmission is controlled and regulated are still not understood. Since the serotonin transporter (5-HTT) is involved in terminating the action of 5-HT that is released from the presynaptic nerve terminal, the regulation of the 5-HTT may be an important step in controlling 5-HT neurotransmission at the synaptic cleft. The present study investigated the effects of reserpine administration on 5-HTT gene expression in the rat brain. Male Sprague-Dawley rats were injected with reserpine (10 mg/kg, i.p.) and sacrificed at 8h, 3d, 7d or 21d after the injection. Control rats were injected with saline and sacrificed either 8h or 21d after the injection. The midbrain region was dissected, RNA isolated and probed for 5-HTT expression using Northern Blotting. Date were analyzed using Super-Anova followed by post-hoc Dunnett's test. While mRNA levels for 5-HTT were unchanged at 8h after reserpine, a significant decrease was noted at 3d and 7d ($F=10$; $p<0.0001$) with mRNA levels returning to control values by 21d. The results of this study provide useful information regarding the role that the 5-HTT may be playing in the homeostatic control of 5-HT neurotransmission at the synapse. (Research funds from USPHS grant NS 31699).

245.11

EXPRESSION, FUNCTION, AND DEVELOPMENT OF A 5-HT TRANSPORTER (SERT) IN THE PLASMA MEMBRANE OF THYROID FOLLICULAR CELLS. H. Tamir*[1,2] K.P. Liu[1], S.H. Hsiung[1], R.D. Blakely[3], A.F. Russo[4], M.S. Clark[4] & M.D. Gershon[2]. 1. NYS Psych. Inst.: 2. Dpt. of Anat. and Cell Biol.P&S New York, NY.10032. 3.Dpt. of Pharmacol. Vanderbilt U. Nashville, TN 37232-6600; 4. Dpt. of Physiol. and Biophys. U. of Iowa Iowa City, IA. 52242.

5-Hydroxytryptamine (5-HT) is synthesized by the neural crest-derived thyroid parafollicular (PF) cells. All PF secretory vesicles co-store 5-HT with calcitonin; therefore, 5-HT and calcitonin are always co-secreted. Because 5-HT stimulates follicular (F) cells and modulates their response to TSH, 5-HT may be a PF → F paracrine transmitter . If so, then a thyroid mechanism, analogous to the SERT of serotonergic neurons, would be required to inactivate 5-HT. Since the thyroid lacks serotonergic nerves, and PF cells do not take up 5-HT, we tested the hypothesis that the SERT is expressed by the putative F cell targets. mRNA encoding SERT was found in human thyroid glands and a rat F cell line (FRTL-5). The FRTL-5 cells also took up ^{3}H-5-HT by a process that was temperature and Na^+-dependent, and antagonized by 5-HT-, but not catecholamine-selective uptake inhibitors. Antibodies to rat SERT immunocytochemically labeled F, but not PF, cells. Transporter-mediated uptake of ^{3}H-5-HT by F cells was found to arise early in development (E13 in mice) and to be maintained in adult life in mice, guinea pigs, and bats. These observations support the idea that 5-HT is a paracrine transmitter in the thyroid. Grant DK19743, NS12969, and NS15547.

245.12

TOPOLOGICAL LOCALIZATION AND DISULFIDE STATUS OF THREE CYSTEINE RESIDUES IN THE SEROTONIN TRANSPORTER Jie-Guang Chen, Shuxian Liu-Chen and Gary Rudnick* Dept. of Pharmacology, Yale University, New Haven, CT 06510

Hydropathy analysis predicts three cysteines (C109, C200, and C209) in extracellular loops of rat serotonin transporter. We mutated these residues, singly and in combination, to either alanine or serine and expressed the mutant transporters in HeLa cells using the vaccinia-T7 transient expression system. Mutation of C109 to alanine had no effect on transport activity or surface expression of the transporter. However, inactivation of transport activity by the impermeant cysteine reagent (2-aminoethyl)methanethiosulfonate (MTSEA) was reduced in the C109A mutant. In the absence of Na^+, MTSEA inactivation increased dramatically, and the effect of C109 replacement was more pronounced. Changing either C200 or C209 to serine resulted in a marked loss of transport activity and decreased surface expression of the fully glycosylated transporter. MTSEA rapidly inactivated the residual transport activity in mutant C200S, suggesting increased accessibility of a previously unreactive cysteine residue. The double mutant C109A-C200S was also very sensitive to MTSEA, but the reactivity of a C200S-C209S mutant was much lower, similar to that of the wild-type transporter. These results suggest that C200 and C209 are linked by a disulfide bond. This was further supported by the observation that surface expression of the transporter was normal in the C200S-C209S mutant. The Na^+-dependence of transport and ligand binding was abnormal in both C200S and C200S-C209S mutants. The results suggest that C109 is in the first external loop and that C200 and C209 are linked by a disulfide bond in the second external loop of the serotonin transporter. Disrupting this disulfide may prevent proper Na^+ binding and produce a free sulfhydryl group that inhibits proper surface expression of the transporter. Supported by grants from USPHS-NIDA.

245.13

SEROTONIN TRANSPORTER CHIMERAS CONTAINING THE THIRD AND FOURTH EXTERNAL LOOP OF THE NOREPINEPHRINE TRANSPORTER. Y. Smicun* and G. Rudnick. Dept. of Pharmacology, Yale Univ. Sch. of Med., New Haven CT 06510

To examine the role of the predicted external loops of the serotonin transporter (SERT) on its function, we created chimeric transporters in which the third and fourth external loops of SERT were exchanged with the corresponding loops of the norepinephrine transporter (NET). We expressed the SERT chimeras in HeLa cells using the vaccinia-T7 expression system and measured uptake of serotonin (5-HT), binding of the cocaine analog β-CIT, and cell surface expression. Transport of 5-HT by the third loop SERT-NET chimera was identical to that of wild-type SERT. The sensitivity of this chimera to the transport inhibitors cocaine, citalopram and desipramine was similar to that of wild-type SERT. These results suggest either that the amino-acid composition of the third loop is not important for the function of SERT, or that the conserved amino-acids in the third loop of the two transporters are sufficient for transport and inhibitor binding. HeLa cells transfected with the fourth loop SERT-NET chimera were impaired in uptake of 5-HT, and membrane vesicles prepared from these cells did not bind β–CIT. The transporter also was not expressed in the cell membrane. These results suggest that the fourth loop chimera does not fold properly.

246.1

ALGORITHMS FOR AUTOMATIC STRUCTURE IDENTIFICATION IN A 3-D RAT BRAIN ATLAS DEVELOPED AS TEMPLATE FOR IN SITU HYBRIDIZATION NEUROANATOMICAL DATABASE A. Danckaert, D. Talabot-Ayer, M. Peitsch, N. Guex, J. Knowles, F. E. Bloom^, E. Merlo Pich.*
Geneva Biomedical Research Institute, CH1228 Geneva, Switzerland, ^Neuropharmacology, The Scripps Research Institute, La Jolla, CA.

Mapping the brain distribution of novel genes is a foundamental step in the process of gene function identification. Since more that 50,000 genes are estimated to be present in the brain, a reasonable way to store, analyze, and compare these information is using neuroanatomical databases. Recent technological developments in the field of medical imaging, computer science, and networking has allowed neuroscientists to expand the concep of brain maps and databases. In the present work we describe the development of a neuranatomical working station, portable on PC and Macintosh computer, whose major goal is to support the correct attribution of areas of specific hybridization signal for a gene product to a given neuroanatomical structure and/or cell group. A 3-D reconstruction of adult Wistar rat brain was obtained by averaging about 600 images collected from 5 animals in both coronal and sagittal cutting planes. The initial 3-D rendering was obtained by defining asimmetric voxel with a resolution between 25 and 120 µm. Sections were stained with cresyl violet and regions of different staining intensities were identified on the captured image, separated by an automatic contour definition algorithm, reconstructed in 3-D, and named according to Paxinos (1986) and Swanson (1992) brain atlas. When a brain section containing in situ hybridization signal adiacent to a cresyl violet stained section is analyzed, an algorithm automatically attributes the signal to a given brain structure by comparing the location of the signal with the location of the structures defined in the "standard" brain, and store it in a database normalized over the "standard" brain. Funding was private.

246.2

CAN POSITRON EMISSION TOMOGRAPHY DETECT CHANGES IN INTRASYNAPTIC DOPAMINE? COMPUTER SIMULATIONS CAN OPTIMIZE THE EXPERIMENTAL DESIGN. E.D. Morris[1], C.A. Heidbreder[1], T.S. Shippenberg[1], M. Ernst[1], B.T. Christian[2] and E.D. London[1]* 1. Brain Imaging Section, NIDA, Baltimore, MD 21224 and 2. Dept. of Radiology, Massachusetts General Hospital, Boston, MA 02114.

Cocaine-induced inhibition of dopamine (DA) re-uptake results in increased intrasynaptic dopamine levels in forebrain regions. Computer simulations of a newly-modified model of receptor-ligand kinetics were performed to optimize the sensitivity of PET studies to changes in DA levels. A compartmental model of dynamic PET activity was developed (Morris et al., 1995): it assesses effects of transient changes in endogenous DA levels on receptor-ligand binding. The model requires knowledge of the inputs of exogenous and endogenous ligands to the system and their kinetic constants in brain tissue. The inputs are: (1) the amount of receptor-ligand which is injected as a single bolus, and (2) the dynamics of free DA in the neighborhood of the receptor. The dynamics of DA in the primate brain were derived from microdialysis assays of endogenous DA in the nucleus accumbens of the rat, before and after exposure to cocaine (Heidbreder et al., 1996). ^{11}C-Raclopride (RAC) was examined as a possible PET tracer. Its plasma input and tissue parameters were derived from the literature (Farde et al., 1989). Computer simulations examined the influence of several design considerations on the PET output: (1) timing of cocaine injection relative to RAC injection, (2) advantages of one DA-receptor ligand over another, (3) scanning duration, and (4) route of administration of cocaine challenge. An optimal design was defined as one that maximizes the weighted sum of squares difference between the simulated PET curves of 'rest' and of 'post-cocaine' experiments. Preliminary findings indicate that the optimal timing of cocaine injection is 5 min. prior to that of RAC. Changes in DA levels are undetectable with PET when cocaine injection follows RAC injection by 15 min. or more. Such simulations can help avoid unnecessary experimental manipulations of animals and facilitate interpretation of negative results.

246.3

125I-AB-MECA LABELS A1 AND A2A ADENOSINE RECEPTORS IN RAT BRAIN. D.R. Weaver* and L.P. Shearman. Lab. Developmental Chronobiology, Pediatrics, Mass. General Hospital, Boston, MA 02114.

Adenosine modulates neuronal activity and neurotransmitter release through interaction with cell surface receptors of A_1, A_{2A}, A_{2B}, and A_3 subtypes. ^{125}I-AB-MECA (N6-4-amino-3-iodo-benzyladenosine-5'-N-methyluronamide) has high affinity for recombinant A_1 and A_3 receptors (Olah et al., Mol Pharmacol 45:978-982, 1994). We studied whether this ligand is useful for autoradiographic localization of A_3 receptors in rat brain. Slide-mounted sections were incubated in binding buffer (50 mM TRIS, 10 mM $MgCl_2$, 1 mM EDTA, 2 U/ml adenosine deaminase), then incubated with ^{125}I-AB-MECA (400 pM) in the same buffer for 90 min at 22° C, washed (10 min, 0°C), dried, and exposed to film for 7-14 d. Non-specific binding was defined with 10 uM NECA.

The autoradiographic distribution of ^{125}I-AB-MECA binding closely resembled the pattern of A_1-receptor binding, with specific binding in cerebellum > hippocampus > thalamus > cortex = striatum. XAC (1 uM) completely blocked specific ^{125}I-AB-MECA binding, indicating binding was primarily to A_1, not A_3 receptors, under these conditions. In the presence of CPX (50 nM, to block A_1 receptors), specific ^{125}I-AB-MECA binding remained only in the neostriatum. Competition studies indicated that striatal ^{125}I-AB-MECA binding in the presence of 50 nM CPX reflected binding to A_{2A} receptors. Our studies indicate that ^{125}I-AB-MECA labels A_1 and A_{2A} adenosine receptors in rat brain. The level of A_3 receptor binding in rat brain appears insufficient to allow autoradiographic detection of this subtype. *Supported by HD29470. 125I-AB-MECA was generously provided by NEN Life Science Products.*

246.4

DISTRIBUTION OF A_{2A} ADENOSINE RECEPTORS (A_{2A}ARs) IN RAT BRAIN: CHARACTERIZATION OF MONOCLONAL ANTIBODIES AND IMMUNOHISTOCHEMICAL LOCALIZATION OF A_{2A}ARs. D.L. Rosin,[1] R. Woodard,[2] P.G. Guyenet[1] and J. Linden[3] Depts. of Pharmacol.,[1] Physiol.[2] and Medicine,[3] Univ. of Virginia, Charlottesville, VA 22908

The purine nucleoside adenosine exerts its diverse effects in the CNS through four receptor subtypes: A_1, A_{2A}, A_{2B} and A_3. To better understand the cellular mechanisms of adenosine's actions, we have developed monoclonal antibodies directed against purified, full length, nondenatured human A_{2A}AR, extended on the amino terminus with hexahistidine and the 8 amino acid FLAG epitope to facilitate purification. Antibodies recognized the purified receptor and not the FLAG peptide by ELISA. Immunohistochemical analysis of HEK293 cells expressing the human ARs or western blot analyis of membranes from transfected HEK293 cells demonstrates that the antibody recognizes the A_{2A} but not the A_1 or A_3 subtypes; western blots revealed a weak (<5%) crossreactivity to A_{2B}AR. A 45 kDa glycosylated protein from rat striatum or A_{2A}-transfected HEK293 cells was recognized by western blot. In rat brain intense A_{2A}-like immunoreactivity (A_{2A}-LI) was found in striatum, nucleus accumbens, and olfactory tubercles in a pattern similar to that reported by binding autoradiography or *in situ* hybridization for the receptor. Circumventricular organs (subfornical organ and area postrema) were also well labeled. A_{2A}-LI in subpostremal nucleus solitarius was markedly reduced following nodose ganglionectomy; these data confirm the presence of A_{2A}ARs on central terminals of rat vagal afferent neurons and suggest a modulatory role for adenosine in cardiopulmonary reflexes. In summary, we have developed and characterized specific anti-A_{2A}AR antibodies; the discrete pattern of distribution of A_{2A}-LI in rat CNS detected with these antibodies is consistent with other measures of receptor localization and supports a role for adenosine in modulating dopaminergic and cardiovascular function. *Supported by HL37942, HL28785, Scottish Rite Schizo. Res. Program and Amer. Heart Assoc.-VA Affiliate.*

246.5

IMMUNOHISTOCHEMICAL STUDIES ON THE LOCALIZATION OF ADENOSINE A1 RECEPTOR IN THE POSTNATAL AND ADULT RAT HIPPOCAMPUS. T. Ochiishi*[1], A. Yukawa[1], H. Miyamoto[1], Y. Saitoh[2], H. Nakata[2], Y. Sekino.[3,4] [1]Biosignalling Dept., National. Inst. of Biosci. & Human Technol., Tsukuba, Ibaraki 305, [2]Dept. of Mol & Cell Neurobiol., Tokyo Metropolitan Inst. for Neurosci., Fuchu, Tokyo 183, [3]PRESTO, Res. Dev. Corp. of Japan, Mitsubishi Kasei Inst.of Life Sci., Machida, Tokyo 194, and [4]Dept. of Neurobiol. & Behav., Gunma Univ., Maebashi 371, Japan.

Adenosine has a potent inhibitory effect through its A1 receptor in the central nervous system. Several studies have recently shown that adenosine acts as one of the regulatory factors for synaptic plasticity such as long-term potentiation. In order to elucidate cellular mechanisms underlying hippocampal plasticity, we focused on function of adenosine A1 receptor in adult and developing rat hippocampus. In this study, we used the rabbit antiserum against the adenosine A1 receptor protein purified from rat brain (Nakata, 1993) and examined when and where the adenosine A1 receptor is expressed during the development and at adult rat hippocampus using immunohistochemical technique.

In the adult rat (3-months of age)brain, immunoreactivities to adenosine A1 receptor were observed in the Ammon's horn and dentate gyrus. In the fields CA1 and CA3, around the cell body of pyramidal cells were immunostained. In addition, a little higher immunoreactivities were observed in the surrounding area of the pyramidal cells and their dendrites of the CA2 field. However, during the development, Ammon's horn was uniformly stained by this antibody until post-natal day 21 (P21). The mature type of immunopositive profiles were established from P21 to P28.

246.6

EARLY POSTNATAL EXPRESSION OF L-HISTIDINE DECARBOXYLASE AND HISTAMINE H_1 RECEPTOR IN THE RAT BRAIN. T. Sallmen, M. Lintunen, K. Karlstedt, H. Fukui, K.S. Eriksson and P. Panula*. Dept. of Biology, Abo Akademi Univ., 20520 Abo/Turku, Finland, Dept. of Pharmacology II, Osaka Univ., Osaka, Japan.

Histaminergic tuberomammillary neurons with developed projections are present in the neonatal rat brain. An other pool of brain histamine is in mast cells, that migrate into the brain during embryonic development. Some of these cells later migrate to the thalamus, but most of them disappear after postnatal days 10-12. The functions of these mast cells are still unknown. In this study the expression of the histamine synthesizing enzyme L-histidine decarboxylase (HDC) and histamine H_1 receptor was studied by *in situ* hybridization in the rat brain on postnatal days 0, 1, 2, and 5. High HDC expression and intense histamine immunofluorescence were found from day 0 in the tuberomammillary cell bodies as in adult rats, but also in the mast cells of the choroid fissure. Histamine-storing mast cells in adult brain did not express HDC. The HDC expression in the mast cells appeared on the day of birth and started decreasing on postnatal day 5. These results identify early mast cells as the only known site of HDC synthesis in the rat brain in addition to neurons. These mast cells differ from those observed in rat tissues in general, as they have both high histamine content and high expression of HDC. Histamine H_1 receptor mRNA was fairly evenly distributed in the rat brain on the day of birth, but the expression level increased in the hippocampus, hypothalamus, and cortex beginning on postnatal day 1, possibly indicating a correlation to the histamine containing mast cells, and the histaminergic neurons in the tuberomammillary nucleus. The contribution of early mast cells to histamine content will be determined by HPLC fluorometry.

Supported by the Academy of Finland, the Sigrid Juselius Foundation, and the Alcohol Research Foundation of Finland.

246.7

EXPRESSION AND CELLULAR LOCALIZATION OF B_2 BRADYKININ RECEPTOR mRNA IN RAT SENSORY NEURONS. S. Yokoyama*, H. Takeda, and H. Higashida. Dept. of Biophysics, Neuroinformation Research Institute, Kanazawa University School of Medicine, Kanazawa 920, Japan

We have previously demonstrated by cDNA cloning that both rat and mouse B_2 bradykinin receptors (BKRs) of the smooth muscle type are expressed in NG108-15 mouse neuroblastoma x rat glioma hybrid cells (BBRC 200: 634-641, 1994). The B_2 BKR of rat origin is identical with those cloned from rat uterus and PC12 pheochromocytoma cells. Using the cDNA as probe, we examined expression and cellular localization of B_2 BKR mRNA in adult rat dorsal root and trigeminal ganglia. In both tissues, RNA blot hybridization analysis identified RNA species of ~6000 and ~4000 nucleotides, consistent with those observed in NG108-15 cells. Reverse transcription polymerase chain reaction (RT-PCR) followed by DNA sequencing confirmed the presence of B_2 BKR mRNA. For *in situ* hybridization study, two digoxigenin-labeled RNA probes were prepared to recognize different parts of B_2 BKR mRNA. In dorsal root ganglia, these probes gave intense signals for B_2 BKR mRNA in neural cell bodies; small neurons were more frequently stained than large neurons. The tendency was less prominent in trigeminal ganglion neurons. These results suggest that the B_2 BKR of the smooth muscle type is involved in regulating pain conduction.

246.8

A NOVEL STRATEGY TO PREPARE FUNCTIONALLY ACTIVE [^{35}S]LEPTIN: IDENTIFICATION AND CHARACTERIZATION OF A HIGH AFFINITY LEPTIN BINDING SITE IN THE CHOROID PLEXUS OF MOUSE AND RAT BRAIN. Xiao-Ming Guan*, Hong Yu, Michael R. Tota, Michael P. Graziano, Lei Xu, Patricia J. Hey, Lex H.T. Van Der Ploeg and Roy G. Smith. Department of Biochemistry and Physiology, Merck Research Laboratories, Rahway, NJ 07065

Leptin is the product of the recently identified *ob* gene, serving an important role in the control of food intake and energy homeostasis. To localize the leptin receptor in the brain, we developed a novel labeling strategy involving a cell-free expression system to produce a radioactively pure, native [^{35}S]leptin probe for receptor autoradiographic studies. Using this radioligand, we identified a high affinity binding site for leptin in the choroid plexus of mouse and rat brains. This binding was specific to leptin, insensitive to GTPγS, and highly concentrated in the choroid plexus. In addition to binding in the wild-type and *ob/ob* mice, equally potent binding of [^{35}S]leptin was observed in the choroid plexus of the *db/db* mouse, an animal model that is proposed to have a defective leptin receptor. Similarly, ZDF rats, another commonly used animal model of diabetes and obesity, displayed leptin binding indistinguishable from wild-type rats. Our data provide functional evidence in support of the notion that insensitivity to leptin stimulation as observed in *db/db* and ZDF animals is not due to a deficiency in ligand binding domain of the leptin receptor, but rather is likely to be the result of abnormality occurring at a site(s) down stream from receptor binding.

Source of support: Merck & Co.

246.9

SPECIFIC [^{125}I]OB PROTEIN BINDING SITES LOCALIZED IN CHOROID PLEXUS, THALAMUS AND HYPOTHALAMUS IN RAT. E.S. Corp*, D.B. Conze, F. Smith and L.A. Campfield. The Bourne Laboratory, Cornell University Medical College, White Plains, NY 10605 and Hoffmann-La Roche, Nutley, NJ 07110.

OB protein (OB) is synthesized in fat, circulates in plasma and reduces appetite and body weight following peripheral or CNS administration in rats and mice. In the brain, OB protein receptors (OB-R) are expressed maximally in the choroid plexus (CP) in the lateral cerebral ventricle. The behavioral potency of OB injected icv and molecular studies indicating the presence of OB-R mRNA in hypothalamus suggest that OB-R are present in hypothalamus. However, the precise anatomical location of OB-R within the hypothalamus is not known. Prior to a survey for specific [^{125}I]hOB binding sites in hypothalamus, we used quantitative receptor autoradiography to determine optimal binding conditions for [^{125}I]hOB in the CP; and additionally, using homologous competitive binding assays, determined binding parameters for [^{125}I]hOB in the CP. Specific binding of [^{125}I]hOB was pH dependent (optimal, pH 6.0). Nonspecific binding was 10 to 12% of maximal binding. [^{125}I]hOB bound with high affinity in the CP; KD, 0.3 ± 0.04 nM; Bmax, 8.5 ± 0.8 fmol/mg tissue. [^{125}I]hOB binding in the CP was insensitive to unlabelled 1 μM insulin, 100 nM NPY, or 100 μM GTP-*y*-S. Relative to binding measured in the CP, comparable levels specific [^{125}I]hOB binding sites were detected in the meninges and surrounding tissue of the cerebral arteries; low to moderate levels of specific binding were measured in the ventrolateral nucleus of the thalamus (14 ± 3% of CP). In the hypothalamus, low levels of specific binding sites were localized and measured in the arcuate (ARC, 7 ± 2 % of CP) and ventromedial nucleus (VMN, 6 ± 1% of CP). Both the VMN and ARC are well-established as neural sites involved in the control of feeding the regulation of body weight. These data suggest that OB-R are present in VMN and ARC where they may mediate OB effects on appetite and body weight.

Supported by Hoffmann-La Roche and the Whitehall Foundation

246.10

DISTRIBUTION OF LEPTIN RECEPTOR (OB-R) mRNA IN THE CENTRAL NERVOUS SYSTEM OF THE RAT. C.C. Cheung, S. Lok, J.L. Kuijper, D.S. Weigle, P.D. Finn*, D.K. Clifton, and R.A. Steiner Depts. PBIO, Ob-Gyn, and Medicine, Univ. Washington, and Zymogenetics, Inc., Seattle, WA 98195

Leptin is a secretory product of adipocytes and is thought to have a physiological role in the regulation of body weight and metabolism. The putative leptin receptor (OB-R) is the protein product of the *diabetic* (*db*) gene in the mouse and *fatty* (*fa*) gene in the rat, and mutations in *db* or *fa* result in obesity, hyperglycemia, and infertility. To learn more about leptin's action in the brain, we used *in situ* hybridization to determine the distribution of OB-R mRNA in rat brain tissue. A ^{33}P-labeled OB-R cRNA probe containing the region encoding the C-terminus of the extracellular domain, the putative transmembrane domain, and the box 1 motif in the cytoplasmic tail was used. We found OB-R mRNA to be highly expressed in the leptomeninges and choroid plexus lining the ventricles, in piriform cortex, and in the molecular layer of the cerebellum. Moderate levels of expression were detected in the thalamus (in the ventral medial, ventral posterolateral, ventral posteromedial, lateral dorsal, and lateral posterior thalamic nuclei). OB-R mRNA was found to be expressed at low to moderate levels in the hypothalamus (in the arcuate, dorsomedial, ventromedial nuclei, and the retrochiasmatic area) and CA1 pyramidal layer of the hippocampus. *Conclusion:* The widespread distribution of OB-R mRNA in the brain implies that the role of leptin extends beyond the regulation of food intake and metabolism and suggests that either leptin or a similar molecule may be expressed as an endogenous ligand in the brain. *(Supported by NIH grant RO1 HD27142)*

246.11

DISTRIBUTION OF [^{125}I]LEPTIN BINDING SITES IN RAT AND MOUSE BRAIN AND PERIPHERAL TISSUES. M.M. Durkin*, E.L. Gustafson, N. Adham, C. Gerald, B. Borowsky, Y. She, D.L. Linemeyer, and T.A. Branchek. Synaptic Pharmaceutical Corp., 215 College Rd., Paramus, NJ 07652.

We have utilized receptor autoradiographic techniques to determine the distribution of [^{125}I]leptin binding sites in rat and mouse brain, as well as in selected peripheral tissues from both species. Incubation of tissue sections with 1 nM [^{125}I]leptin resulted in binding in numerous tissues. The specificity of the binding was ascertained by co-incubation of the tissue with 100 nM purified mouse leptin, which completely inhibited the binding of [^{125}I]leptin in all tissues.

The most intense autoradiographic signals were seen in the choroid plexus of the brain, in the lung, in the spleen, in lymph nodes, and in the thymus. The testes and omental fat tissue were negative, consistent with previous reports (Tartaglia et al., 1996). In order to determine the cell types, [^{125}I]leptin was cross-linked to the tissues using either glutaraldehyde or paraformaldehyde, and the slides dipped in photographic emulsion. The CNS binding was restricted to cells of the choroid plexus, and no labelling was observed in the brain parenchyma. In the spleen, [^{125}I]leptin binding sites were located in the red pulp, and on smooth muscle cells of blood vessels. In the lung, a high level of specific binding was observed in smooth muscle cells of the arterioles, and a lower level in the pulmonary artery. [^{125}I]leptin binding sites in the thymus were found mainly on cells in the medullary region.

The pattern of [^{125}I]leptin binding observed in the present study is mostly consistent with the reported localization of the cloned leptin receptor mRNA described by molecular biological techniques. In addition, we have now determined the cellular identity of the binding sites located in the rat and mouse CNS and periphery.

Funded by Synaptic Pharmaceutical Corporation and Ciba-Geigy Limited.

246.12

NEUROKININ-2 RECEPTORS IN THE GUINEA-PIG GASTROINTESTINAL TRACT: REGIONAL AND CELLULAR DISTRIBUTION [1]A.L. Portbury*, [1]J.B. Furness, [2]H.C. Wong, [3]J.H. Walsh, [3]N.W.Bunnett [1]Department of Anatomy and Cell Biology, University of Melbourne, Parkville, Victoria, 3052, Australia, [2]CURE Gastroenteric Biology Center, VA Wadsworth Medical Center, Los Angeles and [3]Departments of Physiology and Surgery, UCSF, San Francisco.

Results of studies concerning the localisation of the NK2 tachykinin receptor in the gut are ambiguous in that the autoradiographic localisation of the receptor does not correlate fully with the available pharmacological data. In order to clarify the distribution of this receptor in the guinea-pig gastrointestinal tract, we have carried out immunohistochemical studies using a polyclonal antiserum raised against the C-terminal region of the receptor. Immunoreactive nerve terminals, but never nerve cell bodies, were found throughout the myenteric plexus in all regions examined except the stomach and esophagus. NK2 receptor-immunoreactive terminals were rare in the submucous and tertiary plexuses. Reactivity was always confined to the varicose portion of the axon; inter-varicose and terminal parts of the axons were non-reactive. Lesion studies in the small intestine revealed the reactive terminals to be intrinsic in origin and anally directed. NK2 receptors occurred on terminals of NOS interneurons. In addition to nerve terminals, smooth muscle cells were NK2 receptor-immunoreactive. The immunoreactivity was localised mainly to the cell surface where it appeared to be evenly distributed along the entire cell membrane. In most regions examined, NK2 receptor-immunoreactivity was seen on muscle cells of both the longitudinal and circular layers. In the caecum, the taenia displayed strong NK2 receptor-immunoreactivity. A subpopulation of epithelial cells at the bases of the glands in the proximal colon were strongly immunoreactive for the NK2 receptor, as were a minority of epithelial cells in the caecum, distal colon and rectum.

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246.13

VASOPRESSIN/GALANIN NEURONS IN THE BNST AND AME COEXPRESS GALANIN RECEPTOR mRNA. M.A. Miller*, P.E. Kolb, and M.A. Raskind. Department of Psychiatry and Behavioral Sciences, University of Washington, Seattle, WA 98195.

The neuropeptides vasopressin (VP) and galanin (GAL) have been implicated in learning and memory processes. VP and GAL are coexpressed in several regions including the bed nucleus of the stria terminalis/medial amygdala (BNST/AMe) complex. These steroid-sensitive neurons project to the lateral septum (LS) and ventral hippocampus (VH), regions important for mediating the effects of both peptides on memory. GAL, cosecreted with acetylcholine (ACh) into the VH, has been hypothesized to act at a presynaptic receptor to inhibit further ACh release and at a post-synaptic receptor to antagonize the actions of ACh. To determine whether GAL, cosecreted with VP, could also directly regulate BNST/AMe neurons, we have used double in situ hybridization histochemistry to assess the coexpression of GAL receptor mRNA by these cells. GAL mRNA expressing neurons were detected in coronal sections through the BNST and AMe of adult male Wistar rats (n=5) using a digoxigenin-labeled cRNA probe (generously provided by Dr. Henry Friesen, University of Manitoba) and GAL receptor mRNA expressing neurons were detected using a radio-labeled cRNA probe (generously provided by Bristol-Myers Squibb). The majority of GAL mRNA expressing neurons in the BNST/AMe complex coexpressed the GAL receptor. GAL receptor mRNA expressing neurons were also present in the LS and VH. These results raise the possibility that, in addition to exerting post-synaptic actions within the LS and VH, GAL may directly modulate the activity and/or secretion pattern of VP/GAL neurons in the BNST and AMe. Supported by NS33606 (NIH) and AG10917 (NIA).

246.14

AVP V_{1A} RECEPTORS IN THE RHESUS MONKEY BRAIN. D. M. Toloczko[1,3], L. Young[2], and T. R. Insel*[2,3] [1]Department of Anthropology and Organismic and Evolutionary Biology Program, University of Massachusetts, Amherst, MA 01003; [2]Department of Psychiatry, Emory University School of Medicine, Atlanta, GA 30322; and [3]Yerkes Regional Primate Research Center, Atlanta, GA 30329

Binding sites for [^{125}I]Lin-AVP were mapped in the rhesus monkey brain by using receptor autoradiography. Binding was found in layers II-III and V-VI of several cortical fields with labelling most intense within the cingulate, insular, and entorhinal cortices. These same layers were labeled in all prefrontal cortical fields. Subcortical regions with high specific binding included the mammillary bodies, the central nucleus of amygdala, and the olfactory and uncinate fasciculi. Competitive ligand binding results were consistent with binding to a V_{1A} receptor. Furthermore, molecular studies supported a V_{1A} receptor identity. V_{1A} cDNA was amplified by RT-PCR from select rhesus brain regions. Using a probe derived from our clone of the rhesus liver V_{1A} receptor, Southern blots confirmed the pharmacologic characterization. The pattern of binding found in several subcortical regions may be relevant to previous reports of AVP effects on memory. Supported by RR0165.

246.15

VASOPRESSIN, OXYTOCIN AND ANGIOTENSIN II RECEPTORS IN THE BRAIN OF INBRED POLYDIPSIC MICE.

M. Chritin[1], Y Ueta[2], H. Yamashita[2], K. Koizumi[3], J.J. Dreifuss*[1] and E. Tribollet[1]. [1]Dept of Physiology, University Medical Center, 1211 Geneva 4, Switzerland; [2]Dept of Physiology., University of Occupational and Environmental Health, School of Medicine, Iseigoaka 1-1, Yahatanishi-ku, Kitakyushu 807, Japan; ,[3]Dept of Physiol., SUNY Health Sci. Ctr. Brooklyn, NY 11203, USA.

The STR/N strain of mice is characterized by extreme polydipsia and polyuria which are not due to abnormal vasopressin (AVP) production. These impairements are thought to result from central mechanisms leading to an abnormally increased appetite for water. However the nature of the central mechanisms involved has not been elucidated yet. In the present study, we have examined the distribution and density of AVP, ocytocin (OT) and angiotensin II (Ang II) receptors by *in vitro* autoradiography in these two strains. The distribution of the 3 types of receptors was similar in STR/N mice and in ICR mice, but the amount of AVP and Ang II receptors was markedly different in a few areas. A high number of AVP binding sites was found in the periventricular thalamic nucleus of polydipsic mice while this structure was only lighly labelled in control mice. Ang II binding sites were much less numerous in the magnocellular part of the paraventricular nucleus in polydipsic mice than in control ones. On the contrary, the amount of Ang II binding was markedly higher in the area-postrema and nucleus of the solitary tract area in polydipsic mice. These differences were found both in males and in female mice. The amount of OT binding sites was similar in polydipsic and control mice. These data further support the hypothesis that the AVP and Ang II central systems are involved in the mechanisms responsible for polydipsia in STR/N mice.

Swiss National Science Foundation, Grant 31.00-040878.94. OTT Foundation.

246.16

LOCALIZATION OF ESTROGEN RECEPTORS IN THE HIPPOCAMPUS OF MALE AND FEMALE RATS. N.G. Weiland*, C. Orikasa[a], S. Hayashi[a], and B.S. McEwen, Neuroendocrinology, Rockefeller University, New York, NY and [a]Tokyo Metropolitan Institute for Neuroscience, Tokyo Japan.

Several aspects of hippocampal function are altered by estrogen administration including increased agonist binding to NMDA receptors, modulation of GAD mRNA levels, and increased spine and synapse density of CA1 pyramidal neurons. Earlier autoradiographic studies using [^{3}H]-estrogen uptake indicated that estrogen receptors (ER) were present in the hippocampus, but cellular and regional locations were not established. We used immunocytochemistry (ICC) to determine the cellular location of the ER and whether or not sex differences in the distribution of ER exist in the hippocampus and cortex. Young adult male and female rats were gonadectomized for one week. The animals were perfused with acrolein and paraformaldehyde, and ICC was performed (ER polyclonal antibody 1:40,000, Hayashi) on every 6th section (40 μm) from rostral to caudal hippocampus. ER immunoreactivity (ERir) was observed only in interneurons and exhibited both regional and rostral-caudal gradients. No sex differences were observed. The density of ERir cells were greatest in the dentate hilar polymorph region in rostral hippocampus (bregma minus 2.5 mm), fell to low levels in the middle regions, and were high again in the most ventral region of the hilus (bregma minus 4-4.5 mm). The radiatum of dorsal CA1 contained the second most dense population of ERir cells, with the greatest density at bregma minus 4-4.5 mm. ERir cells were scattered in a very low density (0-4 cells/section) in the remaining regions of hippocampus. These data demonstrate a mechanism for a direct action of estrogen in all regions with primary sites in the hilus of the dentate gyrus and CA1 of the hippocampus in both male and female rats. (Supported by NIH grants NS30105 to NGW and NS07080 to BSM.)

246.17

IMMUNOCYTOCHEMICAL (ICC) LOCALIZATION OF NUCLEAR PROGESTIN RECEPTORS (PR) AND ESTROGEN RECEPTORS (ER) WITHIN THE RAT DORSAL RAPHE NUCLEUS (DRN). S.E. Alves*, N.G. Weiland, S. Hayashi* and B.S. McEwen. The Rockefeller University, New York, NY and *Tokyo Metropolitan Institute for Neuroscience, Tokyo, Japan.

Evidence indicates a modulatory role for estrogen (E) and progesterone (P) on serotonergic (5-HT) activity in the rat brain, however the mechanism(s) of action remain unclear. Recently, E-induced PR have been localized within 5-HT neurons of the macaque DRN (Bethea, Neuroendo 60:50-61;1994). In this study, we investigated whether PR and/or ER exist within 5-HT neurons in rat DRN. Young adult female and male Sprague-Dawley rats were gonadectomized, and 1 wk later were treated with either vehicle or estradiol benzoate (EB, 10 μg in oil, sc, 1x day/2 days). Animals were transcardially perfused the next day with acrolein / paraformaldehyde, and sequential, dual-label ICC was performed on adjacent 40 μm sections with either a PR monoclonal (1:1600 Affinity Bioreagents) or an ER polyclonal antibody (1:40,000 Hayashi), followed by a tryptophan hydroxylase (TPH) polyclonal antibody (1:1000 Protos Biotech). Specific nuclear E-induced PR-immunoreactivity (-ir) and ER-ir were seen in both sexes scattered through the rostral DRN, and more discretely located along the lateral wings of this nucleus, and into the surrounding central grey. However, no colocalization of nuclear PR or ER with cytoplasmic TPH-ir was observed in either sex or treatment, indicating that the steroid targets are not 5-HT cells. These findings suggest a species difference in ovarian steroid regulation of 5-HT activity, perhaps indirectly via local interneurons in the rat. We are presently investigating possible sex differences in receptor distribution and attempting to identify the phenotype of the steroid receptor-containing cells. This study was supported by NS07080 to BSM, NS30105 to NGW and an NIH Endocrine Training Grant DK-07313-17.

247.1

DIFFERENTIAL MODULATION OF Ca^{2+} MOBILIZATION EVOKED BY MULTIPLE RECEPTORS LINKED TO PHOSPHOINOSITIDE TURNOVER. E. del Rio*, C.P. Downes # & D.G. Nicholls Neuroscience Institute and #Biochemistry Department, University of Dundee, Dundee, DD1 9SY U.K.

Ca^{2+}-release from intracellular stores has been studied by digital imaging of fura-2 microfluorescence of rat cerebellar granule cells in primary culture. Both the cholinergic agonist carbachol and the glutamatergic agonist 1S,3R ACPD are known to evoke PI-turnover and release of Ca^{2+} from intracellular stores. We now show that stimulation of cerebellar granule cells with agonists for other PI-linked receptors (noradrenaline, histamine and serotonin) leads to distinctive patterns of intracellular Ca^{2+}-mobilization. We also show that at 4 DIV the majority of the cerebellar granule cells in the primary culture population contain receptors for all these agonists, and all these receptors can couple to Ca^{2+}-mobilization.

When responses in polarizing and mildly depolarizing conditions (20 mM KCl) are compared four patterns of response can be distinguished: 1) metabotropic glutamate receptor activation by the agonist 1S,3R ACPD in 20 mM KCl conditions leads to both an enhanced PI-turnover and larger Ca^{2+}-release signals, 2) muscarinic receptor activation by carbachol in 20 mM KCl results in higher PI-turnover without any perceivable change in the size and distribution of the Ca^{2+}-release signal, 3) PI-turnover stimulation by histamine appeared insensitive to 20 mM KCl while the resulting Ca^{2+}-release signal was largely enhanced and 4) in the case of noradrenaline and serotonin PI-turnover was not enhanced by 20 mM KCl but the Ca^{2+}-release signal was moderately, but significantly enhanced in the presence of 20 mM KCl.

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247.2

DISTRIBUTION OF PHOSPHATIDYLINOSITOL 3-KINASE (PI3K) ISOFORMS IN RAT BRAIN BY IMMUNO-HISTOCHEMISTRY AND [^{3}H]WORTMANNIN AUTORADIOGRAPHY AND IDENTIFICATION OF NOVEL PROTEIN INTERACTIONS, K. J. Hurt*, R. Zakhary, and S. H. Snyder. Dept. of Neuroscience, Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205.

PI3K exists as a dimer, usually composed of a p110 catalytic subunit and a p85 regulatory/adapter protein. Upon activation of receptor tyrosine kinases, p110 is recruited to the membrane by p85 association with phosphotyrosines. The kinase catalyzes the formation of phosphatidylinositol-3,4,5-trisphosphate (PIP3) from PI45-bisphosphate (PIP2) and participates in the activation of mitogenic and morphogenic pathways. Distribution of PI3K catalytic and regulatory subunits in adult rat brain has been demonstrated using autoradiographic and immunohistochemical techniques. The differential localization suggests different roles or regulation of PI3K-mediated signaling in different areas of the brain. Investigation of interacting proteins in immunoprecipitation and yeast 2-hybrid experiments has also identified novel interactions of PI3K with known proteins in the brain. (Supported by NIMH T32MH18030)

247.3

PHOSPHATIDYLINOSITOL 4,5-BISPHOSPHATE DECREASES THE ABILITY OF TUBULIN TO ACTIVATE Gαq. I.S. Popova*, E. Greene and M. M. Rasenick. Department of Physiology and Biophysics, University of Illinois, Chicago, IL 60612

We have shown previously that the cytoskeletal protein tubulin is involved in the regulation of phospholipase $C\beta_1$ ($PLC\beta_1$). At low dimer concentrations, tubulin with a GTP analog bound activates $PLC\beta_1$, while at high concentrations it inhibits the enzyme. It is suggested that the interaction of tubulin with both Gαq and $PLC\beta_1$, accompanied by a transfer of guanine nucleotide from tubulin to Gαq, is responsible for $PLC\beta_1$ activation. The $PLC\beta_1$ substrate, phosphatidylinositol 4,5-bisphosphate (PIP_2), binds to tubulin and prevents microtubule assembly. This interaction is thought responsible for the $PLC\beta_1$ inhibition by tubulin, since it might prevent the access of the enzyme to PIP_2. Here we investigate further the effect of PIP_2 on tubulin and the m_1 muscarinic receptor-triggered tubulin-Gαq interaction. First, the ability of tubulin to bind and hydrolyze guanine nucleotide in the presence and absence of PIP_2 has been tested. The incubation of tubulin with PIP_2 decreases the amount of GTP bound to tubulin. At a tubulin to PIP_2 molar ratio of 1:12, a 25% decrease in GTP binding is observed, however, PIP_2 does not affect tubulin GTPase activity. This could be explained by PIP_2 binding to a specific tubulin isotype. In membranes from Sf9 cells expressing m_1 muscarinic receptors, Gαq and $PLC\beta_1$, both carbachol and PIP_2 increase the association of tubulin-[^{32}P]AAGTP (azidoanilido-GTP) with the membrane. Carbachol, but not PIP_2, promotes the transfer of [^{32}P]AAGTP from tubulin to Gαq. When carbachol stimulation is studied in cells expressing m_1 muscarinic receptors and Gαq, but not $PLC\beta_1$, no increase in the association of tubulin-[^{32}P]AAGTP with the membrane, nor in the nucleotide transfer to Gαq are observed. These results suggest a specific interaction between tubulin and PIP_2 which decreases the guanine nucleotide binding to tubulin, but increases tubulin association with the membrane upon m_1 muscarinic receptor stimulation. Thus, cholinergic signaling might modify both lipid and cytoskeletal components associated with the synaptic membrane. Supported by NIMH and Council for Tobacco Res.

247.4

EFFECT OF EXCITATORY AMINO ACIDS ON INOSITOL PHOSPHATE ACCUMULATION IN RETINAL PIGMENT EPITHELIUM. G. Fragoso and A. M. López-Colomé*. Instituto de Fisiología Celular, Universidad Nacional Autónoma de México, Apdo. Postal 70-253, México 04510, D. F., México.

The retinal pigment epithelium (RPE) located between the choriocapillaries and the neural retina, plays a fundamental role in the maintenance of the structural and functional integrity of photoreceptors. We previously reported the presence of specific excitatory amino acid (EAA) receptors in chick and human RPE cells in culture, and in this work we have studied the effects of EAA on the inositol phosphate second messenger pathway in these cells. Primary cultures of RPE cells were prepared from 7-day-old chick embryos. Glutamic acid (L-GLU) at a concentration of 1 mM induced a 200 % increase in [^{3}H]-inositol phosphates (InsPs) accumulation within 15 minutes in cells preincubated with [^{3}H]-myo-inositol in the presence of 10 mM lithium chloride. L-GLU analogues (1 mM) stimulated InsPs accumulation in the following order: L-GLU = NMDA > QA = KA > ACPD. The potency of EAA analogues for stimulating InsPs formation was determined within a concentration range from 10 nM to 1 mM. NMDA-induced response was significant since 5 minutes of stimulation and this effect was dependent on external calcium. The NMDA-induced response was completely blocked by MK-801 (5 μM) and CPP (200 μM) and partially inhibited by the Ca^{2+}-channel blockers verapamil (10 μM), Nifedipine (10 μM) and Dantrolene (30 μM). (+)–α-Methyl-4-carboxyphenylglycine showed effect as blocker and 3,5-Dihydroxyphenylglycine as agonist of the ACPD-response. These data suggest the presence of mGluRs (mGluR1 and/or mGluR5) as well as NMDA receptors in the RPE cells, coupled directly or through the influx of external Ca^{2+}, to phospholipase C activation. The physiological meaning of these effects in RPE remains to be elucidated, but studies under way suggest the possibility of an involvement in functions as cell division or phagocytosis. Supported by Grant 3375-N from CONACyT.

247.5

ELEVATION OF INTRACELLULAR CALCIUM BY CARBACHOL AND DELTA OPIOIDS IN SH-SY5Y CELLS: CHARACTERIZATION OF THE COINCIDENT SIGNAL. A. Yeo, E.P. Kelly, P.J. Roberts* and G. Henderson. Dept of Pharmacology, University of Bristol, Bristol, BS8 1TD, U.K.

In SH-SY5Y cells activation of Gi/Go-coupled receptors does not elevate intracellular free calcium [Ca^{2+}]i but in the presence of ongoing muscarinic Gq-coupled receptor activation then Gi/Go coupled receptor activation now causes a further elevation of [Ca^{2+}]i. Here we show that δ-opioid receptor stimulation releases intracellular Ca^{2+} from the same thapsigargin-sensitive pool as muscarinic receptor activation.

[Ca^{2+}]i was measured in confluent monolayers of SH-SY5Y cells loaded with Fura2. DPDPE (30nM) elevated the peak increase in [Ca^{2+}]i when it was applied simultaneously with carbachol and also elevated the plateau increase in [Ca^{2+}]i when it was applied during a prolonged (5-30min) exposure to carbachol. In the absence of extracellular Ca^{2+} the elevation of [Ca^{2+}]i evoked by a high concentration of carbachol (1mM) was not maintained but declined to the predrug level presumably because the Ca^{2+} stores had been emptied. Emptying of the stores in this way abolished both the elevation of [Ca^{2+}]i evoked by bradykinin (acting at the Gq-coupled B2 bradykinin receptor) and by DPDPE (acting at the pertussis toxin-sensiti e, Gi/Go-coupled δ-opioid receptor).

Western blot analysis revealed that SH-SY5Y cells express only the PLCβ1 and PLCβ3 isoforms of PLCβ with the β1 isoform appearing to be the more abundant. The lack of βγ activatable PLCβ2 and the low level of PLCβ3 in these cells may explain why δ-opioid receptor stimulation alone does not elevate [Ca^{2+}]i.

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247.6

HETEROGENEITY IN CALCIUM SENSITIVITY OF MOUSE CEREBELLAR INOSITOL 1,4,5-TRISPHOSPHATE RECEPTORS. T. MICHIKAWA[1], T. HIROTA[1], S. KAWANO[2], M. HIRAOKA[2], T. FURUICHI[1], and K. MIKOSHIBA*[1,3]. [1]Department of Molecular Neurobiology, Institute of Medical Science, University of Tokyo, Tokyo 108; [2]Department of Cardiovascular Disease., Medical Research Institute, Tokyo Medical and Dental University, Tokyo, 113; and [3]Molecular Neurobiology Laboratory, Institute of Physical and Chemical Research (RIKEN), Tsukuba Life Science Center, Ibaragi 305, Japan

The inositol 1,4,5-trisphosphate (IP_3) receptor (IP_3R) is an IP_3-gated Ca^{2+} release channel localized on the intracellular Ca^{2+} storage sites, such as endplasmic reticulum, in both neuronal and glial cells. It has been shown that the cerebellar IP_3Rs are regulated by cytoplasmic Ca^{2+} in a biphasic manner with the maximum activity of the channels at Ca^{2+} concentration ([Ca^{2+}]) of 200~300 nM. The biphasic Ca^{2+} dependence might provide for both feed-forward and feed-back effects on Ca^{2+} that is believed to be a key property to generate Ca^{2+} waves and Ca^{2+} oscillations.

To analyze the Ca^{2+} dependent regulation of the IP_3R more precisely, cerebellar IP_3Rs reconstituted into planar lipid bilayers were characterized at the single channel level. IP_3-dependent currents (~120 pS in 50 mM Ba^{2+}) were measured in the presence of 2 μM IP_3 and 2 mM ATP by using a voltage-clamp technique after incorporation of mouse cerebellar microsome vesicles into planar lipid bilayers. [Ca^{2+}] in a cytoplasmic side was varied from 0.2 to 1.5 μM while maintaining free Ca^{2+} chelater (HEDTA) concentration at 1 mM. We found that there is a heterogeneity in the Ca^{2+} sensitivity among the cerebellar IP_3Rs. In addition to the channel whose maximum open probability was occurred at ~350 nM [Ca^{2+}], some channels revealed more low Ca^{2+} sensitivity with maximum open probability at 550~750 nM [Ca^{2+}]. The existence of two types of channel with different Ca^{2+} sensitivities provides a basis for complex patterns of intracellular Ca^{2+} signaling.

247.7

STRUCTURAL ANALYSIS OF THE LIGAND BINDING SITE OF THE TYPE 1 INOSITOL 1,4,5-TRISPHOSPHATE RECEPTOR. F. Yoshikawa, M. Morita, T. Monkawa, T. Michikawa, T. Furuichi* and K. Mikoshiba, Department of Molecular Neurobiology, Institute of Medical Science, University of Tokyo, Tokyo 108

Inositol 1,4,5-trisphosphate receptor (IP_3R) is a second messenger inositol 1,4,5-trisphosphate (IP_3) induced Ca^{2+} release channel. To define the structural determinants for IP_3binding of the type 1 IP_3R (IP_3R1), we developed a means of expressing the N-terminal 734 amino acids of IP_3R1 (T734), which contain the IP_3binding region, in *Eschericia coli* (*E. coli*). The T734 protein expressed in *E. coli* exhibited a similar binding specificity and affinity for IP3 as the native IP_3R from mouse cerebellum. Deletion mutagenesis, in which T734 was serially deleted from the N-terminus up to residue 215, markedly reduced IP_3binding activity. However, when deleted a little more toward the C-terminus (to residues 220, 223, and 225), the binding activity was retrieved. Further N-terminal deletions over the first 228 amino acids completely abolished it again. C-terminal deletions up to residue 579 did not affect the binding activity, whereas those up to residue 568 completely abolished it. In addition, the expressed 356 amino-acid polypeptide (residues 224-579) exhibited specific binding activity. Taken together, residues 226-578 were sufficient and close enough to the minimum region for the specific IP_3binding, and thus forming an IP_3binding "core". Site-directed mutagenesis was performed on 41 basic Arg and Lys residues, within the N-terminal 650 amino acids of T734. We showed that single amino acid substitutions for 10 residues, which were widely distributed within the binding core and conserved among all members of the IP_3R family, significantly reduced the binding activity. Among them, three (Arg-265, Lys-508, and Arg-511) were critical for the specific binding, and Arg-568 was implicated in the binding specificity for various inositol phosphates. We suggest that some of these 10 residues form a basic pocket that interacts with the negatively-charged phosphate groups of IP_3.

247.8

INCORPORATION OF RADIOLABELED PLASMA UNSATURATED FATTY ACIDS INTO RAT BRAIN SYNAPSES : EFFECT OF CHOLINERGIC STIMULATION. Collins R. Jones, M.Catherine Bennett*, Toshanari Arai and Stanley I. Rapoport. Lab. of Neurosciences, National Institute on Aging, Bethesda, MD 20892-1582

Cholinergic stimulation has been shown to increase the turnover of arachidonic acid (AA, 20:4) and docosahexaenoic acid (DHA, 22:6) but not palmitic acid (16:0, PAM) in rat brain. We hypothesized that cholinergic stimulation would specifically increase the incorporation of these fatty acids into the synaptosomal fractions of rat brain. The fatty acid method developed in our laboratory allowed us to test this hypothesis under physiological conditions. [^{3}H]AA (1.35 mCi/kg), [^{3}H]DHA (1.35 mCi/kg) or [^{3}H]PAM (20.7 mCi/kg) was infused intravenously for 5 min into awake, adult male rats before and after treatment with arecoline (15 mg/kg) and brain subcellular fractions isolated. Radiolabeled fatty acid incorporation into membrane phospholipid was measured and normalized to plasma exposure. More than 50% of incorporated [^{3}H]AA, [^{3}H]DHA or [^{3}H]PAM was localized to synaptosomal phospholipids with 30% of the radiolabel entering microsomal phospholipid. Cholinergic stimulation with arecoline increased the incorporation of both [^{3}H]AA and [^{3}H]DHA but not [^{3}H]PAM into the phospholipid of synaptosomal fraction by 400% and 100%, respectively. Incorporation of either polyunsaturated fatty acid was not altered in the myelin, mitochondrial or cytosolic fractions. It is likely that these polyunsaturated fatty acids play a role in synaptic membrane signal transduction processes involving phospholipase A2 activation and can be used as probes to measure synaptic activity *in vivo*.

247.9

CALCIUM-INDEPENDENT CYTOSOLIC PHOSPHOLIPASE A_2 (cicPLA$_2$) IS THE MAJOR PLA$_2$ ISOFORM IN VERTEBRATE BRAIN AND IN EXPRESSED IN THE LIMBIC SYSTEM. R. Diaz-Arrastia*, L. Ma, and S. Jones, Dept. of Neurology, University of Texas Southwestern Medical School, Dallas, TX 75235, and Genetics Institute, Cambridge, MA 02140.

Phospholipase A_2 (PLA$_2$) generates a number of putative second messengers which play key roles in normal synaptic plasticity as well as excitotoxicity. It is not clear which PLA$_2$ isoform is responsible for the production of lipid mediators in the brain. We have studied two intracellular PLA$_2$ isoforms which have recently been cloned and characterized. The first, a Ca^{2+}-dependent cytosolic PLA$_2$ (cPLA$_2$) compromises less than 10% of the total lipase activity present in neural tissues, and is expressed predominantly in astrocytes, as shown by immunohistochemistry. The other is a Ca^{2+}-independent cytosolic enzyme (cicPLA$_2$) which can be resolved from the former isoform by standard chromatographic techniques. This enzyme is biochemically and immunologically similar (if not identical) to a cicPLA$_2$ recently cloned from CHO cells (S. Jones, manuscript in preparation). Immunoprecipitation experiments confirm that this is the major PLA$_2$ activity in rat brain homogenates. The enzyme has a molecular mass of 85 kDa as measured by SDS-PAGE and non-denaturing gel-filtration chromatography. It is inhibited by bromoenol lactone (BEL), but unlike other Ca^{2+}-independent PLA$_2$s, it is not specific for plasmalogens and is not activated by ATP. It has a neutral pH optimum and is not specific for sn2-arachidonyl phospholipids. Immunohistochemical localization of cicPLA$_2$ in adult rat brain shows that it is enriched in neuronal cells in the hippocampus and piriform cortex. It is also expressed in other cortical neurons as well as in non-neuronal cells in other parts of the CNS.

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247.10

ARACHIDONIC ACID RELEASE IN RESPONSE TO P_2 PURINOCEPTOR ACTIVATION IN CULTURED ASTROCYTES. W.C. Chen, C.Y. Wen* and C.C. Chen. Institute of Pharmacology and Anatomy, College of Medicine, National Taiwan University, Taipei, Taiwan.

ATP-induced arachidonic acid (AA) release was studied in [^{3}H]AA-prelabeled cultured astrocytes. To characterize the P_2 purinoceptor-mediated effects of ATP, the subtype-specific agonists 2-methylthio ATP (2-MeSATP) and UTP were compared. ATP, UTP or 2-MeSATP induced a dose-dependent increase of [^{3}H]AA release. The order of potency was ATP≥UTP≥2-MeSATP, indicating that ATP interacted with both P_{2U} and P_{2Y} purinoceptors. ATP, UTP and 2-MeSATP induced a rapid increase of $[Ca^{2+}]_i$ and a sustained $[Ca^{2+}]_i$ increase. The Ca^{2+} ionophore-A23187 mimicked the effects of these three agonists on AA rleease. ATP, UTP, 2-MeSATP or A23187-induced AA release was markedly decreased in the absence of external Ca^{2+} in condition the sustained $[Ca^{2+}]_i$ increase induced by these three agonists was abolished. Inorganic Ca^{2+} channel blocker Co^{2+} at 1 mM inhibited the ATP, UTP, 2-MeSATP or A23187-induced AA release. Long-term treatment of the cells with TPA resulted in attenuation of the ATP, UTP or 2-MeSATP response. Western blot analysis showed the expression of PKC$\alpha,\delta,\eta,\theta$ and ζ in astrocytes. The possible involvement of specific PKC isoform in the regulation of P_2 purinoceptor agonists-induced AA release will be explored. However, the P_{2U} and P_{2Y} agonists-induced Ca^{2+} mobilization leading to extracellular Ca^{2+} influx was essential for the AA release induced by ATP, UTP and 2-MeSATP.

247.11

NEURONAL NUCLEOTIDE RECEPTOR LINKED TO PHOSPHOLIPASE C: STIMULATION OF NEURO 2A CELLS BY UTP AND POSSIBLE REGULATION BY PKC SUBTYPE ε. C.C. Chen* and W.C. Chen. Institute of Pharmacology, College of Medicine, National Taiwan University, Taipei, Taiwan.

Incubation of Neuro 2A mouse neuroblastoma cells with UTP and UDP results in a concentration-dependent increase in the accumulation of inositol phosphates (IP) with equal potency and maximal effect, ATP, ADP and 2-MeSATP were much less potent. Cross-desensitization experiments indicated that UTP and ATP may interact with the same population of P_2 purinoceptor-P_{2u}. The UTP- and ATP-induced IP accumulation were not affected by pretreatment of cells with pertussis toxin (PTX), indicating that the P_{2u} purinoceptor in Neuro 2A cells is coupled to PTX-insensitive Gq protein. Short term (10 min) treatment of cells with 1 μM TPA inhibited the UTP and ATP effects, the inhibitory effect was gradually attenuated with increased length of TPA treatment (1.5-6 hr) and was not seen after long-term (24 hr) treatment. Western blot analysis using eight protein kinase C (PKC) isozyme-specific antibodies showed that Neuro 2A cells express PKCα, PKCε, PKCθ and PKCζ. Following TPA treatment of the cells for various times (10 min, 1.5, 3, 6 or 24 hr), translocation of PKCα, PKCε and PKCθ from the cytosol to the membrane was seen after 10 min or 1.5 hr treatment. However, partial and complete down-regulation of both membrane PKCα and PKCθ was seen after 3 and 6 hr treatment, respectively. In contrast, the TPA-induced translocation of PKCε was maintained to 3-6 hr treatment and almost complete down-regulation occurred only after 24 hr treatment. The observed TPA-induced inhibition of UTP or ATP stimulated PI hydrolysis therefore correlated well with the extent of translocation of PKCε, but not of PKCα or θ. This study is the first to show the existence of the P_{2u} purinoceptor in Neuro 2A cells and the possible involvement of neuronal PKCε in regulation of the receptor-mediated PI turnover.

247.12

SUBSTANCE P (SP) STIMULATES PHOSPHOLIPASE D IN CHINESE HAMSTER OVARY CELLS (CHO) EXPRESSING THE NK1 TACHYKININ RECEPTORS. M. Tencé*, Y. Torrens, J.C. Beaujouan and J. Glowinski. INSERM U 114, Collège de France, 75005 Paris, France.

In CHO cells transfected with the rat NK1 receptor cDNA (CHO-NK1), SP activates several intracellular pathways: hydrolysis of phosphatidylinositols, activation of adenylate cyclase and release of arachidonic acid (Nakajima et al., *J. Biol. Chem.* 267:2437, 1992; Garcia et al., *Biochem. Pharmacol.* 48:1735, 1994). We report here that SP and the selective NK1 agonist, [Pro^9]SP, also stimulate a phospholipase D (PLD) activity. In CHO-NK1 cells prelabelled with tritiated myristic acid, [Pro^9]SP (10 nM) induced a sustained production of both tritiated phosphatidic acid and diacylglycerol. This effect resulted from the activation of a PLD since in the presence of ethanol, SP and [Pro^9]SP also stimulated time- and dose-dependently, the formation of tritiated phosphatidylethanol (PEt) (EC_{50}: 2 and 1 nM, respectively). This response exhibited a pharmacological profile typical for NK1 receptor. Indeed, neither Senktide nor [Lys^5,MeLeu^9,Nle^{10}]NKA (4-10), selective agonists for NK3 and NK2 receptors, respectively, were efficient in stimulating the formation of PEt. Furthermore, the response induced by [Pro^9]SP was inhibited by selective NK1 antagonists (RP 67580, SR 140333 and CP 96345). The stimulation of PLD by [Pro^9]SP did not involve a pertussis toxin-sensitive G-protein. In contrast, protein kinase C seemed to play a crucial role in the regulation of PLD activity since inhibition or down-regulation of the kinase suppressed the formation of PEt induced by [Pro^9]SP. This study suggests that PLD activation could be another signal transduction pathway involved in the mechanism of action of SP in central and peripheral tissues expressing NK1 receptors.

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247.13

PHOSPHOLIPASE A_2 ISOFORMS IN RAT HIPPOCAMPAL SYNAPTOSOMES
M.L. Ruehr and R.V. Dorman*
Dept. of Biol. Sci., Kent State Univ., Kent, Ohio 44242

Phospholipase A_2 (PLA_2) dependent-release of arachidonic acid (AA) has been implicated in synaptic plasticity. AA accumulation has been linked to neurotransmitter release from hippocampal mossy fiber synaptosomes (HMFS) and this effect is potentiated by another second messenger, diacylglycerol (Zhang and Dorman, Brain Res Bull 32:437-441, 1993). Here we show the presence of multiple isoforms of PLA_2 in HMFS and Ca^{2+}-dependent translocation of PLA_2, which is predicted to play a role in its activation. HMFS and P_2 synaptosomes were prepared from rat hippocampi, sonicated in buffer containing 0-5 mM Ca^{2+} and separated into cytosolic and membrane fractions. Purity of each fraction was assessed through Western analysis of glucose-6-phosphate dehydrogenase (G-6-PDH), as the cytosol marker, and Ca^{2+}-ATPase, as the membrane marker. We observed, through densitometric analyses, that 92% of the G-6-PDH was associated with the cytosolic fraction, while 99% of the ATPase remained in the membrane fraction. These fractions were then probed with a PLA_2 antibody which labels the catalytic site. This antibody recognized protein bands with apparent molecular weights of 160 and 112 kDa in the cytosolic fraction and 112 and 35 kDa in the membrane fraction of the HMFS. In contrast, only one 80 kDa band was observed in homogenates from CHO cells or rat kidney. This antibody also labeled 112 and 35 kDa proteins in the cytosolic and membrane fractions of P_2 synaptosomes, respectively. In the HMFS, the 160 kDa PLA_2 isoform decreased in the cytosolic fraction as the concentration of Ca^{2+} was increased from 0.05- 5μM, while the 112 and 35 kDa isoforms increased in the membrane fraction under these same conditions. Therefore, it appears that rat HMFS and P_2 synaptosomes contain several isoforms of PLA_2 which are differentially regulated when exposed to Ca^{2+}.

247.14

ACTIVATION OF A NICOTINIC ACETYLCHOLINE RECEPTOR STIMULATES PRODUCTION OF 8-LIPOXYGENASE METABOLITES IN ISOLATED CELLS OF *APLYSIA*.
T. Tieman, J.S. Kehoe, J.H. Schwartz, and S.J. Feinmark*. Department of Pharmacology and the Center for Neurobiology and Behavior, Columbia University, New York, NY, 10032 and Laboratoire de Neurobiologie, Ecole Normale Superieure, Paris, France.

We previously reported that the formation of novel 8-lipoxygenase metabolites, including 8-HETE and 8-KETE, could be induced in the *Aplysia* central nervous system by activation of a nicotinic acetylcholine (ACh) receptor. This receptor induces a fast chloride conductance that is known to be activated selectively by suberyldicholine and blocked selectively by α-bungarotoxin (α-BTX). The 8-lipoxygenase metabolites were quantified by reverse-phase HPLC after the application of Ach (100 μM) or suberyldicholine (100 μM) to neural components from the total nervous system. Formation of these metabolites could be inhibited by α-BTX. We have now shown that these lipids can be produced by isolated neurons which are known to have this particular nicotinic receptor. As few as 20 isolated Medial cells from the pleural ganglion produced measurable 8-lipoxygenase products when exposed to suberyldicholine (100 μM). On the other hand, exposure of Medial cells to either GABA (200 μM) or glutamate (1 mM), both of which induce Cl-dependent responses in these cells, failed to stimulate metabolite formation. In control experiments on an equivalent number of isolated RB cells (known to have only the suberyldicholine- and α-BTX-**insensitive** receptor), suberyldicholine failed to induce production of these metabolites. These findings show that the metabolites are formed directly and selectively by neurons bearing the relevant ACh receptor, and they suggest that an increase in chloride conductance alone is not sufficient to activate the 8-lipoxygenase pathway. These data raise interesting questions as to how a presumably ionotropic receptor could activate lipid metabolism and what role these lipids may play in neural function. Support: JSK, CNRS; JHS, l'Univ. Paris VII, NIMH K05 MH00921; TT and SJF, NINDS P01 NS29832.

247.15

DESENSITIZATION OF M1 MUSCARINIC RECEPTOR-MEDIATED ACTIVATION OF NITRIC OXIDE SYNTHESIS.
A.E. Cuadra* Sheng Zu Zhu and E. E El-Fakahany. Graduate Program in Pharmacology, Dept. of Psychiatry, University of Minnesota, Minneapolis, MN 55455.

The free radical nitric oxide (NO) is an important molecule in relation to various functions such as vascular smooth muscle relaxation, toxicity for invading bacteria, and has been implicated in the establishment of memory. It is therefore important to understand the mechanisms of regulation of this cellular messenger. In our laboratory we have established *Chinese hamster ovary* (CHO) cells which are stably transfected with the human M1 muscarinic receptor and neuronal NO synthase (NOS). M1 receptors cause the release of calcium from intracellular stores through synthesis of inositol trisphosphate. Thus, this pathway is expected to activate the calcium dependent neuronal NOS. Incubation of these CHO cells with the muscarinic agonist carbachol (CBC) resulted in a time dependent activation of neuronal NOS. This response was measured by assaying the conversion of tritiated L-arginine into L-citrulline. This activation of NOS also depended on the concentration of agonist. Desensitization of this response was investigated. Transfected CHO cells were pretreated with 1mM CBC for various time periods, ranging from one hour to 24 hours. Our results show no significant change in agonist induced citrulline formation after one hour of CBC pretreatment versus control. After 3 hours, a significant decrease of the maximal response was observed, being accompanied by a shift of the dose response curve to the right. Cells pretreated with CBC for twenty four hours showed very little increase in citrulline formation upon stimulation of muscarinic receptors.
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247.16

CYCLIC GMP-DEPENDENT PROTEIN KINASES: TARGETS OF NITRIC OXIDE IN THE RAT BRAIN.
A-E-D El-Husseini*, JA Williams, C Bladen, PB Reiner, SR Vincent. Kinsmen Laboratory of Neurological Research, Dept. of Psychiatry, University of British Columbia, Vancouver, B.C. Canada, V6T 1Z3.

Nitric oxide (NO) mediates a variety of biological functions such as neurotransmission, responses to inflammation, and neuronal excitotoxicity. In order to identify potential targets of NO we carried out two studies: First, type II cGMP-dependent protein kinase (cGK II) phosphorylation was measured in rat thalamus after application of NO *in vitro* or by electrical stimulation of the NO synthase (NOS)-containing mesopontine cells in anaesthetized animals. cGKII phosphorylation increased in response to NO *in vivo* as well as *in vitro*, which suggested that this kinase is a target of NO signalling. Second, we investigated the distribution of NOS and both the type I and type II cGKs throughout the brain using NADPH-diaphorase (NADPHd) histochemistry combined with either immunocytochemistry or *in situ* hybridization. Using cGKI specific antibodies, strong staining was observed in Purkinje cells in the cerebellum, and moderate staining in the posterior hypothalamus, olfactory bulb and spinal cord. Except for a few neurons in the olfactory bulb, cGKI neurons were negative for NADPHd. cGKII expression was determined by *in situ* hybridization using ^{35}S-labelled type II antisense riboprobes. High levels of expression were observed in cortex, olfactory bulb, thalamus, tuberal hypothalamus, septum, and brainstem. Neurons expressing cGKII were also negative for NADPHd. Taken together, these data support the notion of NO as a diffusable messenger, as it is produced in neuronal populations that project to or are in close proximity to distinct brain regions expressing targets for NO.
Supported by grants from MRC Canada

247.17

CLONING AND CHARACTERIZATION OF POSTSYNAPTIC DENSITY 93 (PSD-93), AN nNOS INTERACTING PROTEIN
J. Brenman*, S. Craven, and D. Bredt. Dept. of Physiology, UCSF, San Francisco, CA 94143.

NItric oxide formation in brain is efficiently coupled to calcium influx through NMDA type glutamate receptors. To elucidate possible mechanisms responsible for this coupling, we performed yeast two-hybrid screening to identify proteins which interact with nNOS. Two proteins were identified, the postsynaptic density proteins, PSD-93 and PSD-95. Here we report the cloning and characterization of PSD-93. PSD-93 is unique among PSD-95 / SAP-90 family members in its cerebellar postsynaptic distribution. PSD-93 is highly expressed in cerebellar Purkinje neurons. By immunostaining PSD-93 is enriched in Purkinje neuron soma and dendrites. This is in contrast to PSD-95 which is highly concentrated in the presynaptic plexus of cerebellar basket cells. Given that PSD-93 and PSD-95 each contain multiple potential binding sites for nNOS and the NMDA receptor, complexes involving oligomers of PSD-93/95 may help account for the functional as well as physical coupling of nNOS to NMDA receptors.
(sources of support: NIH, MDA, Council for Tobacco Research, and American Heart Association)

247.18

IDENTIFICATION OF PROTEINS FROM RAT BRAIN THAT INTERACT WITH P70 S6 KINASE,
P.E. Burnett*, D.M. Sabatini, S. Blackshaw, and S.H. Snyder. Dept. of Neuroscience, Johns Hopkins University Sch. of Med., Baltimore, MD 21237.

Activation of p70 S6 kinase is a common feature of several mitogen stimulated signal transduction pathways. p70 S6 kinase is involved in control of translation via phosphorylation of the S6 ribosomal subunit. Neuronally relavent pathways include stimulation of PC12 cells by NGF. In an attempt to identify relavent neuronal targets of p70, a two hybrid screen was performed using a rat brain cDNA library.

We have identified two clones which interact specifically with p70. The first is a novel DHR (GLGF/PDZ) domain containing protein. The second is a brain enriched developmentally regulated transcription factor. We have confirmed interactions with *in vitro* binding assays and are investigating their potential as a substrates for p70 kinase activity.
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248.1

LIPOPOLYSACCHARIDE FEVER REQUIRES PRODUCTION OF PROSTAGLANDINS IN THE PREOPTIC AREA

T.E. Scammell*, J.D. Griffin, and C.B. Saper. Dept. of Neurology, Harvard Medical School / Beth Israel Hospital, Boston, MA 02115

Prostaglandin E2 (PGE2) is essential for the production of fever, but the necessary sites of PGE2 synthesis are unknown. Since low doses of PGE2 rapidly produce fever when injected into ventromedial regions of the preoptic area (POA), we hypothesize that these regions are critical sites of PGE2 production during fever. We microinjected ketorolac, a water-soluble inhibitor of PGE2 production, into a variety of preoptic sites and measured its effect on the fever typically produced by intravenous lipopolysaccharide (LPS). Ketorolac inhibits both cyclooxygenase-1 and -2, the rate limiting enzymes in prostaglandin production. Intravenous injection of ketorolac completely blocked LPS fever for over 4 hours. Injection of ketorolac into the ventromedial preoptic area, the organum vasculosum of the lamina terminalis (OVLT), and immediately adjacent sites markedly attenuated LPS fever. In contrast, ketorolac injections more than 500 um from the OVLT had little effect on fever. Vehicle injections, even into the peri-OVLT region, had no effect on LPS fever. These experiments demonstrate that prostaglandins produced in ventromedial preoptic regions surrounding the OVLT play an essential role in LPS fever.

This research was supported by USPHS grants NS 09474, NS 09466, and NS 33987.

248.2

EVIDENCE FOR DIRECT INTERACTION OF INTRAVENOUS LPS WITH THE CENTRAL NERVOUS SYSTEM.

J. K. Elmquist*, T. E. Scammell, and C. B. Saper. Dept. of Neurology, Harvard Medical School/Beth Israel Hospital, Boston, MA 02115.

It is clear that peripheral immune cues alter CNS activity, but the mechanisms by which these signals are conveyed to the brain remain controversial. Recent experimental results indicate that the vagus nerve is essential in transducing immune system signals following intraperitoneal administration of LPS (endotoxin) or cytokines. The role of the vagus nerve following intravenous (iv) administration is less understood but iv LPS induces the expression of Fos-like immunoreactivity (Fos-IR) in nuclear groups of the rat brain involved in regulation of acute phase response. Using adult male Sprague-Dawley rats, we performed intracranial rhizotomies of the left vagus nerve and subsequently challenged the rats with iv LPS. Unilateral interruption of vagal afferents did not prevent brain Fos-IR including that seen in the ventromedial preoptic area, paraventricular hypothalamic nucleus, and the nucleus of the solitary tract. Additionally, we used immunohistochemistry for cyclooxygenase 2 (COX 2), the inducible form of the enzyme responsible for the production of prostaglandins following iv LPS. Administration of iv LPS induced COX 2-like immunoreactivity (COX 2-IR) in perivascular cells throughout the brain. Double label immunohistochemistry revealed that the majority of the cells containing LPS-induced COX 2-IR also stained with a marker for perivascular microglia and meningeal macrophages. Moreover, intracranial vagotomy did not abolish the LPS-induced COX 2-IR in perivascular cells throughout the CNS. Our results indicate that interruption of vagal afferents arising in the left nodose ganglia does not prevent CNS activation by iv LPS. Furthermore, these findings indicate that circulating LPS or cytokines may directly interact with cells in the meninges and perivascular space throughout the brain to initiate the CNS mediated components of the acute phase response. This work was supported in part by USPHS grants NS33987, NSO9474, and MH10832.

248.3

THE THERMOSENSITIVITY OF NEURONS IN THE VENTROMEDIAL PREOPTIC AREA OF THE HYPOTHALAMUS.

J.D. Griffin* and C.B. Saper. Department of Neurology, Beth Israel Hospital and Harvard Medical School, Boston, MA 02115

In response to an i.v. challenge with febrogenic doses of lipopolysacharide (LPS), studies from our lab have demonstrated that Fos immunoreactivity is displayed by neurons in the ventromedial preoptic area (VMPO) of the hypothalamus. In addition, neurons in VMPO show distinct axonal projections to areas such as the anterior perifornical area (APFx). We would suggest that synaptic input from LPS stimulated neurons in VMPO alters the activity of integrative thermosensitive neurons in the APFx, resulting in a shift in the set-point temperature into the hyperthermic range. Further evidence that the majority of neurons in VMPO are GABAergic, and current models of themoregulatory control, suggest that VMPO may have a comparatively high proportion of temperature insensitive neurons which are stimulated by LPS challenge and project to the APFx. The present study has focused on determining the inherent thermosensitivities and morphologies of neurons in VMPO. In a saggittally sectioned tissue slice preparation from the rat hypothalamus, whole-cell recordings were made from neurons in VMPO. Spontaneously active neurons were classified as either warm sensitive ($m \geq 0.8$ $imp \cdot s^{-1} \cdot °C^{-1}$) or temperature insensitive. Neurons which did not show spontaneous firing rates were classified as silent. In addition, recorded neurons were filled with the intracellular stain biocytin (0.5%) to determine the exact location and morphology of each neuron. Neurons in VMPO showed similar variations in temperature sensitivity and morphology as compared to other areas of the hypothalamus, with 55% of the neurons classified as temperature insensitive and 30% as warm sensitive. Temperature insensitive neurons had dendrites which projected rostrally and caudally, while warm sensitive neurons had denrties that projected laterally and medially. The results of this study suggest that there is no significant difference in the relative temerature sentivities or morphologies of neurons in VMPO, compared to other areas of the hypothalamus. (Supported in part by NIH NS09466, NS07291 & AHA 91011)

248.4

Sex differences in response to PGE_2, IL-1β and LPS in conscious and anaesthetised rats.

X. Chen, K. E. Cooper* and Q. Pittman
Neuroscience Research Group, Dept. of Med. Physiol., Univeristy of Calgary, Calgary, Canada

The extrahypothalamic vasopressinergic system has been shown to play a role in reducing febrile temperatures. As this system is less well developed in female rats, we have compared the pyrogenic responses of male and female rats to LPS, PGE_2 and IL-1β. PGE_2 (25 ng/5 µl per rat icv) resulted in higher fevers in conscious and anaesthetised (50 ng/5 µl per rat) females than in their male controls. Both sexes showed dose-dependent febrile responses to IL-1β (0.4, 1 and 2 µg/kg ip), but no appreciable differences in fever were noticed except for a slightly higher response in females in the first phase of fever. This discrepancy was abolished in anaesthetised rats (1 µg/kg iv), so it may arise from behavioural changes to the injection or a different distribution of IL-1β following ip injections. In anaesthetised rats, IL-1β 1 µg/kg iv caused concurrent increases in heart rate and mean arterial blood pressure, with heart rate staying elevated throughout the testing period (5 hrs following iv injection) and blood pressure returning toward baseline as time proceeded. There were no sex differences in these cardiovascular responses. LPS (E. coli, 50 µg/kg ip) evoked the same magnitude of fever in both sexes. Blockade of AVP V_1 receptors (Manning compound, 1 nmol/5 µl icv) did not give rise to exaggerated IL-1β fever in female rats. The results suggest there is a sex-related difference in fever development to PGEs which is not apparent with IL-1β and LPS given peripherally, which may activate pathways that are not entirely PGE-dependent. Supported by MRC.

248.5

TEMPERATURE SENSITIVE HYPOTHALAMIC NEURONS: THERMAL EFFECTS ON $GABA_A$ SYNAPTIC INHIBITION.

P. Wung Burgoon* and J.A. Boulant. Department of Physiology, The Ohio State University, Columbus, OH 43210.

Previous studies suggest that cooling increases the amplitudes and durations of inhibitory postsynaptic potentials (IPSPs), which can decrease firing rate and, thereby, contribute to neuronal temperature sensitivity. Temperature influences the resistance of the postsynaptic membrane, but it also influences presynaptic & postsynaptic kinetics that determine inhibitory postsynaptic currents (IPSCs). To study thermal effects on these synaptic events, whole-cell patch clamp recordings were made in preoptic and suprachiasmatic regions of rat hypothalamic tissue slices. Current-clamp and voltage-clamp recordings examined the effect of temperature on IPSPs and IPSCs, which were reversibly blocked by 10 µM bicuculline. Cooling always increased postsynaptic membrane resistance, which can increase IPSP amplitude. While cooling increased the durations of both IPSPs and IPSCs, it often decreased IPSC amplitudes. The increase in IPSP amplitudes and durations with cooling appears to be due primarily to thermally-induced changes in membrane resistance. However, in some neurons, IPSP amplitudes remained constant during temperature changes. In these cases, cool-induced increases in membrane resistance appear to compensate cool-induced decreases in IPSC amplitude.
(Supported by NIH grant NS14644.)

248.6

ANTEROVENTRAL THIRD VENTRICLE (AV3V) LESION MAY BLOCK FEVER BY INCREASING PLASMA CORTICOSTERONE.

W.S. Hunter*, L.L. Murphy and M.J Propes. Department of Physiology Southern Illinois University, Carbondale IL 62901.

We have demonstrated (1993,96) that AV3V lesions which ablate the organum vasculosum laminae terminalis (OVLT), block endotoxin (LPS) induced fever in guinea pigs and rats. Fever is blocked by dexamethasone pretreatment (Coelho et al. 1992) and is enhanced in rats treated with glucocorticoid antagonist before i.p. LPS injection (Morrow et al., 1993). We hypothesized that AV3V lesion would produce elevation of corticosterone (CS) by releasing inhibition of normal fever suppression mechanisms. To test this, groups of three 300–400 g male Sprague-Dawley rats were implanted with temperature sensitive radio telemetry devices (Minimitter). One rat in each group was sham operated and two had electrolytic lesions stereotaxically placed in the AV3V region. Seven days post-lesion, after 1 h baseline recording, each rat was injected i.p. with 100µg/kg *E. coli* LPS or vehicle. Four hours post-injection the rats were quickly decapitated, blood collected for CS analysis and brains fixed for histological analysis. Of lesioned rats, those with low (<0.6°C) or no fever had highest plasma CS levels (>500 pg/ml). Sham operated or lesioned rats given vehicle exhibited temperature elevation <0.5°C, and CS levels <185 pg/ml. Lesioned rats which fevered with LPS had intermediate CS levels. These data support the hypothesis that rats with AV3V lesions ablating the OVLT exhibit elevated CS sufficient to suppress fever. Funded in part by the Illinois Eagles.

248.7

THERMORESPONSIVENESS OF VENTROMEDIAL HYPOTHALAMIC (VMH) NEURONS TO REPEATED SCROTAL COOLING AND HEATING AT DIFFERENT COLONIC TEMPERATURES Q. Li and J. Thornhill. Dept of Physiology, Col. of Med. Univ. of Sask. 107 Wiggins Rd, Saskatoon, SK. S7N 5E5. Canada.

Our previous studies have shown that neurons within the VMH specifically respond to changes in peripheral (scrotal) temperature of urethane anaesthetized rats. In the present study, we determined if VMH thermoresponsive neurons could retain their same thermoresponsiveness to repeated scrotal thermal stimulation, when colonic temperature was reduced. Extracellular VMH neuronal activity was recorded with glass-micropipettes filled with 0.5 M sodium acetate containing 2% protamine sky blue from urethane anaesthetized male Sprague-Dawley rats with colonic temperature kept at 37°C, 35°C and 33°C during scrotal cooling (ice pack) and heating (the same size pack containing 40°C hot water). With colonic temperature kept at 37°C, all of 9 warm responsive (WRN), 7 cold responsive (CRN) and 17 VMH temperature non-responsive neurons(TNRN) retained their same thermoresponsiveness to a second trial of scrotal cooling and heating. Another 19 WRNs, 11 CRNs and 21 TNRNs were tested at an initial colonic temperature of 37°C, then at 35°C with two trials of scrotal thermal stimulation. Furthermore, 11 of 19 WRNs, 7 of 11 CRNs and 15 of 21 TNRNs were further examined for their thermoresponsiveness when colonic temperature was 33°C to the third trial of scrotal heating and cooling. Again, these VMH WRNs, CRNs and TNRNs retained their same thermoresponsiveness to scrotal thermal stimulation regardless of changes in colonic temperature. These results suggest that thermoresponsiveness of VMH neurons to repeated thermal stimulation of the scrotum is retained even when colonic temperatures are acutely reduced. *Supported by the MRC of Canada.*

248.8

THERMOSENSITIVITY OF A TRANSIENT OUTWARD CURRENT IN HYPOTHALAMIC NEURONS. C. S. Wang and S. R. Kelso*, Dept. of Biol. Sci., Univ. of Illinois at Chicago, Chicago, IL 60607

Whole-cell voltage clamp recordings were conducted in rat preoptic-anterior hypothalamic(POAH) tissue slices. POAH neurons are believed to be the center for thermoregulation in mammals. Sixty-three neurons were classified based on their firing rate response to temperature changes. 76% of the neurons were T-insensitive and 24% warm-sensitive. After determining thermosensitivity, a fast transient outward current was recorded, using TTX (0.5uM) to block Na^+ currents, TEA (10 mM) to block delayed rectifier, and low concentration of Ca^{2+} (0.2 mM) to minimize contributions from Ca^{2+} currents.

Steady-state activation and inactivation of the current were similar to that of I_A in other mammalian central neurons. It was also sensitive to 4-AP. Temperature insensitive cells showed a significantly higher amplitude of peak I_A. Upon temperature elevation, there was a general increase in the amplitude of peak I_A for T-insensitive cells, while the peak amplitude for warm-sensitive cells was decreased. The total current (by integration) of I_A was decreased for both cell types with temperature being raised. However, the percent decrease of I_A for warm sensitive cells is two to three times larger than that for T-insensitive cells. This larger decrease of I_A for warm cells is presumably caused by an increased rate of inactivation of I_A at a higher temperature. This may contribute to the bigger increase in firing rates in warm sensitive cells in response to raising temperature.

248.9

CORE TEMPERATURE AND METABOLIC RESPONSES TO CRF ADMINISTRATION. K.P. Sausen, S.T. Ahlers, C.L. Foreman, M.H. Quesada, C.-M. Staschen, and J.R. Thomas. Thermal Stress/Adaptation Program, Naval Medical Research Institute, Bethesda, MD 20889-5607.

This study compared core temperature (Tcore) and VO2 responses to corticotropin releasing factor (CRF). Eight Long-Evans rats underwent lateral ventricular cannulation and abdominal implantation of a telemetric thermal probe. Tcore and VO2 were recorded for 30 min before and for 90 min after intracerebro-ventricular injection of saline, 0.3 or 3.0µg CRF. All rats received all doses, separated by 1 week. Data were examined as change from preinjection baseline to 10, 30, 60, and 90 min post injection. Tcore fell after CRF administration. Relative to saline, the Tcore drop was significant at 90 min for 0.3µg, and at 60 and 90 min for 3.0µg. VO2 fell over the course of the postinjection period for saline and 0.3µg CRF, but not in the 3.0µg condition. These data suggest a complex relationship between metabolic and thermo-regulatory responses to CRF. Supported by Naval Medical Research & Development Command work unit 62233NM33C30.004-1002.

248.10

NITRIC OXIDE SYNTHASE AND NITROTYROSINE IMMUNOREACTIVITY IN THE RAT FOREBRAIN FOLLOWING HEAT STRESS. **A.M. Zardetto-Smith*, N.W. Kooy, S.J. Lewis and K. C. Kregel. Depts. of Anatomy, Pediatrics, Pharmacology and Exercise Science, Univ.of Iowa, Iowa City, IA 52242.**

This study assessed nitric oxide synthase (NOS)/nitrotyrosine (NT) immunoreactivity (ir) in regions of the forebrain of normothermic rats and rats challenged with heat stress. Conscious male Sprague-Dawley rats were maintained either at normothermic core body temperature (T_c - 38.0°C) or exposed to an ambient temperature of 42°C until T_c reached 41°C. Heated rats were subsequently maintained at 41°C for 30 min. Both groups were returned to their home cages and after 12 h were euthanized with an overdose of pentobarbital (100 mg/kg, ip). After perfusion and post-fixation, brainstem sections were cut using a vibratome and processed for endothelial NOS-ir (ecNOS-ir), inducible NOS-ir (iNOS-ir), neuronal NOS-ir (nNOS-ir), or nitrotyrosine-ir (NT-ir) using the ABC immunoperoxidase technique. Heated rats showed increases in ecNOS-ir over controls in astrocytes near the ependymal lining of the third and lateral ventricles, in astrocytes populating the subfornical organ and median preoptic nucleus, and in astrocytes surrounding blood vessels. Normothermic rats showed nNOS in the supraoptic nucleus and magnocellular paraventricular nucleus, but heated rats showed an increased density of staining in these nuclei. Both normothermic and heat-stressed rats demonstrated iNOS and NT-ir within ependymal cells lining the ventricles; iNOS was also present in ependymoglial cells lining the floor of the superior horn of the lateral ventricle. The results indicate that (i) nNOS may be increased by a physiological level of heat stress; (ii) that ependymal cells may contain significant amounts of iNOS and NT; and (iii) that astrocytes at the ventricular interface may display increased ecNOS in response to hyperthermia. (Supported by NIH AG-12350 to K. Kregel).

248.11

INHIBITION OF CONSTITUTIVE NITRIC OXIDE SYNTHASE CAUSES HYPOTHERMIA, Thomas F. W. Horn* and Floyd E. Bloom. The Scripps Research Institute, Department of Neuropharmacology, La Jolla, CA 92037, USA.

Nitric oxide synthase (NOS) is localized in neurons of hypothalamic nuclei, in the brainstem as well as in the peripheral nervous system, suggesting a role in the regulation of autonomic functions. We tested whether nitric oxide (NO) blockers affect normal and elevated body temperature and gross motor activity in conscious rats using telemetry. The unspecific NOS-blocker L-NAME (50 mg/kg, i.p.) decreased baseline body temperature from 37.2 ± 0.1 C to a minimum of 36.2 ±0.2 (n=12). To distinguish the site of action and rule out effects due to blood pressure changes, we applied the normotensive cNOS-specific blockers 7-Nitroindazole (7-NI) or 3-Br-7-NI. The vehicle for the drug application (arachis oil) did not change normal body temperature when applied i.p. (37.2 ± 0.1, n=12). Body temperature minima due to i.p. 7-NI were observed approx. 90 min after injection in a dose-dependent manner: 37.4 ± 0.1 (1 mg /kg, n=11), 36.3 ± 0.1 (20 mg/kg, n=11, $p<0.01$); 35.9 ± 0.1 (40 mg /kg, n=11, $p<0.01$) and 34.8 ± 0.2 (50 mg /kg, n=11, $p<0.001$). Telemetered gross motor activity (cumulative over 6h post injection) was decreased after 1 mg/kg 7-NI and reached its minimum at 40 mg/kg. 3-Br-7-NI was found to be more potent by causing similar responses at a dose of 10 mg/kg. An i.p. of dose 10mg/kg L-NIL, a specific blocker of inducible NOS failed to alter body temperature. When febrile animals (100 µg E. coli-Lipopolysaccharide, LPS, i.p.), received 7-NI, no fever was observed, whereby the first peak of the biphasic LPS-fever was replaced by a hypothermia of approx. 50% as seen in non-febrile animals. However i.c.v. application of the soluble sodium salt of 7-NI (66 µg/kg in 5 µl, n=9) did not decrease body temperature, suggesting the peripheral nervous system as the site of action.

Our data are evidence that NO is involved in basal thermoregulation via the peripheral nervous system. The consideration of this effect will be important for future physiological and behavioral studies using blocking agents for NOS e.g. in the prevention of neuronal damage due to ischemia. Sup. by DAAD, HFSOP.

248.12

DIFFERENTIAL EFFECTS OF THREE COX INHIBITORS MICRODIALYZED INTO THE PREOPTIC AREA (POA) OF GUINEA PIGS. E. Schic, M. Szekely, A. L. Ungar, and C. M. Blatteis*. Dept. of Physiol. & Biophys., Univ. of Tennessee, Memphis, Memphis, TN 38163.

In guinea pigs, iv LPS promptly induces fever and enhances the production of PGE_2 in the POA, apparently via peripheral, vagally mediated signals to the central noradrenergic system; norepinephrine rapidly augments the local production of PGE_2. If fever and PGE_2 were directly linked, the blockade of PGE_2 synthesis in the POA by locally applied COX inhibitors should prevent the concomitant increases in core temperature (T_c) and preoptic PGE_2, whereas, if PGE_2 were not an obligatory central mediator of fever, blockade of its synthesis should not abolish fever caused by iv LPS. To test this hypothesis, sodium indomethacin trihydrate (indo; 1.0 µg/µl artificial (a)CSF; pH 7.4) was microdialized bilaterally into the POA of conscious guinea pigs from 30 min before until 4 h after the iv injection of pyrogen-free saline (PFS; 0.2 ml) or LPS (2 µg/kg in 0.2 ml PFS). LPS augmented preoptic PGE_2 without raising T_c, and indo pretreatment abolished this rise, indicating that, under these conditions, PGE_2 and fever are not correlated, probably due to inflammatory tissue reactions to the implanted probes. Indeed, when the POA was alkalized with pH 7.8 aCSF, iv LPS produced biphasic increases in T_c and preoptic PGE_2, while sodium meclofenamate (1.2 µg/µl, soluble in pH 7.8) prevented the PGE_2 increase and reduced but did not block the fever caused by iv LPS. If alkaline aCSF potentiates (presumably through enhanced neuronal activity), then acidified aCSF should prevent (by depressing neuronal activity) the LPS-induced rises in PGE_2 and T_c. Indeed, sodium salicylate (10 µg/µl, soluble in pH 3.5) inverted T_c and prevented PGE_2 production caused by iv LPS. These results support the notion that PGE_2 is produced in the POA rather than peripherally in response to iv LPS. They also show that bilaterally implanted microdialysis probes and acid aCSF introduce local reactions that may vitiate the effects expected. (Supported by NIH grant NS-34857.)

248.13

THERMOREGULATORY EFFECTS OF SEROTONIN 5-HT2C RECEPTOR GENE INACTIVATION. A. M. Strack* and L. Tecott. Depts. of Physiol. and Psych., UCSF, San Francisco, CA 94143

Serotonin (5-HT) modulates core temperature by action at multiple 5-HT receptor subtypes. To study the role of the 5-HT2C receptor in thermoregulaton, we have used a 5-HT2C receptor mutant mouse strain (Nature 374: 542, 1995). Male knockout (KO) and wildtype (WT) mice were implanted with radiotelemetric temperature probes i.p. 1 week before experimentation.

m-chloro-phenylpiperazine (mCPP), a 5-HT2C agonist which produced an immediate hypothermia in WT, but not KO mice. Because mCPP inhibits food intake in WT, but not KO mice and core temperature correlates closely with feeding, we hypothesized that the differential temperature response between the groups resulted from differences in food intake. To test this, we fasted WT and KO mice for 24 hrs. Whereas, KO mice had no change in temperature, WT mice had decreased core temperature, beginning ~5 hrs after start of the fast. The differential time course between mCPP-induced and fasting-induced hypothermia suggests regulation of different 5-HT2C receptor mechanisms.

In a subsequent experiment, mice were housed at 5°C for 4 days to determine their responses to decreased environmental temperature. WT mice had decreased peak temperature at night and increased AM nadir temperature, resulting in a damped circadian oscillation in core temperature typical of circadian rhythms following stress. KO mice had a similar decrease in night peak amplitude, however nadir temperatures were higher on day 1 and lower on day 3 compared to control nadir values. The variance in circadian oscillation suggests that putting KO mice in cold unmasks a disruption in a circadian or a temperature feedback system due to lack of 5-HT2C receptors.

We conclude that the 5-HT2C receptor plays multiple roles in temperature regulation at sites yet to be elucidated. *Supported by DK28172 and DA00282.*

248.14

FUNCTIONAL ORGANIZATION OF HYPOPHYSIOTROPIC TRH NEURONS. R. T. Zoeller, R. Lee, M. Curran, D. L. Fletcher, and D. E. J. Blazis*. Department of Biology and Neuroscience and Behavior Program, Univ. Massachusetts, Amherst, MA 01003.

The pituitary-thyroid axis is controlled predominantly by neurons in the hypothalamic paraventricular nucleus (PVN) which synthesize and secrete thyrotropin (TSH)-releasing hormone (TRH). These neurons are clustered in the parvocellular division of the PVN and project uniformly to the median eminence. Cold exposure in rats produces a rapid increase in serum TSH which is dependent upon TRH secretion. We have previously demonstrated that cold exposure rapidly elevates cellular levels of the mRNA encoding TRH, indicating that hypophysiotropic TRH neurons become activated by sensory afferents (adrenergic) mediating the thyrotropic response to cold. However, we were not able to conclusively determine whether all TRH neurons are activated by cold exposure. We have now used two approaches to determine whether all or a subset of TRH neurons in the PVN are activated by cold exposure. First, we have used dual-label *in situ* hybridization to determine that cold exposure does not induce c-*fos* expression in all TRH neurons of the PVN. In addition, we are testing whether TRH mRNA levels become selectively elevated in c-*fos* expressing cells. Second, we have cloned unique regions of adrenergic receptor subtypes to determine the composition of adrenergic receptors in TRH neurons and the proportion of TRH neurons exhibiting adrenoceptor expression. These two approaches should allow us to conclude whether all TRH neurons respond to cold exposure to elicit this neuroendocrine reflex. Supported in part by NSF IBN 9514835.

248.15

THE RELATIONSHIP BETWEEN BODY TEMPERATURE AND ECF STRIATAL DOPAMINE LEVELS IN MICRODIALYSIS STUDIES. T.H. Wu,* I.N. Acworth and T.J. Maher. Div. Pharm.Sci., Mass. Coll. Pharmacy &AHS, Boston, MA 02115 and ESA Inc., Chelmsford, MA 01824.

Ample evidence has implicated a role for dopamine (DA) in the regulation of body temperature in animals. Observed striatal extracellular fluid (ECF) DA levels may be influenced by body temperature changes *in vivo* but also by factors affecting probe recovery in microdialysis experiments. In the present studies, animals were subjected to hyperthermic or hypothermic conditions to raise or lower body temperature and rectal temperature (RT) was monitored. Simultaneously ECF DA was determined in striatum using a 3 mm loop design microdialysis probe. Additionally, the *in vitro* recovery of DA by microdialysis probes was determined under similar temperature conditions as a reference for the *in vivo* studies. Following the elevation of RT in rats for 2 hrs with a temperature-controlled aluminum plate, RT increased to 40.4 °C. Following induced hypothermia for 2 hours with ice packs, RT was decreased to 22.8 °C. The linear relationship between the striatal DA level changes (%) and RT (°C) from baseline was statistically significant ($p < 0.0001$; $R = 0.825$). The slope of the regression line equaled 15.6 % /°C. For the *in vitro* recovery of DA, a linear regression line ($p < 0.05$; $R = 0.854$) existed, but with a much shallower slope (0.67 %/ °C). These studies demonstrate that a linear relationship between RT and ECF striatal DA level changes in rats and that the changes in ECF DA are not mainly the result of changes in probe recovery.

Supported in part by a grant from ESA, Inc.

248.16

CARDIOVASCULAR RESPONSES TO A NOVEL STRESSOR IN CHRONICALLY STRESSED ANIMALS. M. Dallman, S. Bhatnagar, A.I. Basbaum & B.K. Taylor* Dept. of Physiology & Keck Center for Integrative Neurosciences, UCSF, San Francisco, CA, 94143-0444.

We have previously shown that chronic, intermittently cold-stressed animals exhibit elevated levels of adrenocorticotropin and corticosterone upon exposure to a novel stressor, restraint, compared to control animals. This enhanced neuroendocrine responsiveness to a novel stressor occurs despite the negative feedback effects of circulating corticosterone that is released by cold exposure. The present study assessed whether this enhanced responsiveness extends to other stress-sensitive systems, namely the cardiovascular system. Adult, male Sprague-Dawley rats were instrumented with indwelling femoral arterial catheters and 2-3 days later exposed to either 7 days of chronic, intermittent cold stress (4h/day at 4°C; CHR) or left undisturbed (CTL). On the 8th day, blood pressure (BP) and heart rate (HR) were monitored every minute during a baseline period, 30 min of restraint and for 30 min after return to the home cage. We did not observe any differences in baseline BP or HR between CTL and CHR animals. Over the 30 minute restraint period, peak BP and HR responses occurred within 2 min and then decreased similarly in CHR and CTL animals. Interestingly, when returned to the home cage, CHR animals exhibited lower BP and HR responses compared to CTL animals. Previous data have shown that some indices of autonomic responsiveness to restraint (ie., brown fat temperature and plasma epinephrine) are not altered in chronically stressed animals. Thus, the present data support the notion that prior exposure to chronic stress has different effects on autonomic and hypothalamic-pituitary-adrenal responsiveness to novel stressors. *Supported by the Medical Research Council of Canada, DK28172 and DA08377.*

OSMOTIC REGULATION

249.1

MULTISYNAPTIC CONNECTIONS BETWEEN THE LAMINA TERMINALIS AND THE RAT KIDNEY. B.J. Oldfield*, L. Colvill, M. Giles and M.J. McKinley. Howard Florey Institute of Physiology and Medicine, University of Melbourne, Victoria, 3052, Australia.

There is evidence based on cerebral lesions in both sheep and rats that sites in the midline preoptic region can influence renal function. In the present experiments, the retrograde transynaptic transport of the Bartha strain of pseudorabies virus has been used to map the multisynaptic pathways to the kidney. Thirty Sprague-Dawley rats were anesthetised with sodium thiopentone (60 mg/kg) and the left kidney exposed through a flank incision. Six 1.5 µl injections of a suspension of virus were made throughout the renal cortex and rats survived between 1 and 4 days. Identification of the transynaptic spread of the virus throughout the CNS showed infected neurons sequentially in sympathetic preganglionic neurons of the lower thoracic cord (predominantly ipsilaterally), in premotor neurons in the medulla, pons, and paraventricular nucleus of the hypothalamus. At survival periods of 90 hours or longer virus positive neurons were identified in components of the lamina terminalis, the subfornical organ (SFO), organum vasculosum lamina terminalis (OVLT) and median preoptic nucleus as well as adjacent preoptic areas. Some of the infected neurons of the SFO and OVLT were fos-positive following 24 hours of dehydration. The data show the existence of a neural substrate via which osmotic and angiotensin-mediated changes may be detected at circumventricular sites and relayed to the kidney.

Supported by an Institute Block grant to the Howard Florey Institute by the National Health and Medical Research Council of Australia.

249.2

ROLE OF SODIUM IN MODULATING RESPONSES TO OSMOTIC STIMULI IN RAT SUPRAOPTIC MAGNOCELLULAR NEURONS. D.L. Voisin and C.W. Bourque*. Centre for Research in Neuroscience, McGill University, Montreal, Canada, H3G 1A4.

In mammals, changes in natriuresis and diuresis through effects on the secretion of oxytocin and vasopressin are part of the physiological mechanisms which regulate osmolality of the extracellular fluid. Supraoptic neurons, which synthesize and secrete the neurohypophysial hormones, are themselves recognized as osmoreceptors. Recent biophysical studies in our laboratory have provided evidence supporting the involvement of stretch-inactivated cationic channels in the transduction process underlying osmoreception in supraoptic neurons. We have investigated how these osmoreceptor-mediated responses may be modulated by the concentration of sodium ions in the extracellular fluid. Whole-cell recordings obtained from isolated rat supraoptic neurons revealed that excess NaCl was more potent than osmotically equivalent excess mannitol in eliciting membrane depolarization and firing rate increases (n=5). In voltage clamp, increases in external [NaCl] evoked a larger inward current than mannitol, but the underlying increase in membrane conductance was the same (n=5). These data suggest that the permeation characteristics of osmotically-gated cationic channels permit the simultaneous detection of extracellular sodium concentration and osmotic pressure. *Supported by the MRC (Canada) and le Programme Lavoisier (France).*

249.3

BURST FIRING AND ITS MODULATION IN NEURONS OF THE RAT ORGANUM VASCULOSUM LAMINA TERMINALIS (OVLT) IN VITRO. S.J. Gentles* and C.W. Bourque. Centre for Research in Neuroscience, McGill University, Montreal, Canada, H3G 1A4.

The OVLT has been proposed as an interface between the general circulation and the central nervous system. Intracellular recordings from OVLT neurons *in vitro* have previously revealed the presence of a low threshold spike (LTS) upon depolarization from negative potentials [Nissen *et al.* 1993, *Am J Physiol* 264]. In many cells, the presence of an LTS contributes to the generation of ryhthmic bursting activity. Extracellular recordings from OVLT neurons were therefore obtained in explants of rat hypothalamus at 33°C. Electrical activity was monitored under resting conditions, and in response to the application of various drugs (eg. angiotensin II, atrial natriuretic factor, cholecystokinin, GABA, carbachol, NMDA, neurotensin and noradrenalin) and osmotic stimuli. Bursting activity consisting of repeated clusters (2-7 spikes at 40 to 60 Hz) was observed under resting conditions in 19 of 64 spontaneously active neurons. The characteristics of these bursts were modifiable by drug application in 6 of the 19 cells tested. The bursting pattern could also be induced in 5 of the silent cells tested. These results indicate that OVLT neurons can display burst firing consistent with the presence of an LTS and that the expression of the bursting pattern can be modulated by neurotransmitters and osmotic stimuli. *Supported by the Medical Research Council of Canada.*

249.4

IONIC CURRENTS OF CELLS OF THE SUBFORNICAL ORGAN THAT PROJECT TO THE SUPRAOPTIC NUCLEUS, R.F. Johnson, T.G. Beltz, R.E. Wachtel* and A.K. Johnson. Departments of Psychology, Pharmacology and Anesthesiology and the Cardiovascular Center, University of Iowa and the VA Medical Center, Iowa City, IA 52242-1407

The subfornical organ (SFO) activates areas of the brain involved in body fluid regulation when it detects elevated levels of angiotensin II in the blood. In so doing, it transduces the humoral signal to a neural efferent signal. To understand the transduction mechanism, we have started by examining the electrophysiological characteristics of cells of the SFO that project to the supraoptic nucleus (SON). Eleven-day-old rats received injections of rhodamine labeled beads aimed at the perinuclear region of the SON. After three days to allow retrograde transport, the SFO was removed and its cells dissociated and cultured. Whole-cell patch-clamp recordings were obtained from cells, that displayed the retrograde label, for 2-5 days. The typical family of currents observed to a step depolarization protocol consisted of a rapid inward current that rapidly inactivated and was followed by outward current that peaked and then declined to a steady state. The rapid inward current is characteristic of a sodium current and can be blocked with tetrodotoxin. The outward current appears to be a combination of two potassium currents. The first is a delayed rectifier that could be block with tetraethylammonium. The second is a transient A-current that requires a period of hyperpolarization before it can be activated by a step depolarization. Supported by NHLBI HL 14388 and NASA NAGW-4358.

249.5

***c-fOS* PROTEIN (FOS) IMMUNOREACTIVITY IN ANTERIOR CIRCUMVENTRICULAR ORGANS (ACVO's) AND PERIOPTIC HYPOTHALAMIC NUCLEI (POHN) AFTER OSMOTIC CHALLENGE IN LATE GESTATION FETAL SHEEP.** T.J. McDonald*[1], C. Li[1], M.J.M. Nijland[2], A. Caston-Balderrama[2] and M.G. Ross[2] [1]Laboratory for Pregnancy & Newborn Research, Coll. Vet. Med., Cornell University, Ithaca, NY 14853; [2]Perinatal Research Labs, Dept. OB/GYN, Harbor-UCLA Med. Ctr., CA 90502, (DK 43311).

Introduction: Polyhydramnios, a serious complication of pregnancy, is associated with abnormal fetal swallowing, eg. anencephaly, mandibulofacial dysostosis. Information on sensory neurons involved in swallowing comes primarily from studies in adults and implicates ACVO's and POHN. We hypothesized that the same sensory neural structures reported to modulate swallowing in postnatal animals will show Fos activation in the fetal sheep during an osmotic challenge.

Methods: Five fetal sheep brains [3 treated, 2 controls; ≈ 130 days of gestation (dGA)] were immunostained for Fos. Seventy-five min prior to tissue collection the dams were given 20% mannitol (1 ml/kg/min for 10 min i.v.). ACVO's and POHN were scored for amount and intensity of Fos immunostaining as compared to controls.

Results: After the osmotic challenge, Fos increased in all ACVO's and POHN (Table 1) in comparison to controls.

Conclusion: Based on Fos immunostaining, all ACVO's and POHN participate in sensing systemic osmotic stimuli at 130 dGA. We speculate that, as in adults, fetal swallowing in late gestation is influenced by ACVO's and POHN.

SFO	MnPO	OVLT	SON	PVN
+	++	+	+++	++

Table 1. Relative Fos activity of 130 day fetal sheep neurons 75 min after 20% mannitol infusion to dam. Increase in Fos immunostaining compared to controls: + light, ++ moderate, +++ heavy. SFO [subfornical organ]; MnPO [median preoptic nucleus (n.)]; SON [supraoptic n.]; PVN [paraventricular n.].

249.6

CALCITONIN RECEPTORS IN THE RAT SUBFORNICAL ORGAN STIMULATE WATER INTAKE

H. A. SCHMID* and M. RAUCH Max-Planck-Institute for Physiol. & Clin. Res., Kerckhoff-Institute, Parkstr. 1, 61231 Bad Nauheim, Germany.

Although numerous receptors for calcitonin (CT) have been described in the rat subfornical organ (SFO), their possible functional relevance has so far not been investigated. Due to the absence of a functional blood-brain-barrier in the SFO, blood-borne CT can reach and affect these central receptors and might thus cause central effects in addition to its well documented peripheral effect of lowering blood calcium levels. Using a brain slice preparation, the effect of bath applied rat-CT (rCT) and angiotensin II (AngII) on the extracellularly recorded electrical activity of SFO neurons was investigated. Superfusion with rCT (10^{-7} M) activated 62% and AngII (10^{-7} M) activated 85% of SFO neurons tested with both peptides (n=26). With only one exception, all rCT-responsive neurons could in addition be activated by AngII. The threshold concentration for CT was 10^{-9} M and thus similar to the threshold concentration of AngII in this preparation. Because circulating AngII is known to stimulate water intake via its excitatory effect on SFO neurons, the effect of CT on water intake of water sated rats was investigated. Subcutaneous injection of AngII ($2x10^{-4}$ M, 200µl) and rCT ($4x10^{-4}$ M, 200µl) caused drinking in 9 of 11 and 6 of 9 rats, respectively, whereas only 1 out of 14 control rats drank during 60 min after the injection of saline. The cumulative water intake of all rats receiving AngII (2.6 ± 0.5 ml) or rCT (1.0 ± 0.3 ml) was significantly ($p<0.02$; Mann-Whitney rank sum) increased vs. the control (0.07 ± 0.07). These data provide the first direct evidence for an interaction of blood-borne calcitonin with neurons in the brain and suggest a similar effect of AngII and CT on water intake and possibly other SFO mediated physiological responses like salt appetite and release of antidiuretic hormone. Supported by DFG Si 230/1.

249.7

HIGH RESOLUTION IMMUNOGOLD LOCALIZATION OF AQUAPORIN-4, A WATER CHANNEL, IN RAT BRAIN. E. A. Nagelhus, S. Nielsen†, P. Agre‡, and O. P. Ottersen.* Dept. of Anat., Univ. of Oslo, P. O. Box 1105 Blindern, N-0317 Oslo, Norway.†Dept. of Cell Biology, Univ. of Aarhus, Denmark.‡Dept. of Biol. Chem., Johns Hopkins Univ. School of Med., Baltimore, USA.

A new member of the aquaporin family, aquaporin-4, has recently been cloned and found by Northern blotting and in situ hybridization to be strongly expressed in brain (Proc. Natl. Acad. Sci. USA 91:13052-13056, 1994). We have used an affinity purified antibody raised against a synthetic peptide near the C-terminus of the cloned protein to investigate its precise localization. Immunoblotting demonstrated a 29 kDa and a 50-60 kDa band which were both completely ablated using peptide absorbed antibody. Light microscopy revealed strong immunostaining along the entire surface of the brain and particularly ventrally where the most prominent labeling occurred at mesencephalic and diencephalic levels. There was also strong labeling in the cerebellum, area postrema, and nucleus supraopticus. Postembedding immunogold analysis of the latter structures, and of the subfornical organ, spinal cord, and hippocampus, indicated that aquaporin-4 is restricted to glial membranes and to subpopulations of ependymal cells (those associated with the subfornical organ). The density of gold particles were particularly high in perivascular glial processes which displayed a stronger immunolabeling of the membranes facing blood vessels than of the membranes facing the neuropil. This polarization was confirmed by double labeling with different gold particle sizes revealing an inverse polarization in regard to the distribution of the glutamate transporter GLAST (see Neuron 15:711-720, 1995). The glia limitans displayed strong labeling, more intense on the pial surface than on the surface abutting on the neuropil. Following preembedding immunogold cytochemistry the silver enhanced 1.4 nm gold particles were associated with the cytoplasmic aspect of the glial membrane, consistent with an intracellular localization of the C-terminal. Our data indicate that aquaporin-4 is found in glial processes throughout the brain and that it is preferentially targeted to glial membranes associated with brain-blood or brain-liquor interfaces. (Supported by the Norwegian Research Council)

249.8

VASOPRESSIN RESPONSE TO DEHYDRATION IN AGED BROWN NORWAY/FISCHER 344 RATS. C. D. Sladek* and K.L. Swenson. Dept. Physiology, Chicago Med. Sch., North Chicago, IL, 60064.

The plasma vasopressin (pVP) response to 72 hours of water deprivation is attenuated in 30 mo. old Fischer 344 (F344) relative to 4 mo old rats. This appears to reflect an inability to increase VP synthesis. In contrast, elevated pVP has been reported in the Brown-Norway (BN) strain of rats secondary to reduced renal VP responsivity. We evaluated the response to dehydration in the F1 cross of these strains, BN/F344F1, in order to determine the usefulness of this strain for future studies on the mechanisms by which aging impacts on regulation of VPmRNA. The following parameters were evaluated in male BN/F344F1 rats of 4 and 30 mos of age (NIA colony, Harlan SD) following 72 hrs of water deprivation (Dehyd) or water ad libitum (Hyd): change in body wt (ΔBW), hematocrit (Ht), urine osmolality (uOsm), pVP. The results are tabulated below:

AGE mo	(n)	H20 Status	ΔBW %	Ht %	uOsm mosm/kg	pVP pg/ml
4	(8)	Hyd.	0.0±0.4	44±1	1158±115	0.4±0.02
4	(8)	Dehyd.	-8.0±0.3*	50±1*	4440±32*	2.3±0.4*
30	(7)	Hyd.	0.2±0.2	40±3	1535±157	0.5±0.2
30	(6)	Dehyd	-6.1±0.2*	45±2*	2373±486	0.8±0.2

*$p<0.05$ vs hydrated of same age.

In spite of similar dehydration in the 4 and 30 mo old rats as indicated by the ΔBW and Ht, pVP was not elevated in the dehydrated old rats. This suggests that the effect of aging on the VP response to dehydration in BN/F344F1 rats is similar to that observed previously in F344 rats. Supported by NIH grant RO1-AG12701.

249.9

EFFECT OF AGING ON VASOPRESSIN RESPONSE TO DEHYDRATION IN FEMALE FISCHER 344 RATS. J. Catudioc-Vallero* and C. D. Sladek. Dept. Physiology, Chicago Med. Sch., North Chicago, IL, 60064.

The plasma vasopressin (pVP) response to 72 hours of water deprivation is attenuated in male 30 mo. old Fischer 344 (F344) relative to 4 mo old rats. This appears to reflect an inability to increase VP mRNA content and consequently VP synthesis. Since gonadal steroids have been implicated in the regulation of VPmRNA content and gonadal steroid production is differentially effected by aging in males and females, we evaluated the VP response to dehydration in female Fischer 344 rats of 4, 12/13, 19/20, and 28 mos of age in order to determine if gender differences exist in the effect of aging on this response. Daily vaginal smears indicated that all the rats 19 mos of age and older were in pseudopregnancy (constant diestrus). Their responses were compared to young rats in diestrus. The following parameters were evaluated following 72 hrs of water deprivation (D) or water ad libitum (H): % change in body wt (ΔBW), hematocrit (Hct), plasma osmolality (pOsm, mOsm/kg), and pVP (pg/ml); n=4-6. The results are tabulated below:

AGE	4		12 13		19 20		28	
H2O	H	D	H	D	H	D	H	D
ΔBW	5±2	-11±0	1±2	-9±0	0±0	-9±0	0±0	-10±0
pOsm	300±3	313±3	299±2	308±2	304±4	307±2	297±1	316±2
Hct	43±2	50±1	42±2	47±1	40±1	42±2	37±1	41±2
pVP	2±0	14±6	2±1	16±6	2±0	26±13	2±1	12±4

pVP was elevated by dehydration ($F=27$, $p<0.001$) irrespective of age ($F=1$). Thus, gender differences exist in the effect of aging on the VP response to aging in F344 rats. Supported by NIH grant RO1-AG12701.

249.10

INHIBITION OF OSMOTICALLY STIMULATED VASOPRESSIN RELEASE BY 17β-ESTRADIOL AND DIHYDROTESTOSTERONE. K.L. Swenson* and C.D. Sladek. Finch Univ. Health Sci./Chicago Med. Sch., North Chicago, IL.

In previous experiments, testosterone inhibited the osmotically stimulated increase in vasopressin (VP) release from hypothalamo-neurohypophyseal system (HNS) explants. Therefore, experiments were performed to determine which of the active testosterone metabolites, 17β-estradiol (E_2) or dihydrotestosterone (DHT), was responsible for this inhibitory effect on VP. HNS explants were obtained from intact male rats. The explants were perifused in the presence or absence of E_2 (50pg/ml) or DHT (3ng/ml) throughout the entire experiment. After a 4 hour equilibration period, half of all groups were exposed to a ramp increase in osmolality (40mosm total) during the last 6 hours. Perifusate was collected in 20 minute fractions for a total of 10 hours. Both E_2 and DHT significantly inhibited the osmotically stimulated increase in VP as compared to controls (determined by RIA of the perifusate fractions). Therefore, both active testosterone metabolites, E_2 and DHT, inhibited the osmotically stimulated increase in VP release. Steroid receptors have not been localized in VP neurons, suggesting that the results are due to a nongenomic effect of these steroids on the VP neuron or to genomic effects elsewhere in the osmotic circuitry.

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249.11

CENTRAL SEROTONIN INJECTION PRODUCES SUSTAINED INHIBITION OF RENAL SYMPATHETIC ACTIVITY IN VOLUME-DEPLETED RATS, K.E. Scrogin, R.L. Thunhorst* and A.K. Johnson. Departments of Psychology and Pharmacology and the Cardiovascular Center, University of Iowa, Iowa City, IA 52242-1407

Central serotonin (5-HT) injections cause a slowly developing natriuresis in hydrated rats that is dependent on renal sympathetic nerves. In the present study, renal sympathetic nerve activity (RSNA) was recorded in mildly hypotensive, hypovolemic rats to determine if central 5-HT produces sympathoinhibition. Mean arterial blood pressure (MAP), heart rate (HR) and RSNA were recorded in conscious, unrestrained rats. Rats were volume depleted by iv furosemide (4x1 mg/kg) and MAP lowered by iv minoxidil (2 mg/kg). Responses to 0 (n=3), 20 (n=9) or 100 μg (n=8) icv, and 100 μg (n=5) iv 5-HT were then recorded for 90 min. Furosemide increased MAP (8±1 mmHg) and RSNA (35±9% above baseline) and caused a cumulative urine excretion of 15.4±0.5 ml. Minoxidil reduced MAP (-12 mmHg) and increased HR (24±5 bpm) and further increased RSNA (37±10% of pre-minoxidil levels). Dose-dependent reductions in RSNA of 20.9±3.4 and 38.8±6.8% were observed by 90 min after central 5-HT injection. Intravenous injection of 5-HT (100 μg) caused a reduction in RSNA (17±8% below baseline after 90 min) that was significantly less marked than that after the same dose given icv. The data indicate that central 5-HT produces a progressive and sustained renal sympathoinhibition despite the unloading of arterial and cardiopulmonary baroreceptors. The results suggest a role for central 5-HT in long-term modulation of sympathetic-dependent renal function. Supported by NHLBI HL14388 and NASA NAGW-4358.

249.12

CHRONIC LASIX TREATMENT EARLY IN LIFE DISRUPTS DEVELOPMENT. G. Brandt, J. Diaz* and R. McCowen. Dept. of Psychology, University of Washington, Seattle. Wa. 98195.

Male and female Long-Evans Hooded pups were assigned to one of three groups: Furosemide-inject once per day at 60mg/kg of body weight; saline control-inject once per day at 60 mg/kg of body weight; or non-inject controls. Once per day from day zero (day of birth) through day nine, pups were removed from dams, stimulated to induce micturition, and weighed. Pups were then injected or not, according to group assignment and kept in small cages set on a heating pad for 1 1/2 hours (the estimated peak time for action of furosemide). Pups were then once again stimulated to induce micturition, weighed, and returned to their respective dams. On day 15, pups were weighed and decapitated. Dependent measures taken include wet and dry weights for whole body, brain, heart, and spleen; tibia length, and blood glucose levels.

Furosemide-treated animals had significantly smaller whole-body wet weights by postnatal day 3 through day 15, and significantly smaller percent wet-brain-to body weights on day 15. Percent brain water was not different. Tibia length was significantly shorter in furosemide-treated animals. Furosemide-treated animals were hyperglycemic on day 15, while saline-inject and non-inject control animals showed normal blood glucose levels. Blood glucose levels were not different on day 4 or day 10.

These data support the hypotheses that chronic furosemide treatment during critical periods of development results in an impaired rate of somatic growth, and may alter the normal course of brain development and other specific metabolic systems by the alteration of water and electrolyte balance. Further study is needed to determine if the alteration of sodium balance is the cause of abnormal development of specific systems and growth patterns.

249.13

EFFECTS OF ADRIAMYCIN-INDUCED NEPHROTIC SYNDROME ON RENIN-ANGIOTENSIN SYSTEM MEDIATED WATER AND SODIUM INTAKES IN RATS, J. Xu, S.R. Robinson* and A.K. Johnson. Departments of Psychology and Pharmacology and the Cardiovascular Center, University of Iowa, Iowa City, IA 52242-1407

The present study examined whether the renin-angiotensin system (RAS) is involved in the altered drinking behavior in adriamycin-treated rats. Rats were injected with either adriamycin (3.5 mg/kg) or saline through the sublingual vein. Adriamycin-treated nephrotic rats drank more water two weeks after adriamycin injection. Daily saline intake was not significantly different. Nephrotic rats showed reduced water intake following isoproterenol (30 μg/kg, sc), overnight water deprivation and furosemide (10 mg/kg, sc) + captopril (2 mg/kg, sc). Saline intake also was blunted after furosemide + captopril. No difference in drinking was found after hypertonic saline (4%, sc). Before isoproterenol treatment, plasma renin activity (PRA) levels did not differ between groups while aldosterone levels were significantly lower in nephrotic rats. After isoproterenol treatment, elevation of PRA and aldosterone levels was significantly blunted in nephrotic rats. No difference in vasopressin levels between groups was found either before or after isoproterenol treatment. The results suggest that the extracellular rather than intracellular drinking mechanisms are disturbed in nephrotic rats and the RAS might be responsible for the reduced drinking response to acute thirst and sodium intake challenges. Supported by NHLBI HL 14388 and NASA NAGW-4358.

249.14

EFFECTS OF CORTICOSTERONE ON CONTROL OF RENAL SYMPATHETIC NERVE ACTIVITY. D. A. Scheuer* and S. W. Mifflin. Univ. Texas Health Science Center, San Antonio, TX 78284-7764.

Chronic increases in corticosterone (cort) produce hypertension, however the effects of cort on neural control of the circulation are poorly understood. Therefore, experiments were performed in male Sprague-Dawley rats (control (con), n=3, chronic cort treatment , n=5) to determine the effects of cort on baroreflex control of renal sympathetic nerve activity (RSNA). A minimum of 13 days after initiation of cort rats were anesthetized with Inactin (100 mg/kg, i.p.) and instrumented to measure mean arterial pressure (MAP), heart rate (HR) and RSNA. Barorefex control of RSNA was determined, using intravenous infusions of phenylephrine and nitroprusside to change arterial pressure at the rate of 1-2 mmHg/sec, before and 2 and 3 hrs after administration of the glucocorticoid type II receptor antagonist Mifepristone (Mif; 30 mg/kg s.c.). Reflex curves were analyzed using a 4 parameter logistic function. Baseline MAP (124±2 vs. 114±3 mmHg) and HR (427±6 vs. 369±6 bpm) were greater ($P<0.05$) in cort vs. con rats. RSNA, as a percentage of maximum, was 70±5 and 72±4% in con and cort rats, respectively. In cort rats MAP was significantly decreased 2 hrs (111±2 mmHg) and 3 hrs (109±4 mmHg) after Mif. Despite the reduction in MAP, HR (426±12 and 433±18 bpm) and RSNA (77±7 and 75±8%) were not significantly elevated 2 and 3 hrs after Mif. The pressure at midrange of the RSNA baroreflex curve in cort rats was 134±3 mmHg before Mif, and was shifted to the left (121±2 and 118±2 mmHg; $P<0.05$) 2 and 3 hrs following treatment of cort rats with Mif. In con rats Mif did not significantly change baseline MAP, HR or RSNA, or reflex control of RSNA. These results suggest that chronic elevations in cort modulates sympathetic regulation. (Supported by AHA, TX Affiliate)

249.15

NORADRENERGIC NEURONS IN VENTROLATERAL MEDULLA INTEGRATE NEUROENDOCRINE FUNCTION OF VASOPRESSIN

S.Shioda*, M.Iwase, I.Homma, S.Nakajo, K.Nakaya, T.Yada, A. Takaki, Y.Nakai Dept. Anat. (S.S., Y.N.) & Physiol. (M.I., I.H.) Sch. Med. and Lab. Biol. Chem. Sch. Pharmac. Sci. (S.N., K.N.), Showa Univ., Tokyo 142; Dept. Physiol. Sch. Med. (T.Y.), Kagoshima Univ., Kagoshima 890; Dept. Physiol. Fac. Med. (A.T.), Kyusu Univ., Fukuoka 812, Japan.

Although the pathway arising from noradrenalin (NA) neurons in the medulla is involved in the regulation of vasopressin (VP), the precise mechanism of action and biological significance of NA still need to be investigated. NA neurons of A1 cell group in the medulla made synaptic inputs to hypothalamic VP neurons as revealed by combined tract tracing with immunocytochemistry. Unilateral electrical stimulation (80-90 μA at 50 Hz for 10 min) of A1 cell group induced bilateral increase of Fos-like immunoreactivity (F-LI) in hypothalamic neurosecretory neurons. Strong F-LI was visible in many VP neurons but rarely in oxytocin neurons as revealed with double immunostaining. *In situ* hybridization revealed that high level of VP mRNA was noted in the hypothalamus at 12 h and reached its peak at 24 h after stimulation. One μM NA increased cytosolic Ca^{2+} in isolated VP neurons. Dibutyryl cAMP also increased cytosolic Ca^{2+} in VP neurons. An inhibitor of protein kinase A (H89) inhibited NA-induced increase in cytosolic Ca^{2+} in VP neurons. These findings suggest that NA stimulates Ca^{2+} signaling pathway, which is at least partly mediated by cAMP-PKA pathway, and that the cAMP and Ca^{2+} systems participate in secretion and gene transcription of VP in response to physiological stimuli. (Supported in part by Ministry of Education, Science, and Culture of Japan)

249.16

SWELLING-INDUCED, 85 kDA cPLA$_2$ MEDIATED ARACHIDONIC ACID RELEASE IN THE HUMAN NEUROBLASTOMA CELL LINE CHP-100.

S. Basavappa*[1], S. F. Pedersen[2], N. K. Jørgensen[2] and E. K. Hoffmann[2]. [1]University Laboratory of Physiology, University of Oxford, Oxford OX1 3PT, UK and [2]August Krogh Institute, University of Copenhagen, DK-2100 Copenhagen Ø Denmark.

Upon exposure to hypoosmotic stress, cells initially swell and undergo regulatory volume decrease (RVD). Utilizing the pH sensitive fluorescent dye BCECF, a decrease in intracellular pH (pH_i) after hypoosmotic stress was noted in CHP-100 cells. In cells loaded with ^{3}H-Arachidonic acid (^{3}H-AA), exposure to hypoosmotic solution increased release of ^{3}H-AA by 250 $\pm$ 19% (n=19) as compared to control cells. Swelling-induced release of ^{3}H-AA was significantly inhibited by 15 min pretreatment with the potent inhibitor of the 85 kDA cPLA$_2$ AACOCF$_3$, a trifluormethyl ketone analogue of arachidonic acid (AA) (n=10, $p<0.05$) while the PLC inhibitor U73122 did not significantly alter the swelling-induced ^{3}H-AA release (n=6). However, pretreatment with NPPB, which blocks Cl^- permeability and the RVD response, significantly increased ^{3}H-AA release by 423 + 68% (n=6). In single cells loaded with Fura -2, exposure to 50 and 200 μM AA resulted in an elevation in intracellular Ca^{2+} level ($[Ca^{2+}]_i$). Interestingly, cells initially acidified during exposure to AA, but then gradually alkalinized. The effect of AA on anion permeability was assessed in cells loaded with ^{36}Cl. Subsequent exposure to hypoosmotic stress rapidly increased efflux of ^{36}Cl and this efflux was significantly inhibited by 200μM AA. In addition, AACOCF$_3$ significantly ($p<0.05$) blocked the swelling-induced ^{36}Cl efflux. Thus, the present studies indicate that arachidonic acid plays a key role during RVD.

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249.17

SHRINKAGE-INDUCED ACTIVATION OF Na/H EXCHANGE: ROLE OF MYOSIN PHOSPHORYLATION AND CELL DENSITY.

R.W. Putnam, L.D. Shrode, J.D. Klein, W.C. O'Neill, P.B. Douglas and R.W. Fyffe*. Dept. Physiol. & Biophys., Wright State Univ. Sch. of Med., Dayton, OH 45435.

The effect of cell shrinkage on the Na^+/H^+ exchanger was studied in cultures of C6 glioma cells. In fully confluent cultures (4 days after plating), shrinkage resulted in a rapid alkalinization of 0.6 pH unit at an initial rate of 0.300 $\pm$ 0.018 pH/min (n=4). This alkalinization was dependent on the magnitude of cell shrinkage, resulted in a shift of the pK' of the exchanger for internal H ions, was inhibited by ML-7 (an inhibitor of myosin light chain kinase) and involved increased phosphorylation of an 18 kD protein (that co-localizes with myosin light chain, MLC). These data suggest that in C6 cells, like in primary astrocytes (Shrode et al., *Am. J. Physiol.* 269:C257-C266, 1995), shrinkage-induced activation of Na^+/H^+ exchange involves phosphorylation of myosin light chain. Interestingly, little shrinkage-induced alkalinization (only 0.044 pH unit at a rate of 0.096 $\pm$ 0.006 pH/min; n=6) was seen in subconfluent cultures of C6 glioma cells (2 days in culture, about 50% confluent). Na^+/H^+ exchange was present in these cells since they showed amiloride-sensitive pH_i recovery in response to NH_4Cl-induced acidification. The lack of shrinkage-induced alkalinization persisted in subconfluent cells that were growth arrested (serum starvation) or that were differentiated by exposure to 0.25 mM di-butyrl cAMP. However, MLC was still phosphorylated in these subconfluent C6 cells upon shrinkage. These data indicate that a step in shrinkage-induced activation of the Na^+/H^+ exchanger between phosphorylation of MLC and the exchanger is missing in subconfluent cultures of C6 glioma cells. [Supported by JDF grant (to RWP) and NIH HL-47449 (to WCO).]

249.18

RAT BRAIN INTERSTITIAL VOLUME FRACTION AND N-ACETYL-ASPARTATE(NAA) MEASURED BY MICRODIALYSIS DURING ACUTE HYPONATREMIA.

T.N. Sager, J.A. Lundbæk and A.J. Hansen* Neuropharmacology, Novo-Nordisk A/S, , DK-2760 Maaloev, Denmark.

In vitro studies indicate that brain interstitial volume fraction (IVF) is decreased during acute hyponatremia. However it is unknown, how brain IVF reacts *in vivo*. Acute hyponatremia in rats was induced by i.p. injection of distilled water. (10%+5% of body weight). This led to a 20% decrease in plasma [Na+] after 1 h. We have previously shown that the realtive loss (RL) of mannitol is directly related to IVF[1] and, hence, RL of ^{3}H-mannitol in the striatum was measured in the following hours using microdialysis. NAA has been implicated in nerve cell volume regulation and microdialysis was further used to measure interstitial [NAA]. The RL of ^{3}H-mannitol did not change during the course of the experiment indicating that the IVF remained unchanged. No change in [NAA] was observed either. These results compliments earlier findings using the same animal model where the IVF (using the TMA microlelectrode technique) and brain water were determined. Despite a 20 % decrease in plasma osmolality the brain swelled only 10 % demonstrating volume regulation. The present results show that NAA has no role in the this response. The fact that IVF remained unchanged suggests that the interstitial space and the cells are equally responsible for this brain volume regulatory response.

[1]Fink-Jensen, A., T.N. Sager, and A.J. Hansen: In vivo recovery of brain microdialysis determined by use of mannitol. Submitted.

NEUROENDOCRINE REGULATION: SUPRAOPTIC NUCLEUS

250.1

MASKING OF A LOW THRESHOLD Ca^{2+} SPIKE BY AN A-TYPE K^+ CURRENT IN RAT SUPRAOPTIC NEURONS.

T.E. Fisher* and C.W. Bourque. Centre for Research in Neuroscience, Montreal General Hospital and McGill University, Montreal, Canada, H3G 1A4.

In many mammalian neurons, T-type calcium currents mediate a low threshold, calcium dependent spike following depolarizations from negative potentials. Yet although the magnocellular neurosecretory cells (MNCs) of the rat supraoptic nucleus have a T-type current, their voltage-current relationship shows outward rather than inward rectification. To examine the interactions that underlie the near threshold behaviour of the MNCs, we compared the biophysical properties of a transient, A-type K^+ current (I_A) and low threshold Ca^{2+} currents (I_{Ca}) using whole-cell patch clamp of acutely isolated MNC somata. While I_A and I_{Ca} activated at the same voltage (-60 mV), I_A was larger in amplitude (e.g. at -50 mV 551$\pm$55 pA, n=16; vs 92$\pm$9 pA, n=36) and activated more rapidly (e.g. times to peak amplitude at -50 mV of 7.2$\pm$0.3 ms vs 33$\pm$1 ms). Furthermore, blockade of I_A by addition of 10 mM 4-aminopyridine converts the outward rectification of MNC somata seen in current clamp to an inward rectification resulting in the activation of low threshold spikes. These data suggest that the electrogenic effect of calcium influx at low voltages is masked by the I_A and that the role of the T-type current may depend, rather, on intracellular effects of calcium ions such as the activation of calcium dependent conductances.

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250.2

SHORT-LATENCY EFFECTS OF PROTEIN SYNTHESIS INHIBITION.

B.C. Wilson*, T.M. Saleh & G.J. Pittman. Neuroscience Research Group, University of Calgary, Calgary, Alberta, Canada T2N-4N1.

An antisense oligonucleotide (oligo) directed against oxytocin (OT) mRNA has been shown to have specific, short-latency effects on oxytocin-related lactational variables when injected into the SON of conscious, lactating rats. The mechanism(s) of action of antisense oligos are poorly understood, but it is known that these oligos interfere with translation in protein synthesis. We wondered if the same short-latency effects would result if translation was blocked using a protein synthesis inhibitor, anisomycin (AN). To determine an effective dose of AN, FOS immunohistochemistry following ip CCK (100 ng/kg) was carried out on 2 groups of male rats injected 4 h previously with 500 ng or 2 μg AN into each SON. The higher dose of AN, but not the lower dose, abolished CCK-induced FOS expression. Using conscious, lactating dams, bilateral injections were made on three subsequent days of lactation: Day 8 (0.5 μL aCSF; control), Day 9 (500 ng or 2 μg AN dissolved in 0.5 μL aCSF; experimental); Day 10 (0.5 μL control) into the SON through guide cannulae. Four hours post-injection, 10 pups were re-introduced into the cage of each dam and assessments were made of maternal behaviour, pup weight gain and the number of milk-ejections occurring in a 30 min period after suckling was established. Both dosages of AN had no significant effect ($p>0.05$) on maternal behaviour or litter weight gain (though weight gain was reduced). The number of milk-ejections observed in 30 mins was significantly ($p<0.05$) reduced in a dose-dependent manner. These data demonstrate that blocking protein synthesis results in short-latency effects similar to those of antisense at a time before pituitary OT content is depleted. Thus, interference with translation may affect cellular secretory functions.

Supported by the Heart and Stroke Foundation of Canada.

250.3

CNQX BLOCKS EXCITATORY RESPONSES OF RAT SUPRAOPTIC NEURONES EVOKED BY SUPRACHIASMATIC STIMULATION IN VITRO. L.-N. Cui, R. E. J. Dyball and J. Li* Dept of Anatomy, Univ. of Cambridge, Downing Street, Cambridge CB2 3DY, UK. and Dept of Physiology, Univ. Coll. London, Gower Street, London WC1E 6BT, UK.

Secretion of vasopressin and oxytocin from magnocellular cells in the paraventricular and supraoptic nuclei (SON) of hypothalamus displays a circadian variation. Since the suprachiasmatic nucleus (SCN) of the hypothalamus represents the endogenous biological clock in mammals, it may also regulate the activity of magnocellular cells. We therefore investigated the projection from the SCN to the SON using an electrophysiological approach *in vitro*.

Coronal and horizontal hypothalamic slices from young male Wistar rats were perfused with artificial cerebrospinal fluid (ACSF). Single unit extracellular recordings were made from rat SON neurones, while stimuli (<1mA peak-to-peak) were delivered at 1 Hz to the SCN through a bipolar stimulating electrode. The responses of SON neurones to the stimulation was analyzed by creating peri-stimulus time histograms. A total of 46 cells were recorded of which 25 (54.3 %) responded to SCN stimulation; 17 neurones were excited and 8 neurones were inhibited. The excitatory responses were investigated further by adding CNQX (5 - 20 μM), an antagonist of non-NMDA type of glutamate receptors, to the ACSF. In 4 of the 5 SON neurones tested, CNQX blocked the excitatory response. While the LD-APV (10 - 40 μM), an antagonist of the NMDA type of glutamate receptor, had no effects on the 3 neurones tested.

The results suggest that there are functional projections from the SCN to the SON and that the pathway is mediated by the non-NMDA type of glutamate receptor.

We are grateful for the financial support of the MRC.

250.4

MORPHOMETRIC STUDY OF OXYTOCIN AND VASOPRESSIN NEURONS IN THE SUPRAOPTIC NUCLEUS: MODULATION DURING LACTATION. J.E.Stern* and W.E.Armstrong, Dept of Anat. & Neurobiol., Univ. Tenn., Memphis, TN 38163.

The aims of the present study were to determine whether the cytoarchitecture of identified oxytocin (OT) and vasopressin (VP) neurons in the supraoptic nucleus (SON) differed either as a function of cell type or lactation. *In vitro* intracellular recordings were obtained from hypothalamo-neurohypophysial explants prepared from virgin or lactating female rats. Neurons were intracellularly labelled with neurobiotin and reconstructed in three dimensions for morphometric analysis. SON dendrites exhibited little branching and there were no differences in the number of branches or spine density between cell type or as a function of lactation. Most of the dendrites projected ventrally toward the glial lamina and the total dendritic length per cell ranged from 912-1567 μm. Axons were identified by their smaller diameter, beaded appearance and dorso-caudal trajectory, and were typically cut at the dorsal surface of the explant. The axon arouse either directly from the soma (63%) or from a proximal dendrite (37%), but this difference did not vary between OT and VP cells. Only one case of an axon collateral was observed. During lactation, a significant enlargement of the somal area as well as an increase in the number of primary dendrites was observed in both cell types. Further analysis revealed that this increase was due to an increase in the number of short (<200 μm) dendrites. The apparent dendritic volume of OT neurons was greater than that of VP neurons in both groups. In the case of OT neurons, a decrease in the average dendritic length was observed during lactation. Since apparent dendritic volume was unaffected, this suggests OT dendrites become shorter and fatter during lactation. On the other hand, the average dendritic length was not changed in VP neurons during lactation. These results suggest that there is dendritic reorganization among OT neurons during lactation which would accompany the well known synaptic reorganization.

Supported by NIH NS 23941 (WEA) and the Neuroscience Center for Excellence.

250.5

ELECTROPHYSIOLOGICAL AND MORPHOLOGICAL CHARACTERISTICS OF SUPRAOPTIC PERINUCLEAR ZONE NEURONS. W.E.Armstrong* and J.E.Stern, Dept of Anat. & Neurobiol.; Univ. Tenn., Memphis, TN 38163.

Parvocellular neurons are sparse in the supraoptic nucleus (SON), but neurons in the area immediately surrounding the nucleus are thought to serve as interneurons. Cytoarchitectonically, this perinuclear zone (PZ) is ill-defined and contains several cell types, including GABAergic neurons thought to mediate baroreceptor-induced inhibition of vasopressin release. We recorded intracellularly from dorsal PZ neurons in ventral hypothalamic explants from male and female rats, characterized some basic membrane properties, and filled a subset with biotin tracers in order to study their dendritic and axonal trees. Electrophysiologically, PZ neurons (n = 23) were distinguished from SON neurons by having faster Na^+ action potentials, little subthreshold transient outward rectification, and strong subthreshold transient inward rectification reminiscent of low-threshold spiking neurons described elsewhere in the brain. The shape of the low threshold spike varied considerably across neurons, such that in some cells only a pair of Na^+ spikes were evoked, whereas in others a more extended burst was produced. Morphologically, the recovered PZ neurons were diverse; both bipolar or multipolar neurons were found, some with smooth and others with very spiny dendrites. In general, PZ neurons had more extensive dendritic trees than SON neurons, and most exhibited dendrites projecting into the SON. However, only a few of the filled PZ neurons had axonal projections into the SON. Some axons branched extensively within the PZ. Many PZ neurons had an axonal projection rostro-medial to the SON, in the region of the ventral preoptic area. These data suggest that while PZ neurons have common functional characteristics which distinguish them from SON neurons, several morphological subtypes are represented in this group, and only a small subset projects axons to the SON. Supported by NIH NS 23941 (WEA) and the Neuroscience Center for Excellence.

250.6

MODULATION OF GABA CURRENTS IN HYPOTHALAMIC NEUROSECRETORY NEURONS BY THE NEUROACTIVE STEROID ALLOPREGNANOLONE. **A. Fáncsik* and J.G. Tasker, Dept. of Cell & Molecular Biology, Tulane University, New Orleans, LA 70118.**

The release of the hypothalamic hormones, oxytocin and vasopressin, is regulated by circulating levels of gonadal steroids and their metabolites. The progesterone metabolite allopregnanolone (3α-hydroxy-5α-pregnan-20-one) has been shown to modulate γ-aminobutyric acid A ($GABA_A$)-receptor mediated ionic currents in numerous cultured and acutely dissociated neuronal preparations. Allopregnanolone enchances GABA currents in the nerve terminals of the neurohypohysis (Zhang and Jackson, J. of Neuroendocrinol. 6:533, 1994). The aim of the present study was to determine the receptor-mediated effects of allopregnanolone on the magnocellular somata in the hypothalamic supraoptic nucleus (SON). Whole-cell voltage-clamp recordings were used in coronal slices of rat hypothalamus to record spontaneous GABA-mediated inhibitory postsynaptic currents (IPSCs) in SON magnocellular neurons. IPSCs were blocked by the $GABA_A$-receptor antagonist bicuculline, and the decay phase of the average IPSC was best fitted by a double exponential. Bath application of allopregnanolone (1μM) caused an increase in the decay time constant of the average IPSC in 9 of 9 cells tested, which was accompanied by a decrease in the IPSC amplitude in 6 of the 9 cells. Intracellular application of allopregnanolone through the patch pipette had no effect in 9 of 10 cells, suggesting that the steroid acts at an extracellular membrane site. However, the internal application of allopregnanolone completely blocked the action of the steroid applied in the bath in 7 of 8 cells through an unknown mechanism. These results suggest that allopregnanolone may affect the kinetics of opening and closing of $GABA_A$- receptor channels in SON magnocellular neurons.

This study was supported by the LA American Heart Association and the NINDS.

250.7

$GABA_B$ RECEPTOR-MEDIATED INHIBITORY ACTIONS IN RAT HYPOTHALAMIC SUPRAOPTIC NEURONS. N. Ibrahim, H. Yamashita*, I. Shibuya, N. Kabashima & Y. Ueta Dept. of Physiol., Univ. Occup. & Environ. Health, Kitakyushu 807, Japan

GABA-immunoreactive neurons have been identified in the perinuclear region of the supraoptic nucleus (SON) and GABA potently inhibits the electrical activity of SON neurons. The inhibitory action of GABA has been thought to be mediated exclusively by $GABA_A$ receptors. In this study, effects of GABA via $GABA_B$ receptors have been investigated. Using extracellular recording, rat hypothalamic slices containing SON magnocellular neurons were continuously perifused with a solution containing the $GABA_A$ antagonist, picrotoxin (50 μM) and exposed to the $GABA_B$ agonist, baclofen (10 nM - 10 μM). SON neurons were inhibited by baclofen in a dose-dependent manner. The inhibitory effects of baclofen were reversed by the selective $GABA_B$ antagonist, 2 hydroxy-saclofen (2OH-saclofen) also in a dose-dependent manner. Moreover, 2OH-saclofen alone potentiated the firing rate of SON neurons. Analysis of synaptic currents with a slice patch-clamp technique revealed that baclofen inhibited SON neurons both postsynaptically and presynaptically. These results indicate that $GABA_B$ receptors participate in the inhibitory action of GABA in the supraoptic magnocellular neurons.

250.8

EXTERNAL CALCIUM DEPENDENCE OF SYNAPTIC TRANSMISSION TO SUPRAOPTIC NEURONS IN THE HYPOTHALAMIC SLICES OF THE MOUSE. E. Honda, K. Inenaga*, H. Yamashita[1], S. Nakamura and T. Hirakawa Dept. Physiology, Kyushu Dental Col., Kitakyushu 803 and [1]Dept. Physiology, Univ. Occup. Environ. Health Sch. of Med., Kitakyushu 807 Japan.

Excitatory postsynaptic currents (EPSCs) were recorded from neurons of the supraoptic nucleus (SON) of trimmed slice preparations of mouse hypothalamus, with blind patch clamp. The effects of perfusion of Ca free medium on the evoked EPSCs by focal stimulation dorsal or dorsolateral to the SON, the spontaneous EPSCs (sEPSCs) and miniature EPSCs (mEPSCs) were examined. The evoked EPSCs, sEPSCs and mEPSCs were almost blocked by the glutamate antagonist kynurenic acid (1 mM). Both the number and the amplitude of the sEPSCs were not significantly changed by tetrodotoxin. While the evoked EPSCs were completely abolished by the Ca free medium, the sEPSCs and mEPSCs still remained. Both the number and the amplitude of the sEPSCs and mEPSCs were not significantly changed by perfusion of the calcium free medium. When the extracellular potassium concentration was increased from 3 to 21 mM, the mEPSC frequency increased. The increased mEPSC frequency was suppressed by the perfusion of calcium free medium but the basal level of the mEPSC frequency persisted. These results imply that glutamatergic synaptic transmission to the hypothalamic neurosecretory cells, which are due to voltage-dependent (evoked EPSCs) and voltage-independent mechanisms (sEPSCs and mEPSCs), have high and low sensitivity to external Ca concentration, respectively. In previous studies, the Ca free medium was used for blockade of synaptic transmissions in hypothalamic neurons. The present study suggests that the Ca free medium is not suitable for that kind of purpose.

250.9

EFFECTS OF PACAP ON VASOPRESSIN RELEASE, $[Ca^{2+}]_i$ AND IONIC CURRENTS IN RAT HYPOTHALAMIC SUPRAOPTIC NEURONS. I. Shibuya*, J. Noguchi, N. Harayama, N. Kabashima, Y. Inoue, K. Tanaka, Y. Ueta, M. Nomura & H. Yamashita Dept. of Physiol., Univ. Occup. & Environ. Health, Kitakyushu 807, Japan

Pituitary adenylate cyclase activating polypeptide (PACAP) is localized in the magnocellular part of the supraoptic and the paraventricular nuclei (SON and PVN), however, the physiological function of PACAP is poorly understood. We have confirmed that PACAP type-I receptor mRNA is localized particularly in these nuclei by *in situ* hybridization histohemistry. To elucidate the role of PACAP in these nuclei, we measured vasopressin (AVP) release, cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$), membrane potential and ionic currents in SON neurons of rats. PACAP (10^{-12}-10^{-7} M) induced AVP release in SON slice preparations and Ca^{2+} rise in isolated SON neurons. The PACAP-induced Ca^{2+} rise was strongly dependent on extracellular $[Ca^{2+}]_i$. Moreover, PACAP caused depolarization and increased firing of action potential in the current-clamp mode of the patch-clamp technique and induced inward currents in the voltage clamp mode at concentrations as low as 10^{-13} M. These results suggest that PACAP may play an important role in the physiological function of SON neurons via paracrine and/or autocrine.

250.10

Ca^{2+} RELEASE FROM INTERNAL STORES: ROLE IN GENERATING DEPOLARIZING AFTERPOTENTIALS IN SUPRAOPTIC NUCLEUS NEURONS Z. Li* and G.I. Hatton. *Dept. of Neuroscience, University of California, Riverside, CA 92521.*

An increasing body of evidence indicates that Ca^{2+} induced Ca^{2+} release is involved in modulation of Ca^{2+}-dependent afterhyperpolarizations, neurotransmitter release from nerve terminals, expression of immediate-early genes, long-term potentiation and Ca^{2+} oscillations. In this study, influences of Ca^{2+} release from internal stores on the generation of depolarizing afterpotentials (DAPs) were investigated in magnocellular neurons of rat supraoptic nucleus (SON) using whole-cell patch recording techniques in brain slices. Recorded from more than half of the cells encountered, DAPs following evoked single spikes had an amplitude of 3.00 ± 0.19 mV (mean ± sem) and lasted for 1.02 ± 0.06 s. Their sizes usually increased with the number of preceding spikes, but could be reduced or eliminated when intervals between consecutive current pulses evoking tens of spikes were short. DAPs were eliminated by removal of external Ca^{2+} (n = 25), and significantly reduced by bath applications of nifedipine or ω-conotoxin (n = 16). Blockade of Ca^{2+} release from internal stores by perifusion with ryanodine or dantrolene (n = 14), or direct diffusion of ruthenium red (n = 5) into cells suppressed DAP amplitudes by ~50% and shortened their durations in all 19 cells tested. Depletion of Ca^{2+} internal stores by perifusion with thapsigargin or cyclopiazonic acid also inhibited DAPs by ~50% (n = 10). Along with reduced DAPs, elimination of phasic patterns of firing was also observed. In contrast, caffeine, an agent known to enhance intracellular Ca^{2+} release, amplified DAPs and promoted phasic firing in 7 of 8 cells tested. These results suggest that Ca^{2+} influx via high-voltage activated Ca^{2+} channels in SON cells triggers ryanodine receptor-mediated Ca^{2+} release from internal stores. This process enhances DAPs and promotes phasic firing in SON cells, and thus increases vasopressin release. Supported by NINDS grant NS16942.

250.11

ROLE OF PREOPTIC AREA IN THE REGULATION OF SUPRAOPTIC NEURONAL ACTIVITY IN RATS. Q. Z. Yang* and G. I. Hatton. Department of Neuroscience, University of California Riverside, CA 92521

Neuroanatomical studies indicated the existence of direct projections from the median preoptic nucleus (Sawchenko & Swanson, 1983), medial preoptic area (MPO) and lateral preoptic area (LPO) (Weiss & Hatton, 1990). Experiments were undertaken to provide evidence for the existence of a synaptic connection between the supraoptic nucleus (SON) and the preoptic area. In horizontally cut brain slices including SON and the preoptic areas, intracellular and whole cell patch-clamp recordings were used to define sites in which electrical stimulation would evoke monosynaptic responses in SON neurons. Stimulation of the LPO usually evoked nearly equal numbers of fast IPSPs (10 cells) and EPSPs (9 cells) in 19 SON neurons. Stimulation of the MPO evoked more EPSPs (8/11) than IPSPs (3/11) in 11 SON neurons. These evoked IPSPs were blocked by bicuculline (10-20 μM) or picrotoxin (50 μM), suggesting $GABA_A$ receptor mediation. Of the remaining excitatory responses, most were blocked by non-NMDA receptor antagonists (NBQX or CNQX) at 3-10 μM. In a few cases (3 SON neurons) evoked EPSPs persisted in CNQX (10 μM) but were blocked by tropisetron (20 μM), a selective 5-HT_3 serotonin receptor antagonist. Nanodrop applications of glutamate (10 mM) in the LPO and MPO usually increased firing frequency of the SON neurons for 10-15 s or a brief reduction followed by long-duration increase in firing frequency. These results support previous anatomical findings and suggest that the inputs from preoptic area play an important role in regulating activity of the SON neurons. Supported by NINDS grant NS 16942.

250.12

TAURINE IN RAT NEURAL LOBE: LOCALIZATION IN ASTROCYTES AND SELECTIVE RELEASE BY HYPOOSMOTIC STIMULATION. S. Miyata*, O. Matsushima# and G.I. Hatton. Dept. of Neuroscience, University of California, Riverside, CA 92521; #Dept. of Biological Science, Faculty of Science, Hiroshima University, Higashi-Hiroshima, 724 Japan.

Taurine, a generally inhibitory amino acid, is known to be present in the mammalian brain, but its physiological roles are unclear. In this study, taurine localization was accomplished using a well characterized monoclonal antibody against taurine itself. At the light microscopic level, taurine immunoreactivity was seen almost exclusively in the pituicytes, the neural lobe's resident astrocytes, and the cellular distribution pattern matched that of GFAP immunoreactivity previously described for these cells. Electron microscopy revealed strong immunoreactivity in the cell bodies and processes of these glia and, in addition, weak taurine immunoreactivity was sometimes found within neurosecretory vesicle-containing axonal processes. Using HPLC, hypoosmotic stimulation (270 mOsm/kg) of neural lobes *in vitro* resulted in preferentially increased taurine release into the medium and reduced tissue content, compared to glutamic acid, glutamine or GABA, as measured by HPLC. These results suggest that taurine is mainly present in neural lobe astrocytes which are known to engulf the axons and terminals when demand for peptide release is low. Further, taurine's preferential released by hypoosmotic stimulation possibly acts to inhibit oxytocin and vasopressin release from the terminals via its known GABA-like effects. Supported by NINDS grant NS09140.

250.13

TARGETED REDUCTION OF OXYTOCIN (OX) GENE EXPRESSION: ELECTROPHYSIOLOGICAL AND IMMUNOCYTOCHEMICAL STUDIES OF SUPRAOPTIC NUCLEUS (SON) NEURONS. G.I. Hatton*, W. S. Young III#, Q.Z. Yang, S. Miyata and Z. Li. Dept of Neuroscience, Univ. of California, Riverside, CA 92521; #Lab of Cell Biology, NIMH, Bethesda, MD 20892

Magnocellular OX and vasopressin neurons in rat, but not cat, display distinct firing patterns and certain Ca^{2+}-dependent membrane potentials. Comparable data for the mouse are lacking, and the recent availability of mice with targeted reduction of OX gene expression permitted studies of possible influences of OX on intrinsic cellular properties. Whole-cell and sharp electrode recordings were made from SONs in slices from mutant and wild-type mice. In whole-cell mode, action potentials (mean ± sem) = 95 ± 4.8 mV, resting membrane potentials = 58.3 ± 1.6 mV, and input resistance = 0.94 ± 0.12 GΩ. Hyperpolarizing current injection induced voltage responses suggestive of time-independent inward rectification. Depolarization to resting levels from - 90 mV revealed neither low threshold spikes nor obvious delayed return to baseline, and there was little spike frequency adaptation to long depolarizing current pulses. Lucifer Yellow dye injections often yielded dye coupled neurons. Continuous and phasic firing patterns, depolarizing afterpotentials, plateau potentials and afterhyperpolarizations were also observed. Calbindin-D_{28k} immunoreactivity tended to be heavily located in the normally OX-rich SON areas. Except for OX immunostaining, neurons from wild-type and mutant mice shared similar properties. Our studies provide preliminary data on SON neurons of normal and OX gene mutant mice, showing that firing patterns are rat-like and supporting the hypothesis that Ca^{2+} binding proteins, but not hormone content and local OX release, are important in generation of firing patterns and Ca^{2+}-dependent membrane potentials. Supported by NINDS grants NS09140 & NS16942.

250.14

METABOTROPIC GLUTAMATE RECEPTORS AND THE CONTROL OF VASOPRESSIN SECRETION FROM MAGNOCELLULAR NEUROENDOCRINE CELLS. Adriana Serje, Zheng Fan, Walid Al-Ghoul, Robert Greenwood* and Rick Meeker,. Department of Neurology, University of North Carolina, Chapel Hill, NC 27599

Vasopressin (VP) neuroendocrine cells are extensively innervated by glutamatergic terminals and are activated by agonists to each major type of ionotropic glutamate receptor. However, it has been surprisingly difficult to elicit robust secretion of VP in vitro by pharmacological activation of these receptors. To evaluate the potential contribution of metabotropic glutamate receptors (mGluRs) we used in situ hybridization to measure mGluR subtype mRNAs in the supraoptic nucleus (SON) and in vitro stimulation to evaluate the ability of agonists to evoke VP release. Three subtypes of mGluRs were found to be expressed in the SON in vivo in the following order of abundance: mGluR3 > mGluR1 >> mGluR7. RT-PCR confirmed the expression of mRNA for mGluR1 and mGluR3 in primary cultures which were used for stimulation studies. Addition of the mGluR agonist, 1S, 3R ACPD, resulted in a modest but significant release of VP which averaged 1.03 ± 0.43 pg over prestimulation baseline. Inactive isomer and medium controls averaged -0.56 ± 0.66 and -0.36 ± 0.29 pg, respectively. The drug-induced release was largely due to the response of approximately one third (10/33) of the cultures which averaged 3.80 ± 0.47 pg. This response is at least as great as the response induced by activation of ionotropic receptors and indicates that mGluRs of the mGluR1 subtype probably play an equally important role in the control of VP secretion by neuroendocrine cells.

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250.15

INHIBITORY ACTION OF TAURINE ON RAT SUPRAOPTIC MAGNOCELLULAR NEURONS.

N. Hussy*, C. Deleuze, M.G. Desarménien & F. Moos. Biologie des Neurones Endocrines, CNRS UPR 9055, CCIPE, rue Cardonille, 34094 Montpellier 5, France.

Taurine is likely to be involved in the regulation of cellular osmotic balance. In the supraoptic nucleus (SON), taurine is predominantly concentrated in glial cells (Decavel & Hatton, 1995). To understand its role in the osmotic regulation of the SON, we studied the effects of taurine on SON magnocellular neurons from adult male rats. Taurine (0.1-10 mM), applied onto whole-cell voltage-clamped acutely dissociated neurons activated a Cl^- current, as did GABA (30 µM) and glycine (100 µM). The current activated by 1 mM taurine was inhibited by strychnine (1 µM, a concentration that specifically antagonized glycine receptors) but was unaffected by the $GABA_A$ receptor antagonist gabazine (3 µM). Moreover, taurine- (at 1 mM) and glycine-activated currents were not additive. At higher concentration (10 mM), taurine was a weak agonist on $GABA_A$ receptors. Thus, taurine activates predominantly glycine receptors, with an EC_{50} of 406 µM (established in the presence of gabazine). *In vivo* extracellular recordings of SON magnocellular vasopressin (AVP) neurons were obtained on anaesthetized rats. Drugs were pressure-applied via double-barreled pipettes in the vicinity of the recorded neuron. Taurine (1 mM) inhibited the phasic activity of AVP neurons, decreasing the mean frequency of firing during active phases as well as the duration of the active phases. This effect was stronger in normally hydrated than in dehydrated rats. Strychnine (300 nM) triggered a phasic pattern on quasi-silent AVP neurons recorded in water-loaded rats and enhanced phasic activity in normally hydrated rats. Preliminary *in vitro* results indicate that hypoosmotic solutions can elicit the release of taurine in the SON. These results suggest that taurine, acting mainly through glycine receptors, plays an important role in the regulation of SON magnocellular neurons, and more specifically in the inhibition of AVP neurons in hypoosmotic condition, a situation likely to induce release of taurine from SON glial cells.

250.16

AP-1 DNA-BINDING ACTIVITY IN RAT SUPRAOPTIC NEURONS (SON) FOLLOWING FLUID IMBALANCE.

J.T. McCabe* and K. Wang. Anatomy & Cell Biology, Uniformed Services University of the Health Sciences, Bethesda, MD 20814.

The protein products of c-*fos* and c-*jun*, and certain related leucine zipper-containing transcription factors, form heterodimers (Fos-Jun and Jun-Jun family member heterodimers) and homodimers (Jun family members) that bind to the DNA consensus sequence, AP-1. Since the AP-1 DNA binding site is known to be essential for the transcription of certain genes, Fos and Jun dimerization and sequence-specific DNA binding represents a crucial link between cell stimulation and subsequent alterations in gene expression. Hypertonic saline injection causes a profound increase in mRNA levels and protein products in the SON [NEUR. ABST. '94, 1567 & '95, 875]. Using the gel shift DNA binding assay, AP-1-like DNA binding activity was determined in SON tissue nuclear extracts. SON tissue samples obtained from control (no injection) and hypertonic saline-stimulated rats contained a significant level of AP-1-like DNA binding activity and the observed band was equivalent to what was seen from incubation with HeLa cell extracts. Preincubation of SON extracts from osmotically stimulated animals with c-Fos antibody resulted in a supershifted band, while no Fos antibody-supershifted band was observed in tissue samples from non-injected rats.

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250.17

DIFFERENTIAL INDUCTION OF TRANSCRIPTION FACTOR FAMILIES IN THE SUPRAOPTIC NUCLEUS (SON) AND ITS AFFERENTS. S.M. Luckman*. Dept. Neurobiology, The Babraham Institute, Babraham, Cambridge, CB2 4AT, U.K.

Infusion of saline at differing tonicity leads to the acute induction of c-*fos* and *jun* B mRNAs in the SON that correlates to the osmotic stimulus (Luckman *et al.*, 1996). C-*jun*, is repressed by infusion of 0.35M or 0.75M saline, but is co-induced at higher levels of stimulation (1.5M saline). To investigate this phenomenon further c-*fos* was compared to *nur*77 and *egr*1, in both the SON and in putative afferent neurones of the nucleus tractus solitarii (NTS) following saline infusion or a single injection of CCK. Intravenous infusions were made into conscious, free-moving rats (n=4 per group). The results of *in situ* hybridization are given as the mean number of silver grains per cell in all cells containing >20 grains within six counting frames per structure.

	fos/SON	*fos*/NTS	*nur*/SON	*nur*/NTS	*egr*/SON	*egr*/NTS
0.15M	36±5	32±2	55±10	38±2	47±2	40±2
0.75M	124±4*	36±1	96±10	40±3	88±6*	38±2
1.5M	373±25*	60±6*	172±19*	63±5*	98±8*	42±3
CCK	58±6	72±4*	85±11	88±5*	45±5	42±4
ANOVA	<0.0001	<0.0001	<0.001	<0.0001	<0.0001	>0.05

*P<0.01 Dunnett's post-hoc comparisons to 0.15M group

Analysis of the dorsal SON, where oxytocin neurones predominate, detected an induction of c-*fos* by CCK (36±6 and 75±4; P<0.002, Student's t-test), not apparent in the ventral SON (35±4 and 41±3). There was no significant induction of *nur*77 or *egr*1 in the dorsal SON by CCK (62±14 and 103±1, P=0.08; 47±1 and 45±5, P>0.05), though the lack of significance for *nur*77 may have been due to a number of neurones expressing high levels of this mRNA in the control group. These results show the differential induction of three transcription factor families in magnocellular neurones and their putative afferents depending on the stimulus. The infusion of 0.75M does not activate the NTS, while 1.5M saline or CCK do. The additional afferent input to the SON may be required to allow the previously observed co-induction of c-*jun*. (BBSRC funded).

250.18

FOS EXPRESSION IN THE HYPOTHALAMIC MAGNOCELLULAR NEURONS OF PARTURIENT RATS. T. Kiyohara*, S.-H. Lin, S. Miyata and T. Nakashima. Dept. of Applied Biol., Kyoto Inst. of Technol., Kyoto, 606, Japan.

In previous study, numerous Fos-positive hypothalamic magnocellular neurons were observed in parturient rats (2 hr after the first pup delivery) not in gestating ones, and Fos expression was limited in the period of early lactation. In the present experiment, Fos immunoreactivity was examined in animals of virgin, early stage of parturition, late stage of parturition and lactation using dual immunocytochemistry for Fos and oxytocin/vasopressin neuropeptides. Further, expression of c-fos mRNA in the hypothalamus of these groups were verified with *in situ* hybridization. In parturient rat brains (1 hr after the first pup delivery), there were few Fos-positive neurons in both supraoptic and paraventricular nuclei. No difference was seen between non-pregnant females and early parturient ones. During the late stage of parturition, there were significant increase in the number of Fos-positive oxytocinergic and vasopressinergic neurons. These results suggest that activation of magnocellular neurons which reflcted in Fos expression is not critical for parturition itself, but may implicate in the expression of maternal behavior.

250.19

GRADED RECRUITMENT OF MEDULLARY CATECHOLAMINE AND HYPOTHALAMIC NEUROENDOCRINE CELLS BY HEMORRHAGE. D.W. Smith, K.M. Buller and T. A. Day*, Dept. of Physiology & Pharmacology, University of Queensland, AUSTRALIA 4072.

Hemorrhage stimulates pituitary release of vasopressin (VP), oxytocin (OT) and ACTH. Caudal medulla A1 noradrenaline cells play a crucial role in the VP response, but the role of medullary catecholamine cells in ACTH and OT responses is less clear. Using the c-*fos* technique we have now systematically investigated the recruitment of both hypothalamic neuroendocrine and medullary catecholamine cells by hemorrhage and have tested the effects of A1 lesions on hypothalamic cell responses. Rats were subjected to hemorrhages of 0, 4, 8, 12 or 16 ml/kg and 2 h later sacrificed and the brain processed for Fos, TH, PNMT, OT and VP immunolabelling. Only hemorrhages of 12 or 16 ml/kg produced significant hypotension (BP 50-75 mmHg for 15-45 min). These hemorrhages also significantly increased c-*fos* expression in A1, A2, C1 and C2 catecholamine cells, VP and OT neurosecretory cells, and cells of the medial parvocellular (MP) paraventricular nucleus, the latter being the primary location of tuberoinfundibular corticotrophin releasing factor cells. Only VP cells of the supraoptic nucleus responded to milder hemorrhages (8 ml/kg). Unilateral ibotenic acid lesions along the rostrocaudal extent of the A1 cell group one week prior to a 12 ml/kg hemorrhage produced moderate reductions in the responses of all three classes of hypothalamic neuroendocrine. This data supports a role for the A1 cell group in the relay of hemorrhage-related information to hypothalamic neuroendocrine cells but the large recruitment of other medullary catecholamine populations, notably A2 cells, suggests need for further study of their involvement. (Supported by NHMRC and NHF.)

250.20

CATECHOLAMINERGIC AFFERENTS TO THE MEDIAL SEPTAL AREA IN THE RAT. F.Condé*, F.Chéruel and A.J.Baertschi. Lab. of Neuroendocrinology, University Paris-XI, 91405-Orsay (France), and Centre Médical Universitaire, Geneva (Switzerland).

Recent results of our laboratory suggest that the rostro-ventral part of the medial septal area (MSA) is involved, through its catecholaminergic innervation, in the splanchnic osmotic regulation of vasopressin. The present study was designed to determine the identity of the catecholaminergic neurons projecting to MSA. Afferent neurons were retrogradely labeled by fluorogold (FG) injections in the MSA. Their catecholaminergic content was checked by immunocytochemistry using antisera directed against tyrosine hydroxylase (TH, Eugene Tech), the first enzyme in the catecholamine synthesis, or against phenylethanolamine N-methyltransferase (PNMT, Y.Tillet, CNRZ, Nouzilly,France), the enzyme which converts noradrenaline (NA) to adrenaline (A). The later enzyme was used to separate neurons of the C1-C2 (A) groups from the A1-A2 (NA) groups in the medulla. Our results show that no adrenergic innervation reaches MSA, as none of the FG-positive neurons located in the C1, C2 and C3 areas were PNMT-positive. The noradrenergic innervation of MSA originates in A2 area (nucleus of the tractus solitarius), and A6 (locus coeruleus), where all FG-positive cells were TH-positive, and in ≅ 60% of the A1 cells of the ventrolateral medulla. The dopaminergic innervation of MSA originates from the ventral tegmental area (A10 group), in which only ≅ 60% of the FG-positive cells were also TH-positive. Although in the incerto-hypothalamic dopaminergic system (A11, A13, A14 groups) FG- positive cells were intermingled among the TH-positive cells, none of them were TH-positive. These and previous results suggest three possibilities how splanchnic osmoreceptors can regulate vasopressin secretion: 1) via spinal inputs and a relay in A1 and MSA; 2) via vagal inputs and a relay in A2 and MSA; 3) via afferents projecting in A10 and a relay in MSA.

251.1

MODULATION OF BAROREFLEX FUNCTION BY ENDOGENOUS CATECHOLAMINES IN THE NUCLEUS OF THE SOLITARY TRACT (NTS) OF THE SPONTANEOUSLY HYPERTENSIVE (SH) RAT. A.P. Riley, L.F. Hayward, and R.B. Felder * Cardiovascular Center, Dept. of Internal Med, University of Iowa, Iowa City, IA 52242.

Both noradrenaline levels and α-2 binding sites in the dorsal medulla are reduced in the SH rat compared to the normotensive Wistar Kyoto (WKY) rat. Previous studies have demonstrated that pharmacological blockade of endogenous catecholamines (eCAT) in the NTS of normotensive rats attenuates baroreflex function. In the present study the role of eCAT in the NTS on baroreflex function was examined in SH rats. Baroreflex curves were produced by stimulating the left aortic depressor nerve with increasing frequencies (2-15 Hz, 0.2 ms, 8V, for 10 sec) in urethan anesthetized SH and WKY rats (10-12 week old). Ipsilateral microinjection of idazoxan (IDAZ; 1000 ng/100 nl), an α-2 receptor antagonist, in the caudal NTS significantly attenuated baroreflex control of mean arterial pressure (MAP; $p<0.001$, see Table for MAP data) but not renal sympathetic nerve activity (RSNA) in WKY rats (n=4). α-2 blockade in SH rat significantly attenuated baroreflex control of MAP ($p<0.001$, n=4; see Table) and RSNA ($p<0.003$). At the highest frequency of baroreceptor activation (15 hz), α-2 blockade attenuated baroreflex control of MAP to a greater extent in the SH vs WKY rat (59±8% vs 20±3 %, respectively; $p<0.01$). These results demonstrate that eCAT in the NTS play an important role in baroreflex neurotransmission in both normotensive and SH rats, and suggest that the SH rat may be more sensitive to changes in eCAT in the NTS compared to the WKY rat. Supported by HL 29302 (RBF).

	Stimulation frequency			
	2 hz	5 hz	10 hz	15 hz
WKY control	-11±4	-24±7	-27±5	-32±8
WKY/IDAZ	-9±3*	-14±4*	-18±6*	-26±7*
SHR control	-7±1	-20±4	-32±3	-41±3
SHR/IDAZ	-6.5±1*	-10±2*	-15±2*	-16±3*

Numbers are changes in MAP±SEM (mmHg); * signficantly different from control.

251.2

PRESSOR RESPONSE OF CHEMOREFLEX IS NOT BLOCKED BY BILATERAL MICROINJECTION OF IONOTROPIC OR METABOTROPIC ANTAGONISTS INTO THE COMMISSURAL NTS. A.S. Haibara, L.G.H. Bonagamba and B.H. Machado*. Dept. Physiology., School of Medicine of Ribeirão Preto, USP, 14090-900, Ribeirão Preto, SP, Brazil.

We have previously shown that bradycardic response of chemoreflex is blocked by NMDA antagonist into the commissural NTS. In the present study we investigated the effects of blockade of ionotropic or metabotropic receptors in the lateral portion of the commissural NTS (0.5 mm lateral to midline) on the cardiovascular responses of the chemoreflex induced by potassium cyanide (KCN, 20 µg/rat, i.v.) in unanesthetized rats. Bilateral microinjections of 6,7-dinitroquinoxaline-2,3-dione [DNQX (0,5 nmoles/100 nl), a non-NMDA antagonist], kynurenic acid [KYN (10 nmoles/100 nl), an ionotropic antagonist] or α-methyl-4-carboxyphenylglycine [MCPG (2,5 nmoles/100 nl), a metabotropic antagonist) were performed into the NTS through guide cannulas previously implanted. In relation to the cardiovascular responses of chemoreflex: a) microinjection of DNQX or KYN produces a significant reduction on the pressor response (+64±4 vs +34±7 mmHg and +50±2 vs +30±3 mmHg, respectively), b) only KYN abolished the bradycardic response (-225±28 vs -18±7 bpm) and c) MCPG produces no changes in the pressor (+46±9 vs +43±10 mmHg) and bradycardic (-233±20 vs -208±34 bpm) responses. These data show that: 1) the bradycardic response of chemoreflex is blocked (KYN) but the pressor response is attenuated by KYN or DNQX and 2) MCPG produced no effects on the chemoreflex. We conclude that the cardiovagal component of the chemoreflex is mediated by ionotropic receptors (NMDA), while the pressor response is not blocked by ionotropic or metabotropic antagonists into the commissural NTS. Supported by FAPESP and CNPq.

251.3

EFFECTS OF GLUTAMATERGIC BLOCKADE IN THE NUCLEUS TRACTUS SOLITARII (NTS) ON CARDIOVASCULAR RESPONSES TO VOLUME LOAD IN ANESTHETIZED RATS. D.S.A. Colombari*, E. Colombari, O.U. Lopes and S.L. Cravo. Dept. of Physiology, UNIFESP-EPM, São Paulo, SP 04023-900, Brazil.

The central pathways involved in the renal vasodilation induced by volume load (VL) have not been elucidated yet. Therefore, the purpose of this study was to verify the role of NTS glutamatergic receptors on the renal vasodilation induced by VL. Experiments were performed in adult male Wistar rats (280 - 320 g) anesthetized with urethane. The femoral artery and vein were cannulated for mean arterial pressure (MAP) recording and drug infusion, respectively. VL was induced either by iv infusion of 3% dextran or 4% Ficoll (1% bw, 0.4 mL/min). Renal blood flow (RBF) was recorded by Doppler flowmetry and expressed as the percentage of the baseline. Renal relative vascular conductance (RVC) was calculated by dividing Doppler shift (kHz) by the MAP (mmHg) and also expressed as percentage of the baseline. In control animals (N=9) VL produced a transient hypertension (+23 ± 4 mmHg). RBF and RVC rose by 171 ± 18% and 133 ± 12% of baseline after 10 min, respectively, and remained elevated for 60 min (163 ± 28% and 159 ± 25%, respectively). Ten min after bilateral microinjection of kynurenic acid (7.3 nmol/100 nL, N=6) into the NTS, VL produced a transient hypertension (+33 ± 4 mmHg) but renal vasodilation was no longer observed. The increase in RBF after 10 min was reduced to 119 ± 8% of baseline and the initial increase in RVC was abolished (87 ± 8%). After 60 min RBF and RVC increases were not different from control (129 ± 8% and 128 ± 11%, respectively). When saline was inject into NTS (N=6), VL produced responses comparable to that observed in control animals. These data suggest that renal vasodilation induced by VL involve the activation of glutamatergic receptors in the NTS, probably associated with baroreceptor and/or cardiopulmonary primary afferents. **Financial support:** CAPES, FAPESP, Brazil.

251.4

MICROINJECTION OF AN INHIBITOR OF HEME OXYGENASE (HO) INTO THE NUCLEUS TRACTUS SOLITARII (NTS) INCREASES BLOOD PRESSURE IN CONSCIOUS RATS. [1]E. Colombari*, [2]R.A. Johnson, [2]M. Lavesa, [1]D.S.A. Colombari, [3]W.T. Talman, and [2]A. Nasiletti. [1]Dept. of Physiol.,UNIFESP-EPM, São Paulo, SP 04023, Brazil. [2]Dept. of Pharmacol.,NYMC, Valhalla NY 10595, USA. [3]Dept of Neurol., Univ. of Iowa & VAMC, Iowa City, IA, 52242, USA.

HO is an enzyme which degrades heme to form biliverdin and carbon monoxide (CO). Previous studies have shown that systemic administration of zinc deuteroporphyrin 2,4-bis-glycol (ZnDPBG, 45 mol/Kg, i.p.), an inhibitor of HO activity, increases blood pressure in rats. Since ZnDPBG-induced increases in arterial pressure and can be prevented by alpha-1-adrenergic or ganglionic blockade, it suggests that ZnDPBG-induced increases in blood pressure may include a central component. It has been suggested that HO-derived CO may serve to modulate NTS function and that this low molecular weight gas may be a neuromodulator. The purpose of our study was to ascertain if HO-mediated formation of CO in the NTS may act locally to influence blood pressure. We used awake Sprague-Dawley rats to determine the blood pressure effects of ZnDPBG (4.5nmol/100nL) and CO (saturated in 100 nL of saline), microinjected into the NTS. Unilateral and bilateral microinjections of ZnDPBG increased arterial pressure (+16±4 and +31±4 mmHg, respectively), but did not affect heart rate. Unilateral microinjection of CO did not affect arterial pressure but did normalize the blood pressure in rats made acutely hypertension by unilateral microinjection of ZnDPBG. This suggests that (1) a HO product being generated in the NTS can serve to lower blood pressure and (2) that in the NTS, local administration of exogenous CO exerts a vasodepressive action that is prominent in the presence of an inhibitor of endogenous CO production. We conclude that a product of HO, presumably CO, formed in the NTS may contribute to the regulation of blood pressure. Support: NIH HL 32205; HL 14388; HL 34300; HL 36670, HL 18579.

251.5

FINE STRUCTURE AND PLASTICITY OF PHENYLEPHRINE-SENSITIVE NEURONS IN THE NUCLEUS OF THE SOLITARY TRACT (NTS). C.A. Peto, R.K.W. Chan and P.E. Sawchenko.* The Salk Institute, La Jolla, CA 92037.

Intravenous phenylephrine (PE) gives rise to patterns of Fos expression in the NTS that conform to the central distribution of the carotid sinus and aortic depressor nerves. This has been exploited to permit ultrastructural characterization of neurons presumed to comprise the first station in the processing of arterial baroreceptor afferent input, and to probe for stimulation-induced structural alterations. Freely moving rats were submitted to PE-induced hypertension (2.1-2.5 µg/kg/min, 25 min, iv), perfused at varying intervals, and sections through the "baroceptive strip" of the NTS prepared for preembedding immunolocalization of Fos-ir. PE-induced Fos-ir in the NTS was detectable at 30 min, and peaked at 2 hr, after the challenge. Labeled neurons formed a continuous strip extending from the dorsal aspect of the commissural NTS (NTScom) to the dorsal and dorsolateral subnuclei at the level of the area postrema (NTSap). Ultrastructurally, PE-sensitive neurons in these regions were small, round to ovoid in shape, with scant cytoplasm, and a generally unremarkable organelle complement. Barosensitive neurons were distinguished by extensively invaginated nuclei and highly developed Golgi apparati; Fos-ir cells in the NTSap differed from those in NTScom in their moderately well-developed rough ER, more highly developed Golgi and less convoluted nuclei. Synaptic input to PE-sensitive neurons was sparse, and provided by terminals containing predominantly small, clear synaptic vesicles that formed some asymmetric, though predominantly symmetric, junctions evenly distributed over cell somata and primary dendrites. Structural alterations of Fos-ir cells seen over the 2 hr time course prompted comparisons between animals exposed to PE infusion for shorter (25 min) vs longer (2-3 hr) intervals. Prolonged stimulation was accompanied by accentuation of nuclear invaginations, marked accumulation of heterochromatin at their apices, and evidence of further development and activation of the Golgi, including increased incidence of vesicular budding, with electron lucent vesicles ostensibly dispersing and accumulating within nuclear invaginations. These may represent adaptations to facilitate changes in gene expression and/or to maintain neurotransmitter availability in the face of a persisting challenge. (supported by HL-35137)

251.6

EFFECTS OF CAROTID SINUS NERVE TRANSECTION ON PHENYLEPHRINE-INDUCED FOS EXPRESSION IN THE NUCLEUS OF THE SOLITARY TRACT. R.K.W. Chan* and P.E. Sawchenko The Salk Institute, La Jolla, CA 92037.

We have previously described patterns of phenylephrine (PE) -induced Fos expression in the nucleus of the solitary tract (NTS) that conform to the central distribution of carotid sinus and aortic depressor afferents. To test whether Fos-immunoreactive (-ir) NTS neurons comprise the recipients of first-order baroreceptor inputs, and to identify the subregions supplied by individual afferents, we examined the effects of bilateral carotid sinus nerve transections (at 4-30 days after surgery) on the distribution and neurotransmitter specificity of NTS neurons exhibiting Fos-ir in response to sustained hypertension (25-35 mmHg) induced by intravenous PE infusion (2.1-2.5 µg/kg/min, 25 min) in freely moving rats. Bilateral transection of carotid sinus nerves dramatically reduced (by 70.4%) the number of Fos-ir neurons in the dorsal 'baroceptive strip' of commissural NTS (NTScom) at the level of the calamus scriptorius, a region innervated by the carotid sinus nerve. By contrast, only a moderate attenuation (26.0%) of the PE-induced Fos response was observed in the dorsal and dorsolateral aspects of NTS at the level of the area postrema level (NTSap), a region supplied by aortic depressor afferents. As was the case in PE-challenged sham-operated animals, Fos-ir barosensitive neurons in the NTSap displayed markers for nitric oxide. Saline-infused barodenervated animals did not display Fos expression in the NTS. Outside the NTS, carotid sinus denervation resulted in a moderate decrease (30.4%) in PE-induced Fos expression in presumed depressor neurons of the caudal ventrolateral medulla, a majority of which displayed markers of a GABAergic phenotype (GAD 67 mRNA). These effects of partial barodenervation were evident in animals challenged 4-14 days after surgery. Rats tested at 30 days displayed Fos responses that were not distinguishable from controls, and that were unaffected by re-stripping of the carotid sinus region. These findings support the view that Fos-ir neurons in the NTScom comprise the recipients of first-order baroreceptor inputs carried by the carotid sinus nerve. In addition, the results provide a basis from which to explore the mechanisms underlying recovery of baroreflex function following longer term carotid sinus denervation. (supported by HL-35137 & AHA-California Affiliate)

251.7

WHOLE CELL RECORDINGS REVEAL AN NMDA RECEPTOR MEDIATED COMPONENT IN SENSORY AFFERENT TRANSMISSION IN THE NUCLEUS OF THE SOLITARY TRACT (NTS). M.L. Aylwin, J.M. Horowitz*, and A.C. Bonham, Univ. of California, Davis, CA 95616

The participation of NMDA receptors in sensory afferent transmission in the NTS has been difficult to establish. We identified both NMDA and non-NMDA receptor-mediated synaptic currents in NTS neurons that received input from the solitary tract (ts) by examining ts-evoked excitatory post synaptic currents (EPSCs) at voltage steps from -90 to +60 mV. Rat transverse medullary slices (300 μm thick) were superfused with (in mM) NaCl 125; KCl 2.5; $MgCl_2$ 1.0; NaH_2PO_4 1.25; $NaHCO_3$, 25; dextrose, 10; $CaCl_2$, 2.0; picrotoxin 0.1; bicuculline 0.01; bubbled with 95% O_2 / 5% CO_2. Glass electrodes (3 - 6 MΩ) contained (in mM) CsF 145; NaCl 5.0; $MgCl_2$ 1.0; EGTA 10; Hepes 10; $MgATP_2$ 3; NaGTP 0.2; pH 7.4. TS stimulation at 1 - 7 V, 0.1 ms, 0.5 Hz evoked EPSCs with onset latencies of 2.2 - 7.7 ms in 19 medial NTS neurons. 17 of these 19 neurons exhibited EPSCs having both a fast and slow component (Hestrin et al., J. Physiol. 1990). The fast component (peak at 5 - 6 ms) had an amplitude linearly related to voltage and was blocked by the non-NMDA receptor antagonist, NBQX (3 μM; n = 2). The slow component (measured at 20 ms after the peak) was not linearly related to voltage, was revealed at potentials positive to 0 mV, and was abolished by the NMDA receptor antagonist, AP5 (50 μM; n = 8). 7 of these 8 neurons had onset latencies of < 4 ms. AP5 had no effect on the fast component. Thus, both NMDA and non-NMDA receptors coexist on the same NTS neurons and are activated by primary afferent input from the ts.

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251.8

EXCITATORY VESTIBULAR NUCLEUS PROJECTIONS TO NUCLEUS TRACTUS SOLITARIUS: IMPLICATIONS FOR VESTIBULAR-AUTONOMIC INTERACTION.

Z. Zhou* and C.-S. Poon. Harvard-MIT Division of Health Sciences and Technology, M.I.T., Cambridge, MA 02139.

The vestibular system plays an important role in maintaining stable arterial blood pressure during changes in posture and is responsible for eliciting motion sickness related vomiting. The nucleus tractus solitarius (NTS) is a region critical for autonomic and cardiorespiratory regulation and the triggering of emesis. Previous studies using neuroanatomic tracing and single-unit recording in vivo have shown that the NTS receives projections from the vestibular nucleus (VeN) in rabbit and cat. In the present study, we used an in-vitro brainstem slice preparation from Sprague-Dawley rats (1-3 weeks of age) to examine the synaptic connection between VeN and NTS. Whole-cell patch recordings were obtained from NTS neurons under voltage clamp (holding potential ≈ -65 mV) at a level slightly rostral to obex. Electrical stimulation (0.1 ms impulses, 8-20 V) of the caudal medial VeN elicited monosynaptic postsynaptic currents (EPSCs) in medial and intermediate NTS neurons. No inhibitory postsynaptic currents were observed. The EPSC responses induced by VeN stimulation were almost totally blocked by application of CNQX (10-20 μM), an antagonist for non-NMDA receptors. Thus, synaptic connections between the VeN and NTS are excitatory and are mediated in part by the neurotransmitter glutamate acting on non-NMDA receptors. (ONR grant N00014-95-0414 and NIH grant HL50641)

251.9

ESTIMATION OF CARDIAC BAROREFLEX GAIN BY DRUG AND SPECTRAL METHODS IN NORMOTENSIVE AND HYPERTENSIVE RABBITS. Head GA, Lukoshkova EV, Godwin SJ, Janssen BJA, Gaudet EA, Malpas, SC, Sheppard, K* Baker Medical Research Institute, Prahran, Victoria.

Power spectral analysis is now being used in the clinic to estimate baroreflex gain but there has been relatively little validation. We compared the gain of the baroreceptor-heart rate reflex estimated by "invasive" techniques and spectral analysis methods in conscious normotensive and hypertensive rabbits. In normotensive rabbits and animals made hypertensive for 1 week with an i.v. infusion of angiotensin II, cardiac baroreflex gain was assessed by infusion of phenylephrine and nitroprusside or caval cuff and compared to the gain estimated from the average transfer function between MAP and HR. In an initial study in conscious rabbits with an implanted renal sympathetic nerve electrode we found that the major sympathetic oscillations were between 0.2 and 0.4 Hz. In normotensive rabbits the baroreflex gain assessed by the average transfer gain between MAP and HR in this frequency region (coherence greater than 0.5) was higher than the gain obtained with the drug method (7.9 ± 0.6 vs 6.2 ± 0.5 b/min/mmHg respectively, P <0.05). In animals with baroreflexes assessed by phenylephrine and caval cuff, the gain was higher than for the spectral method (8.0 ± 0.4 vs 6.4 ± 0.3 b/min/mmHg, respectively, P<0.05). Estimates by each method were reproducible when measured on 5 separate occasions. Hypertensive rabbits had significantly reduced baroreflex gains compared to normotensives but now gain was similar for both spectral and drug methods (4.2 ± 0.3 vs 4.4 ± 0.7 b/min/mmHg respectively). In sino-aortically denervated rabbits baroreflex gain estimated from the spectral method was much reduced and with very low coherence (0.2 ± 0.03) between MAP and HR These studies show that the spectral power for MAP and HR in the frequency range between 0.2 and 0.4 Hz can be used effectively to estimate changes in baroreflex sensitivity for a group of normotensive or hypertensive rabbits but absolute gain values for the methods depend on the technique used.

251.10

SEROTONIN$_3$ RECEPTOR AGONISTS INTO THE NTS INHIBITS THE BRADYCARDIA BUT PRODUCED NO CHANGES IN THE RESPIRATORY AND SYMPATHETIC RESPONSES TO CHEMOREFLEX ACTIVATION. C. Sévoz, J.C. Callera[1], M.B. Emerit*, B.H. Machado[1], M. Hamon and R. Laguzzi. INSERM U.288, C.H.U. Pitié-Salpêtrière, Paris, France; [1]FMRP-USP, Ribeirão Preto, Brazil.

We previously demonstrated that stimulation of 5-HT$_3$ receptors in the NTS blocks the cardiovagal component of both baro-and Bezold-Jarisch reflex in anesthetized rats, and of both baro and chemo-reflex in unanesthetized rats. In the present study we analysed the effects of intra-NTS microinjections of 5-HT or a 5-HT$_3$ receptor agonist, 1-(m-chlorophenylbiguanide. CPBG) upon the cardiovascular and respiratory responses elicited by carotid body chemoreceptors stimulation (CBS) in anesthetized rats. CBS was performed by KCN (40 μg/Kg, i.v.) or by intra-carotid administration of satured CO_2 saline in spontaneously breathing or in artificially ventilated rats, respectively. Local microinjections of 5-HT (2.5 and 5 nmol, n=6 for each dose) or CPBG (300-1200 pmol, n=6 for each dose) blocked in a dose-dependent manner, the atropine sensitive cardiac chemoreflex response (-94±14.5 vs -5.3±2.3 bpm for CPBG 1200 pmol). However, neither 5-HT nor CPBG affected the reflex increase in respiration and in lumbar sympathetic nerve discharge elicited by CBS. The inhibitory effect of 5-HT and CPBG upon the cardiovagal chemoreflex response was blocked by the prior intra-NTS microinjections of 5-HT$_3$ antagonists: zacopride (100 pmol, n=6) or ondansetron (100 pmol, n=6). These data taken together with those of our previous studies indicate that 5-HT$_3$ receptors within the NTS play a key modulatory role in the reflex control of cardiac activity. Supported by INSERM, FAPESP and USP/COFECUB.

251.11

THE DIAGONAL BAND OF BROCA (DBB) INHIBITS BAROREFLEX-INDUCED DECREASES IN RENAL SYMPATHETIC NERVE ACTIVITY. S.L. Bealer*, Dept. Physiology, Univ. Tennessee, Memphis, TN 38163.

The DBB is a CNS site which mediates baroreflex-induced changes in vasopressin neuron activity. The present experiments determined if neurons in the DBB were also involved in control of changes in renal sympathetic nerve activity (RSNA) during increased arterial pressure (AP). RSNA and AP were evaluated in chloralose-anesthetized rats during iv infusions of four doses of phenylephrine (5-20 μg/kg/min) before and following injection of 2μl isotonic saline or lidocaine (LIDO; 2%) in the DBB. The slope of the regression line comparing changes in AP and RSNA (% control) during iv phenylephrine was used as an estimate of baroreflex sensitivity. Prior to DBB injection, both groups had a similar relationship between AP and RSNA (Saline, -1.5 ±0.3; LIDO, -1.2±0.3). Following saline treatment this relationship was similar to control values (-1.2±0.4), while LIDO injections resulted in a significant increase in baroreflex sensitivity (-2.3±0.3). These data show LIDO injections in the DBB enhance baroreflex sensitivity of RSNA and suggest that this brain region tonically inhibits sympathoinhibition observed during acute increases in AP. (Supported by grants USPHS HL-25877 and American Heart Association 91015600).

251.12

ANGIOTENSIN POTENTIATES SOLITARY TRACT AFFERENT RESPONSES IN MEDIAL NUCLEUS TRACTUS SOLITARII (mnTS) NEURONS RESPONSIVE TO GLUTAMATE BUT NOT TO SUBSTANCE P. K. L. Barnes* and D. M. DeWeese.
Research Institute, Cleveland Clinic Foundation, Cleveland, OH 44195.

Angiotensin (Ang) II may regulate neurotransmission in the mnTS via presynaptic Ang receptors that modulate the release of L-glutamate (Glu) from solitary tract (TS) afferents at mnTS synapses. This study evaluated the capacity of Ang II and substance P (SP) to modulate excitatory postsynaptic potentials (EPSPs) evoked by TS stimulation in mnTS neurons at the level of the area postrema, using whole-cell patch clamp recordings in horizontal rat medulla slices. In 9 neurons responsive to Ang II and Glu, but unresponsive to SP, the peak height and duration of TS-evoked EPSPs were significantly increased after Ang II (1 μM). Either tetrodotoxin (TTX, 10 μM, n = 3) or the kainate/AMPA antagonist DNQX (100 μM, n = 3) blocked both the EPSPs produced by TS stimulation and all responses to Ang II. Ang II did not alter the EPSPs generated by TS stimulation in SP-responsive mnTS cells, and synaptic block with TTX failed to prevent Ang-induced excitation in these neurons. Thus TS-evoked EPSPs mediated by postsynaptic kainate/AMPA receptors are potentiated via presynaptic Ang receptors in those mnTS neurons responsive to Ang II and Glu, but unresponsive to SP. In contrast, mnTS neurons responsive to SP appear to have postsynaptic Ang II receptors, as we showed previously, which do not modify TS-evoked responses in these cells. These data reveal that Ang II may evoke hypotension and bradycardia in the mnTS via presynaptic Ang receptors that potentiate the release of Glu from baroreceptor afferents that synapse on mnTS neurons. (Support by NSF BNS-9109673).

251.13

MORPHOLOGY OF GABAERGIC NEURONS WITHIN THE NUCLEUS OF THE SOLITARY TRACT (NTS) RECEIVING AORTIC NERVE INPUTS. Steve Mifflin* and Jing Zhang, University of Texas Health Science Center, San Antonio, TX 78284.

Immunocytochemical studies have revealed a large number of GABAergic neurons within NTS. The afferent inputs to these cells and therefore their functional role remains conjectural. To determine if GABAergic cells within NTS receive aortic nerve (AN) inputs, we recorded responses of NTS neurons to AN stimulation intracellularly in barbiturate anesthetized rats using electrodes filled with 2M K-citrate and 1.5-5% Neurobiotin. The AN evoked input was characterized as monosynaptic (MS; the second of 2 stimuli separated by 5ms evoked an input; minimal onset latency variability) or polysynaptic (PS). After characterization of the AN input, Neurobiotin was injected into the cell and avidin-Texas Red used to reveal the labelled cell; a polyclonal GABA antibody and standard immunocytochemical procedures were used to reveal GABA. Recordings were obtained from 5 MS and 12 PS neurons receiving an AN input. No MS evoked cells contained GABA, while 6 of the PS units did. Excitatory post-synaptic potential onset latencies in milliseconds were 4.0±1.1 (MS), 18.1±1.7 (PS, GABAergic) and 18.8±2.9 (PS, non-GABAergic). There was no difference between the PS units which contained GABA, PS cells which did not contain GABA and MS cells comparing; soma surface area at its largest in a single section in μm^2 (252±34; 194±48; 258±55); number of dendrites branching off of the soma (2±.4; 2±.8; 3±.4); the ratio of long to short axis in µm (27±3 to 14±1; 22±2 to 12±2; 27±4 to 13±1) or subnuclear location within NTS. These results indicate that some GABAergic neurons within NTS receive polysynaptic aortic baroreceptor inputs. There is no obvious difference in somatic morphology between AN activated cells which contain GABA and those which do not. Supported by HL-41894.

251.14

INFLUENCE OF SELECTIVE EXCITATORY AMINO ACID RECEPTOR AGONISTS ON NUCLEUS OF THE SOLITARY TRACT (NTS) NEURONS RECEIVING AORTIC NERVE (AN) INPUTS. Jing Zhang* and Steve Mifflin, Univ of TX Hlth Sci Ctr San Antonio, TX 78284.

The influence of agonists for excitatory amino acid receptors (EAAs) on the discharge of AN evoked NTS neurons were investigated in pentobarbital anesthetized, paralyzed and artificially ventilated rats. NTS neurons receiving an AN input were classified as monosynaptic neurons (MSNs, second of two stimuli separated by 5 ms evoked discharge; minimal onset latency variability) or polysynaptic neurons (PSNs). EAA agonists were applied by microiontophoresis at currents of 5, 10, 20 & 40 nA. At these "doses", glutamate (100 mM) increased the firing rate (>1 Hz) of some MSNs (5/9) and PSNs (6/8). There was no difference in glutamate potency between PSNs and MSNs (ANCOVA, P=0.18). AMPA (10 mM, n=13, 6 MSNs & 7 PSNs), kainate (10 mM, n=8, 5 MSNs & 3 PSNs), *trans*-ACPD (selective metabotropic receptor agonist, 100 mM, n=9, 5 MSNs & 4 PSNs) excited AN evoked NTS neurons with similar potencies as glutamate. In contrast to non-NMDA and metabotropic EAA agonists, NMDA (100 mM) was more potent on PSNs than on MSNs (ANCOVA, P<0.01). The effects of the EAA agonists on AN non-evoked NTS neurons were also tested in present study. All EAA agonists stimulated ADN non-evoked NTS neurons (P<0.05) and *trans*-ACPD was less potent than other EAA agonists (ANCOVA, P<0.05 vs each of other agonists). Our results indicate: 1. Not every NTS neuron receiving an AN input is sensitive to EAA agonists, suggesting that EAAs might not be the only transmitters used in baroreceptor afferent integration. 2. NMDA receptors are likely to play a role in the baroreflex at the level of PSNs, not MSNs. 3. Metabotropic receptors could mediate and/or modulate afferent integration by both MSNs and PSNs. Supported by HL-41894.

251.15

ULTRASTRUCTURAL CIRCUITRY OF CARDIORESPIRATORY REFLEXES: ARE THERE MONOSYNAPTIC PROJECTIONS FROM THE NUCLEUS OF THE SOLITARY TRACT (NTS) ON TO CARDIOINHIBITORY NEURONS IN THE NUCLEUS AMBIGUUS? V. J. Massari*, T. A. Johnson, W. Coleman, J.-M. Lauenstein, and P. J. Gatti, Dept. Of Pharmacology, Howard University College of Medicine, Washington, D.C. 20059.

Primary afferent cardiorespiratory neurons send a central projection into the NTS. When cardiorespiratory reflexes (such as the baro- and chemoreceptor reflexes) are initiated, efferent parasympathetic preganglionic effects on the heart are in large part mediated by neurons in the ventrolateral nucleus ambiguus (NA-VL). These neurons project to the heart via the vagus nerve. This study was designed to define the neuroanatomical circuitry connecting the NTS and the NA-VL. Five anesthetized cats received microinjections of an anterograde tracer into the NTS. After ten days a retrograde tracer was injected into the left atrial cardiac ganglion which selectively controls atrio-ventricular conduction. Three days later medullary tissues were processed for the light and electron-microscopic histochemical visualization of both tracers. By light microscopy, anterogradely labeled terminals in the ventrolateral medulla were seen surrounding retrogradely labeled negative dromotropic neurons in the rostral NA-VL. Labeled axons and terminals were also observed at the electron microscopic level in the NA-VL. Labeled axons were both myelinated (600-800 nm diameter) and unmyelinated (200-500 nm). Labeled terminals commonly contained numerous pleomorphic vesicles and several large dense core vesicles. Some labeled terminals formed axo-dendritic and axo-somatic synapses on negative dromotropic neurons. This is the first ultrastructural demonstration of a monosynaptic connection between neurons in the NTS and a functionally associated cardioinhibitory neuron in the NA-VL. Supported by a Collaborative Core Unit Award from Howard Univ. Grad. School and a grant from the Office of the President, Howard University.

251.16

FUNCTIONAL TOPOGRAPHIC DISTRIBUTION OF NEURONS OF THE PVH WHICH PROJECT TO THE NTS IN STRESSED RATS. Danielle Champagne* and Guy Drolet, Unité d'Hypertension, Centre de Recherche du CHUL, Université Laval, Québec, Canada, G1V 4G2.

The nucleus tractus solitarii (NTS) and the paraventricular nucleus of the hypothalamus (PVH) have major roles in the integration of endocrine and autonomic responses to stressful challenges. The NTS sends afferent projections to the PVH, suggesting that these neurons may be involved in conveying cardiovascular information to the PVN during stress. However, little is known about the functional relevance of these neuronal pathways with respect to stress. In this study, we have attempted to map specific subdivision of the PVH which are involved in the stress response, and which makes direct projections to the NTS, by combining Fos immunocytochemistry with retrograde tracing. The highest number of afferent neurons to the NTS were found in both, the ventromedial parvicellular part of the PVH (PVHmpv) and the ventral portion of the dorsomedial parvicellular part of the PVHmpd. Fewer retrogradely labeled neurons were also seen in the lateral parvicellular part of the PVH (PVHlp) and in the dorsal parvicellular part of the PVH (PVHdp). Exposure to neurogenic stress induced the activation of cell populations (Fos-ir) which were localized heterogeously throughout the PVH. The distribution of PVH neurons which were double-labeled for Fos-ir and the retrograde tracer was restricted to some parts of PVH. The proportion of double-labeled neurons, Fos-ir/WGA-Au, represented 25% (PVHmpv), 25% (caudal PVHmpd) and 50% (caudal periventricular zone) of the total number of retrogradely labeled neurons within the PVH. There were no double Fos-ir/WGA-Au cells in other levels of the PVH. The present study revealed the existence of different populations of neurons in the PVH that project directly to the NTS. The population of neurons which are activated by stress may provide important afferent information to the NTS for the integration of homeostatic responses to stressful challenges and, this may represent potential sites of dysregulation involved in the development of hypertension induced by chronic exposition to stress. *Supported by the MRC & HSFC.*

251.17

ENHANCED CARDIAC BAROREFLEX AND ATTENUATED ISOPROTERENOL RESPONSIVENESS FOLLOWING FOOD RESTRICTION IN AORTIC COARCTATION HYPERTENSION. J.M. VanNess, M.E.Freeman*, and J.M. Overton. Nutrition, Food and Movement Sciences, Department of Biological Science, Florida State University, Tallahassee, FL, 32306.

The purpose of this study was to examine the effect of short term food restriction on cardiac baroreflex function and beta-adrenergic responsiveness in aortic coarctation (AC) hypertension. AC hypertension was induced in 40 female Sprague-Dawley rats (222±6 g) using a blunt 21 gauge needle as a guide. Four days later, rats were assigned to an *ad libitum* fed group (CON) or a food restricted group (FR) that received 60% of the food consumed by the CON group. Three weeks later carotid and jugular cannulae were implanted under halothane anesthesia for the measurement of pulsatile blood pressure (MAP) and infusion of drugs. FR rats had significantly lower resting MAP (119±4 vs. 150±7 mmHg) and heart rate (HR; 369±6 vs. 409 ±7 bpm) than CON rats three days after cannulation. The HR and MAP responsiveness to bolus injections of isoproterenol (ISO) were examined after either ganglionic blockade (GB; 30 mg/kg hexamethonium, 0.1 mg/kg atropine) or without any pretreatment. FR rats had attenuated increases in HR and decreases in BP to ISO following GB (0.025, .05, 0.1, and 0.2 µg/kg) or without any pretreatment (0.025, .05, and 0.1 µg/kg). For example: 0.05 µg/kg ISO increased HR 92±12 bpm and decreased MAP 27±3 mmHg in CON rats, the same dose increased HR by only 51±7 bpm and decreased MAP 14±2 mmHg in FR rats. Similar responses to ISO were seen in animals following GB. Cardiac baroreflex function was assessed by alternating hypertensive episodes using bolus injections of phenylephrine (PE; 0.25-1.0 µg/kg) and hypotensive episodes using sodium nitroprusside (NP; 2.5-15 µg/kg). FR rats had significantly greater reflex bradycardia in response to PE (slope= -1.42 vs -0.58 bpm/mmHg). The reflex tachycardia in response to NP was not altered by FR (slope= -1.56 bpm/mmHg in FR vs. -1.41 bpm/mmHg in CON). These results indicate that FR may attenuate the HR and MAP responsiveness to a given level of sympathetic stimulation, and enhance cardiac baroreflex function.

JMV is a Graduate Student Research Fellow, Amer. Heart Assoc., FL Affiliate.

251.18

BAROREFLEX-INDUCED ENDOGENOUS NITRIC OXIDE RELEASE FROM RVLM IN NORMOTENSIVE AND HYPERTENSIVE RATS. D.M. Liu*, R.P. Chao, A. L. Chiou and Y. Wang. Dept. of Pharmacology, National Defense Medical Center, Taipei, Taiwan, 100

Rostral ventral lateral medulla (RVLM), an integrated area of sympathetic activity in the brain stem, relays the neural transmission of the baroreceptor reflex. We and others previously found NMDA receptor is involved in baroreflex during bilateral carotid artery occlusion (BCCO). In this study, we first examined the interactions of NMDA and NO in the RVLM of urethane-anesthetized rats. The pressor area of RVLM was identified by electrical and chemical (NMDA) stimulation -induced hypertensive response. Extracellular NO concentration was measured through Nafion- and porphyrine- coated carbon fiber electrodes and *in vivo* chronoamperometry. We found that local application of NMDA to the RVLM elicited NO release and pressor response. Similarly, microinjection of NO donor sodium nitroprusside (SNP) or S-nitroso-N-acetyl-penicillamide (SNAP) into RVLM resulted in a dose-dependent pressor response. To determine whether endogenous NO is involved in the baroreflex, we directly measured the NO concentration in RVLM during carotid clamping. We found that BCCO induced NO release and hypertension simultaneously. The increases of NO release and pressor response were reversibly antagonized by topical application of lidocaine to the carotid sinus nerve, suggesting that BCCO-induced NO release in RVLM is mediated by baroreflex, not by local ischemia. Furthermore, BCCO-induced endogenous NO release in SHR was significantly higher than that in WKY. Collectively, NO may serve as a mediator in baroreflex to integrate the sympathetic outflow in RVLM. The discrepancy of evoked NO release may differentially regulate the neurogenic vasomotor function in WKY and SHR. (This study is supported by a grant from the National Science Council of R.O.C.).

251.19

THE CENTRALLY ACTIVE NO-DONOR, ISOSORBIDE DINITRATE ATTENUATES THE VAGAL BRADYCARDIA EVOKED BY THE BEZOLD-JARISCH REFLEX. J. G. P. Pires*, A. L. Domingues, M. T. Souza, A. L. T. Cardoso, S. R. Silva and H. A. Futuro-Neto. Dept. of Pysiological Sciences, Biomedical Center, UFES, Vitória, ES-29040, Brazil.

Centrally produced NO is implicated in the modulation of autonomic functions. Previous studies had shown that i.v. nitroglycerin rapidly releases NO in the CNS. This study was designed to investigate the effects of isosorbide dinitrate (ISB), another NO donor agent, on the vagal bradycardia evoked by the Bezold-Jarisch (BJ) reflex. Male Wistar rats, 260-350 g, were pretreated with ISB, either chronically (1 mg kg^{-1} d^{-1}, i.p., 10 days; n=7) or acutely (1 mg kg^{-1}, i.p.; 24, 19, 14 and 2 h before the test; n=7). Controls (C) received saline. The animals were anesthetized (urethane, 1.2 g kg^{-1} i.v., after ether induction) and instrumented for recording blood pressure (BP) and heart rate (HR). Left jugular vein was cannulated for drug administration. Once BP and HR had stabilized a BJ reflex was evoked with phenylbiguanide (PBG; 10 μg kg^{-1} i.v.) and repeated twice at 15-min interval. The rats were then treated i.v. with atenolol (At; 1 mg kg^{-1}); after 10 min, three additional reflex bradycardias were elicited with PBG. Statistical differences were assessed by Dunnett's test after ANOVA. Results showed that acute treatment with ISB attenuated the reflex bradycardia (overall, after At: -173 ± 29 bpm *vs* -262 ± 16 bpm in C; $p<0.05$). The chronic administration of ISB, however, did not alter the BJ reflex (overall, after At: -250 ± 20 bpm *vs* -256 ± 21 bpm in C). These results are compatible with the hypothesis that NO plays a role in the central control of autonomic functions. The lack of influence of chronically administered ISB implies that tolerance was achieved. It has recently been shown that L-NAME, a NOS inhibitor, potentiates the BJ reflex (Araújo et al., *Braz. J. Med. Biol. Res.* 28:1009, 1995). Taken together with the present data, we suggest that locally released NO is able to inhibit the cardiac vagal motoneurons in rats. *Supported by FCAA and CNPq.*

251.20

AORTIC AFFERENTS INFLUENCE THE SYMPATHETIC T-RHYTHM RECORDED FROM RAT TAIL BLOOD VESSELS. M.P. Gilbey and C.D. Johnson*. Dept. Physiol., Royal Free Hosp. Sch. Med., London NW3 2PF, U.K.

Our studies have shown that the dominant rhythm (T-rhythm) in unitary sympathetic activity recorded from rat caudal artery and lateral vein is often non-respiratory-related (Johnson & Gilbey, J. Physiol.: 476, 437-442 1994; 483, 103P-104P, 1995; 489, 160P-161P, 1995). The rhythm commonly presents as bursts of discharges (≈ 0.8 Hz). Here we have examined the influence of aortic nerve stimulation on the T-rhythm.

In anaesthetized rats (vagotomized, ventilated and paralysed) sympathetic unit activity was recorded from blood vessels using a focal recording technique (Johnson & Gilbey, 1994). Central respiratory drive was monitored via phrenic nerve activity. A stimulating electrode was placed on the left aortic nerve.

A short train of stimuli to the aortic nerve (ten 1 ms pulses at 100 Hz, voltage 2 x threshold for depressor response (0.5-10 V) delivered every 7 s for 47 trials per test) produced a transient, complete suppression of unit activity in both artery (latency to onset 590 ± 30 ms S.E.M., n=7) and vein (520 ± 30 ms, n=6) that entrained the T-rhythm in artery (7/7) and in vein (5/6). Continuous trains of stimuli (1 ms pulses, 50 Hz for 5 mins) slowed the frequency of the T-rhythm recorded from the artery during the first minute (from 0.65 ± 0.04 Hz to 0.54 ± 0.07 Hz, 3/3) which was sustained throughout stimulation (2/3). None of the units showed cardiac-related rhythmicity.

We conclude that although the sympathetic units did not have cardiac-related activity, discharges can be influenced by aortic nerve afferents (that have mainly a baroreceptor function in the rat). We suggest that the baroreceptor influence on sympathetic activity may be mediated via the modulation of the T-rhythm.

Supported by the Wellcome Trust

251.21

β-ENDORPHIN INHIBITION OF ENDOGENOUS NOREPINEPHRINE RELEASE FROM THE A2 NORADRENERGIC NUCLEUS: RECEPTOR SPECIFICITY AND LOCALIZATION. J.A. Carr* and M.M. D'Souza. Dept. Biol. Sci., Texas Tech Univ., Lubbock, TX 79409.

We used an *in-vitro* approach to determine the opioid receptor subtype mediating the inhibitory effects of β-endorphin (βE) on endogenous norepinephrine (NE) release from the A2 area of the caudal dorsomedial medulla (CDMM) in rats. The Na^+ channel blocker tetrodotoxin (TTX) was used to examine the role of propagated action potentials in βE-inhibition of evoked NE release. The inhibitory effect of βE on K^+-induced NE release from CDMM slices was mimicked by the μ-opioid receptor agonist [D-Ala^2, N-Me-Phe^4, Gly-ol^5]-enkephalin but not the δ-opioid receptor agonist [D-$Pen^{2,5}$]-enkephalin (DPDPE) or the *k*-opioid receptor agonist U-50488. The inhibitory effect of βE was blocked in a dose-dependent fashion by the μ-opioid receptor antagonist CTOP (Cys^2, Tyr^3, Orn^5, Pen^7 amide). Tetrodotoxin inhibited NE release evoked by 25 mM K^+ in a dose related fashion but had no effect on basal NE release. The inhibitory effects of βE were not observed in the presence of TTX. These results suggest that βE acts on μ-opioid receptors to inhibit K^+-evoked NE release from the A2 cell group. The fact that βE-inhibition of evoked NE release was abolished in the presence of TTX suggests that the μ-opioid receptors involved are not located on noradrenergic nerve terminals.

Supported by a Grant-in Aid from the American Heart Association.

SUBCORTICAL VISUAL PATHWAYS I

252.1

LOCALIZATION OF CALCIUM BINDING PROTEIN CALRETININ IN THE CAT SUPERIOR COLLICULUS. C.-J. Jeon*, H.-W. Yang and M.-H. Jeon. Department of Biology, College of Natural Sciences, Kyungpook National University, Taegu, 702-701, S. Korea.

Previously, two calcium binding proteins calbindin-D28K and parvalbumin have been localized in the cat superior colliculus (SC) (Mize et al., 1991; 1992). The present study describes the distribution of another calcium binding protein calretinin and compares this labeling to that of calbindin-D28K and parvalbumin in the cat SC. Calretinin has been localized with antibody immunocytochemistry.

These experiments reveal two important features of SC organization. First, densely labeled calretinin-like immunoreactive fibers were found within the zonal and upper superficial gray layer. These fibers were varied in size and were distributed through the medial-lateral and rostral-caudal extent of the SC. A few varicosities and terminal swellings were also found on many of these fibers. Second, no calretinin labeled cells were found in this dense band of fibers. By contrast, anti-calretinin labeled cells formed two laminar tiers in the deeper SC. The first one was found within the deep optic and upper intermediate gray layers and the other one within the deep gray layer. The predominant anti-calretinin labeled cells were small to medium sized round, vertical fusiform, or stellate neurons. Some stellate neurons in the intermediate gray layer showed very large dendritic field with varicosities along dendrites. The very large projection neurons in the deep layers were not labeled by this antibody.

The present and previous results show that the three calcium binding proteins calbindin-D28K, parvalbumin, and calretinin have a unique neuronal sublaminar organization in the cat SC. Supported by KPNU 95 grant.

252.2

LOCALIZATION OF TWO CALCIUM BINDING PROTEINS CALBINDIN-D28K AND CALRETININ IN THE RABBIT SUPERIOR COLLICULUS. J.-K. Pyun[1], M.-H. Jeon[1], H.-W. Yang[1], W.S. Lee[2]* and C.-J. Jeon[1]. [1]Department of Biology, Kyungpook National University, Taegu, 702-701, and [2]Department of Pharmacology, College of Medicine, Pusan National University, Pusan, 602-739, S. Korea.

Calcium plays a key role in signal transduction. We have localized two calcium binding proteins calbindin-D28K and calretinin and studied the distribution and morphology of labeled neurons in the rabbit superior colliculus (SC). Calcium binding proteins have been localized with antibody immunocytochemistry.

Anti-calbindin-D28K labeled neurons were concentrated in two layers: one within the upper superficial gray layer, and a second within the intermediate gray layer. Scattered calbindin labeled neurons also were found in the deep layers. The vast majority of calbindin labeled cells were small to medium sized round, vertical fusiform and stellate neurons. Some stellate neurons in the intermediate gray layer showed very large dendritic field with numerous prominent varicosities along each dendrite.

The morphology and distribution of anti-calretinin labeled neurons were similar to those of calbindin-D28K. However, the number of calretinin labeled neurons was much lower than that of calbindin-D28K in both superficial and intermediate layers. Calretinin immunoreactivity in the deep superficial gray layer consisted of many fibers which were not labeled antiserum against calbindin-D28K. These were small caliber fibers with a few varicosities along its course.

The two calcium binding proteins calbindin-D28K and calretinin thus reveal distinct distribution patterns in the rabbit SC. Supported by KPNU 95 grant.

252.3

NITRIC OXIDE SYNTHASE AND CHOLINE ACETYLTRANSFERASE ARE CO-LOCALIZED IN NEURONS PROJECTING TO THE PATCH-CLUSTER SYSTEM OF THE CAT SUPERIOR COLLICULUS. G.D. Butler, C.A. Scheiner, R.H. Whitworth*, and R.R. Mize, Dept. of Anatomy and Neuroscience Center, Louisiana State University Medical Center, New Orleans, LA 70112

Nitric oxide synthase (NOS), identified by nicotinamide adenine dinucleotide phosphate diaphorase (NADPH-d) histochemistry, is found in fiber patches in the intermediate gray layer (IGL) of the cat superior colliculus (SC). These patches have characteristic patterns like those known to contain acetylcholine. We have used choline acetyltransferase (ChAT) antibodies and NADPH-d histochemistry to determine directly if the two substances are colocalized in the cat SC. We also confirmed the origin of these fibers from the pedunculopontine tegmental nucleus (PPTN) by injecting biocytin into the right PPTN of two cats. A computer-based microscope plotter was used to map the distribution of single and double labeled neurons in the PPTN and fibers in SC. NADPH-d labeled fibers formed two tiers of patch-like fibers, one superficial and the other deeper within the IGL. Adjacent sections labeled by ChAT produced a similar pattern of fiber labeling. Superimposed computer plots showed that the patches labeled by the two substances overlap. Double labeled sections also revealed fibers within the patches that contained both labels. In addition, NADPH-d and ChAT were colocalized in 94.6% of neurons in three sections through the PPTN. Counts of NADPH-d and ChAT labeled cells in adjacent sections suggested that NADPH-d labeled neurons are a substantial subset of the total ChAT population because the number of NADPH-d labeled neurons were 68.5-80.1% of ChAT neurons in these sections. In summary, our results provide direct evidence that neurons in the PPTN that project to the IGL contain both ChAT and NADPH-d (and thus presumably nitric oxide). Supported by NIH NEI-02973 and a Louisiana State Board of Regents Graduate Student scholarship.

252.4

LOCALIZATION OF KAINATE AND N-METHYL-D-ASPARTATE RECEPTOR SUBUNITS IN THE CAT SUPERIOR COLLICULUS USING ANTIBODY IMMUNOCYTOCHEMISTRY R.R. Mize* and G.D. Butler, Department of Anatomy and the Neuroscience Center, Louisiana State University Medical Center, New Orleans, LA 70112.

Physiological evidence suggests that the quisqualate/kainate and n-methyl-d-aspartate (NMDA) receptors are localized at the same post-synaptic sites opposite glutamate containing synaptic terminals and that both are necessary to mediate long-term potentiation. To date, there is limited ultrastructural evidence that demonstrates directly the localization of these receptors at specific synaptic sites. We have employed electron microscope immunocytochemistry using silver intensified diaminobenzidine to localize the NMDAR1 subunit of the NMDA receptor and the GLUR5-7 subunits of the kainate receptor. We localized these receptor subunits in the superficial layers of the cat superior colliculus (SC) where numerous glutamate-containing synaptic terminals from the retina and visual cortex are known to terminate. Retinal terminals were identified by normal morphology and cortical terminals by degeneration after cortical lesion of areas 17-18. Antibody labeling to the NMDAR1 subunit was found along post-synaptic densities and internalized within profiles that were opposite synaptic terminals of retinal origin containing round vesicles and pale mitochondria and degenerating terminals of cortical origin. Post-synaptic profiles labeled by NMDAR1 included conventional dendrites and presynaptic dendrites which themselves contained pleomorphic vesicles and formed synapses with other profiles. GLUR5-7 antibody also labeled post-synaptic densities on both conventional dendrites and presynaptic dendrites opposite retinal and cortical terminals. Both antibodies also labeled receptor proteins at non-synaptic sites. These results show indirectly that the kainate and NMDA receptor proteins are likely colocalized at post-synaptic sites related to both major excitatory afferents to SC. Supported by NIH EY-02973.

252.5

SUBCORTICAL NUCLEI CONTRIBUTING AXONS TO THE CRITICAL ZONE OF THE COMMISSURE OF THE SUPERIOR COLLICULUS: AN HRP STUDY IN THE CAT.

W.E. Todd & A.C. Rosenquist*
Dept. of Neuroscience, Univ. of Penn., Phila., PA 19104

Transection of a critical zone of the commissure of the superior colliculus (CSC) restores visual orienting to a cat previously rendered hemianopic by a large unilateral visual cortical ablation (Wallace et al., *J. Comp. Neurol.* 284:429-450, 1989). The critical zone lies from approximately 58% to 68% of the distance between the posterior commissure (0%) and the caudal pole of the superior colliculus (100%). Transections of the CSC rostral or caudal to the critical zone do not lead to recovery of visual orienting (Wallace et al., *J. Comp. Neurol.* 284:429-450, 1989).

Over 40 subcortical nuclei project through the CSC (Edwards et al., *J. Comp. Neurol.* 184:309-330, 1977). In order to determine which subcortical nuclei send axons through the critical zone, we transected various regions of the CSC and injected HRP into the transection site. HRP is taken up by cut axons and transported retrogradely to their parent cell bodies. Analysis of the location of labelled cells revealed that two regions project almost exclusively through the critical zone of the CSC: the substantia nigra pars reticulata and a subregion of the pedunculopontine nucleus. Interestingly, destruction of either of these two nuclei contralateral to a cortical lesion has been shown to result in recovery (Wallace et al., *J. Comp. Neurol.* 296:222-252,1990; Durmer et al., *Soc. Neurosci. Abstr.* 20: 1187, 1994).

(Supported by EYO2654.)

252.6

SMALL VOLUME RETROGRADE TRACER INJECTIONS INTO THE SUPERIOR COLLICULUS OF THE CAT REVEAL AN ANATOMICAL SUBSTRATE FOR RECOVERY OF THE VISUAL ORIENTATION RESPONSE IN THE HEMIANOPIC CAT

J.S. Durmer* & A.C. Rosenquist
Dept. of Neuroscience, Univ. of Penn, Phila., PA 19104

Previous anatomical studies in the cat have shown multiple projections from brainstem nuclei to the superior colliculus (SC). Lacking in the literature is a systematic study of crossed SC afferents. In particular, the regional and topographical organization of crossed collicular afferents from the substantia nigra pars reticulata (SNpr) and the pedunculopontine nucleus (PPN) to the SC have not received much attention.

We have previously reported that in a cat with the unilateral removal of all known visual cortical areas (a hemianopic cat) transection of the commissure of the superior colliculus (CSC), destruction of a critical zone of the contralateral SNpr or ablation of a critical zone of the contralateral PPN, results in the return of visual orientation behavior in the previously hemianopic visual field (Wallace et al., *J. Comp. Neurol.* 296:222-252, 1990; Durmer et al., *Soc. Neurosci. Abstr.* 20:1187, 1994). Thus, recovery seems to depend on elimination of a contralateral projection to the SC.

This study demonstrates a topographically organized distribution of contralaterally projecting cells utilizing small volume injections of the fluorescent retrograde tracers diamidino yellow in one SC and fast blue into the mirror symmetric position of the other SC. By varying the rostrocaudal, lateromedial and dorsoventral position of these injections, the complex nature of contralateral SC afferents is revealed.

(Supported by EY02654 & 5-T32-EY-07035-17)

252.7

INACTIVATION OF THE CAT SUBSTANTIA NIGRA PARS RETICULATA AND SENSORY RESPONSIVENESS.

V.M. Ciaramitaro* & A.C. Rosenquist
Dept. of Neuroscience, Univ. of Penn, Phila., PA 19104

Permanent destruction of the rat and cat substantia nigra pars compacta (SNpc) produces sensory inattention in the ipsilateral field (Feeney and Wier, *Brain Res.* 178:329-346, 1979; see Schultz, *Prog. Neurobiol.* 18:121-166, 1982 for review in rat). Reversible pharmacological inactivation of the cat substantia nigra pars reticulata (SNpr) also produces neglect of visual-auditory targets (Boussaoud & Joseph, *Exp. Brain Res.* 57:297-304, 1985), suggesting that destruction of the SNpr should also produce sensory inattention .

We compared permanent versus reversible inactivation of the cat SNpr on tests of orienting to visual, auditory and somatosensory stimuli. We have confimed and extended previous findings. Unilateral injections of the $GABA_A$ agonist, muscimol, produced unresponsiveness to visual, auditory and somatosensory stimuli over several hours, with visual impairments being the most enduring. Thus, while responses to auditory or somatosensory stimuli were present, animals failed to respond to visual stimuli, suggesting that results are not simply due to a motor impairment. Injections of saline and the $GABA_A$ antagonist, bicuculline methiodide, were ineffective.

In contrast, we have failed to observe visual unresponsiveness after ibotenic acid lesions of the SNpr, but see some evidence for auditory and/or somatosensory unresponsiveness. However, motor biases prevented testing for several days post-lesion and deficits could have resolved in this time via compensatory mechanisms.

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252.8

EFFECTS OF ANGIOTENSIN II ON THE VISUAL RESPONSES OF THE RAT'S SUPERIOR COLLICULUS DURING NEONATAL DEVELOPMENT.

A. Marois, S. Darveau, and C. Casanova*. École d'Optométrie, Université de Montréal, Québec, Canada, H3C 3J7.

We recently reported that Angiotensin II (Ang II) yielded a strong suppressive effect on visual neuronal activity in the superior colliculus (SC) of adult rats (Merabet *et al.*, 1994. *Neuroreport* 5: 2649). Since the density and proportion of angiotensinergic receptors vary during post-natal development, we were interested in characterizing and comparing the physiological effects of Ang II in neonatal rats at various stages of development (PND 10 to 40).The effect of Ang II on baseline and visually evoked collicular activity was studied in 6 groups of rats (10-15, 15-20 days, etc...). Experiments were carried out on pups anesthetized with urethane (25%). A recording-injecting microelectrode filled with the peptide was lowered into the superficial layers of the SC to record visual evoked potentials (VEP, Grass photostimulator). Ang II was injected at a concentration of 10^{-6}M (volume of 30-40 nl at a rate of 10nl/mn). Preliminary results indicated that, in most cases, injection of Ang II provoked a reduction in the amplitude of the VEP (mean ± SD= 70 ± 11%), with no change in the latency of the potential. These effects were restricted to the superficial layers of SC, supporting autoradiographic studies which indicated that angiotensinergic receptors were distributed in the dorsal collicular layers. The strength of inhibition appeared to vary with age, being more pronounced in younger animals. In contrast to adult rats, recovery of the responses after injection of 10^{-6}M Ang II was often either delayed or absent. These results suggest that, as for adult animals, Ang II has a suppressive effect on the activity of the superficial collicular layers in neonatal rats. In addition, there are some indications that the strength of the physiological action of Ang II may vary according to the stage of development, and therefore be related to the change of receptor density and/or proportion. *Supported by MRC of Canada, FCAR, and FRSQ.*

252.9

HABITUATION AND DISHABITUATION OF DEEP TECTAL NEURONS IN THE MONGOLIAN GERBIL (*Meriones unguiculatus*): NOVELTY DETECTION. M. G. Bigel*, C. G. Ellard and S. Reinis, Dept. of Psychology, Univ. of Waterloo, Waterloo, Ontario, Canada.

Employing standard electrophysiological recording, three unique experiments were conducted to investigate the characteristics of habituation and dishabituation in deeper layer neurons of the superior colliculus (SC) of the Mongolian Gerbil. Results showed that habituation is common to neurons in the deeper layers of the SC and occurred as a result of repeated visual stimulation. It was found that neurons habituated at different rates but that not all neurons habituated to repeated stimulation. Further, it was found that a habituated neuron could not be dishabituated by changing the location of a stimulus within the neuron's receptive field or by changing the shape of the stimulus alone. However, a neuron's response could be reinstated by increasing stimulus intensity alone. There was no correlation between a neuron's rate of habituation and its location in the SC nor was there any relation between the strength of a cell's response (number of spikes fired at stimulus onset), its depth or rate of habituation. It was concluded that changing the intensity of a stimulus alone was sufficient to dishabituate a neuron. Results of this study suggest that neuronal habituation is a robust phenomenon related to novelty detection at the behavioural level and that the ability to detect novelty is dependent on unique features of the new stimulus. Research supported by an NSERC grant to C.G.E.

252.10

VISUAL RESPONSES AND TOPOGRAPHY IN THE SUPERIOR COLLICULUS (SC) OF THE NEWBORN MONKEY. B.E. Stein*, M.T. Wallace & J. G. McHaffie. Dept. of Neurobiology and Anatomy, Bowman Gray School of Medicine, Wake Forest Univ., Winston-Salem, NC 27157

The newborn rhesus monkey, like the newborn human, can make eye/head movements in response to visual stimuli. Despite the fact that these early behaviors are likely to involve the circuitry of the SC, little is known about the functional state of this structure at birth. In order to examine the organization of the visual representation at birth, single-unit extracellular recordings were done in the superficial SC of two newborn animals. In contrast to similar studies in an altricial species such as cat, both single and multiunit visual responses were readily recorded from these layers within hours of birth. A systematic series of electrode penetrations revealed a well organized, adult-like visuotopic map. In most respects, the response properties of neonatal SC neurons paralleled those of the adult: they responded robustly to stationary and moving stimuli, exhibited near adult incidences of velocity selectivity, surround inhibition and within-field spatial summation/inhibition, and were unselective for stimulus features such as shape, orientation and direction of movement. Neonatal SC neurons differed substantially from the adult in only two respects: receptive field size and latency. Neonatal receptive fields were on average 37% larger than in the adult, and response latency was more than twice (204%) as long (159.9 ± 36.5 ms vs. 78.5 ± 14.6 ms). These results demonstrate a surprising maturity of visual information-processing capabilities of the SC in the newborn monkey, which is likely to be necessary to subserve its visual orientation capabilities. Supported by NIH EY06562.

252.11

DEVELOPMENT OF AUDITORY RESPONSIVENESS, TOPOGRAPHY, AND FUNCTIONAL CONVERGENCE WITH VISUAL INPUTS IN CAT SUPERIOR COLLICULUS (SC). M.T. Wallace* and B.E. Stein. Dept. of Neurobiology and Anatomy, Bowman Gray School of Medicine, Wake Forest Univ., Winston-Salem, NC 27157

Auditory inputs play a critical role in the orientation functions mediated by the SC. Their role is accomplished not only by evoking responses to auditory cues, but also by their influence over visual responses. In the adult SC, auditory neurons respond robustly and with short latency, and have discrete spatiotopically-organized receptive fields (RFs). Despite the fact that the nature of auditory responses and the alignment of auditory and visual maps are key features in determining cross-modality interactions, little is known about their development in cat. Auditory SC development was examined in animals from 1-135 days postnatal (dpn). The first auditory neuron was seen at 5 dpn. Early neurons responded weakly, had long latencies (>100ms), habituated rapidly and lacked RFs. Auditory maturation was most accelerated from 21-45 dpn, when a coarse spatiotopic map became evident and was refined (RFs shrank by nearly 400%). During this period auditory responses became more robust, latencies shortened, auditory-visual map alignment became evident, and many neurons became overtly responsive to both auditory and visual cues. Although covert interactions among auditory and visual afferents are likely to be essential in guiding cross-modality alignment, there was a temporal disparity between map alignment and the ability of auditory cues to alter visual responses. These findings are consistent with the hypothesis that the mechanisms guiding map alignment are necessary but not sufficient for normal multisensory integration. Supported by EY06562.

252.12

CROSS-MODAL ORIENTATION BEHAVIORS: DEVELOPMENTAL COMPENSATION FOLLOWING NEONATAL LESIONS OF ASSOCIATION CORTICES. L.K. Wilkinson*[1], M.T. Wallace[2] and B.E. Stein[2]. Dept. Psychology[1], Virginia Commonwealth Univ., Richmond, VA 23298 and Dept. Neurobiology and Anatomy[2], Bowman Gray School of Medicine/Wake Forest Univ., Winston-Salem, NC 27157.

The ability of the cat superior colliculus (SC) to integrate sensory inputs from different modalities in order to direct attentive and orientation behaviors is dependent on corticotectal influences; most notably, those from the anterior ectosylvian sulcus (AES) (Wilkinson et al., 1992; Wallace and Stein, 1993). However, if AES is removed at birth, corticotectal influences from the rostral lateral suprasylvian area (rLS) increase to compensate for the absent AES (Wilkinson et al., 1995). This compensation results in normal multisensory orientation behaviors . Surprisingly, in the present experiments, unilateral removal of AES and rLS at 5-7 days postnatal (dpn) degraded, but did not eliminate, the development of multisensory orientation behaviors. Thus, a significant enhancement in orienting to a visual stimulus which elicited 50% correct responses when presented alone occured when this stimulus was paired with a spatially coincident neutral auditory stimulus (AES-rLS ablated animals=84% correct responses; normal animals=99%). Conversely, a significant decrease in correct responses occurred when the auditory stimulus was spatially disparate (AES-rLS ablated animals=6% correct responses; normal animals=1%). Thus, it appears that the compensatory hierarchy for normal multisensory behavior contains additional component(s) beyond AES and rLS. The source of these influences remain to be identified. Supported by NIH grant EY06562

252.13

NEONATAL PATTERN DEPRIVATION FAILS TO DISRUPT NORMAL MAP ALIGNMENT OR CROSS-MODALITY INTEGRATION IN CAT SUPERIOR COLLICULUS (SC) J.G. McHaffie*, M.T. Wallace and B.E. Stein. Dept. Neurobiol. & Anat., Bowman Gray Sch. of Med., Winston-Salem, NC 27157.

The development of a spatiotopic auditory map and its alignment with the visuotopic map in the SC is known to depend on early visual-auditory experience. Presumably, such early cross-modality interactions are also essential if normal integration is to develop. The present experiments were initiated to determine whether patterned visual stimuli are necessary for: 1) the development of aligned visual, auditory, and somatosensory maps; 2) the appearance of multisensory (i.e., cross-modal) neurons; and 3) the maturation of normal cross-modality integration. Unimodal and multisensory neurons (n=29) were recorded in the SC of adult cats (n=2) whose eyelids were opened after having been sutured closed since before normal eye opening. Immediately after opening the eyes, it was apparent that visual, auditory, and somatosensory receptive fields (RF's) were topographically organized and aligned with one another. Multisensory neurons were frequently encountered (n=22) and had RF's that were in close spatial register. Visual-nonvisual cross-modality interactions also appeared normal: two modality-specific stimuli within their respective RF's produced response enhancement, but when one of these stimuli was outside its RF, either no enhancement or response depression was produced. The magnitude of the enhancement was greatest for minimally effective cues and when the peak periods of the two modality-specific discharge trains were overlapping. These data indicate that experience with patterned visual stimuli is not a critical factor in the maturation of cross-modality (i.e., visual-nonvisual) processes in the SC. Supported by NIH EY 06562, the Forsyth County United Way, and a BGSM Venture Grant

252.14

THE DISTRIBUTION AND POSTNATAL DEVELOPMENT OF NMDAR1 IMMUNOREACTIVITY IN THE CAT SUPERIOR COLLICULUS (SC). K.K. Anstrom*, J.G. McHaffie and B.E. Stein. Department of Neurobiology & Anatomy, Bowman Gray School of Medicine, Winston-Salem, NC 27157.

Recent studies indicate that NMDA receptor-mediated events play a role in sensory processing and crossmodal integration in the cat SC. The distribution and postnatal ontogeny of NMDA immunoreactivity were demonstrated with an antibody to the R1 subunit, the constant element of the NMDA receptor. In adults, the superficial laminae displayed dense neuropil label with a dorsoventral gradient, as well as homogeneously distributed, small-soma NMDAR1(+) neurons. In the deeper laminae, label was restricted to a mediolateral band of large-soma neurons in the upper aspects of the intermediate (SGI) and scattered neurons in the deep (SGP) laminae. Following HRP injections into the brainstem, virtually all HRP-labelled neurons were also NMDAR1(+), indicating that deep laminae output neurons, known to be involved in crossmodal integration, preferentially express NMDA receptors. In newborns, only a few labelled neurons were found in lateral SGI; the superficial laminae were devoid of label save for some cellular labelling in their most dorsal aspects. By PND 7, the adult pattern had been acheived in SGI and a few labelled neurons appeared in SGP; the superficial laminae had acquired light, homogeneous neuropil label. Between PND 10 and 24, NMDAR1(+) neurons increased in SGP to resemble the adult distribution; superficial neuropil label became denser but did not display a dorsoventral gradient until PND 46. Thus, superficial laminae NMDAR1 immunoreactivity undergoes a protracted period of postnatal maturation, whereas the deeper laminae acquire their adult-like pattern at or before the onset of crossmodal integration. Sponsored by NIH EY06562.

252.15

ROLE OF NMDA RECEPTORS IN MULTISENSORY INTEGRATION IN THE SUPERIOR COLLICULUS. J.H. Graham *, J.W. Vaughan, S.J. Schaafsma, M.T. Wallace, B.E. Stein. Dept. Neurobiology & Anatomy, Bowman Gray School of Medicine/Wake Forest Univ., Winston-Salem, NC 27157.

The superior colliculus contains unimodal neurons that respond to visual, auditory, or somatosensory stimuli and multisensory (MS) neurons which can integrate combinations of these inputs and increase their overall response levels. In the present studies the effects of glutamatergic agonists and antagonists on these different sensory responses were investigated in the cat. Iontophoretically applied NMDA was found to be capable of enhancing responses to modality-specific stimuli and to multisensory combinations of stimuli in more than 80% of the MS neurons studied. The NMDA antagonist AP5 was able to antagonize NMDA's effects, and when applied alone, AP5 was able to reduce responding to both modality-specific and multisensory stimuli below control levels in the majority of these MS neurons, as reported by Binns and Salt (1996). Subpopulations of MS neurons were also encountered in which AP5 tended to increase multisensory integration. Furthermore, NMDA receptor activation and antagonism had greater effects on the unimodal responses of MS neurons than on their multisensory responses. Auditory responses in unimodal and MS neurons appeared less likely to be altered by NMDA or AP5, and the variability seen in this study as well as by others indicates that NMDA receptor activation and inactivation may be affecting auditory responses indirectly. Supported by NIH grant EY 06562 and NS 22543.

252.16

SPATIAL ORGANIZATION OF MULTISENSORY CELLS IN THE DEEP LAYERS OF CAT SUPERIOR COLLICULUS (SC). D.C. Kadunce*, J.W. Vaughan and B.E. Stein. Department of Neurobiology & Anatomy, Bowman Gray School of Medicine/ Wake Forest Univ., Winston-Salem, NC 27157.

The receptive fields (RFs) of multisensory neurons in cat superior colliculus (SC) are surprisingly large given their presumptive role in target location. However, cross-modality RF heterogeneity might provide greater spatial precision than would be predicted by RF size. As a test: 1) stimuli (e.g., visual-auditory) were paired at various coincident RF locations; and paired so that 2) one stimulus was fixed at a location within the RF and the influence of the other was at progressively greater disparities. 92% of the cells revealed the presence of a "best area" within the RF where multisensory responses and cross-modality integration were most effective. Similarly, the majority (62%) of neurons showed that multisensory integration was maximal when the spatial disparity between the two stimuli was minimized despite the fact that all stimuli were within their excitatory RFs. It is likely that these factors covary so that RF heterogeneity and the size and location of the neuron's best area may provide a better picture and offer greater insights toward understanding multisensory integration than absolute RF size. Supported by NIH grant NS 22543 and ONR N000 1495C 0092

252.17

NEUROACTIVE SUBSTANCES IN PARABIGEMINAL CELLS WHICH PROJECT TO SUPERIOR COLLICULUS IN 13-LINED GROUND SQUIRRELS. R. J. Garcia-Mercado and N. Lugo*. Department of Anatomy and Institute of Neurobiology, University of Puerto Rico Medical Sciences Campus, San Juan, Puerto Rico

The parabigeminal nucleus (Pb) has extensive reciprocal connections with the superficial layers of the superior colliculus (SC). Somata of parabigeminal neurons have been shown to contain substance P (SP), somatostatin (SS), neuropeptide Y (NPY), GABA, ChAT and tyrosine hydroxylase (TH). In the present experiments we sought to determine whether the parabigeminal cells projecting to SC are associated with particular neuroactive substances. Parabigeminal cells projecting to SC were retrogradely labeled by injecting fluorescent microspheres into the superficial layers of SC of anesthetized ground squirrels. After survival periods of two weeks to allow transport of the microspheres, the squirrels were again anesthetized and intracardially perfused with phosphate buffer (pH 7.4) followed by 4% paraformaldehyde in buffer. Brain sections 40 µm thick were cut frozen on a sliding microtome and processed for immunofluorescence or with DAB-nickel enhanced avidin biotin complex (ABC) method. Cells containing microspheres were immunoreactive to antibodies to ChAT, NPY and SP. Most of these cells were ChAT positive. We could not demonstrate that cells reactive for antibodies to SS, GABA, TH contained microspheres. The GABA reactive cells were very small (10-15 µm diameter) but SS- and TH-reactive cells were as large as other cells which did contain microspheres. This suggests that the SS- and TH-reactive cells may be interneurons or may project to targets other than SC. (Supported by NIH Grant MH-48190).

252.18

EFFECTS OF ANGIOTENSIN II (AII) IN THE SUPERIOR COLLICULUS (SC) OF HAMSTER. W.R. Bauer*, Y. Zhang, R.W. Rhoades and R.D. Mooney. Dept. of Anatomy and Neurobiology. Medical College of Ohio, Toledo, OH 43699

The superficial layers of the SC have a high density of AII receptors. Our *in vivo* studies suggested that these modulate visual activity of SC neurons by postsynaptic mechanisms. To test whether AII had pre- or postsynaptic effects, we made intracellular recordings from superficial SC neurons, using an SC slice preparation in which EPSPs were evoked by stimulation of the attached optic tract. Depolarizing postsynaptic responses in these neurons were evoked by micropressure applications of glutamate during synaptic blockade with a low Ca^{2+}-high Mg^{2+} superfusion solution. In 18 of 22 neurons (82%), AII (1 µM) reduced EPSP amplitude by an average of 51 ± 16 (s.d.)% ($p<0.001$) and reduced glutamate depolarizations by an average of 40 ± 21% ($p<0.001$). These effects were correlated ($r=0.66$; $p<0.01$). In the remaining 4 neurons (18%), application of AII increased the EPSP amplitude by 183 ± 71% ($p<0.05$), but had no effect on glutamate-evoked depolarization in 3 of these cells and produced a 20% increment in the fourth. As previously reported, there was no correlation between the effects of AII on EPSP amplitude and those on membrane potential. There was no significant difference in the effects of AII on membrane potential between periods of superfusion with normal Krebs solution and with low Ca^{2+}-high Mg^{2+} solution. Thus, the suppressive effects of AII in the SC appear to be mediated at a postsynaptic site; whereas the facilitation, observed in only a few neurons, may involve presynaptic modulation.

Supported by NS32540.

252.19

NADPH-DIAPHORASE POSITIVE NEURONS IN THE SUPERFICIAL LAYERS OF THE SUPERIOR COLLICULUS (SC) ARE ALTERED IN AGED RATS. A. Vercelli * and S. Jhaveri, *Dept. Anatomy, Pharmacology and Forensic Medicine, Univ. of Torino, Torino, Italy, and Dept. Brain & Cognitive Sciences, Mass. Institute of Technology, Cambridge, MA 02139.*

The rat visual system exhibits a protracted maturation of the pattern of NADPH-d expression: adult-like patterns are seen around the time of eye opening. NADPH-d positive SC neurons are also vulnerable to denervation: monocular enucleation on P0 results in marked alterations in their dendritic fields (Vercelli et al., Neurosci. Abst. 1995). Here we present evidence on the morphology of NADPH-d expressing neurons in the retinorecipient tectal zones of aged rats.

Five albino rats, aged 20-26 months of age, were perfused with 4% paraformaldehyde, their brains postfixed for 4 h and 50 µm thick sections cut coronally. Tissue was reacted with 1 mg/ml NADPH, 0.2 mg/ml NBT and 1% Triton in PB for 2 h at 37°C. Control sections from normal rats (6-12 months) were processed similarly. Care was taken to ensure that the intensity of the NADPH-d reaction was the same in the aged animals as in normal controls.

In 4 of the 5 aged rats, density of reactive SC neurons was significantly reduced when compared to normals. This decrease was especially evident in the medial and lateral SC. Dendrites of NADPH-d containing cells were shorter and appeared to have fewer vertically-oriented branches. Whether this reflects decreased expression of NADPH-d, or results from an actual atrophy of the dendrites remains to be determined. There was also an increase in the percent of neurons with horizontally oriented dendrites.

These results demonstrate profound age-related changes in the morphology of the SC neurons. The possibility that these changes are a consequence of corresponding alterations in the cells of the retina is being investigated.

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253.1

ANALYSIS OF VISUALLY EVOKED FIELD POTENCIALS IN THE OPTIC TECTUM OF THE PIGEON . J. C. Letelier*, C. Madrid, G. Marin, D. Morales, J. Mpodozis, C. Rozas and M. Velasco. Laboratorio de Neurobiología y Biología del Conocer, Facultad de Ciencias, Universidad de Chile, Santiago, Chile.

The pigeon's optic tectum is a highly organized 14 layers structure, receiving 2.6 million contralateral retinal fibers. Retinal axons end at layers 2 to 7, while tectal output is mainly made of cells located in layers 10 to 13. Here we analyze, in the anesthetized pigeon, the synaptic events following the visual activation of the tectal retinal afference, using multiunit recordings of incoming retinal fibers and visually evoked Local Field Potencials (LFP). LFP were obtained at different tectal depths, spaced by 50 µm. Brief flashes, delivered monocularly, were used as visual stimuli.

At the superficial layer 1 the LFP begins, after a 20 ms latency, with a large negative phase followed by many secondary negative peaks located at regular intervals within the next 50 ms. This polyphasic profile shows more peaks, increasing amplitudes and shorter latencies at higher flashes intensities. At 300-350 µm, which corresponds to the end of the retinorecipient layers, the LFP profile shows an inversion of all its negative components, thus, in layers 8 to 14 it begins with a large positive peak. At any depth, the LFP profile reflects mostly the synaptic currents associated with retinal synapses, since flashes do not trigger action potentials in tectal cells. In the pigeon the ganglion cell population has five conduction velocity groups, consequently the interpretation of the origin and changes of the various LFP components is complex. Multiunit recordings show that retinal fibers, in response to flashes, give a quasi-periodic train of bursting activity lasting 20-50 ms with frecuencies in the 20-60 Hz range. Each burst generates a peak in the LFP similar to the one obtained using single shock electrical stimulation of the optic nerve. The overall complex LFP profile, as well as its changes according to depth, are then the combination of peaks generated by these retinal bursts. These characteristics of the LFP profiles provide a physiological marker of retinorecipient layers and of the retinal activity which produce them (Supported by FONDECYT 1950687).

253.2

SOME INTRIGUING PROPERTIES OF THE ONGOING VISUAL ACTIVITY IN THE TECTO-FUGAL VISUAL PATHWAY OF THE AWAKE PIGEON. J. Mpodozis*, J. C. Letelier, C. Madrid, G. Marin, D. Morales, C. Rozas and M. Velasco. Laboratorio de Neurobiología y Biología del Conocer, Facultad de Ciencias, Universidad de Chile, Santiago, Chile.

The retinotectofugal pathway is considered the main system mediating visual perception in birds. At least 90% of retinal ganglion cells project to the tectum. In acute preparations, retinal and tectal cells exhibit complex movement sensitive receptive fields. Here we report the first results concerning the response properties of retino-tecto-fugal neurons in the awake and head-restrained pigeon.

Extracellular recordings reveal that a) the spontaneous ongoing activity in the tectum is extremely low when the bird is looking at a static visual scene, b) when an object is moved against a background in the visual field, tectal cells respond vigorously, as expected for movement sensitive cells, but they are not stimulated with large and coherent motion of the visual background, c) as many other birds, pigeons perform, once a second, large cyclotorsional saccades with 3 to 6 cycles of 30Hz oscillations. During these saccadic oscillations most tectal cells remain silent even when the image moves in the retina at speeds encompassing the normal range of stimulating speeds.

These result are intriguing as pigeons are capable of complex static visual discriminations that appear mediated by the tecto-fugal system. Furthermore, this tectal lack of activity is also puzzling, as tectal cells, including the main output located in layer 13, receive a direct retinal input that should be active in these conditions. These results open two non-trivial possibilities: a) the retina is self silenced during saccades and whole field motion b) an inhibitory, non-retinal, intra or extra tectalsignal affects the processing of retinal activity at tectal level. (Supported by FONDECYT 1950687).

253.3

ON THE MOSSY FIBRE PROJECTION FROM THE NUCLEUS OF THE BASAL OPTIC ROOT TO THE VESTIBULOCEREBELLUM IN PIGEONS: COLLATERALS TO THE VESTIBULAR AND CEREBELLAR NUCLEI B. Linkenhoker, A.F. Kingstone* and D.R.W. Wylie. Department of Psychology, University of Alberta, Edmonton, Alberta, Canada, T6G 2E1.

In the generalized anatomy of the cerebellar circuit, mossy fibres (Mfs) terminating in the granule layer of the cerebellar cortex often send collaterals to nuclei which receive input from Purkinje cells in the zone to which the Mfs are projecting. In pigeons, the nucleus of the basal optic root (nBOR) has been shown to project as Mfs to folium IXc,d of the vestibulocerebellum. It has also been demonstrated that Purkinje cells in the pigeon vestibulocerebellum project to the vestibular and cerebellar nuclei. Given these facts, one might expect that the Mfs arising from nBOR send collaterals to the vestibular and cerebellar nuclei. In this study, we made small injections of biotinylated dextran amine (BDA) into nBOR of three pigeons which resulted in the anterograde labelling of Mfs projecting to folium IXc,d. In all three cases, Mf collaterals were observed to terminate heavily in the cerebellar nuclei. Most of these terminals were adjacent to the fourth ventricle in the medial margin of the lateral cerebellar nucleus, and the ventral margin of the medial cerebellar nucleus. Terminal labelling was also found in cerebellovestibular process. In one case, a Mf collateral terminated in the tangential nucleus, superior vestibular nucleus and the descending vestibular nucleus. This research was supported by grants from NSERC and AHFMR to D.R.W.W..

253.4

PROJECTIONS OF THE NUCLEUS OF THE BASAL OPTIC ROOT IN PIGEONS: A STUDY USING THE ANTEROGRADE TRACER BIOTINYLATED DEXTRAN AMINE D.R.W. Wylie* and B. Linkenhoker. Dept. of Psychology, Univ. of Alberta, Edmonton, AB, Canada, T6G 2E1.

The nucleus of the basal optic root (nBOR) of the Accessory Optic System has been implicated in the visual control of compensatory head and eye movements. Previous anatomical studies have shown that the nBOR projects, (i) bilaterally to the vestibulocerebellum, oculomotor complex (OMC), inferior olive and the interstitial nucleus of Cajal, (ii) ipsilaterally to the pretectal nucleus lentiformis mesencephali, and (iii) to the contralateral nBOR. In this study, we made small injections of biotinylated dextran amine (BDA) into nBOR in three pigeons. In addition to confirming the projections listed above, we found numerous others. Bilateral projections to the vestibular nuclei, pontine nuclei (caudally), nucleus Darkschewitsch, central grey, red nucleus and mesencephalic reticular formation were found. The cerebellar nuclei received ipsilateral projections as did several subnuclei of the dorsolateral thalamus, and the lateral geniculate nucleus. Sparse projections were also observed to the ipsilateral tectum, several pretectal nuclei, and several sites in the mesencephalon. We also found a different projection pattern to the OMC than that previously reported. Whereas previous studies found projections to the ventral subdivision (OMv) and dorsolateral subdivision (OMdl) on the ipsilateral and contralateral sides respectively, we found a bilateral projection to the OMv and a contralateral projection to OMdl and the dorsomedial subdivision of the OMC. This research was supported by grants from NSERC and AHFMR to D.R.W.W..

253.5

SYNAPTIC TARGETS OF CROSSED ISTHMOTECTAL AXONS IN *XENOPUS:* IMPLICATIONS FOR PLASTICITY. K. K. Rybicka and S.B. Udin*, Dept. of Physiology & Biophysics, SUNY, Buffalo, NY 14214.

Visual activity during development is required for the ipsilateral visual input to Xenopus tectum to come into register with the contralateral map. Correlated activity is hypothesized to be the cue by which a Hebbian process stabilizes isthmotectal synapses (which relay ipsilateral visual input) when they are located near to retinotectal synapses with matching visual field locations. In order to test this hypothesis, we have begun to identify the tectal cells upon which isthmotectal axons synapse and to determine whether retinotectal axons terminate on the same dendrites.

Isthmotectal axons were labeled with HRP, and tectal cells were labeled by post-embedding immunogold reaction with antibodies to GABA. Ultrastructural examination reveals that some isthmotectal axons terminate on GABA-positive dendrites. Other axons contact unlabeled postsynaptic structures that were consistent in appearance with the GABA-poor zones of GABA-positive dendrites.

To determine whether retinotectal axons synapse onto the same dendrites, we surveyed all of the processes post-synaptic to filled isthmotectal axons. The most common non-isthmic inputs were GABA-positive. Surprisingly, we found no inputs with typical retinotectal features of round, clear vesicles, pale mitochondria, and asymmetric synapses. These results indicate that retinotectal-isthmotectal interactions are more complex than originally assumed.

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253.6

RELATIVE INPUT FROM IPSILATERAL AND CONTRALATERAL NUCLEUS ISTHMI TO DIFFERENT LOCI OF THE FROG OPTIC TECTUM DEPENDS ON RETINOTOPIC LOCATION. E.A. Dudkin and E.R. Gruberg*. Biology Dept., Temple Univ., Phila., PA 19122.

The inferior visual field of each eye of *Rana pipiens* extends from about 60^{o} contralaterally past the midline in front to 180^{o} ipsilaterally in the back. When input from the left nucleus isthmi is cut and the right eye is intact but occluded, the animal no longer responds to prey stimuli in the right (contralateral) inferior visual field. An equivalent statement can be made for the right n. isthmi input and the left eye.

To explore the anatomical basis for such a result we have injected HRP at single, circumscribed locations in tectal lobes, and counted the number of ipsilateral and contralateral n. isthmi cells that are backfilled. At tectal injection sites representing the inferior *contralateral* visual field, an average of 4.1 times more labeled cells are found in the contralateral nucleus isthmi than in ipsilateral n. isthmi. At tectal injection sites representing the inferior *ipsilateral* monocular visual field, an average of 3.4 times as many labeled cells are found in ipsilateral n. isthmi as in contralateral n. isthmi. After unilateral optic tract cut (which also severs contralateral isthmotectal input), animals respond to prey stimuli in the superior visual field for 2 to 3 days post surgery; after this time responsiveness diminishes rapidly. Similar results are seen after unilateral optic nerve cut. At tectal HRP injection sites representing the superior visual field, approximately the same number of labeled cells are found in the ipsilateral and contralateral n. isthmi. Our results suggest that there is a threshold input from n. isthmi that enables the frog to respond to prey stimuli in different parts of the visual field. Supported by NIH grant EY04366.

253.7

BINOCULAR INTERACTIONS AND VISUAL FIELD SPECIFIC PROJECTIONS IN THE TECTO-ROTUNDAL PATHWAY OF THE PIGEON. O. Güntürkün*, B. Diekamp, B. Hellmann and C. Theiss. AE Biopsychologie, Fakultät für Psychologie, Ruhr-Universität Bochum, 44780 Bochum, Germany.

The pigeon's retina projects topographically onto the contralateral tectum (TO) with the superior retina, pointing to the lower visual field, being represented in the inferior TO. The tectal projections to the n. rotundus (Rt) were assumed to be mainly ipsilaterally organized with no binocular interactions at rotundal level. We reanalyzed this system with anatomical and electrophysiological techniques.

Ventral TO-injections of biotinylated dextranamine labelled a dense and bilateral network of fibers and terminals throughout the entire Rt and triangularis (T). In contrast, dorsal TO-applications always exhibited a different tracing pattern with most fibers and boutons being restricted to the T. These results, showing visual field specific projections to the Rt, are supported by data of intra-TO heterogeneities of cell- and glutamate receptor-densities: the ventral half of layer 13 had a significantly higher neuron density than the dorsal half. Concomitantly, AMPA-Glu R2/3-like cells were more numerous in the ventral TO. No AMPA Glu R1- or R4-like neurons could be detected in layer 13. Thus, dorsal and ventral TO (representing upper and lower visual fields) differ in their internal structure and their Rt-projections with glutamate being a key transmitter in this system.

In recordings from the Rt, neurons responsive to a contralateral whole-field stimulus showed robust excitatory and inhibitory responses, while ipsilateral stimuli had nearly always no effects. However, a substantial number of cell responses to a contralateral stimulus were modulated by additional ipsilateral stimuli. These binocular responses showed either fascilitatory (binocular spike rate > contra + ipsi spike rate) or inhibitory interactions. Our data indicate for the first time on single unit level that rotundal cells receive binocular input with a number of cells showing complex modes of integration of these two afferents.

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253.8

3D-MORPHOLOGY OF INTRACELLULARLY LABELED NEURONS IN THE TECTOFUGAL PATHWAY OF THE CHICK. H. Luksch and H.J. Karten*, Dept. of Neuroscience, UCSD, San Diego, La Jolla, CA, 92093.

In birds, visual information is processed by two parallel systems, the thalamofugal and the tectofugal pathway, which can be homologized with the mammalian geniculo-cortical and colliculo-pulvino-cortical pathways, respectively. Recent studies (Karten et al., in prep.) have shown that elements of the tectofugal pathway in the stratum griseum centrale (SGC) can be further subdivided on the basis of the termination fields in their target structure, n. rotundus: type 1 cells project to the anterior portion, whereas type 2 cells project to the posterior part of n. rotundus. In this study we analyzed the three-dimensional morphology of these neurons and additional elements of the tectofugal pathway by intracellular staining. In chick hatchlings, fluorescent beads were pressure-injected stereotactically into different nuclei of the tectofugal pathway. After survival of three days, animals were sacrificed and brains were sliced on a vibratome. Backlabeled neurons were identified with an epifluorescence microscope, impaled under visual control and filled with biocytin. Filled neurons were reconstructed with camera lucida and using confocal microscopy, and dendritic fields were measured. To determine input onto labeled cells, retinal afferents were traced using cholera-toxin B subunit.

In the SGC of the chick optic tectum, cell types defined by their connectivity have intriguing morphological differences. Type 1 is found in the superficial SGC and has very large dendritic fields (3 to 4 mm) that can cover up to one third of the tectum with distal dendrites arborizing in layer 5b; these dendrites have specialized terminal structures that contact retinal afferents. Type 2 is found in the deeper SGC and has dendritic fields of comparable width, but these dendrites never reach layer 5b. In the n. rotundus, neurons have conspicuous claw-like dendritic endings that presumably contact tectal efferents; this connection is currently being investigated. Additionally, the morphology of the small retinal ganglion cells that presumably project onto SGC-type 1 cells will be demonstrated. Our findings will be discussed in relation to the physiological characteristics of neurons of the tectofugal pathway.

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253.9

AGE-RELATED CHANGES IN CALCIUM-BINDING PROTEINS EXPRESSION ON CHICK ACCESSORY OPTIC NUCLEUS. C.A.B. Toledo*[1]; S. Medeiros [1], and L.R.G. Britto [2]. [1]Dept. of Physiol. Sciences, Federal Univ. of Santa Catarina, Florianópolis, SC, and [2]Dept. Physiol. Biophys., Inst. Biomed. Sci., Univ. São Paulo, SP, Brazil.

Some calcium-binding proteins have been implicated in the buffering of cytosolic calcium. It is possible that this action might differ according to the age of the animal. In this study, immunocytochemical techniques were used to analyze the distribution of the calcium-binding proteins (CBP) calbindin (Calb), calretinin (CaR), and parvalbumin (PV) during aging on bird's accessory optic nucleus (nucleus of the basal optic root, nBOR). Four young (two weeks old) and four old adult (ten-twelve months) chickens (*Gallus gallus*) were perfused, the brains posfixed, cryoprotected, frozen, and cut at 30 µm in the coronal plane. Monoclonal antibodies against Calb and PV (Sigma) and an antiserun against CaR (Chemicon) were used with conventional immunoperoxidase and immunofluorescence techniques to detect and quantify those proteins. Usually, many cells with somata sizes ranging from 10 to 25 µm (PV + and CaR +) and 5 to 10 µm (CalB+) were found into nBOR. Comparing old and young birds, no diferences regarding size and shape were seen. However, we detected a notable decrease of the number of cells and fibers exhibiting CBP immunoreactivity in old animals. That reduction was not observed for other immunopositive nuclei used as immunohistochemical controls. The results were consistent and observed in all of those three proteins but it appeared to be more evident for PV. These data indicate that some cytosolic process can be altered in the chick nBOR during aging and it could include metabolic changes involving CBPs.

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253.10

VISUALIZATION OF BASAL OPTIC TRACT TERMINALS BY ANTEROGRADE TRACERS. J. Martin, N. Kogo and M. Ariel.* Dept. of Anatomy & Neurobiology, Saint Louis Univ., St. Louis, MO 63104.

The present study examined the light microscopic appearance of direction-sensitive retinal ganglion cell axons terminating in the turtle's basal optic nucleus (BON). These axons were labelled by injections into the basal optic tract (BOT) that was visualized on the ventral surface of the in vitro turtle brainstem. Either biotinylated dextran amine was iontophoresed extracellularly into the BOT or neurobiotin was injected intracellularly into physiologically identified axons. After up to 16 hours for tracer transport, the brains were fixed in 4% paraformaldehyde, sectioned parasagittally at 100 µm, soaked in ABC-Triton, reacted with diaminobenzidine and counterstained.

Analysis of extracellularly filled retinal axons revealed about three times more axons just inside the BON's rostral border than at its caudal border and thick (2-4 µm) axons within 100 µm from the ventral border with thin (<2 µm) axons positioned throughout the nucleus. Of all the labelled axons, only thin axons extented caudally from the nucleus, perhaps indicating some labelling of non-BOT fibers. The intracellular filled axons were morphologically similar to the extracellular findings of size and position within the BON. All had thick main axons that run along the BON's ventral surface, from which primary branches extended obliquely within the BON. From the primary branches, smaller branches arborize to form beadlike varicosities or boutons that clustered within a region of 50 µm or less. These clusters may account for areas of synaptic contact onto BON cells of specific direction preferences. (Supported by NS 33190 to MA).

253.11

DIRECTION-SENSITIVE IPSP INPUT TO THE ACCESSORY OPTIC SYSTEM OF TURTLE. N. Kogo* and M. Ariel. Department of Anatomy and Neurobiology, Saint Louis Univ., St. Louis, MO 63104.

We described last year the unitary EPSP inputs to the Basal Optic Nucleus (BON) of turtle using a reduced in vitro brainstem preparation in which the retinas remained attached but many dorsal structures were removed, i.e. tectum, cerebellum and dorsal thalamus. In that preparation, we observed IPSPs on only few occasions. However, when an intact brainstem preparation is used, we find that the probability of spontaneous IPSPs in the BON cells is dramatically increased. To see the IPSPs clearly, we added QX314 to the patch pipette solution to block Na^+ spikes so that the membrane potential could be depolarized in the current clamp mode. Holding the membrane potential at -25 to 0 mV, large spontaneous IPSPs were regularly observed.

When the contralateral optic nerve was stimulated during this recording, a prolonged IPSP was also observed after the fast EPSP. This evoked IPSP did not follow high frequency stimulation, suggesting that it is mediated by a polysynaptic pathway. More importantly, during retinal stimulation by a moving pattern, IPSPs occurred more frequently during motion that was opposite the preferred direction of the EPSP input. When bicuculline was applied to the bath solution of the brain chamber (separate from the retinal chambers), these IPSPs disappeared and the visual response to pattern motion became entirely direction-sensitive EPSPs. During lidocaine application to the contralateral retina, spontaneous IPSPs still occur. These results suggest that brainstem dorsal structure(s) may mediate an inhibitory input to BON. Direction-sensitive responses are also found in the pretectal lentiform nucleus, making it a likely source of this inhibitory input. (NS 33190 to MA).

254.1

TEMPORAL SENSITIVITY OF COLOR OPPONENT NEURONS IN HUMAN VISUAL AREAS MEASURED WITH FMRI S.A. Engel*[1], and B.A. Wandell[2], [1]Dept. of Psychology, UCLA, Los Angeles, CA; [2]Dept. of Psychology, Stanford University, Stanford CA

Primate cortex contains opponent color neurons that are excited by some wavelengths of light and inhibited by others (e.g. blue vs.yellow). Previously, we have used fMRI to detect the presence of opponent neurons by measuring the amount of stimulus contrast needed to generate a criterion response for various colored stimuli. For example, more contrast is needed to generate a criterion response to a mixture of blue and yellow than to either light alone. Here we report that the relative sensitivity of these mechanisms changes with the temporal frequency of the stimulus. Subjects viewed color contrast reversing patterns that alternated with a uniform field at 1/42 Hz. The patterns reversed at either 1 Hz, 4 Hz, or 10 Hz. Eight planes of fMRI data were acquired in the occipital lobe every three seconds (TE = 40, TR = 750, FA = 70). Responses were measured as the amplitude of the 1/42 Hz harmonic that best fit the fMRI signal. Responses were averaged within areas that were identified as V1 or V2 based on functional maps of their retinotopic organization. At low temporal frequencies (1 Hz) data from areas V1 and V2 indicate the presence of opponent colors neurons. For example, more contrast is needed to produce a criterion response to a sum of blue and yellow stimuli than to either stimulus alone. At higher temporal frequencies (10 Hz) data from areas V1 and V2 mainly reflect the activity of L+M cone (luminance) neurons. Hence, the relative amount of fMRI signal arising from blue-yellow opponent neurons decreases rapidly with increasing temporal frequency. This low-pass temporal sensitivity, present in V1 and V2, resembles the results of psychophysical measurements of human opponent colors mechanisms.

Supported by EY03164 and the McDonnell-Pew Foundation.

254.2

CHROMATIC PROPERTIES OF NEURONS IN STRIATE CORTEX OF THE MACAQUE MONKEY. **A. Hanazawa*, I. Murakami, H. Komatsu, Lab. of Neural Control, Natl. Inst. for Physiol. Sci., Myodaiji, Okazaki 444, Japan.**

Chromatic properties of neurons were studied in striate cortex of a macaque monkey performing a visual fixation task. Color selectivity of neurons was studied by using stimuli evenly distributed on the C.I.E. xy-diagram and was characterized with respect to summation of cone signals. Seventeen of seventy-eight neurons (22%) showed color selectivity which coincided with an assumption of linear summation (fitting to a model, $p < 0.01$). Most of the other neurons were tuned to a limited extent of hue or saturation with a manner indicative of some nonlinear processing.

We found that many striate neurons have sensitivity to center-surround combination of colors or to color contrast. The responses of most neurons (20/22) to a small stimulus were suppressed by a large surrounding stimulus. Usually, such suppression was caused by the same and near colors as that of the center stimulus. In addition to this, we also observed suppression by distant colors. In some neurons, suppression was always induced by particular colors regardless of the color of the center stimulus. In other neurons, the surrounding color that induced suppression depended on the center color.

These results suggest that striate cortex plays an important role in constructing sharp tuning to the color from opponent color representation and in detecting color contrast.

Supported in part by a grant from the HFSPO and by JSPS fellowship for Japanese Junior Scientists.

254.3

MODULAR ARCHITECTURE OF CELLS PROJECTING FROM PRIMARY VISUAL CORTEX (V1) TO AREA MT J.D. Boyd* & V.A. Casagrande. Depts of Cell Biology & Psychology, Vanderbilt University, Nashville, TN 37232

Within primate V1, patterns of anatomical connections form the structural bases for parcelating different types of visual information. Thus, connections differ radially between layers and tangentially between cortical modules defined by high and low cytochrome oxidase (CO) activity. CO blobs and interblobs are centered within layer IIIB and cells in these zones send projections to different compartments of area V2. Although CO periodicities that align with the CO blobs have been reported to occur in cortical layers above and below layer IIIB, consistent differences that relate to the periodicities in these other layers have not been identified. In this study we examined the tangential organization of cells in V1 projecting to area MT in the bush baby, a prosimian primate.

Injections of cholera toxin B conjugated to colloidal gold (CTB-Au) were made in four bush babies. Cortical hemispheres were either flattened and cut tangentially or blocked and cut parasagitally. Sections were reacted for CTB-Au to identify retrogradely labeled cells in V1 and/or CO to identify CO compartments and layers. A few sections were stained for myelin to verify that injections were in MT.

The main finding was that V1 cells projecting to area MT formed a patchy mosaic in which the highest concentrations of retrogradely labeled cells were located in layer IIIC (IVB of Brodmann) directly below the centers of the CO blobs in IIIB. As predicted from the work of others, MT injections also resulted in bands of labeled cells in V2 with wider center to center spacing than the clusters in V1, as well as clusters of labeled cells in area DL with similar spacing to that of V2. These findings are in agreement with some reports indicating that layer IIIC consists of at least two anatomical compartments, only one of which projects to MT. At present, the functional significance of these compartments remains to be determined. Supported by EYO1778, EYO3778, EYO8126, AND HD15052.

254.4

EXCITATORY AND INHIBITORY SYNAPTIC CIRCUITS OF PARALLEL PATHWAYS TO THE CO BLOBS OF PRIMATE VISUAL CORTEX. Y. Ding*, J.D. Boyd & V.A. Casagrande. Depts of Cell Biology & Psychology, Vanderbilt University, Nashville, TN37232-2175

The cytochrome oxidase (CO) rich blob compartments in the primary visual cortex (V1) of primates have been shown to be physiologically and anatomically distinct. These compartments within cortical layer III are unique in that they receive direct thalamic input from the koniocellular (K) lateral geniculate nucleus (LGN) pathway and indirect input via cortical layer IV from the parvocellular (P) and magnocellular (M) LGN pathways. How are these parallel streams integrated within the CO blobs? To answer this question we compared the types of synapses in owl monkeys formed by K axons labeled via injections of WGA-HRP into the LGN with synapses formed by P and M target cells in layer IV labeled via injections of PHA-L. The neurochemical content of both pre- and postsynaptic profiles were identified by postembedding immunocytochemistry for GABA and glutamate. Samples were restricted to CO blobs within cortical layer IIIB. This study demonstrates that all K axons and majority of axons from layer IV within the CO-blobs use glutamate, the major excitatory amino acid as a neurotransmitter. However, K axons and layer IV axons exhibit differences in synaptic relationships to CO blob cells. Two thirds of the K axons terminated on dendritic spines, the majority of which could be identified as glutamatergic. The remaining K axons terminated on dendritic shafts of which about half contained GABA, the major inhibitory amino acid neurotransmitter and the remaining contained glutamate. In contrast, axons from layer IV terminated mainly on dendritic shafts, slightly more than half of which were positive for glutamate. Thus, within the CO blobs the vast majority of direct K pathway axons terminate on excitatory neurons, whereas more than a third of layer IV axons (presumably providing the indirect input from the P and M pathways) terminate on inhibitory interneurons. Taken together, these results suggest that the K pathway and the P and M pathways use different strategies to contribute to the processing of visual information within layer III. Supported by EY01778, EY08126, and HD15052.

254.5

PATCHY CONNECTIONS FROM AREA 17 TO AREA 21A COLOCALIZE WITH CYTOCHROME OXIDASE BLOBS IN CAT VISUAL CORTEX. Bevil Conway, Jamie Boyd and J. Matsubara* Depts Ophthalmology and Anatomy, Univ of Brit Columbia, Vancouver, B.C

We have shown that in cat area 17, the Y- and W-cell projections from the LGN terminate in the lower and upper portions of the cytochrome oxidase (CO) blobs, respectively. We also reported that the cells projecting from area 17 to posterior medial lateral suprasylvian (PMLS) are clustered within the lower portions of the CO blobs. Together, these results suggest that a modular organization to the Y-type processing stream exists in cat area 17, and beyond into PMLS. In this study we report that another group of cells, those in area 17 that project to 21A, is also patchy and colocalizes with the upper portions of CO blobs in cat visual cortex. After tracer injections in 21A, labeled pyramidal cells in area 17 were located throughout L 2/3, but slighly denser in lower L2 /upper L3 and lower L3, reflecting a bilaminar organization in the coronal plane. In flattened sections of area 17, the labeled cells were arranged in tight patches with an interpatch spacing ranging 0.5-0.9 mm. Alternate serial sections stained for CO revealed that the labeled cells were localized to the CO blobs. Since the pattern of patchy labeling arising in area 17 from injections in 21A so resembled the labeling arising after PMLS injections, we undertook double labeling studies to determine whether the same populations of cells in area 17 projected to both areas 21A and PMLS. FITC- and Texas Red-labeled dextrans were injected into 21A and PMLS, respectively, and revealed that separate populations of pyramidal cells projected to each area.

Thus, this study suggests that area 17 is organized into at least two output-defined modules that are marked by CO histochemistry and demonstrates the presence of novel forms of columnar organization that may be related to the segregation of efferent processing streams in area 17 and beyond into extrastriate visual cortex of the cat. (This work funded by MRC).

254.6

INVESTIGATION OF POTENTIAL CO-LOCALIZATION OF CYTOCHROME OXIDASE WITH NOS OR NMDAR1 IN MACAQUE V1 and V2 NEURONS. B.J. Anderson* and M.T.T. Wong-Riley. Dept. of Cellular Biology & Anatomy, Med. Coll. of Wis., Milwaukee, WI 53226.

Nitric oxide (NO) is a gaseous intra- and inter-cellular messenger in the CNS. NO production can be triggered by the activation of NMDA and other glutamate receptors. Previously, we have shown that cytochrome oxidase (C.O.)-rich non-pyramidal neurons in layers 2/3 of macaque V1 received glutamatergic synapses on their somata. The present study addressed whether neurons immunoreactive for NOS or NMDA receptor subunit 1 (NMDAR1) had high levels of C.O. activity. Macaque striate cortical sections were reacted for C.O. histochemistry followed by immunogold silver staining for NOS or NMDAR1. We found two populations of NOS-immunoreactive (ir) neurons: (a) a relatively rare population of intensely-labeled, small-to-medium sized neurons with low levels of C.O. activity; and (b) moderately-labeled neurons that varied in size, location, and C.O. levels. Medium-sized cells of the latter group had moderate to high levels of C.O. in puffs, but low to moderate levels of C.O. in interpuffs. In layer 4B, large NOS-ir non-pyramidal neurons were darkly reactive for C.O. In 4C, some, but not all, NOS-ir neurons were rich in C.O. In layers 5 and 6, C.O.-rich cells were often NOS-ir, but C.O.-poor ones could be either NOS-positive or negative. NMDAR1-ir neurons were most common in layer 2, where C.O. activity was relatively low. A few NMDAR1-ir in layers 4, 5 and 6 had moderate to high levels of C.O. In V2, NOS was heterogeneously distributed among C.O.-rich and -poor cells. In layers 3/4, cells intensely stained for NOS were again low in C.O., while cells with moderate amounts of NOS had moderate levels of C.O. However, cells darkly reactive for C.O. did not exhibit NOS-ir. Scattered neurons in layers 2, 3, and 4 were NMDAR1-ir with variable C.O. levels. Our data suggest that NOS-ir and NMDAR1-ir neurons in V1 and V2 have variable demands for oxidative metabolism. Supported by NIH R01EY05439.

254.7

HUMAN BRAIN NITRIC OXIDE SYNTHASE: ISOLATION, SUBCLONING, SEQUENCING, AND IN SITU HYBRIDIZATION OF TRANSCRIPTS IN MACAQUE STRIATE CORTEX. M. Wong-Riley* and Z. Huang. Dept. of Cellular Biology & Anatomy, Medical College of Wisconsin, Milwaukee, WI 53226.

We have previously shown that cytochrome oxidase-rich zones in the macaque striate cortex contained higher levels of neurochemicals related to excitatory glutamatergic synapses, such as NMDA receptors and nitric oxide synthase (NOS). As a first step to determine if the expression of NOS in the macaque visual cortex is regulated at the transcriptional level, we isolated NOS cDNAs from a human occipital cortex cDNA library by PCR, subcloned them into the plasmid pCR™ vector (InVitrogen), and transformed into OneShot competent cells (InVitrogen). Sequence analysis confirmed that human brain NOS cDNAs were isolated. Riboprobes were generated by *in vitro* transcription, labeled with digoxigenin or with α-^{35}S-thioUTP (uridine 5'-[α-^{35}S-thio] triphosphate), and applied to monkey brain sections by *in situ* hybridization. Results indicate that NOS mRNAs were restricted to specific neurons in the primary visual cortex. Sense controls showed no labeling. Studies are underway to determine if NOS gene expressions are regulated by neuronal activity. Thus, adult primate visual neurons have the transcriptional machinery to produce NO, an important intra- and inter-cellular messenger, which is likely to play a critical role in excitatory synaptic circuitry within the striate cortex. Supported by NIH grant R01EY05439.

254.8

THE DISTRIBUTION OF NADPH-DIAPHORASE/NITRIC OXIDE SYNTHASE (NOS) CELLS WITHIN THE LAYERS AND FUNCTIONAL COMPARTMENTS OF PRIMATE V1 AND V2. A. E. Wiencken and V. A. Casagrande*. Dept. of Cell Biology, Vanderbilt University, Nashville, TN 37232-2175

Nitric oxide, a proposed neurotransmitter, is speculated to be involved in a large number of neural processes. Data gathered by our lab and others have demonstrated that high levels of NADPH-diaphorase (a histochemical marker for NOS) co-localize with high cytochrome oxidase (CO) activity within the layers and functional compartments of V1 and V2 in several primate species. In V1, NADPH-diaphorase is dense within layer IV and the CO blobs in layer III, while in V2, NADPH-diaphorase activity colocalizes with the thick and thin CO stripes. Our objective was to determine which cells were responsible for NOS positive neuropil activity in the functional compartments of V1 and V2 and to further characterize these cells. We used both direct and indirect histochemical methods as well as immunocytochemistry to identify NOS containing cells and processes in V1, V2, the LGN and pulvinar of bush-babies, owl monkeys, and squirrel monkeys. CO histochemistry and Nissl staining were used to identify layers and compartments in adjacent sections. Results show that NADPH/NOS cell staining is absent from the LGN and pulvinar suggesting that NOS positive neuropil staining in V1 and V2 arises from cortical cells. Two main classes of non-pyramidal NADPH/NOS neurons can be identified in V1 and V2 of all three species; a more numerous lightly staining population with small somata, and a darkly staining population that occurs at low density. With the exception of layer IV of V1, which contains a lower density of darkly stained NADPH/NOS positive cells, no relationship was found between the distribution of NADPH/NOS cells and the dense NADPH-diaphorase positive neuropil in V1 and V2. Asymmetrical dendritic/axonal distributions of the larger, more densely labeled NADPH/NOS cells is one possible source of the cortical neuropil staining. However, the low density of the darkly labeled NADPH/NOS cells, as well as the relatively high density of the darkly stained network of axons that could be observed in the functional compartments of V1 and V2 are suggestive of another extrinsic source. Supported by grants EY01778; EY07135; EY08126; and HD15052.HD15052.

254.9

COVERAGE OF THE VISUAL CORTEX BY NITRIC OXIDE GENERATING PROFILES. F. Hester*, J. Gemmill, P.R. Montague† and M.J. Friedlander. Neurobiology Research Center, University of Alabama at Birmingham and Division of Neuroscience, Baylor College of Medicine†.

Nitric Oxide (NO) has been proposed to act as a diffusible chemical messenger in the central nervous system. Endogenous cortical NO production that results from N-methyl-D-aspartate receptor (NMDAR) activation enhances the subsequent release of other neurotransmitters, including glutamate and norepinephrine (Montague, et al., Science, 263:973, 1994). Thus, we proposed that NO can functionally link cerebral cortical synapses. This requires substantial coverage of the cortex by NO-generating cellular processes from a small subpopulation of nitric oxide synthase (NOS) positive neurons. We have quantified the volumetric coverage of the cortex by NADPH diaphorase positive neuronal varicosities in three-dimensional space. Two adult tri-colored guinea pigs were perfused with 2% glutaraldehyde. Vibratome sections through visual cortex (area 17) were made at 100 µm, tissue was processed for NADPH diaphorase activity, sections were post-fixed in 1% OsO_4, dehydrated in 100% ethanol, embedded in Spurr's plastic (without propylene oxide) and polymerized. This procedure maintained the 3-dimensional integrity of the tissue (< 5% shrinkage in any plane). Using a 100x oil immersion objective and 3 dimensional digitizing microscope workstation, the X- Y- and Z- coordinates were digitized for each NADPH diaphorase positive neuronal varicosity (n=2271 varicosities, 6 sections) in 100 µm cubes of supragranular, granular and infragranular cortical layers. The average density is 1.1, 0.9 and 1.0 x 10^6 varicosities per mm^3 in cortical layers 2/3, 4 and 5/6, respectively with nearest neighbor distributions of 14.9 ± 0.5 µm, 22.2 ± 0.8 µm and 20.7 ± 0.8 µm. Thus, the visual cortex is richly covered by NADPH diaphorase positive profiles suggesting the capacity for generation of a substantial NO signal in a cortical volume.

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254.10

ORIENTATION MAPS OF SUBJECTIVE CONTOURS IN CAT V1 AND V2. B.R. Sheth*, J. Sharma and M. Sur, Department of Brain and Cog. Sci. MIT, Cambridge, MA 02139.

We investigated responses to subjective contours in visual cortical areas V1 and V2 in adult cats, using optical imaging of intrinsic signals and single-unit recording. The imaging experiments demonstrate that V2 contains a map of the orientation of subjective gratings characterized by a modular representation of orientations and singularities at the intersection of orientation domains. Neurons that prefer similar subjective and luminance orientations are clustered separately from neurons that prefer different orientations, leading to an elaborate pinwheel-like representation of neurons with adjacent response preferences. V1 contains fewer neurons that respond to subjective gratings but these are also organized in modular fashion. Our single-unit studies show that many more cells in V2 (7 of 28 cells) compared to V1 (1 of 28 cells) respond to the identical orientation of luminance and subjective edges. However, many cells in V1 (9 of 28 cells) are partially responsive to subjective edges. Electrode penentrations made normal to the cortical surface reveal that most neurons in the same column respond in a similar manner to subjective contours. These data suggest that a strong response to subjective edges does not arise *de novo* in V2; instead, considerable preprocessing of the perceptual signal takes place in V1. Thus, V1 and V2 process subjective contours in tandem, via single neurons that are organized into maps specialized for the representation of subjective edges. Supported by EY07023 (MS), a McDonnell-Pew Fellowship(BRS) and a Fogarty Fellowship (JS).

254.11

RESPONSE PROPERTIES OF NEURONS IN AND NEAR PINWHEEL-CENTERS IN ORIENTATION PREFERENCE MAPS OF CAT VISUAL CORTEX. I. Gödecke[1], P.E. Maldonado*[2], C.M. Gray[2], and T. Bonhoeffer[1]. [1]Max-Planck-Institut für Psychiatrie, 82152 München-Martinsried, FRG, [2]Center for Neuroscience, University of California, Davis, CA 95616, USA

In the mammalian visual system, optical imaging techniques have shown that neurons responding best to different orientations form highly ordered patches which are organized in a pinwheel-like fashion around "orientation centers". While these imaging techniques have the advantage of getting information about large areas of cortex they have a limited spatial resolution which is at best 100 µm. This circumstance hinders gaining information about the detailed arrangement of neurons and their response properties which is of particular interest in some unique locations in cortical maps such as pinwheel-centers. Do neurons show reduced orientation selectivity the closer they are to the centers? Or are these centers literally singularities where adjacent neurons are precisely tuned, but to radically different orientations?

In order to answer these questions, we have employed the tetrode technique which allows accurate discrimination of individual neuronal spike trains from multiple single unit recordings. We could record up to eight different cells from the vicinity of the tetrode tip, within an estimated radius of 65 µm. In our experiments we used intrinsic imaging to obtain orientation preference maps from area 17 and 18 in seven cats. Subsequently, tetrodes were utilized to precisely determine the response properties of single neurons in- and outside of orientation centers. These recordings revealed that neurons in pinwheel-centers are as selective for stimulus orientation as those in other locations of the cortex. The scatter of the preferred orientation, however, is markedly higher than in the rest of the cortex. In 19% of the recording sites in pinwheel centers (N=81) the largest difference in orientation preference for all cell pairs exceeded 60°, compared to 7% at recording sites outside such centers (N=76). In 8 orientation centers, we found cell pairs with orthogonal preferences (> 80°). This indicates that the orientation centers can be considered "singularities" where iso-orientation patches of all orientation preferences converge onto a single point. Supported by the Max-Planck-Gesellschaft and by the NIH # MH46428.

254.12

SEPARABILITY OF STRENGTH AND LATENCY IN THE RESPONSES OF STRIATE CORTICAL COMPLEX CELLS TO STIMULUS ORIENTATION AND CONTRAST T.J.Gawne* and B.J.Richmond. Lab of Neuropsychology, National Institute of Mental Health, Bethesda, MD 20892.

Previously we showed that the response strength of complex cells is primarily a function of stimulus orientation, while the response latency is more strongly affected by the cue that defined the stimulus, e.g., contrast (Gawne et al., 1994). However, the separation of response strength to orientation and latency to contrast is not perfect, especially at low contrasts. Latency in particular is strongly affected by both orientation and contrast. We report here that the relationships between the response parameters strength and latency, and the stimulus parameters orientation and contrast, can be accurately modeled as two equations in two unknowns.

$$Magnitude = a_1\cos(\theta) + a_2\log_{10}\alpha + a_3 \quad (1)$$

$$Latency = b_1(2 - \log_{10}\alpha)\cos(\theta) - b_2\log_{10}\alpha + b_3 \quad (2)$$

where *Magnitude* is in spikes/sec, θ is the orientation angle from 0 to π, α is the contrast in percent, and *Latency* is in ms.

We recorded from 37 striate cortical complex cells in an awake primate that were presented with flashed, stationary bars of contrast from 5 to 94%, and of 12 evenly distributed orientations. This model accounted for 61.2±2.3% of the variance of the response strength and 67.5±3.4% of the variance of the response latency, the same accounted for by a general ANOVA model using many more parameters (23). The nervous system could make use of response latency and strength together, solving the problem of two equations in two unknowns to eliminate the problem of a less than perfect correspondence between strength and orientation, and latency and contrast. Support: IRP/NIMH/NIH.

254.13

A MODEL OF SIMPLE-CELL ORIENTATION TUNING: FEEDFORWARD TUNING AND CORRELATION-BASED INTRACORTICAL CONNECTIVITY. A.E. Krukowski*, N.J. Priebe and K.D. Miller, Graduate Groups in Biophysics and Neuroscience, W.M. Keck Center for Integrative Neuroscience, and Department of Physiology, UCSF, San Francisco, CA 94143-0444.

We study a model 2-D sheet of layer IV simple cells in cat primary visual cortex, to determine which neuronal and synaptic characteristics and which connectivity schemes are consistent with observed functional responses. We use conductance-based integrate-and-fire models of regular-spiking (adapting) excitatory and of fast-spiking inhibitory cells, as well as four time-varying synaptic conductances (ampa, nmda, gabaA and gabaB). Inputs come from 2-D LGN sheets of ON and OFF cells, modeled as contrast-dependent filters producing instantaneous firing rates from which Poisson spike trains are generated.

We first consider tuning in the absence of intracortical connections. Gabor-shaped receptive fields with experimentally constrained aspect ratios and numbers of subfields robustly account for the sharpness of afferent orientation tuning reported by Ferster et al. (1996), who studied tuning of the 1st harmonic of voltage response to sinusoidal gratings. While we replicate these results, we also find that the mean (0th harmonic) responses are not tuned. Thus, the spike rate of simple cells receiving only feedforward geniculate input would not be well tuned, particularly at high contrast which creates high mean input levels.

Intracortical connectivity must suppress this mean input, and achieve contrast invariance in orientation tuning. We are studying schemes in which intracortical connectivity is determined by the degree of correlation between two cells, as would result from Hebb-type learning rules. Intracortical patterns of excitatory and inhibitory connectivity then have similar orientation tuning, as observed experimentally; differences primarily involve spatial phase rather than orientation. Preliminary results show that such schemes can achieve realistically tight, contrast-invariant orientation tuning. We are exploring whether other experimental constraints on an orientation model can also be satisfied.

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254.14

THE RESTRUCTURING OF VISUAL CORTICAL ORIENTATION PREFERENCES AFTER A LESION DESCRIBED BY A LAMINAR MAP MODEL. D. M. Gorinevski[1]*, F. Wörgötter[1], U. T. Eysel[1], W. v. Seelen[2]. Dept. of Neurophysiol.[1], Inst. for Neuroinformatics[2], Univ. of Bochum, 44780 Bochum, Germany.

A linear model based on two continuous scalar neural fields for thalamic and cortical neural activities was developed to simulate maps of cortical orientation selectivity as revealed by optical recording of intrinsic intracortical signals in the visual system of the cat. We restricted the system to a steady state as a possible fit for the slow intrinsic imaging signals and used inhomogeneously distributed anisotropic interaction kernels to represent the functional anatomy of the thalamo-cortical and the intracortical connections. A radial basis function network was used for the approximation of stimuli, cortical signals and intracortical interactions. Under these assumptions we could reproduce the well-known parameters of cortical maps such as realistic receptive fields and point-spread functions by re-transformation of the resulting integral equation to an equivalent form with solely feed-forward interactions applying the Galerkin method. The geometrical organization of the experimentally recorded high definition maps of orientation preference and tuning strength was then approximated by the modelled interaction geometry. We have simulated the changes in the mapped preferred orientation and tuning strength induced by thermal intracortical lesion. These changes were characterized by the loss of intracortical inputs from the area destroyed by the lesion and could be modelled. Consequently, we were able to quantify the relative weights of the thalamo-cortical and the intracortical interaction components in the model.

Thereby, we introduced a quantitative decomposition of the measurable intracortical functions into these two components, which normally cannot be quantified directly because they are interacting with each other. This way, the model covers the aspect of sub- and intracortical connectivity not only in the existing orientation maps but it can also to some degree explain intracortical plasticity after lesioning.

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254.15

SPATIO-TEMPORAL PATTERNS OF SPONTANEOUS ACTIVITY AND ORIENTATION TUNING IN THE SURROUNDING OF EXCITOTOXIC LESIONS IN THE CAT VISUAL CORTEX. G. Schweigart*, U.T. Eysel. Dept. of Neurophysiology, Ruhr-Universität Bochum, D-44780 Bochum, Germany.

Based on the hypothesis that single cell properties in the visual cortex are dependent on the functional state and anatomical integrity of the cortical neuronal network we were interested in the intracortical spatio-temporal activity pattern following small localized excitotoxin injections.

Single cells were recorded extracellularly in the anesthetized adult cat visual cortex (area 17 and 18) with a linear array of 7 tungsten in glass electrodes with inter-electrode distances of 1 mm. Control recordings of spontaneous activity, RF maps and orientation tuning were obtained for each electrode position prior to injection of 1-2 µl 1% ibotenic acid close to one of the recording electrodes. The cats were kept at background illumination for 12 hrs and the activity was continuously measured. Thereafter RFs were remapped and orientation and direction tuning was again tested with moving light bars.

Immediately after injection the spontaneous activity at the nearest electrodes displayed short episodes of high frequency discharges followed by a complete loss of activity. At electrodes 2-3 mm away from the injection site significant increases of average activity, characteristic bursts, and transient periods of synchronization between the still active recording sites were observed. When retested between 12 and 24 hrs RF size appeared unchanged while the orientation and direction specificity was significantly reduced or abolished at electrodes with increased maintained activity close to the lesion. With increasing time post lesion a sharpening of orientation tuning and recovery of direction specificity was often observed at these sites accompanied by a significant reduction of background activity. Most interestingly the preferred orientations recorded at the very same sites before and after lesioning could display remarkable shifts.

The reappearance of specificity combined with a shift of preferred orientation in given orientation columns indicates lesion-induced cortical plasticity associated with the early phase of functional recovery.

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254.16

FMRI SIGNAL AMPLITUDES REPRESENT STIMULUS CONTRAST IN HUMAN VISUAL AREAS V1, V2, AND VENTRAL OCCIPITAL CORTEX. M.R. Peters, B.J. Anderson, K.A. Williams, D.P. Miller*, and E.A. DeYoe. Dept. of Cellular Biology and Anatomy, Medical College of Wisconsin, Milwaukee, WI 53226. *Div. of Psychology, Carthage College, Kenosha, WI 53140.

The human psychophysical contrast response function (CRF) is a power function with an exponent of 0.7. However, most neuronal CRFs in macaque V1 are best described as hyperbolic functions (Albrecht & Hamilton, 1982). To identify potential sites for the locus of the contrast magnitude percept, FMRI signal amplitudes from twelve levels of stimulus contrast were cross-correlated with functions representing physical and psychophysical contrast magnitudes, and functions similar to those described for neuronal CRFs.

Subjects estimated contrast magnitude at the beginning of 16 sec presentations of each of twelve sinusoidal gratings. Each grating was presented five times and followed by a 16 sec presentation of a uniform field with the same space average luminance as the gratings. Averages of FMRI signal amplitudes for gratings in ascending order of contrast were cross-correlated with: 1) log of the corresponding psychophysical estimates, 2) log of stimulus contrast and 3) hyperbolic reference functions. FMRI signal amplitudes from voxels in V1, V2 and ventral occipital cortex (lingual gyrus and along collateral sulcus) cross-correlated well with all three reference functions ($r>.82, p<.001$). Although functions correlating best varied from voxel to voxel, clusters of voxels with high correlations were found in the same or similar locations in V1, V2 and ventral occipital cortex regardless of response function used. Correlations were not as strong outside of these areas.

Supported by NIH EY10244 (to EAD) and Chapman Foundation of Milwaukee (to MRP).

254.17

FUNCTIONAL MRI IN ANESTHETIZED SQUIRREL MONKEYS Stacy S. Klinke*, Jingna Wei, Donald Devo and Michael J Quast Univ. Of Texas Med. Branch, Galveston, TX 77555-1143

In order to demonstrate functional brain imaging in anesthetized primates, quantitative measurements of regional cerebral blood volume (rCBV) in squirrel monkeys were calculated using contrast enhanced magnetic resonance imaging (MRI) techniques. Three female *Saimiri Sciureus* monkeys were anesthetized with isoflurane in N_2O/O_2. Monkeys were positioned on a 6.5 cm. proton surface coil with fiber optic lights directed at both eyes for photic stimulation. High resolution multislice spin echo (SE) imaging was performed on a 4.7 T MRI system (Varian, Palo Alto, CA) before and after intravenous injection of a superparamagnetic iron oxide contrast agent (Advanced Magnetics, Inc., Cambridge, MA) during periods rest and photic stimulation. Our in plane spacial resolution of 0.23 mm/pixel was sufficient to resolve the stripe of Gennari, which had higher blood volume than other cortical layers in the primary visual cortex. The rCBV's (ml/100g tissue) from selected brain regions are reported (mean±SE):

Region	CBV (off)	CBV (on)	% change
V1 cortex	1.96±0.26	2.28±0.28	16.3
Lateral geniculate	3.08±0.48	3.57±0.52	15.9
Superior colliculus	1.72±0.31	2.07±0.28	20.4
Non-vis. thalamus	1.92±0.19	2.11±0.14	9.9

Statistically significant changes were detected in the visual cortex ($p=.0171$) and V1 grey matter ($p=.0085$) using a one-way ANOVA. Regionally specific alterations in cerebral blood volume were observed in anesthetized squirrel monkeys during visual stimulation using high resolution rCBV imaging. Supported in part by the Whitaker Foundation.

254.18

CONTRAST- AND CONTEXT-DEPENDENT MODULATION OF SINGLE-CELL RESPONSES ELICITED IN CAT AREA 17 BY COMPOUND STIMULI K. Mizobe, T. Kasamatsu *, U. Polat and A.M. Norcia The Smith-Kettlewell Eye Research Institute, San Francisco, CA 94115

Visuocortical cells change response magnitude when their classical receptive field (CRF) is concurrently stimulated with a second stimulus outside the CRF. The biological significance of this interaction is mostly unknown. Recently, collinear (i.e., co-axial and co-oriented) stimuli are shown to reduce the contrast detection threshold of foveally viewed targets in human psychophysics (Polat & Sagi, 1994) and to augment the amplitude of human VEPs at low contrast (Polat & Norcia, 1996).

Recording from area 17 of anesthetized and paralyzed cats, here, we studied how single cortical cells modified their responses when stimulated by contrast reversal of collinearly presented Gabor patches. A Gabor patch was optimally fitted to the CRF in isolation or together with two flanking high-contrast Gabors. The latter had either the same or different orientation as that of the center patch and was presented outside the CRF. The contrast threshold was estimated by a contrast-sweep method. We found: 1) Significant modulation, including both facilitation and suppression, of the CRF responses was obtained when the three Gabors were arranged collinearly. 2) For many cells the effect was clearly contrast-dependent, with facilitation at near-threshold contrast of the target and suppression at supra-threshold contrast. The latter may be mediated through both $GABA_A$ and $GABA_B$ receptors (Mizobe et al., Abstr. Soc. Neurosci., 21, 1995). *Elongated collinear-interaction fields* maintained by short- and long-range lateral connections may underlie perceptual grouping. Supported by SKERI and EY06579.

254.19

CONTRAST ADAPTATION EFFECTS MODELED AS THALAMOCORTICAL AND INTRACORTICAL SYNAPTIC TRANSMISSION CHANGES. D.C. Somers*, E.V. Todorov, A.G. Siapas, and S.B. Nelson°, Dept. of Brain & Cognitive Sciences, MIT, Cambridge, MA and Dept. of Biology°, Brandeis University, Waltham, MA.

Visual cortical neurons exhibit reduced responsiveness after exposure to high contrast stimuli. Several proposed adaptation mechanisms, such as cellular "fatigue" and inhibitory synaptic processes, fail experimental tests. Here, we investigated effects of activity-dependent synaptic efficacy within a cortical circuit model using analysis and simulation; transmission failure probability ("synaptic fatigue") at each thalamocortical and intracortical synapse varied with its recent average synaptic activity level. Prior to investigating adaptation effects, cortical connections were tuned as follows: orientation-selective, cortical excitation strongly amplified low contrast thalamocortical input while inhibitory inputs increased at high contrasts to yield saturating contrast response functions (CRFs). Reduction of efficacy at thalamocortical synapses was observed to shift CRFs rightward along the log contrast axis. Fatigue of intracortical excitatory synapses lowered CRF slope and inhibitory synaptic fatigue raised saturation response levels. Combination of these adaptation mechanisms into a single model yielded a contrast gain control mechanism (cf. Ohzawa et al. '85) which centered the steepest (and most sensitive) part of the CRF near the adapting contrast level for a broad range of contrasts. The synaptic fatigue model accounts for data that cellular fatigue and inhibitory pool models cannot: adaptation effects are stimulus-specific and hysteresis effects for staircase presentation of stimulus contrasts were larger than orientation hysteresis effects (cf. Bonds '91); all efficacies changed during contrast sweeps, but only cortical synapses changed during orientation sweeps. McLean & Palmer (ARVO '96) recently reported that contrast adaptation is eliminated by blockade of pre-synaptic glutamate autoreceptors. Our results are consistent with that finding; disabling changes in excitatory transmission abolished adaptation. However, best model results were obtained with fatigue of all synaptic classes. Thus, we predict that adaptation stimuli yield increased responses under blockade of glutamate autoreceptors and that saturation responses decrease under blockade of GABA autoreceptors only.

254.20

Calcium coding and computation in cortical pyramidal neurons: spike adaptation, contrast Enhancement, and decorrelation. Xiao – Jing Wang* Center for Complex Systems and Department of Physics, Brandeis University, Waltham, MA 02254, USA.

Based on data from recent dendritic recording and calcium imaging experiments, and by computer simulations of a two-compartment conductance model, we investigate the interplay between voltage-dependent electrical activity and the intracellular calcium dynamics in cortical pyramidal neurons. A main characteristic of these cells is spike adaptation when subject to a constant stimulus. The time course of spike adaptation can often be approximated by a mono-exponential law, $f(t) = A + B\exp(-t/\tau_{adap})$, where $f(t)$ is the instantaneous firing rate. By assuming that the spike adaptation is produced mainly by a calcium-dependent potassium conductance (g_{AHP}), we derive this exponential law semi-analytically, and relate the adaptation time constant (τ_{adap}) with cellular parameters such as g_{AHP} and the calcium decay time constant. By the same token, we introduce the notion of "calcium modes" when the calcium conductances are distributed over a multi-compartmental dendrite.

The spike adaptation property endows the pyramidal neurons with interesting computational functions. With our model three phenomena are demonstrated here. (1) When the input consists of a periodic train of pulses, the response of the cell is sharply tuned to the lower input frequencies, similar to the observed contrast adaptation in the visual cortical neurons. (2) If an input is random and correlated in time, the signal is decorrelated in the cell's output ("efficient coding" and "novelty detection"). (3) In the presence of two or several inputs of slightly different amplitudes, the cell can selectively respond to the strongest input and suppresses the others ("selective attention").

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VISUAL CORTEX: STRIATE IV

255.1

THE LATERAL-POSTERIOR COMPLEX: ITS INVOLVEMENT IN STIMULUS-DEPENDENT OSCILLATIONS AND ITS RELEVANCE TO THE BINDING HYPOTHESIS, S. Molotchnikoff and S. Shumikhina* Département de sciences biologiques, Université de Montréal, C.P. 6128, succursale centre-ville, Montréal, Québec, H3C 3J7, Canada.

The lateral posterior complex (LP-P) is a vast thalamic structure which may represent an interface linking virtually all striate and extra-striate areas through afferent and efferent connecting fibers. Hence the LP-P is in a key position to influence visually evoked cortical responses. We propose that the **LP-P is a major player in the neuronal computation which leads to binding several components of a single image**. The general strategy is to record single cell evoked activity in the visual cortex with stimuli suitable to generate γ-range oscillation within their responses. In a second step the LP-P is deactivated with GABA micro-injections. Experiments are carried out on anaesthetised cats. Results are summarized as follows: 1) the LP-P cells exhibit oscillations (γ-range) which are tied to specific properties of the target. 2) A blockade to the LP-P results in a) a sharp decline of the cortical stimulus dependant oscillation, b) a decrease of oscillatory synchronisation between two cortical sites, and c) an absence of differential responses when additional targets are introduced in the visual field. Indeed when the LP-P is depressed, the magnitude of cortical discharges remains unaffected regardless of the number of targets applied. This latter effect may be due to LP-P influences on the impact of the receptive field's periphery. The LP-P may both modulate the cortical stimulus-dependant oscillations and contribute to the formation of synchronised cellular assemblies.

Supp: NSERC and FCAR

255.2

SYNCHRONOUS ACTIVITY BETWEEN PRIMARY VISUAL AND SENSORIMOTOR CORTEX IN THE AWAKE BEHAVING CAT. C. Chiang*, A. von Stein, and P. König, The Neurosciences Institute, 10640 John J. Hopkins Drive, San Diego CA 92121.

Previous work in anesthetized cats has demonstrated that the brain may use temporal coding to process vision, where synchronous neural activity in visual cortex serves to bind related features of a scene into a perceptual whole. It has been proposed that such synchronicity may be a common feature to other aspects of brain function as well. However, evidence that the temporal coding scheme is relevant to actual behavior has been largely indirect (Roelfsema et al., 1995).

Here, we examined the activity of primary visual cortex (area 17) and sensorimotor association cortex (area 7) during a visuomotor paradigm in the awake cat. Cats were alternately presented with two different visual stimuli and trained to respond by either watching one (condition 1, pure vision) or by pressing a lever after seeing the other (condition 2, vision and movement required). During the task, we used intracortical electrodes to record local field potentials simultaneously in both area 17 and area 7. Cross-correlation analyses revealed that activity in area 17 and area 7 is more synchronous during condition 2 than during condition 1 (average relative modulation amplitude for slow frequency ranges = 0.22 ± 0.03 for condition 2, 0.11 ± 0.03 for condition 1; $p = 0.02$). Also, activity in one area almost always preceded activity in the other, as the majority of these correlations did not occur with zero phase lag. In addition, for both area 17 and area 7, autocorrelogram amplitude was consistently lower in condition 2 than in condition 1 (condition 2 = 29% of condition 1 for area 17, 56% for area 7), indicating that synchronization of activity within each area was also greater during the part of the task requiring both vision and movement. Our results provide direct physiological evidence that temporal coding may be used to link the neural substrates involved in visuomotor integration.

Supported by Neurosciences Research Foundation.

255.3

EXPECTANCY DRIVEN SYNCHRONIZATION BETWEEN PRIMARY VISUAL CORTEX AND PARIETAL CORTEX IN CATS A. von Stein*, C.Chiang, P.König, The Neurosciences Institute, 10640 John J. Hopkins Drive, San Diego CA 92121.

Findings on visual cortical interactions indicate that neurons of functional ensembles unite into synchronous patterns of activity. So far this synchronization has been shown to be stimulus-induced, reflecting stimulus-specific properties. If synchronization however is a true correlate of perception, it is not enough to show that it can be reproducably induced by stimuli. The formation and ignition of cell ensembles should be dependent on the meaning of a stimulus, i.e. its behavioral relevance and the cat's contextual expectancy.

To determine the role of synchronization for this "top-down"- guided binding into cell ensembles, we recorded local field potentials from different cortical layers and areas in an expectancy paradigm in behaving cats. Cats were trained to attend and discriminate two different visual stimuli. They learned to respond to the one by watching it and to the other by pressing a lever after seeing. From time to time unexpected stimuli were intermingled and their response observed. We found two types of cortical responses. Strong synchronizations between different layers of one column were induced independent of stimulus type and expectancy. They had zero phase lag and expressed in the high frequency ("gamma") range, resembling the classical synchronizing response to visual stimuli. For the synchronization between areas, we found a consistent synchronization between area 17 and sensorimotor associative areas during the motor-response associated stimulus. These interareal synchronizations had their prominent peaks in the slow frequency ranges (e.g. 4-7 Hz) and showed significant phase lags indicating that the associative area might lead over the primary visual area. They seem to be higher between supragranular layers than between infragranular layers. If gamma band synchronizations were present, they had zero phase lag. Our findings indicate that the slow frequencies might play a role in long range cortical interactions. Phase lagged slow frequency synchronizations induced by the animal's expectancy might reflect the top-down driven binding of ensembles into meaningful entities.

Supported by Neurosciences Research Foundation.

255.4

SYNCHRONIZATION OF VISUAL RESPONSES BETWEEN THE CORTEX, LGN AND RETINA IN THE ANESTHETIZED CAT. M. Castelo-Branco, S. Neuenschwander, S. Herculano and W. Singer* Max-Planck-Institut für Hirnforschung, Deutschordenstraße 46, 60528 - Frankfurt a. M., Germany.

Periodic activity is often associated with the synchronization of spatially distributed responses in the cortex. Recently, oscillatory patterning within a broad range of frequencies was described for visual responses in retinal ganglion cells which is reliably transmitted by the lateral geniculate nucleus (LGN), raising the question of how oscillatory inputs contribute to synchronous oscillatory responses in the cortex.

We have made simultaneous recordings from visual areas 17 and 18 as well as the retina and LGN to examine how the occurrence of synchronization in the cortex follows subcortical oscillatory responses. A sliding window correlation analysis was employed to evaluate the development of synchronization of oscillatory responses over time.

Strong synchronization of oscillatory activity may occur between cortical, geniculate and retinal responses, even when the receptive fields are far apart. There is, however, a marked difference between areas 17 and 18 in the incidence of correlation with geniculate and retinal ganglion cells. Twenty-two out of 63 pairs of cells between area 18 and LGN showed significant correlated activity (35%), in contrast to only 4 out of 223 LGN-A17 pairs (1.8%). A similar difference occurs between A18 and A17 and the retina: only 1 out of 22 retina-A17 pairs correlated significantly their responses (4.5%), while 8 out of 15 retina-A18 pairs showed correlated oscillatory activity (53%).

The finding that oscillatory responses in the cortex, LGN and retina are often unrelated excludes a simple feedforward mechanism for cortical synchronization. However, the observation of strong correlated activity between area 18 and the retina and LGN suggests that oscillatory input may indeed contribute to the oscillatory patterning of neuronal responses in the cortex.

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255.5

FAST OSCILLATIONS IN V1 OF AWAKE MONKEY: SYNCHRONIZATION DEPENDS ON CORTICAL DISTANCE, ON ANGLE BETWEEN PREFERRED ORIENTATIONS AND ON STIMULUS ORIENTATION. A. Frien, R. Eckhorn and H. J. Reitboeck*. Neurophysics, Dept. Physics, Philipps-Univ., D-35032 Marburg, Germany.

Fast synchronized oscillations of visual neurons (γ-range: 30-100 Hz) have been proposed to provide a temporal label for linking different features of a visual object into a perceptual entity [Eckhorn et al. 1988, Biol. Cybernetics 60: 121-130]. In the present work we studied the coupling strength among oscillatory signals generated in pairs of spatially separate groups of neurons according to their cortical distance the angle between their preferred orientations and the respective stimulus orientations. Neural group activities were recorded as multi unit activities (MUA) and local field potentials (LFP, 1-141 Hz) from 101 positions (up to 7 electrodes in parallel) from the upper layers of V1 in an awake, fixating monkey. Pairs of recordings were classified according to their horizontal cortical distance (in steps of 0.75 mm up to 4.5 mm) and to the angle between the preferred orientations (0°-30°, 30°-60°, 60°-90°). Coupling strength of recording pairs was quantified by the peaks in the γ-range (i) for the coherence and (ii) for normalized cross-spectra. For comparison to overall activity we used the center peaks of cross correlograms. Visual stimuli were sinusoidal gratings (1.33 cycl/°, high luminance contrast), drifting at v = 1.5 °/s in 8 different directions.

We found 4 major results: 1.) LFP and MUA coherence (1-141 Hz) declined with cortical distance. 2.) Coherence of fast oscillatory components of MUA strongly depended on stimulus orientation. 3.) The low frequency components of MUA coherence did almost not depend on stimulus orientation. 4.) For a given cortical distance the highest values of coherence in the γ-range were found at stimulus orientations corresponding to the average (half angle) among the preferred orientations at the two recording sites. While the cross-spectra revealed qualitatively similar results the center peaks of cross-correlograms yielded only weak dependence on stimulus orientation. This indicates together with (2.) and (3.) that the stimulus specific lateral interactions are due to couplings among fast oscillatory and not among the broad-band signal components. (Supported by DFG: Ec 53/6-1,2 to R.E.)

255.6

CORTICAL FEATURE-BINDING: NEURONAL OSCILLATIONS CAN OCCUR WITH MS-TIME-SCALE SYNCHRONY, DESPITE LONG AXON CONDUCTION DELAYS, WHEN INTERNEURONS FIRE SPIKE DOUBLETS. R.D. Traub*, J.G.R. Jefferys & M.A. Whittington IBM Watson Res. Ctr., Yorktown Heights, NY 10598, University of Birmingham School of Medicine, Birmingham B15 2TT, U.K. and Imperial College School of Medicine at St. Mary's, London W2 1PG, U.K.

Synchronized neuronal oscillations in the gamma frequency band (30-70 Hz) have been implicated in feature-binding in the visual cortex, by Singer & Gray among others. Consistent with this notion, synchrony of neuronal oscillations occurs at distances at least 7 mm within a hemisphere and across the corpus callosum. The cellular mechanism of long-range synchronization is mysterious, given that axon conduction delays may be several ms or more at such distances. We propose a simple model, generalized from hippocampal and neocortical slice studies showing that interneuron networks alone can generate gamma oscillations (Whittington, Traub & Jefferys, *Nature* 373 (1995) 612-615). The model consists of a chain of interconnected cell groups with a) both tonic and fast (AMPA receptor-mediated) excitation of interneurons by pyramidal cells; b) GABA-A receptor-mediated inhibition of both pyramidal cells and interneurons; and c) axonal conduction delays are negligible *within* cell groups, but between neighboring cell groups can be up to 5 ms. When AMPA-receptor-mediated excitation of interneurons is sufficient to induce spike doublet firing, but not otherwise, gamma oscillations occur that are synchronized on the ms time scale, from one end of the chain to the other. Consistent with data from the Eckhorn lab, oscillations in large neuronal ensembles occur at lower frequencies than in small ensembles. Thus, known or testable properties of neurons and of local synaptic circuits can, in principle, account for gamma oscillations that are tightly sychronized over distances of many mm.

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255.7

RELATION BETWEEN OSCILLATION FREQUENCY OF THE EEG AND OF SYNCHRONOUSLY DISCHARGING CELL ASSEMBLIES. M.H.J. Munk*, S. Herculano and W. Singer, Max-Planck-Institut für Hirnforschung, Deutschordenstraße 46, Frankfurt a.M. 60528, Germany.

Activating the mesencephalic reticular formation (MRF) in anesthetized cats desynchronizes the EEG and facilitates synchronization of distributed neuronal responses evoked by coherent light stimuli. This suggests that arousal leads to enhanced coupling among cortical neurons and increases the size of synchronously active cell ensembles. Here we investigate with multielectrode recordings from A17 and A18 of anesthetized cats putative relations between ensemble size and oscillation frequency. When the EEG is dominated by low frequencies (around 4 Hz), light responses are unreliable and show no stimulus-related synchronization of discharges. When the EEG shifts into the alpha-range (10-14 Hz), light responses become vigorous and auto-correlograms of multi-unit responses may exhibit a robust modulation in the range of 70-100 Hz, indicating that local clusters of neurons synchronized their responses in this high frequency range. Long-distance correlations in this frequency range also occur, even across the hemispheres. After MRF stimulation, when the EEG is desynchronized, the responses of local clusters of neurons engage in stable synchronous, oscillatory discharges, with frequencies lowered to the range of 30-50 Hz, and long-distance correlations become frequent. Interestingly, in the cases where the frequency range remained 80-100 Hz during MRF activation, the oscillatory modulation did not increase, although long-range synchronization could become enhanced. These results suggest that increasing the size of synchronously active cortical cell assemblies reduces the oscillatory frequency of the responses, which is compatible with theory. The high frequency oscillations seen during alpha-activity could reflect either oscillatory input from the retina that is unmasked by the lack of cortically generated oscillations, or the dynamics of local cortical micro-ensembles.

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255.8

MODIFICATION OF DISCHARGE PATTERNS OF NEOCORTICAL NEURONES BY INDUCED OSCILLATIONS OF THE MEMBRANE POTENTIAL M. Volgushev*, M. Chistiakova and W. Singer Ruhr-Universität Bochum, Dept. Neurophysiology, D-44780 Bochum and Max-Planck-Institute for Brain Research, Deutschordenstr. 46, D-60528 Frankfurt/M. Germany.

Neuronal networks have the tendency to engage in oscillatory activity. Here we report that oscillatory modulation of the membrane potential by current injection affects substantially the discharge patterns of neocortical neurones in vitro. Whole-cell recordings were made from layer 2-3 pyramidal cells in slices of the rat visual cortex. Oscillations of the membrane potential were induced by injection of sine-wave currents. With injection of suprathreshold currents spikes occurred preferentially at the positive peaks of the oscillations with a phase shift that depended on the time constant of the membrane, the actual membrane potential and the amplitude of the oscillatory modulation. When paired with subthreshold modulation of the membrane potential, small EPSPs evoked discharges only during a narrow window of the oscillation cycle while large EPSPs elicited trains of spikes that were phase locked to the oscillations. The timing of responses to synaptic activation depended only little on the timing of afferent stimulation but was essentially determined by the oscillation cycle. Thus, an oscillatory modulation of the membrane potential gates transmission of synaptic input with high temporal precision, introduces variable phase-dependent delays between synaptic input and the postsynaptic response and generates precisely timed responses, the frequency of which depends on the oscillation frequency rather than on the amplitude of the synaptic event. We propose that these effects contribute to the synchronization of activity of distributed neurones.

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255.9

POSSIBLE CELLULAR MECHANISMS FOR AROUSAL-INDUCED HIGHER FREQUENCY OSCILLATIONS: ACETYLCHOLINE AND ACPD INDUCE REPETITIVE BURST FIRING IN VISUAL CORTICAL NEURONS D.A. McCormick* and L.G. Nowak Section of Neurobiology, Yale Univ. Sch. Med., New Haven, CT 06510.

Arousal from slow wave sleep is associated with the abolition of slow, synchronized oscillations, such as spindle and delta waves, and the occurrence of higher frequency (20-70 Hz) oscillations in thalamocortical systems. Recordings in vivo demonstrate that these higher frequency oscillations can be associated with repetitive burst firing in unique populations of cortical neurons. Here we investigated the possibility that repetitive higher frequency burst firing may be induced by activation of cholinergic or glutamate metabotropic receptors.

Intracellular recordings from the cat or ferret primary visual cortex maintained in vitro revealed a sub-population of neurons, including layer V pyramidal cells, that generate trains of "single" spikes upon depolarization. Local application of acetylcholine, the muscarinic agonist methylcholine, or the glutamate metabotropic receptor agonist ACPD resulted in depolarization and the activation of single spike activity, followed by the generation of repetitive burst discharges with an interburst discharge frequency of 15-50 Hz. The regularity, but not the occurrence, of these burst discharges was inhibited by reducing $[Ca^{2+}]_o$ to 0.5 mM and raising $[Mg^{2+}]_o$ to 8 mM, suggesting that these events are not generated solely by the activation of Ca^{2+} conductances.

We suggest that the activation of a muscarinic receptors by ascending cholinergic fibers, and glutamate metabotropic receptors, perhaps from cortico-cortical interconnections, can induce repetitive burst firing in a subpopulation of cortical neurons thereby setting the stage for the generation of synchronized neuronal oscillations. Supported by NIH and NSF.

255.10

PERCEPTION RELATED SYNCHRONIZED OSCILLATIONS IN MONKEY STRIATE CORTEX IN A BINOCULAR RIVALRY TASK. M. Kottmann and R. Eckhorn*. Deptm.Physics, Neurophysics, University Marburg, D-35032, Germany.

Recent investigations revealed that single unit spike rates in monkey visual cortex can correlate with the perceived orientation of rivalling gratings (Leopold & Logothetis 1996, Nature 379:549). In the present investigation we asked whether the previously observed synchronized cortical oscillations (35-80 Hz) occur also during binocular rivalry (BR) in a perception related way. BR was induced in a dichoptic view of two overlapping Gabor-gratings (width 1.2°, 3 cycl/°, lumin. 69 cd/m^2; max. contrast 0.96, monitor frame rate 94 Hz) presented at orthogonal orientations for 400 ms (to prevent perceptual switching). While the monkey (macaca mulatta) kept his fixation (±0.5°) he reported his perception by pressing a key (rewarded by juice). The overall correctness of the reports was established by changing the gratings relative contrast randomly and plotting the respective psychometric function (which proved to be similar to those of 3 human subjects). Responses of single- (SUA), multiple-units (MUA) and local field potentials (LFP, 1-140 Hz) were recorded in parallel by 7 µ-electrodes and were characterized with respect to orientation tuning, ocularity, average response power, oscillation index, and synchronization among spatially separate positions. We found perception related activity, including synchronized oscillations, in many recordings with imbalanced contrast among the gratings. With identical strength of stimulation to the left and right eye (to which the monkey reported the orientations at equal probability) many recording sites had MUA and LFP power that was significantly perception related in correspondence to the single cell results of Leopold & Logothetis. More interestingly, under these conditions oscillatory signals and especially their spatially synchronized components (cortical range: 4 mm) were significantly correlated with the perceptual responses. We conclude that synchronized oscillatory signals do not only occur specifically coupled to stimulus- and RF-properties but also to internal perceptual states without physical changes in stimulation. (Supported by DFG grant Ec 53/7 to R.E.)

255.11

OPTICAL IMAGING REVEALS PATTERNS OF BINOCULAR INTERACTION IN CAT PRIMARY VISUAL CORTEX. Frank Sengpiel[1]*, Imke Gödecke[2], Colin Blakemore[1] and Tobias Bonhoeffer[2]. [1]University Laboratory of Physiology, Oxford OX1 3PT, UK, and [2]Max-Planck-Institut für Psychatrie, 82152 München-Martinsried, Germany

For most cells in cat area 17, the response to an optimally oriented drifting grating presented to one eye is tonically suppressed when an orthogonally oriented grating is shown to the other eye. Such *interocular suppression*, which might underlie binocular rivalry, probably results from intracortical inhibition (Sengpiel et al., *Vision Res.* 35, 179-195).

We used optical imaging of intrinsic signals to visualize patterns of responses in area 17 of anaesthetized paralysed cats to dichoptically presented drifting gratings. When gratings of orthogonal orientations were shown to the two eyes with simultaneous onset, activity maps did not differ significantly from those obtained by summing responses to each of the two gratings presented monocularly. In contrast, dichoptic stimulation (duration, 3.5 sec) that was preceded by 2 sec of monocular 'conditioning' presentation of one of the two gratings resulted in a response non-linearity. Compared with the monocular control response to the conditioning grating alone, the presence of the second, rivalrous grating caused not only the expected activation of regions of cortex preferring its particular orientation, but also a reduction in activity that was most pronounced in regions responding most strongly to the orientation of the first grating. Maps were similar, whether the contra- or the ipsilateral eye was stimulated first, although in the latter case facilitation and suppression elicited by stimulation of the other eye were slightly stronger.

Our data indicate that the pattern of interocular suppression generated by a particular stimulus combination mostly reflects the layout of orientation domains and to a much lesser degree the variations in ocular dominance.

Supported by the Max-Planck-Gesellschaft and the Medical Research Council

255.12

DEVELOPMENT OF FACILITATORY AND SUPPRESSIVE BINOCULAR INTERACTIONS IN THE CAT PRIMARY VISUAL CORTEX. Tobe C.B. Freeman, Frank Sengpiel, and Colin Blakemore. SPON: Brain Research Association. University Laboratory of Physiology, Oxford OX1 3PT, U.K.

Binocular neurons in the cat's primary visual cortex (V1) generally show enhanced responses when stimulated through both eyes with optimally oriented gratings of appropriate retinal disparity. However, the response to an optimal grating in one eye is usually suppressed if an orthogonal grating is presented to the other eye (Sengpiel et al. 1995. Vision Res. 35:179-95). We have examined the time-course of development of both facilitatory and suppressive binocular interactions.

We recorded from V1 in kittens aged two to five weeks. For each cell, the dominant eye viewed an optimally oriented drifting grating while moving gratings of various orientations were presented intermittently to the other eye, so as to obtain an orientation tuning curve of interocular interaction. We also tested for disparity selectivity by varying interocular phase.

Interocular suppression was seen in kittens as young as two weeks of age. However, the average strength of suppression (for orthogonally oriented gratings) during the third and fourth weeks was significantly less than that observed in the adult ($p < 0.05$; two-tailed *t*-test) and not until five weeks did it reach adult levels. Binocular facilitation was also observed throughout this time. Interestingly, the orientation tuning of binocular facilitation was significantly wider at three weeks than at four weeks, when it almost reached adult levels ($p < 0.01$). It is likely that the maturation of binocular facilitation (as well as of monocular orientation tuning) reflects, in part, the refinement of clustered horizontal connections in V1. The modest increase in interocular suppression might depend on the strengthening of inhibitory connectivity that is less topographically specific and is present surprisingly early.

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255.13

SUPPRESSIVE BINOCULAR INTERACTIONS IN THE PRIMARY VISUAL CORTEX (V1) OF INFANT RHESUS MONKEYS. Y. Chino*, E.L. Smith III, S. Hatta, & H. Cheng. College of Optometry, University of Houston, Houston, TX 77204-6052.

Although it has been shown that neurons in primate V1 are sensitive to interocular image disparities as early as the 6th postnatal day, little is known about the nature of early cortical binocular interactions. In this investigation, we compared binocular and monocular responses in 244 cells of 6 anesthetized and paralyzed infant rhesus monkeys ranging in age between 6 days and 16 weeks and in 239 units of 6 adult monkeys. Following the determination of a cell's optimal monocular parameters, we measured the responsiveness of individual neurons to the relative interocular spatial phase disparities of dichoptically presented sine wave gratings. The peak and mean binocular response amplitudes were compared to each cell's monocular responses. Prior to the age of 4 weeks, binocular responses were dominated by suppressive interactions even if cells were sensitive to interocular spatial phase disparities. Binocular suppression was particularly robust when orthogonally oriented gratings were employed. The results suggest that intracortical binocular suppression in V1 may contribute to the lower overall binocular response amplitudes found in infant monkeys.

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255.14

OPTIMIZATION PRINCIPLES FOR CORTICAL MAPPINGS: OCULAR DOMINANCE COLUMNS. G.J. Goodhill*. Sloan Center for Theoretical Neurobiology, The Salk Institute, La Jolla, CA 92037.

A common intuitive principle for understanding the structure of cortical maps is that "similar features are represented nearby in the cortex". Particular mathematical versions of this principle are implemented in certain self-organizing algorithms. However, these algorithms embody only a small subset of possible assumptions regarding the measurement of feature similarity, closeness in the cortex, and the degree of topography of a mapping. For instance, in models that assume that features can be represented by points in a low-dimensional space, it is not possible to independently vary the similarity between each pair of features. Such assumptions are generally made for computational convenience rather than biological relevance.

An alternative approach is to first choose measures of similarity, closeness and topographic match, and then optimize directly using techniques such as simulated annealing. This permits investigation of the consequences of a wider variety of assumptions. I explore this approach for the simple case of the ocular dominance column map in primary visual cortex. Recent theoretical (Goodhill, *Biol. Cybern.* 69:109 (1993)) and experimental (Löwel, *J. Neurosci.*, 14:7451 (1994)) work suggests that the strength of between-eye correlations partly determines ocular dominance column periodicity in the cat. Here I use an optimization framework to investigate the influence on periodicity of other parameters that govern the correlational structure of visual stimulation.

Supported by the Alfred P. Sloan Foundation.

255.15

MAPPING OCULAR DOMINANCE COLUMNS IN HUMAN V1 USING FMRI. R. S. Menon*, S. Ogawa and K. Ugurbil, The John P. Robarts Research Institute, London, ON Canada N6A 5K8, Lucent Technologies Bell Laboratories, Murray Hill, NJ USA 07974 and Centre for Magnetic Resonance Research, University of Minnesota, Minneapolis, MN USA 55455.

GRASS goggles (Quincy, MA) flashing at 10 Hz were used for photic stimulation, modified so that all light emitting diodes (LEDs) but one were masked out on each side. 13 normal subjects were used. Binocular stimulation ("B" state) sandwiched between periods of darkness ("D" state), was used to identify primary visual cortex. Monocular stimulation using the left ("L" state) or right ("R" state) flashing LED was used to deliniate ocular dominance columns. Each of the states, B, D, L and R were typically 1 minute in duration. All fMRI experiments were performed using an in-plane resolution of 391 by 391 microns on a 4 T whole-body human imager (Varian/Siemens) [TE=30 ms, TR=50 ms, slice thickness=3 mm, Flip Angle~22° 256x256]. Cross-correlation analysis (corr. coeff. >0.5) was used to identify pixels that responded to left or right eye photic input. Only the images with the alternating monocular stimulation were used for the correlation with the reference waveforms. Binocular stimulation was tested for using a simple t-test of the B minus D states. We found that in certain areas of V1, the correlation analysis detected pixels that oscillated in synch with the left-right stimulus paradigm. At the correlation values used, NO pixels were detected outside the brain suggesting these are not random. In addition, these pixels are found as alternating clumps of pixels along the line of Gennari visible in some of our images, suggesting that they are confined to layer 4C of V1. We note that the Blood Oxygen Level Dependent (BOLD) signal within a column does not return to zero during stimulation of the alternate eye suggesting that there are both specific and non-specific components to the fMRI maps.

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255.16

FUNCTIONAL EYE DOMINANCE DOMAINS IN V1 OF SPECIES LACKING ANATOMICAL SEGREGATION OF LGN AFFERENTS. J.D. Pettigrew*, A.W. Roe†, and K. Fritsches. Vision Touch and Hearing Research Centre, Univ. of Queensland, 4072, Australia. †Section in Neurobiology, Yale Univ. School of Medicine, New Haven, CT.

It is known that abnormal visual experience during postnatal development can change V1 ocular dominance column organization, even in its spatial period and form (1,2,3). Altered visual experience during development can also induce the formation of ocular dominance domains in species which normally lack clear anatomical segregation, such as marmosets, squirrel monkeys, owl monkeys, sheep, owls, and flying foxes. We now examine the possibility that visual experience can dynamically modulate ocular dominance patterns in such animals.

We have investigated this possibility using optical imaging and electrophysiology in species normally lacking anatomical segregation of LGN afferents in layer IV of V1 (the flying fox and the marmoset monkey). Anesthetized, paralyzed animals were presented with visual stimuli of varying orientations in different sequences and periods of ocular stimulation. Functional segregation of ocular dominance domains were examined in relation to the parameters of interocular stimulation. Their relation to the observed orientation and direction selective domains were also examined. Preliminary evidence indicates that certain visual stimulation paradigms can lead to functional segregation of ocular dominance domains and that these domains exhibit variable periodicity. These results suggest some constraints in the underlying pattern of segregation, whether this develops normally or whether it is induced. 1. Lowel, J. Neurosci 14:7451-7468, 1994. 2. Roe et al., Neurosci Abstr 21:1752, 1995. 3. Livingstone, J Neurosci, 16:2806-2906 1996.

255.17

MOTION-STEREO INTEGRATION AND THE PULFRICH-LIKE PHENOMENA N. Qian* and R. A. Andersen. Center for Neurobiology and Behavior, Columbia University, 722 W. 168th St., New York, NY 10032; Division of Biology (216-76), Caltech, Pasadena, CA 91125.

Many psychophysical and physiological experiments indicate that visual motion analysis and stereoscopic depth perception are processed together in the brain. However, little computational effort has been devoted to combining these two visual modalities into a common framework based on physiological mechanisms. We propose such an integrated model in this presentation. We have previously developed a physiologically realistic model for binocular disparity computation (Qian, 1994, *Neural Computation*, 6:390–404). Here we demonstrate that under the general assumptions that V1 cells are well tuned to spatiotemporal frequencies and there exists a phase difference (and/or positional shift) between the left and right receptive fields of a binocular cell, our stereo vision model can be combined naturally with motion energy models to achieve motion-stereo integration. As an application of the model, we show that it gives a unified explanation of the classical Pulfrich depth illusion and its more recent generalizations to dynamic noise patterns and stroboscopic stimuli. In these illusions, one sees depth in a physically flat spatiotemporal stimulus when a neutral density filter is placed in front of one of the two eyes. The filter is known to create a temporal delay in the neuronal responses of the covered pathway. Unlike previous explanations, our model can account for different variants of the Pulfrich effect without introducing any *ad hoc* assumptions. Central to our explanation is our demonstration that model cells with the general physiological properties of real visual cortical cells treat a temporal delay as an equivalent binocular disparity regardless of whether the spatiotemporal stimulus contains coherent motion or not.

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255.18

DETECTION OF FIRST AND SECOND ORDER MOTION

A. Grunewald* and H. Neumann, Division of Biology, California Institute of Technology and Abteilung Neuroinformatik, University of Ulm.

Psychophysical studies suggest that there are different mechanisms for detection of first and second order motion. Recent physiological evidence suggests that simple cells in area 17 of the cat respond to first order motion, but not to second order motion, while complex cells respond to both types of motion (Zhou & Baker, 1993). Simple cells are phase sensitive, while complex cells are not. Does phase sensitivity explain ability to detect first or second order motion? We developed a neural model of motion detection that accounts for both types of motion detection. ON and OFF channels independently process contrast information. Simple cells are phase sensitive because they receive ON and OFF inputs at different spatial regions. Responses are sharpened through interactions between cells tuned to opposite phases. Complex cells are phase insensitive because they receive ON and OFF input at the same spatial regions. Responses are sharpened through feedforward center-surround interactions. Complex cells inhibit simple cells, thus sharpening their direction selectivity. Simple cells respond to first order motion, but not to second order motion through their phase sensitivity. Complex cells respond to any changes in ON or OFF responses. Thus they respond to first and second order motion. The model also shows that a reversal of contrast polarity in apparent motion does not imply separate ON and OFF motion detection (Wehrhahn & Rapf, 1992).

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255.19

MODELS OF APPARENT MOTION AT THE SINGLE NEURON LEVEL - COMPARISONS TO VISUAL CORTEX NEURONS. C.L.Baker Jr.*McGill Vision Research Unit, Dept. of Ophthalmology, McGill University, Montreal, Canada.

Many standard models of motion detection use a linear combination of responses of spatiotemporally band-pass filters with spatial and temporal offsets. Such quasi-linear models can account for some aspects of directional sinewave grating and reverse-correlation measurements in visual cortex neurons.

Direction selectivity in single cortical neurons can also be obtained with "apparent motion" of a bar-shaped stimulus presented in each of two successive spatial positions, separated by a stimulus onset asynchrony (SOA) and a spatial displacement. The band-pass spatial filtering of standard quasi-linear models correctly predicts a cyclical dependence on displacement, with a reversal of preferred direction for displacements larger than about half the period (Baker & Cynader,1986).

However the dependence of a neuron's direction selectivity on SOA does not generally show a similar cyclical variation or reversal for large SOA values (Baker & Cynader,1988). This lack of temporal reversal is very difficult to reconcile in a general way with quasi-linear models in which the temporal filtering is entirely band-pass, but is predicted by models which nonlinearly combine temporally low-pass (sustained) with temporally band-pass (transient) filters. The latter temporal signals might arise from lagged and non-lagged cells of the lateral geniculate nucleus (Saul & Humphrey,1992).

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255.20

RECONSTRUCTION OF MOTION TRAJECTORIES FROM THE DYNAMIC POPULATION REPRESENTATION OF NEURONS IN CAT VISUAL CORTEX. D. Jancke*, A.C. Akhavan, W. Erlhagen, G. Schöner, H.R. Dinse. Institut f. Neuroinformatik, Theoret. Biol. Ruhr-Univ. Bochum RUB, Germany and Centre de Recherche en Neurosciences Cognitives, CNRS, Marseille, France.

The representation of moving stimuli in primary visual cortex has been subject of many studies of individual cells and their spatio-temporal receptive field structure. Tuning to movement parameters such as velocity and movement direction has been observed. However, it has been impossible to reconstruct from individual cell responses the cortical representation of moving stimuli, and thus to link spatial information and movement information. This is important, however, in order to assess the influence of cooperative interactions on the representation of moving stimuli.

We have estimated the representation of a moving stimulus, a square of light (0.4 degrees) moving at various velocities (4.4, 8.8, 15., or 42 degrees/sec) by populations of neurons (extra-cellular recordings in adult anesthetized cat, foveal representation in primary visual cortex). The receptive fields of single cells were first measured to determine the retinal location represented by each neuron. The responses of all neurons to a moving stimulus (presented for all neurons at identical retinal coordinates independent of their RF locations) were analyzed in short time slices and represented as levels of activation on the retinal space, with each neuron contributing at the location of its RF center. The peak of this time-dependent population representation can be tracked and directly compared to the stimulus motion on the retina.

We found that the population response follows the stimulus on the retina with a velocity dependent delay. At 8.8 deg/sec the peak position in the population representation closely matches the current position of the stimulus with near zero delay. For faster speeds a phase lag, for slower speeds a phase advance is observed. We interpret this finding as evidence for contributions of dynamic cooperativity to the representation of moving stimuli in primary visual cortex.

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AUDITORY SYSTEMS: CENTRAL PHYSIOLOGY—BRAINSTEM

256.1

COMPARISON OF ACOUSTIC RESPONSE PROPERTIES OF UNITS RECORDED INTRACELLULARLY AND EXTRACELLULARLY FROM DORSAL COCHLEAR NUCLEUS (DCN) OF UNANESTHETIZED DECEREBRATE GERBILS (*Meriones unguiculatus*). J. Ding, A.M. Berglund [1] and H. F. Voigt*Dept. of Biomedical Engineering, Boston University, Boston, MA 02215-2407, and [1]Dept. of Otolaryngology, Harvard Medical School, Boston, MA 02114-3096.

The gerbil DCN is a complex laminated neural structure, consisting of many cell types with diverse responses to acoustic stimulation. In an effort to associate response properties with cell morphology, we have been conducting intracellular recording and marking experiments on unanesthetized decerebrate gerbils using sharp glass electrodes. In this study, the effects of electrode impalement on the response properties of DCN units were investigated. To do so, various response measures of units recorded intracellularly were compared with those recorded extracellularly using the same type of recording electrodes. Only intracellular units with resting membrane potentials <-50 mV and action potentials >40 mV were included. Units were classified according to the response map scheme. Forty three (43) intracellular units (9 type I/III, 9 type II, 25 type III) were compared with 87 extracellular units (19 type I/III, 10 type II, 58 type III). Type II units have significantly weakened acoustic responses when recorded intracellularly. Their mean thresholds to best frequency (BF) tone bursts and to broadband noise were increased by 40-50% and the mean maximum BF- and noise-driven rates were decreased by nearly 60%. Intracellularly recorded type I/III units have significantly lower maximum BF-driven rates. The only significant difference for type III units is an enhanced noise response when recorded intracellularly. These results suggest that the intracellular microelectrode affects these various unit types differently. Weaker acoustic responses most likely result from membrane disruption, but heightened responses may be related to chloride-channel interference by the KCl recording solution.

Work supported by NIDCD and Boston University.

256.2

NEURONAL CONTROL OF TRANSCRIPTION OF A SYNAPTIC TRANSPORTER, GLYT2, IN THE DORSAL COCHLEAR NUCLEUS OF RAT EVOKED BY CHANGES IN AUDITORY PRIMARY AFFERENT ACTIVITY. N.H. Barmack* and H.-Y. Qian. R.S. Dow Neurol. Sci. Inst., Portland, OR 97209.

Synaptic transporters, found in both neurons and neuroglia, provide a mechanism for the termination of the action of synaptic transmitters by removing the transmitters from the synaptic cleft. However, it is possible that neuronally-expressed transporters, could themselves be regulated by neuronal activity. Such a mechanism could adjust the discharge rate of a postsynaptic neuron around an optimal operating point over a period of hours. The regulation of transporter transcription by neuronal activity was investigated in the present experiment.

Neurons within the dorsal cochlear nucleus (DCN) of the rat express both the neurotransmitter, glycine, and a synaptic transporter, GLYT2. The DCN receives its major afferent input from the auditory nerve. We have manipulated the activity in this nerve by: 1) Cutting the nerve, 2) Detaching the middle ear bones from the tympanic membrane, thereby preventing spontaneous acoustic stimulation, and 3) Selectively stimulating the intact auditory system with pure tones (10 kHz and 40 kHz; 80-90db). Forty-eight hours after each of these procedures was performed, the rats were anesthetized, the brainstem was removed and prepared for hybridization with an oligonucleotide probe that was inverse complementary to the cDNA sequence 1978-2012 of rat GLYT2.

Cutting the auditory nerve or removing the ossicular chain caused a reduction in the level of GLYT mRNA in the ipsilateral DCN relative to the contralateral DCN. This reduction was greater in the deeper layer of the DCN. Conversely, stimulation for 24-48 hours with 10 kHz or 40 kHz signals, caused spatially discrete increases in GLYT2 mRNA that matched locations within the DCN predicted on the basis of the tonotopic map of the DCN. These data provide evidence for neuronal regulation of GLYT2. Abnormal neuronal regulation of GLYT2 might account, in part, for symptoms associated with tinnitus. *Supported by NIDCD DC02557.*

256.3

CALCIUM SIGNALING IN DENDRITES OF DORSAL COCHLEAR NUCLEUS NEURONS. S.C. Molitor* and P.B. Manis, Otolaryngology-HNS, The Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205.

To understand the calcium dynamics associated with activity in dorsal cochlear nucleus (DCN) neurons, we studied the temporal and spatial properties of calcium transients elicited by somatically initiated action potentials in pyramidal and cartwheel cells. Thin brain slices, 200-250 μm thick, were prepared from P13-P18 rat pups. Whole-cell voltage- and current-clamp recordings were made from selected cells using electrode solutions supplemented with the calcium indicators Calcium Green-1 (150 μM) or Fluo-3 (250 μM). Fluorescence images of the proximal dendritic arbors were sampled while performing physiological manipulations.

In pyramidal cells, calcium transients could be evoked by single action potentials, and summated when trains of 2-16 action potentials were elicited. Action potentials evoked calcium transients in primary, secondary and tertiary dendrites; in general the fractional fluorescence change increased with distance from the soma. The amplitude of the fluorescence signals became smaller with distance from the soma when the soma was voltage-clamped with an action potential in the presence of TTX, indicating that the depolarization from the action potential actively propagated into the dendritic tree.

Cartwheel cells generate bursts of action potentials superimposed upon a slow depolarization. Individual bursts generated large fractional changes in the fluorescence signal which could be measured over all of the visible portions of the dendritic tree. Preliminary evidence suggests that the fractional change in fluorescence at dendritic branch points can be larger than in nearby regions of the unbranched dendrite.

Somatically generated action potentials produce increases in calcium levels in the proximal dendrites of DCN pyramidal and cartwheel cells. One effect of such changes in free calcium levels would be to communicate the ongoing activity of the cell, wherever initiated, throughout the dendritic tree.

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256.4

A POSSIBLE VENTRAL COCHLEAR NUCLEUS (VCN) SOURCE OF INHIBITION UPON T-STELLATE CELLS. M.J. Ferragamo*, N.L. Golding and D. Oertel. Dept. of Neurophysiology, Univ. of Wisconsin, Madison, WI 53706

To examine interconnections which underlie reponses to acoustic stimuli of stellate cells *in vivo*, we explored responses to shocks of the auditory nerve with intracellular recordings from anatomically identified cells in parasaggital slices of the murine cochlear nucleus. Stimulation of the auditory nerve evoked not only early excitation as previously reported (Oertel et al., 1990), but also a long train of inhibitory postsynaptic potentials (IPSPs). Trains of IPSPs lasted tens to hundreds of milliseconds. IPSPs were more consistent when the nerve was stimuated repetitively (100 ms duration, 100 Hz), when Mg^{+2} was removed or with addition of 10μM bicuculline. Strychnine (1 μM) abolished IPSPs revealing them to be glycinergic. Amino-5-phosphono-valeric acid (APV; 100μM), an NMDA receptor antagonist, also blocked IPSP trains, indicating that NMDA receptors contribute to driving the neurons responsible for the late, long duration inhibition observed in t-stellate cells. IPSP trains were observed in isolated VCN slices, indicating that inhibitory interneurons lie in the VCN.

In VCN interneurons, located just below the granule cell layer at the DCN border, auditory nerve stimulation evoked late, long duration (tens to hundreds of milliseconds) depolarizations which often resulted in trains of spikes whose timing resembled that of the trains of IPSPs in t-stellate cells. Late depolarizations were enhanced by either stimulus trains or Mg^{+2} removal, were blocked by APV, and were voltage dependent. These cells have long dendrites and an extensive local axonal arborization which are both organized across the tonotopic axis of the VCN. We speculate that this input may be imposing wide inhibitory side bands upon choppers observed *in vivo* (Rhode and Smith, 1986).

(Supported by NIH grant DC00176)

256.5

PROPERTIES OF A LOW-THRESHOLD POTASSIUM CURRENT IN VENTRAL COCHLEAR NUCLEUS NEURONS. J.S. Rothman and P.B. Manis*, Otolaryngology-HNS, The Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205.

A low-threshold potassium current, which is present in a subpopulation of ventral cochlear nucleus neurons and activates at potentials below -50 mV, was investigated in terms of activation and inactivation kinetics, as well as 4-aminopyridine (4AP) sensitivity. In these experiments whole-cell voltage-clamp recordings were taken from acutely isolated ventral cochlear nucleus neurons of pigmented guinea pigs. All currents were measured in the presence of TTX and 10 μM cadmium.

4AP sensitivity was measured by computing the change in slope conductance at the knee of current activation as a function of drug concentration (0.01-4.0 mM). The dose response curve was then fitted with a Hill equation, giving an estimate for Ki of 75 μM.

Activation kinetics were investigated by hyperpolarizing cells to potentials near -100 mV and then stepping to potentials near -50 mV. Under these conditions, currents showed sigmoidal-rise kinetics and were best fit with an exponential function ($\tau = 4.18 \pm 0.75$ msec) raised to a power of 2.3 ± 0.3.

In a number of cells, the low-threshold potassium current also showed voltage-dependent inactivation, where inactivation was always incomplete. The magnitude of the low-threshold current measured near -50 mV varied depending on the size of a 100 msec prepulse; larger hyperpolarizing prepulses elicited larger currents at -50 mV. Steady-state current-voltage relationships as a function of prepulse potential were fit to a Boltzmann equation. Results show an average half-inactivation voltage of -65 mV and an equivalent gating charge in the range 1.5 to 2.8. The non-inactivating portion of the current ranged from 40 to 70% of the current that could be elicited by a -110 mV prepulse.

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256.6

EFFECTS OF SYNCHRONOUS DEPOLARIZATION ON GLYCINE-INDUCED CURRENTS IN DEVELOPING RAT AUDITORY BRAINSTEM NEURONS. K.H. Backus*[1] and E. Friauf[2], [1] Dept. Gen. Zool., Univ. Kaiserslautern, 67653 Kaiserslautern, [2] Center Physiol., Univ. Frankfurt, 60590 Frankfurt, Germany.

The maturation of both excitatory and inhibitory synaptic connections in the auditory brainstem appears to be activity-dependent. Whereas several mechanisms for the development of *excitatory* synapses have been proposed, there is, at present, no conclusive concept for *inhibitory* synapses. Interestingly, the reversal potential of inhibitory synapses changes considerably during development, possibly due to a change of the Cl^- equilibrium potential towards more negative values. This results in a parallel shift from depolarizing to hyperpolarizing transmitter action. Thus, inhibitory synapses may stabilize through a similar mechanism as excitatory synapses do. In order to investigate possible effects of synchronous depolarizing activity on glycine-induced currents, we applied the perforated patch-clamp technique to neurons in acute brainstem slices of young rats. We used gramicidin as the membrane-perforating agent, which allowed the recording of whole-cell currents without impairing the intracellular Cl^- concentration. At a holding potential of -60 mV, glycine application (0.1-1 mM) induced current responses which reversed between -70 and -40 mV. When depolarizing pulse trains were applied in the presence of glycine, attempted to simulate coincident excitatory inputs, the reversal potential of the glycine-induced currents shifted in the positive direction. We conclude that the simultaneous activation of glycine receptors and excitatory input may cooperatively drive Cl^- into the neurons and consequently lead to an acute positive shift of the Cl^- equilibrium potential, thus resulting in a pronounced depolarization when glycine is subsequently released. The context-dependent shift of the Cl^- gradient could acutely assist in the specific strengthening of glycinergic synapses. Supported by the DFG (SFB 269).

256.7

INTRINSIC MEMBRANE PROPERTIES AND FIRING CHARACTERISTICS OF NEURONS IN THE VENTRAL NUCLEUS OF THE LATERAL LEMNISCUS STUDIED IN RAT BRAIN SLICE. S.H. Wu*. Laboratory of Sensory Neuroscience, Institute of Neuroscience, Carleton Univ., Ottawa, Canada K1S 5B6.

The ventral nucleus of the lateral lemniscus (VNLL) is a distinct group of neurons in the lateral lemniscus. The VNLL receives inputs mainly from the contralateral ventral cochlear nucleus and contains a variety of morphological cell types. It has been shown that the VNLL not only gives rise a set of parallel pathways to the auditory midbrain, it is also important for analyzing and encoding of auditory temporal information. In this study we have examined the membrane properties and discharge pattern of VNLL neurons in a rat brain slice preparation. Brain slices (400 μm) were taken through the VNLL of young albino rats (18-37 days old) and were maintained in a small chamber perfused with warm oxygenated saline solution. Intracellular recordings were made with 4 M potassium acetate filled glass micropipettes inserted into the VNLL under direct visual control. Responses to an intracellularly injected current pulse showed that cells in the VNLL can be classified into two groups. One cell type was characterized by nonlinear current-voltage relationships and suprathreshold depolarization elicited only one action potential. The other cell type was characterized by a roughly linear current-voltage relationship and generated multiple action potentials with either a single fast after hyperpolarization (AHP) or a two-component AHP (fast and slow) in the suprathreshold range. The discharge rate of most cells was constant for the duration of the current pulse (60-100 ms), but some cells showed accommodation. Steady-state hyperpolarization of VNLL neurons could change their response to a subsequent depolarization, resulting in a longer latency to the first spike or a longer first interspike interval. These results suggest that the intrinsic membrane conductances of VNLL neurons may contribute to the generation of the temporal firing patterns observed in response to acoustic stimuli in *vivo*. Supported by NSERC.

256.8

ELECTROPHYSIOLOGICAL PROPERTY OF OLIVOCOCHLEAR NEURONS IDENTIFIED BY VITAL STAINING IN THE RAT. KIYOHIRO FUJINO[1,2]*, KONOMI KOYANO[1], and HARUNORI OHMORI[1]. Dept. of [1]Physiol. and [2]Otolaryngol., Fac. of Med., Kyoto Univ., Kyoto 606-01, JAPAN.

Membrane properties of cochlear efferent (olivocochlear; OC) neurons of the rat (postnatal day 5-11) were investigated using whole-cell patch clamp method in slice preparation. OC neurons were retrogradely labeled with Texas Red injected into the cochlea, and alive neurons were clearly identified under a fluorescent microscope during experiment. Two groups of lateral and medial OC neurons (LOC, MOC) demonstrated distinct membrane properties. In current clamp experiments, both neurons showed tonic firing pattern in response to the depolarizing current pulses. However LOC neurons had a longer first spike latency than that of MOC neurons. The latency became longer when the membrane potential was hyperpolarized, and became shorter when the membrane potential was depolarized or 4-AP was applied extracellularly. In voltage clamp experiments, transient outward currents were observed in both groups of the neurons. LOC neurons had two components of the currents; fast inactivating current (I_A) with inactivation time constant (τ_h) of 70-120 ms, and slow inactivating current (I_{KD}) with τ_h of 500-2000 ms, while MOC neurons had only the fast inactivating current (I_A) with τ_h of 20-60 ms. The half inactivating potentials (V_h) of these currents were; -34.8 mV for $I_{A\ LOC}$, -38.5 mV for $I_{KD\ LOC}$, and -75.0 mV for $I_{A\ MOC}$. The I_A of both neurons were sensitive to 4-AP, and the I_{KD} of LOC was sensitive to TEA. The difference in the expression of these currents should be responsible for the difference in firing properties of these neurons. (Supported by a grant in aid from Japanese ministry of education to K.K. (ϕ7680892).)

256.9

INTRINSIC MEMBRANE PROPERTIES SUPPORT THE "CHOPPER" FIRING PATTERN: AN IN VITRO STUDY OF THE LATERAL SUPERIOR OLIVE (LSO). T.J. Adam*, D.W.F. Schwarz, and P.G. Finlayson, Rotary Hearing Center, Dept. of Surgery (ENT), Univ. of British Columbia, Vancouver, B.C., Canada. V6T 2B5.

The "chopper" response pattern is characterized in vivo by a precise first spike latency and subsequent regular firing. We investigated intrinsic membrane properties that support this pattern, recorded intracellularly from LSO neurons in rat brainstem slices. Injection of depolarizing current pulses (200 ms) led to regular firing with precise (± 0.1 ms) first spike latency. Firing rate and the precision of spike timing increased, and latency decreased, with current strength. There was a linear rate-current relationship. Rate accommodation, observed during the pulse, was more pronounced with stronger depolarizations. At subthreshold levels we observed a depolarizing hump preceding a plateau with rectifying properties attributed to voltage activated and Ca^{2+}-dependent K^+ conductances. The hump was resistant to application of tetrodotoxin (0.6 μM) and persisted in Ca^{2+}-free extracellular solution containing the Ca^{2+} channel blocker Co^{2+} (50 μM). It is, therefore, probably not caused by the activation of an inward current. Nevertheless,the threshold for firing of the first spike was always reached during the hump which contributes to the exceptional latency precision of LSO neurons. Application of Ca^{2+} channel blockers in Ca^{2+}-free media, and of the K^+ channel blockers 4AP (50μM to 2 mM) and TEA (1 to 20 mM), indicated the contribution of several K^+ conductances to spike repolarization and afterhyperpolarization which also caused the regularity and accomodation of firing. These results indicate that the "chopper" pattern is largely caused by intrinsic properties of LSO neurons that do not depend on synaptic inputs.

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256.10

INTEGRATION TIME OF BINAURAL COINCIDENCE DETECTION. P. X. Joris*, Lab. voor Neuro- en Psychofysiologie, Univ. of Leuven, B-3000 Leuven, Belgium, and Dept. of Neurophysiology, Univ. of Wisconsin, Madison, WI 53706.

The abilitity of humans to detect interaural time differences (ITDs) as small as 20 μs contrasts sharply with their inability to follow fast temporal changes in this binaural cue, reflected in estimates of binaural integration time ~ 100 ms (Grantham, D.W., J. Acoust. Soc. Am., 72:1178-1184, 1982). The source of this "binaural sluggishness" has not been studied physiologically.

Responses were obtained from ITD-sensitive single cells in the inferior colliculus of pentobarbital-anesthetized cats. Waveforms (4 kHz wide low-pass noise) with a sinusoidally varying interaural correlation were created digitally and presented over a calibrated, closed acoustic system. Interaural correlation was modulated from full correlation to anticorrelation at frequencies between 1 and 1000 Hz. Sensitivity to this modulation was measured by a synchronization index measured from period histograms binned at the modulation frequency.

All cells followed changes in interaural correlation. The magnitude and phase of synchronization were consistent with ITD-sensitivity to unmodulated correlated, uncorrelated, and anticorrelated stimuli. Synchronization magnitude decreased with increasing modulation frequency, with cutoff-frequencies ~ 1 order of magnitude higher than published psychophysical cutoffs. The highest frequency giving significant modulation was 500 Hz. Some cells showed an unexpected frequency doubling and sharp ITD-tuning to both correlated and anticorrelated stimuli, presumably based on envelope information.

These results suggest that binaural sluggishness does not originate in the binaural coincidence detector, but at a level above the inferior colliculus.

Supported by NIH grant DC-00116.

256.11

SOUND CONDITIONING ENHANCES RESPONSES AND PERIPHERAL EFFECTS OF OLIVOCOCHLEAR NEURONS. S.G. Kujawa, M.C. Brown* and M.C. Liberman. Dept. of Otology & Laryngology, Harvard Medical School and Eaton-Peabody Laboratory, Massachusetts Eye & Ear Infirmary, Boston, MA 02114.

Sound conditioning by repeated exposure to moderate-level noise reduces damage from subsequent high-level exposure and enhances cochlear distortion product otoacoustic emissions (DPOAEs) and compound action potentials (e.g., Kujawa and Liberman, Abstr. ARO XIXth Mtg., 1996). The mechanisms underlying these conditioning-related phenomena are unknown; however, the olivocochlear (OC) system has been implicated in similar protective effects (Zheng et al., Abstr. ARO XIXth Mtg., 1996). In the present experiments, we investigated the behavior of the OC pathway in sound conditioned animals. Guinea pigs were conditioned by exposure to noise (2-4 kHz band, 85 dB SPL, 6hr on/18hr off, for 10 consecutive days). Five days after exposure, conditioned animals and untreated controls were tested via DPOAE-based assays of sound-evoked OC effects in the cochlea (Liberman et al., J. Acoust. Soc. Am., in press) or via direct measurement of the responses of single OC neurons. The DPOAE-based assays showed increased OC-mediated effects on cochlear output in conditioned animals. Single OC neuron responses to moderate- and high-level sound (monaurally or binaurally presented) also were enhanced in conditioned animals, although thresholds and spontaneous rates did not differ significantly from controls. Results suggest that sound conditioning strengthens the OC reflex by enhancing the responsiveness of OC neurons. This reflex enhancement may contribute to sound conditioning-related protection from acoustic overexposure.

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256.12

INHIBITORY INFLUENCE OF THE SUPERIOR OLIVARY COMPLEX ON THE AUDITORY NERVE RESPONSE RECORDED IN UNANESTHETIZED RATS. E. G. Meloni*, J. L. Beaton, and M. Davis, Dept. of Psychiatry, Yale Univ. Sch. of Med., New Haven, CT.

Efferent innervation of the rat cochlea originates within subdivisions of the superior olivary complex (SOC) of the brainstem including the ventral nucleus of the trapezoid body, lateral superior olive, and a region surrounding the lateral superior olive (Vetter & Mugnaini, 1992) comprising the olivocochlear efferent system. Despite the well described anatomy of this system, the role of the olivocochlear efferent system in modulating auditory transmission remains unclear. In a series of three experiments we used lesion and chemical inactivation and activation techniques to assess the role of this system on auditory function in unanesthetized rats.

In Experiment 1, animals received bilateral electrolytic lesions of the SOC and a bundle of four 25 m nichrome wires was implanted into the ventral cochlear nucleus for extracellular recording of the compound action potential generated by the auditory nerve (N1 component). One week later, potentials were recorded from awake animals using a 10 kHz tone at different stimulus intensities in the presence and absence of background noise. A between-subjects analysis indicated a significant enhancement of the auditory nerve response in the SOC lesioned animals compared to sham lesioned animals. In addition, background noise masked this response to a greater extent in the SOC lesioned animals than shams. In Experiments 2 and 3, bilateral infusion cannulas were implanted into the SOC along with recording electrodes in the ventral cochlear nucleus. One week later, baseline potentials were recorded from awake animals followed by an infusion of lidocaine (10%, 0.5 ml) or kainate (15 pmol) into the SOC. Chemical inactivation of the SOC by lidocaine enhanced the auditory nerve response whereas chemical activation of the SOC with kainate reduced the response. Taken together, these data suggest that the olivocochlear efferent system exerts an inhibitory influence on auditory transmission in unanesthetized rats. [Supported by MH47840, MH00004 and AFOSR F49620-93-1-0293 DEF]

256.13

BILATERAL COCHLEAR ABLATION ALTERS SYNAPTIC EFFICACY IN DEVELOPING GERBIL LSO NEURONS. V. C. Kotak* and D.H. Sanes, Center for Neural Science, New York University, NY, NY, 10003.

Functional denervation of inhibitory *or* excitatory transmission by unilateral cochlear ablation clearly alters synaptic transmission at developing gerbil lateral superior olivary (LSO) neurons (Kotak & Sanes, J Neurosci, 16:1836, 1996; ARO Abst. 19:474, 1996). In this study, we assessed synaptic function following simultaneous inhibitory *and* excitatory denervation before hearing onset. Bilateral cochlear ablations were performed at P7 (under hypothermia) to decrease spontaneous glutamatergic and glycinergic transmission at LSO. Whole-cell voltage-clamp recordings were then obtained from LSO in brain slices from control and manipulated animals at P10-13. Evoked excitatory and inhibitory postsynaptic currents (EPSCs & IPSCs) were obtained at -55 mV with QX-314 and cesium in the pipet solution. To assess the efficacy of the remaining afferents to LSO, minimum amplitude EPSCs & IPSCs were analyzed. Since EPSCs and most IPSCs were inward, they were differentially characterized by their reversal potential (E_{epsc} ~ 0 mV, E_{ipsc} ~ -30 mV; n=16). The average minimum EPSC amplitude (±SEM) was significantly reduced in manipulated neurons compared to controls (-7.3 ± 0.9 pA vs -10.2 ± 0.9 pA; t=2.1; df=20; p=0.04). Similarly, the average minimum IPSC amplitude was significantly smaller in neurons from ablated animals, compared to controls (-6 ± 1 pA vs -10.1 ± 0.9 pA; t=2.98; df=19; p=0.007). In contrast, preliminary recordings indicate that NMDA receptor-mediated synaptic currents are more prominent in neurons from ablated animals, as assessed with AP-5. These results suggest that while afferents may die or weaken following denervation, an up-regulation of NMDA receptors can, nevertheless, potentiate excitatory transmission. (Supported by NIH and NSF.)

256.14

SYNAPTIC EXCITATION AND INHIBITION IN THE LATERAL SUPERIOR OLIVE. X.W. Fu*, S.H. Wu, and J.B. Kelly. Laboratory of Sensory Neuroscience, Institute of Neuroscience, Carleton Univ., Ottawa, Canada K1S 5B6.

The lateral superior olive (LSO), one of the major nuclei of the superior olivary complex, is known to receive bilateral innervation and, as such, forms a neural network for the early comparison of binaural differences involved in sound localization. In the present study, the synaptic excitation and inhibition of the LSO neurons were investigated in a brain slice preparation of the auditory brainstem. Brain slices (400 μm) were cut in the frontal plane through the LSO of albino rats (14-22 days old) and maintained *in vitro*. Whole-cell patch recordings were made from LSO neurons while the fibers of the trapezoid body were stimulated with a bipolar tungsten electrode. Confirmation of the morphology of neurons in the LSO was obtained by biocytin staining. Recordings were made from three morphological types of neurons: fusiform, multipolar and small round cells. In 16 LSO neurons the ipsilateral and contralateral excitatory responses were completely blocked by the AMPA antagonist, CNQX (10 μM). The EPSPs in another 3 LSO neurons had two components, one sensitive to CNQX and the other to APV. Cyclothiazide (100 μM), which blocks desensitization of AMPA receptor, potentiated the EPSPs and increased the incidence of suprathreshold responses. Stimulation of the contralateral trapezoid body elicited an IPSP, with a reversal potential of about -76 mV. It was unaffected by the GABA receptor antagonist, picrotoxin (25 μM), but was blocked by the glycine receptor antagonist, strychnine (0.25-1 μM). These data indicate that the excitation of LSO could be mediated by an excitatory amino acid acting on both AMPA and NMDA receptors. Contralateral inhibition of LSO is mediated through strychnine-dependent glycine receptors. There was no correspondence between types of synaptic responses mediated by different receptors and the morphological class of biocytin-labeled neurons. Supported by NSERC.

256.15

SEROTONERGIC MODULATION OF INHIBITORY AND EXCITATORY SYNAPSES IN GERBIL LATERAL SUPERIOR OLIVE. K. K. Fitzgerald* and D. H. Sanes. Center for Neural Sci., New York Univ., New York, NY 10003.

The lateral superior olive (LSO), a brainstem auditory nucleus, receives ipsilateral excitation from the cochlear nucleus and contralateral glycinergic inhibition via the medial nucleus of the trapezoid body (MNTB). Since alterations of synaptic activity in the gerbil LSO during postnatal development produce profound changes in synaptic efficacy and morphology (Sanes & Takacs, 1992; Kotak & Sanes, 1995), it was of interest to examine modulation of synaptic transmission in this nucleus. Immunohistochemical staining in gerbils (*Meriones unguiculatus*, P10-16) demonstrated that MNTB contains serotonin (5-HT)-positive puncta, suggesting the possibility that 5-HT is a modulator in the MNTB/LSO circuit.

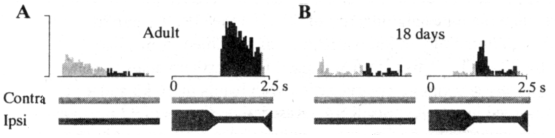
Using whole-cell voltage-clamp recording from LSO neurons in brain slices from P7-12 gerbils, we examined the ability of 5-HT (100 μM) to modulate spontaneously occurring IPSCs, MNTB-evoked IPSCs, and ipsilaterally evoked EPSCs. The most pronounced effect of 5-HT was a dramatic increase in the frequency of spontaneous IPSCs in LSO neurons from P7-8 animals (mean increase = 1.6 ±0.6 Hz, $p<.05$, N=8), suggesting a presynaptic facilitatory action of 5-HT on MNTB neurons. 5-HT produced differential effects on evoked synaptic transmission. Evoked EPSCs were substantially depressed (mean reduction = 51%, N=4). In contrast to the serotonergic facilitation of spontaneous inhibitory transmission, the effects of 5-HT on evoked IPSCs were mixed; both depression and facilitation were observed. These results suggest that 5-HT may be an important modulator of inhibitory and excitatory synaptic transmission during development, and may also regulate interaural level difference coding in the adult LSO.

Supported by NIH and NSF.

256.16

THE ROLE OF INHIBITION IN THE MATURATION OF INTERAURAL LEVEL DIFFERENCE PROCESSING. S.K. Thornton*, M.N. Semple and D.H. Sanes
Center for Neural Science, New York University. N.Y. 10003.

Development of binaural properties in the central auditory system has rarely been studied quantitatively *in vivo*. We have examined maturation of interaural level difference (ILD) processing of neurons in the anaesthetized gerbil inferior colliculus (IC). Tonal stimuli at best frequency were delivered dichotically via calibrated, closed systems. Responses of single EI (contralaterally excited, ipsilaterally inhibited) neurons were recorded at three ages: 17-18, 24-25 and >40 days after birth. Maximum discharge rate increased with age, but other excitatory measures (including thresholds, latencies, dynamic ranges, and rate-level slopes) were similar at all ages. In contrast, inhibition was relatively weak in 17-25 day animals. For example, the slopes (spikes/dB) of ILD functions were shallower and more irregular than those recorded in adults. These immaturities may further restrict processing of complex binaural stimuli. To test this hypothesis, we modulated (trapezoidally) the SPL at the inhibitory ear.



Compared to the control condition (left panel A&B), decreasing ipsilateral SPL initiated a long-lasting "release" from inhibition in adults (right panel, A) and a more abbreviated release in young gerbils (right panel, B). This protracted maturation of inhibition may allow binaural coding properties to become optimized in response to changing acoustic parameters, including head size.

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256.17

POPULATION-INTERVAL MODELS FOR BINAURALLY-CREATED PITCHES. P.A. Cariani.* Eaton-Peabody Laboratory, Massachusetts Eye and Ear Infirmary, 243 Charles St., Boston, MA 02114.

Pitches created by binaural interaction (dichotic combination tones, single & multiple phase-delay pitches, dichotic repetition pitch) have traditionally been explained using spectral-pattern models that analyze "rate-place" profiles of interaural coincidences in the medial superior olivary nucleus (MSO) of the auditory brainstem. Here, we propose an alternative, population-interval model, in which the binaural pitches that are heard always correspond to the most frequent interspike interval present in the MSO.

Computer simulations and our own physiological studies indicate that similar population-interval models can account for a wide range of monaural "periodicity pitches" at the level of the auditory nerve (AN) and cochlear nuclei (CN). Anatomical and physiological evidence suggests that MSO cells act as interaural coincidence detectors that cross-correlate frequency-tuned CN inputs whose timing patterns faithfully preserve those of each auditory nerve. MSO discharge patterns thus largely reflect the time structure of spike train cross-correlations for corresponding fibers in each AN.

(1) Two successive harmonics (600, 800 Hz) presented to different ears produce a dichotic combination pitch at their fundamental F0 (200 Hz). AN fibers over a wide range of characteristic frequencies (CFs) phase-lock to each tone with similar latencies, such that binaural spike train inputs will "beat" with each other at the MSO, coinciding most frequently at time intervals of 1/F0 ms. (2) Noise that is interaurally out-of-phase save for one in-phase frequency band at f_ϕ, produces a pitch at f_ϕ. For each CF, noise generates many intervals at 1/CF in AN fibers. Many more coincidences will be produced for CFs that correspond to f_ϕ (same interaural spike latencies), than for other CFs, producing many more intervals at $1/CF_\phi = 1/f_\phi$. For multiple-phase delays at f_ϕ, $2f_\phi$, $3f_\phi$, intervals at $1/f_\phi$, $1/2f_\phi$, $1/3f_\phi$ are mainly produced, as well as some multiples, resulting in a predominant population interval at $1/f_\phi = 1/F0$. (3) When noise is presented dichotically with interaural delay τ, a repetition pitch at $1/\tau$ is heard. Since AN discharges follow noise envelopes, a similar spike pattern follows on the delayed side, so that coincidences will amplify stimulus periodicities of τ in each CF-channel, and intervals at τ will prevail.

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256.18

THE CONTRIBUTION OF THE DORSAL NUCLEUS OF THE LATERAL LEMNISCUS TO BINAURAL RESPONSES IN THE RAT'S INFERIOR COLLICULUS: INTERAURAL TIME DIFFERENCES. S. A. Kidd and J. B. Kelly*. Lab. of Sensory Neuroscience, Institute of Neuroscience, Carleton University, Ottawa, Ontario, Canada K1S 5B6

Previous studies from our laboratory have shown that the dorsal nucleus of the lateral lemniscus (DNLL) makes a substantial contribution to the suppression of binaural responses in the contralateral central nucleus of the inferior colliculus (ICC) (Li and Kelly, 1992). Pharmacological blockade of neural activity in DNLL results in a partial release from the ipsilateral inhibition normally produced by interaural intensity differences (IIDs). The present study was undertaken to determine the effects of DNLL blockade on ICC responses to interaural time differences (ITDs). Single unit responses to binaurally presented clicks were recorded from anesthetized rats before and after local injection of the excitatory amino acid antagonist, kynurenic acid (2 mM in Locke's solution), into the DNLL. Before injection binaural responses were strongly inhibited by ITDs that favored the ipsilateral ear. Binaural suppression was evident at both short (0-1ms) and long (1-20 ms) ITD intervals. After injection of kynurenic acid the extent of binaural suppression was greatly reduced in the contralateral ICC for both short and long ITD values. The data support the idea that the DNLL is important for shaping binaural responses and that it contributes to both short term and persistent inhibition in the contralateral inferior colliculus (Carney and Yin, 1989; Fitzpatrick et al., 1995; Yang and Pollak, 1994; Yin, 1994).

This research was supported by a grant from NSERC (Canada).

256.19

MODULATION OF AUDITORY CELLS BY EYE AND EAR POSITION IN THE SUPERIOR COLLICULUS OF THE BEHAVING CAT. Luis C. Populin* and Tom C. T. Yin. Neuroscience Training Program and Dept. of Neurophysiology. University of Wisconsin. Madison, WI 53706.

The superior colliculus (SC) is a model system for studying sensorimotor interactions. It appears to contain maps of visual and auditory space as well as motor maps. However, the visual map is retinotopically organized while the auditory map is based upon head coordinates. Studies in monkeys have shown that the maps shift with eye position (Jay and Sparks, 1987) and thus appear to be motor, rather than sensory, maps. Comparable studies in the cat are less clear.

To address this problem, we studied auditory responsive cells in the SC in cats using a variety of behavioral tasks that include fixation, saccades, delayed saccades and sensory probe trials to visual and/or acoustic targets.

Most auditory responsive cells in the intermediate and deep layers of the SC responded best to broadband noise stimuli presented from a region of the contralateral sound field and more strongly when the cat was not fixating a visual target. Many responded to both visual and auditory stimuli and some responded prior to saccades. Of particular interest is that the discharge of some cells varied with the angle of gaze: their responses to identical acoustic stimuli changed as a function of eye position.

While at first glance these results concur with those described in the monkey, they should be interpreted with caution since, unlike the monkey, the cat moves its ears extensively. Measurements of pinna position show that the cat systematically moves its ears as the visual fixation changes. Thus, the changes in pinna position associated with changes in eye position could account for the modulation.

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256.20

EFFECTS OF INJECTIONS OF GABA AGONISTS INTO THE PEDUNCULOPONTINE NUCLEUS (PPN) ON THE P13 MIDDLE LATENCY AUDITORY EVOKED POTENTIAL IN THE RAT. H. Miyazato*, R.D. Skinner and E. Garcia-Rill. Dept. of Anatomy, University of Arkansas for Medical Sciences, Little Rock, AR 72205.

The human P1 (or P50) middle latency auditory evoked potential, which is characterized by: 1) sleep state dependence, 2) rapid habituation, and 3) blockade by scopolamine, is a useful tool in assessing psychiatric disorders. In anxiety disease and schizophrenia it is dishabituated, and in narcolepsy its amplitude is reduced. In our rat model, the potential having the same characteristics as the human P1 occurs at a latency of 13 msec (P13). In order to better characterize the generation of this potential, intracranial injections of neuroactive compounds were made into the PPN, the presumed source of this potential.

Under anesthesia, adult male Sprague-Dawley (n=5) rats were implanted with epidural screws at the vertex and the auditory cortex (ACx) for EEG recording. For microinjections, 22 ga stainless steel guide canulae were implanted in the PPN bilaterally. In alert rats, averaged evoked potential responses following auditory stimuli (32 trials, 103 dB rarefied clicks 100 μsec in duration, 0.2 Hz) were made before and after intracranial injections (0.1 μl). Injections of GABA or the GABA agonist, muscimol, were found to reduce the amplitude of the P13 potential in a dose-dependent manner. While GABA (1-5 mM) greatly reduced the P13 potential briefly (5-10 min), muscimol in doses of 1, 5, or 10 mM reduced the P13 potential for durations of 30, 60 and 100 min, respectively. The Pa potential recorded from the ACx (lemniscal pathway) was not affected.

These results suggest that the manifestation of the vertex-recorded P13 response can be blocked by activation of a known inhibitory GABAergic synapse at the level of the PPN, providing further evidence that the P13 potential is generated, at least in part, by PPN outputs.

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257.1

CLONING OF A cDNA FROM RAT OLFACTORY EPITHELIUM ENCODING AN IP_3 RECEPTOR PROTEIN. D. L Kalinoski*, C. DellaCorte, L.C. Johnson, D. Restrepo, J. Jackson, and R.Dotson. Monell Chemical Senses Center, Philadelphia, PA.

Inositol 1,4,5-trisphosphate has been implicated as a second messenger in responses to selected odorants. Biophysical studies (Lischka 1995) have provided evidence of direct gating of plasma membrane channels by IP_3 in rat olfactory neurons. Antibodies directed against the IP_3R typeI localize to cilia of olfactory neurons and recognize proteins of Mr 260, and 120 KD in Western blots (DellaCorte, 1995). An IP_3 photoaffinity analogue specifically labeled proteins of 120 kD in the same preparation, suggesting multiple forms of the IP_3 R may be present in olfactory neurons (Restrepo, 1992). To examine the molecular identity of the olfactory IP_3 R a partial cDNA with homology to known IP_3Rs was amplified from ciliary RNA by RT-PCR. This cDNA was used for Northern analysis and cDNA library screening. Hybridization with bands of 10 kb and 4 kb was observed in rat olfactory RNA. Restriction digests of 32 positive clones obtained from the library screen have been isolated and inserts of 250 bp to ~3 kb identified. Studies are being conducted to determine the sequences of these clones, and to establish whether multiple isoforms of the IP_3R are encoded in olfactory neurons. (Supported by NIH DC-01228 and DC-00566)

257.2

CHARACTERIZATION OF GCAP IN RAT OLFACTORY TISSUES. C. Moon*, P. Jaberi and G.V. Ronnett , Dept. of Neuroscience, Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205.

The activation of photoreceptor guanylyl cyclase (GC) is the critical step to restore cGMP level from light-induced cGMP hydrolysis[1]. The regulation of photoreceptor GC is mediated by a soluble activating protein that can sense changes of the intracellular calcium concentration[2]. Guanylyl cyclase activating protein (GCAP) is suggested to be a member of calcium-dependent activator of photoreceptor GC[3,4]. Two GCAPs, GCAP-1 and GCAP-2 have been identified to date, and both GCAPs can stimulate rod outer segment GC or recombinant retGC in low calcium concentration[4-6]. The calcium-dependent stimulation effect is abolished by increase of calcium concentration.

Our laboratory has identified novel pGCs in rat olfactory cilia and the pGCs can be activated and produce cGMP by various odorants. However, the precise regulatory mechanism of olfactory pGC has not been determined yet. Preliminary immunohistochemical study by using anti-GCAP-1 antibody showed positive identification of GCAP in rat olfactory tissue. Moreover, purified GCAP-1 could potentiate cGMP production in lower calcium concentration from our pilot experiment using isolated rat olfactory cilia. Taken together, our preliminary observation strongly suggests that GCAP exists in olfactory tissue and regulate olfactory pGC in calcium-dependent mode.

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SUPPORTED BY NIDCD F32 (DC00234-01)

257.3

T-TYPE Ca^{2+} CURRENT SERVES FOR SPIKE GENERATION NEAR THE THRESHOLD LEVEL IN THE OLFACTORY RECEPTOR CELL. F. KAWAI, T. KURAHASHI & A. KANEKO* Dept. of Inform. Physiol., Natl. Inst. for Physiol. Sci., Okazaki, 444, Japan;

The graded receptor potential of olfactory cells is encoded into spike trains that travel to the olfactory bulb. Contrary to the widely accepted concept that the action potential of olfactory cells is a Na^+ spike, we recorded action potentials from the isolated newt olfactory cell even in a Na^+-free solution. To understand the ionic mechanism of spike generation, membrane currents were analyzed under the whole-cell patch clamp condition. We found that the transient inward current activated by membrane depolarization was a mixture of Na^+ and Ca^{2+} currents (I_{Na}, I_{Ca}). I_{Ca} was activated at membrane voltages more positive than -70 mV. The half-maximal activation was obtained at -44 mV, about 10 mV more negative than that of I_{Na}. The decay phase of I_{Ca} or I_{Na} could be fitted well by a single exponential function with a time constant of 9 - 21 msec (I_{Ca}) or 0.9 - 1.6 msec (I_{Na}). I_{Ca} was blocked by 0.1 mM Ni^{2+}, but unaffected by either 0.1 mM Cd^{2+} or 1 μM nifedipine. These observations suggest that I_{Ca} is a T-type Ca^{2+} current. To evaluate the relative contribution of I_{Na} and I_{Ca} to spike generation, receptor cells were stimulated by current injection under the current clamp mode. The spike generated by injection of the least effective current disappeared by adding 0.1 mM Ni^{2+} to the medium, but an increase in current intensity generated a new spike in Ni^{2+}-containing solution. These observations indicate that T-type Ca^{2+} current serves for spike generation near the threshold level.

Supported by HFSP Grant and Grant of Japanese Government. FK is a COE fellow.

257.4

ODORANT-INDUCED OUTWARD CURRENT IN FROG OLFACTORY RECEPTOR NEURONS (ORNs) IS MEDIATED BY CYCLIC AMP. R.Y.K. Pun* and S. J. Kleene. Univ. of Cincinnati, Cincinnati, OH.

It is well established that odorant-induced responses in ORNs of vertebrates are mediated via the activation of cAMP-gated channels. The odorant-activated response usually has a long latency, taking several hundred msec before a response occurs. This latency presumably is related to the time it takes for the cAMP level to increase sufficiently to act on the cAMP-gated channels after the activation of the second messenger cascade. Although odorant-induced hyperpolarizations and outward currents have been reported, invariably, direct application of cAMP has produced an inward current. Whether such odorant-induced outward currents are mediated via cAMP-gated channels or other second messenger systems has never been established. We characterized the outward current responses to the odorant isoamylacetate (10 mM) in frog ORNs, and compared them to responses elicited by 8-cpt-cAMP, a potent analog of cAMP, using the perforated-patch recording technique.

At -60 mV, ORNs that responded with an outward current to odorant application also responded to the cAMP analog (500 μM) with an outward current. When compared on the same cell, the odorant-induced response was larger in amplitude than that evoked by 8-cpt-cAMP, but the duration of the odorant response was shorter. Both odorant and 8-cpt-cAMP responses had short latencies (less than 100 msec), and both responses had similar voltage dependency: the amplitudes of the responses increased when the holding potential was more negative and decreased when membrane potential was less negative. Our results indicate that both depolarizing and hyperpolarizing odorant responses in vertebrate ORNs may be mediated by cAMP.

257.5

OLFACTORY CELL CULTURES AND TISSUES CONTAIN mRNA FOR THE D1, D2-LONG - AND D2-SHORT DOPAMINE RECEPTORS, N.L. Koster*[1], N. M. Richtand[1,2], and S. K. Pixley[1], [1]Dept. of Cell Biology, Neurobiology and Anatomy, [2]Dept. of Psychiatry, College of Medicine, Univ. of Cincinnati, Cincinnati, Ohio 45267.

Olfactory receptor neuron (ORN) axons transfer information to the olfactory bulb (OB). One subset of the OB neurons contacted, dopamine-producing interneurons, may affect this transfer by presynaptic modulation. The radiolabeled dopamine receptor (DAR) ligand, ^{3}H spiperone, binds to only those regions of the adult rat OB that contain ORN axons (Nickell et al., *NeuroRep* 2:9). DARs increase the ability of the OB neurons to discriminate between odorants, based on effects observed after systemic application of spiperone (Wilson and Sullivan, *J. Neurosci.* 15:5574). The DAR subtypes are grouped into "D1-like" DARs (D1 and D5) and "D2-like" DARs (D2, D3, D4). The gene coding for the D2 DAR is alternatively spliced to yield a D2-long and a D2-short form.

To determine the expression and cell-type specificity of olfactory DARs, we examined olfactory tissues, ORN-containing cultures (Pixley, *Neuron* 8:1191) and dissociated OB cultures. Using the reverse transcriptase polymerase chain reaction, we detected the mRNA for the D1, D2-long and D2-short DARs in olfactory mucosa and olfactory bulbs from newborn rats. Messenger RNA for the D1, D2-long, and (in most cultures) the D2-short DAR subtypes was detected in 15 day, ORN-containing cultures. Cultured ORNs labeled with an antibody to the D2/D3 DAR (#AB1502, Chemicon). D2-long and D2-short mRNA was detected in two out of three OB cultures. Some D2/D3 DAR immunopositive OB cells also immunolabeled for tyrosine hydroxylase, the rate-limiting enzyme in dopamine production.

This research was supported in part by NIDCD NIH grants DC00150 to NLK and DC00347 to SKP.

257.6

LONG-LASTING ADAPTATION OF OLFACTORY SENSORY NEURONS IS SELECTIVELY IMPAIRED BY INHIBITORS OF THE CO/cGMP SECOND MESSENGER SYSTEM. T. Leinders-Zufall* and F. Zufall. Section of Neurobiology, Yale Univ. Sch. of Med., New Haven, CT 06510.

While much information has been obtained over the past years on the mechanisms of how odorants excite olfactory receptor neurons (ORNs) very little is known about the molecular steps leading to olfactory adaptation. By applying repeated odor stimuli and using perforated patch recordings under voltage-clamp from isolated salamander ORNs we have elicited long-lasting adaptation (LLA) of the odor response. LLA is clearly coupled to the stimulus strength and is characterized by a strong reduction in peak current amplitude to a given stimulus. This reduced odor responsiveness can continue for several minutes after a brief 100-ms long odor pulse but recovers completely so that the effect can be triggered several times. One manifestation of LLA is a shift to the right of the stimulus-response curves, together with a substantial reduction in saturating odor currents. Because the properties of LLA closely match the effects of exogenous carbon monoxide (CO) or cGMP, we tested the consequences of a variety of pharmacological blockers of the cGMP second messenger system. LLA can be uncoupled from excitation and entirely be prevented in the presence of agents that act as inhibitors of the CO-producing enzyme heme oxygenase-2, such as zinc protoporphyrins. In contrast, specific blockers of nitric oxide synthase have no effect on LLA. A series of control experiments ruled out that the effects of the CO pathway blockers were due to unspecific actions at the level of sGC or at the CNG channels. It is also demonstrated that Ca^{2+} entry is required for LLA to occur; LLA can be abolished by reducing the external Ca^{2+} concentration to ≤ 1 μM. These findings demonstrate that distinct second messenger pathways acting in parallel in a given ORN mediate different functions of olfactory transduction, excitation and long-lasting adaptation. The results strongly suggest that endogenous CO is released in an individual ORN in response to odor stimuli of a given strength resulting in cGMP formation, tonic activation of the CNG channels and Ca^{2+} entry, thus mediating one form of sensory adaptation. Supported by NIDCD grant RO1 DC02227.

257.7

SUPPRESSION OF ODORANT-INDUCED AND cAMP-INDUCED CURRENTS IN OLFACTORY RECEPTOR NEURONS OF THE NEWT. H.Yamada and K.Nakatani*, Institute of Biological Sciences, University of Tsukuba, Tsukuba, Ibaraki 305, Japan

It has been reported that odorants can suppress the odorant-activated current. However, little is known about the mechanism of this phenomenon. We have studied this question by making recordings from an isolated newt olfactory receptor neuron in macro-patch whole cell configuration. We employed a rapid bath solution change system as well as intra-pipette perfusion. Using the above techniques, we observed that odorants suppressed the odorant-induced current at various holding potentials, which confirms the result reported by Kurahashi et al (1994). To avoid the intracellular effects of Ca^{2+}, we used the positive holding potentials to observe outward current for most of the rest of the experiments. Upon perfusing cAMP in the pipette, an outward current was observed. The step of odorant stimulus suppressed this cAMP induced current and the conductance decreased. On the other hand, odorant stimulus did not cause any conductance change in the absence of cAMP in the pipette. During the intra-pipette cAMP perfusion, the current recovered to the initial level after the end of each odorant stimulus. After stopping the cAMP perfusion, which means no additional supply of cAMP, the current did not recover to the initial level. This effect accumulated with multiple stimuli. Furthermore, upon adding IBMX in the bath, we observed a current induced by cAMP that, we think, resulted from the inhibition of PDE. This current was also suppressed by an odorant stimulus. Our results suggest that the decrease in intracellular cAMP concentration may underlie the mechanism of suppression. (Supported by *Toray Science Foundation, Japan.*)

257.8

THE ROLE OF BINDING INTERACTIONS IN THE TRANSDUCTION OF COMPLEX ODORANT MIXTURES. L.R. Gentilcore[1], R.A. Gleeson*[2], and C.D. Derby[1]. [1]Department of Biology, Georgia State University, Atlanta, GA 30302-4010. [2]The Whitney Laboratory, University of Florida, St. Augustine, FL 32086.

Individual odorants bind to specific receptor sites and activate specific transduction pathways. When odorants are combined in a mixture, the resultant response of an olfactory receptor neuron (ORN) is usually less than the sum of the responses to the individual odorants. It has been suggested that the predictability of response magnitude of ORNs to mixtures is dependent not only on the transduction processes activated by each component in the mixture, but also the inhibitory interactions between components at the ligand receptor site, termed binding interactions (Daniel *et al.*, 1996). In the spiny lobster, biochemical and electrophysiolgical studies suggest that binding interactions can shape the responses of ORNs to binary mixtures (Olson and Derby, 1995; Daniel *et al.*, 1996). However, since most biologically relevant odors are complex mixtures, we examined the binding interactions of complex mixtures containing up to 7 compounds.

Biochemical techniques were used to examine the binding of two radiolabeled compounds, adenosine 5'monophosphate (AMP) and taurine, in the presence of mixtures of up to 6 non-competitors (AMP, betaine, L-cysteine, L-glutamate, ammonium chloride, DL-succinate, and taurine) and up to 2 competitors (6-chloropurine and xanthosine 5'monophosphate for AMP; hypotaurine and β-alanine for taurine). We asked the following questions: 1) To what degree do different mixtures inhibit binding of AMP and taurine to their receptor sites? and 2) Does the degree of binding inhibition caused by a mixture result from the sum of the individual effects of that mixture's components? Our results indicate that the magnitude of inhibitory binding due to a mixture is dependent on the composition and intensity of the mixture, rather than the sum of the binding interactions of single components and the radioligand. Further, we find that inhibition by mixtures can be as great as 25 % for AMP and 50 % for taurine.

Supported by grants NIH R01DC00312 and NSF IBN 9109783

257.9

ODORANT-SENSITIVE ADENYLYL CYCLASE IS FUNCTIONALLY INHIBITED BY ODORANT IN PRESENCE OF FORSKOLIN, S. Sinnarajah, P. I. Ezeh*, S. Pathirana, L. J. Myers, and V. Vodyanoy. IBDS, Dept. of Physiology and Pharmacology, College of Veterinary Medicine, Auburn, AL 36849.

To characterize the odorant-olfactory receptor-G_{olf} second messenger pathway, the adenylyl cyclase (AC) activity of ciliary membranes was analyzed by cAMP radioimmunoassay (PerSeptive Diag.). Olfactory cilia from the olfactory epithelium of the rat (Sprague-Dawley) were isolated by sucrose gradient centrifugation, and exposed to a mixture of odorants containing equal parts of ethyl butyrate, eugenol, (+)carvone, and (-)carvone. The basal AC activity was found to be very low. In the presence of 10 μM GTP and 2 mM ATP odorants at concentration between 10 and 1600 μM induced a dose dependent production of cAMP. The dose response curve revealed low and high affinity binding sites with apparent K_d and $(AC\ activity)_{max}$ equal to 1.8 ± 0.5; 34.1 ± 2.5 μM, and 23. 3 ± 3.0; 50.6 ± 6.4 $nMmin^{-1}mg^{-1}$,respectively. G_{olf}-antibodies (Santa Cruz Biotech.) inhibited the AC activity induced by odorants with the apparent K_d for the inhibition reaction and $(inhibition)_{max}$ estimated as 0.2 ± 0.05 μM and 88.0 ± 7.0 %, respectively. AC could be directly activated by 10 μM forskolin alone; however the AC activity induced by forskolin could be significantly inhibited by addition of 10 μM odorant mixture.This functional inhibition of AC activity by odorants could be significantly abolished by 0.2 μM of G_{olf}-antibodies. Because the AC inhibition in the presence of forskolin was controlled by odorants and G_{olf}-antibodies, and could not be controlled by G_i, or βγ-subunits we hypothesize that in presence of forskolin, AC is functionally inhibited by odorants through direct interaction of components of receptor-G_{olf} pathway with AC, or cross-talk with other endogenous enzymes. This mechanism of inhibition may be important for understanding of attenuation of odorant-stimulated cAMP levels in olfactory neurons. Supported by FAA grant 93-G-058.

257.10

ODORANT-REGULATED K^+ CONDUCTANCE IN RAT OLFACTORY NEURONS. F.W. Lischka, J.H. Teeter and D. Restrepo*. Monell Chemical Senses Center and Department of Physiology, University of Pennsylvania, Philadelphia, PA.

Stimulation of olfactory receptor neurons (ORNs) by odorants elicits elevations in the concentration of adenosine 3',5'-monophosphate (cAMP) and inositol-1,4,5-trisphosphate ($InsP_3$) causing opening of cation channels permeable for Ca^{2+} resulting in an increase in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$). It has been proposed that the cAMP-mediated increase in $[Ca^{2+}]_i$ elicits opening of Ca^{2+}-activated Cl^- channels causing cell depolarization, while the $InsP_3$-mediated increase in $[Ca^{2+}]_i$ causes opening of Ca^{2+}-activated K^+ channels eliciting cell hyperpolarization. However, if both cAMP and $InsP_3$ act through an increase in $[Ca^{2+}]_i$, the question arises as to why cAMP does not stimulate opening of both the Ca^{2+}-activated K^+ as well as the Cl^- channels . To explore this issue we recorded odorant-stimulated currents in rat ORN under voltage clamp conditions using the perforated patch clamp technique. While some ORN responded to odorants known to increase cAMP production with stimulation of a current displaying the characteristics of the cAMP-gated nonspecific cation conductance, in a large percent of the cells (72%) the odorants suppressed a voltage-dependent K^+ conductance. The odorant-regulated K^+ conductance was inhibited by internal Cs^+ and external TEA, and its IV relationship displayed a negative slope region at positive potentials. Elevation of cAMP levels by brief stimulation with IBMX did not suppress the K^+ conductance . These experiments suggest that the regulation of a K^+ conductance by odorants plays a role in olfactory transduction. (Supported by NIH DC-00566).

257.11

HOMOMERIC CYCLIC NUCLEOTIDE-GATED CHANNELS FORMED BY CLONED RAT OLFACTORY SUBUNIT 2 ARE GATED BY NITRIC OXIDE. M.-C. Broillet * and S. Firestein, Department of Biological Sciences, Columbia University, New York, NY 10027.

We have previously reported that NO can directly activate the cyclic nucleotide-gated (CNG) channel from salamander olfactory neurons independently from the cAMP second messenger cascade. Its action could be via the chemical modification of SH groups. The native CNG channel is thought to be an hetero oligomer of rOCNC1 and rOCNC2 subunits. In cloned reconstituted CNG channels, the rOCNC1 subunits form a homomeric channel that can be activated by cAMP. The second subunit, rOCNC2 is either not expressed at the membrane or cannot be activated as a homomeric channel by cAMP. Nevertheless, we have transfected human embryonic kidney (HEK) 293 cells with the second subunit rOCNC2 of the rat olfactory CNG channel. Patch-clamp recordings under excised inside-out configuration were made 1 and 2 days after transfection. No CNG activated conductances could be recorded when cAMP (50-500 μM) was perfused. However, after cAMP had been washed out, the nitric oxide donor, S-nitrosocysteine (SNC, 100 μM) was added to the cytosolic solution, resulting in single channel openings and long bursts of openings. The single channel conductance of the NO-gated rOCNC2 is smaller than that of the native CNG channel or the rOCNC1 subunit homomer, or of the heteromeric reconstituted rOCNC1 and rOCNC2 channel. The open channel shows multiple poorly resolved levels of conductance. At least four are apparent in the amplitude histogram. A difference in the P region amino acid sequence between the rOCNC1 and the rOCNC2 channels (E342 versus D234) might explain this channel behavior. These results suggest that rOCNC2 subunits do form a channel expressed at the membrane of the 293 cells. This channel can be gated by NO, which might be its physiological ligand. The possibility of using NO to activate the second subunit of the CNG channel should aid in understanding the contribution of this subunit to the wild type channel.

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258.1

CANDIDATE LIGAND-BINDING SITE IN HUMAN OLFACTORY RECEPTORS LOCATED BY MOLECULAR MODELS AND POSITIVE SELECTION MOMENTS. Michael S. Singer[1*], Yitzhaq Pilpel[2], Doron Lancet[2] and Gordon M. Shepherd[1]. Section of Neurobiology[1], Yale School of Medicine, New Haven, CT 06510; and Membrane Research and Biophysics[2], Weizmann Institute of Science, Rehovot 76100 Israel.

Ben-Arie *et al* ('93, *Hum Mol Gen 3*, 229) have recently reported sequences for members of a cluster of putative olfactory receptors (ORs) on human chromosome 17. In the absence of reliable functional assays for human OR specificity, we have followed previous analyses with two computational methods: molecular models and positive selection moments. Molecular models of several human ORs (such as 17-2, 17-4, 17-23) were built and automatically docked with over 70 odor molecule structures. The results point to odor molecules that may show preferential affinities for particular receptors and identify residues that may interact with the odor molecules. Positive selection moments, calculated for the α-helical sixth transmembrane domain (TM6), were next used to identify residues that have been preferentially diversified by positive selection and are thus likely to interact with odor ligands. The results suggest that residues 622 and 625, which are commonly serines or threonines, could form critical H-bonds with odor ligands. The studies present new methods for the synthesis of evolutionary and structural data and provide leads for future studies of OR function.

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258.2

OLFACTORY NEURON CELL LINES: A WINDOW INTO THE ROLE OF CELL SURFACE CARBOHYDRATES IN NEURITE OUTGROWTH.

A.C. Puche*, P.F. Bartlett+, V. Lapatis+, and B. Key., Dept. Anatomy and Cell Biology, University of Melbourne, Parkville 3053 Victoria, Australia. + Walter and Elisa Hall Institute, Parkville 3053 Victoria, Australia.

The primary olfactory projection does not form a precise point-to-point topographical map between the olfactory neuroepithelium and the surface of the olfactory bulb. Near neighbour relationships between individual primary sensory olfactory neurons (PONs) in the olfactory neuroepithelium are not maintained by their axonal projections in the olfactory bulb. However, subpopulations of PONs dispersed throughout the neuroepithelium project to discrete regions of the olfactory bulb. Our laboratory has identified a number of PON subpopulations that are distinguished by expression of a specific array of cell surface carbohydrates. We hypothesis that this 'chemical code' imparts guidance information directing axon growth to the topographically appropriate region of the olfactory bulb. Olfactory sensory neuron cell lines were generated by retroviral infection and used to assess the role of carbohydrates in neurite outgrowth. Dissociated cells of the clonal cell lines 4.4.1 and 4.4.2 were grown on a substrate of neoglycoproteins (bovine serum albumin derivatized with either β-lactose, α-fucose, α-galactose, β-galactose, or N-acetyl-lactosamine). Lactose and N-acetyl-lactosamine were strong and specific neurite outgrowth promoters in both cell lines, whereas fucose promoted neurite growth from only the 4.4.1 cell line. Primary cultures of olfactory neurons also respond specifically to immobilised N-acetyl-lactosamine and the blood group B carbohydrate. Additionally, neoglycoproteins used for *in vivo* ligand binding histochemistry on the adult olfactory bulb revealed the presence of carbohydrate receptors in the nerve fibre layer. These results suggest that olfactory sensory neurons are capable of responding to a specific subset of carbohydrates, and that complimentary carbohydrate receptors are present in the olfactory pathway. Interactions between these molecules may influence axon growth/guidance in the olfactory system.

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258.3

UP-REGULATION OF NGF mRNA IN NASAL MUCOSA FOLLOWING EARLY UNILATERAL NARIS OCCLUSION IN RATS. J.S. Stewart[1*], D.M. Cummings[2], B.K. Fiske[2], P. Neff[2], P.C. Brunjes[2], & J.B. Tuttle[2]. [1]Washington and Lee Univ., Lexington, VA & [2]Univ. of Virginia, Charlottesville, VA

Gong, et al. (J. Comp. Neurol. *344*) reported a developmentally regulated pattern of NGF receptor immunoreactivity (NGFr-IR) in the olfactory system, with up-regulation of NGFr-IR in the olfactory nerve and mucosa following chemical lesion of the ipsilateral epithelium. After unilateral naris occlusion in rats on postnatal day 2 (P2), Gómez-Pinilla, et al. (Devel. Brain Res. *48*) found increased NGFr-IR in ipsilateral olfactory bulbs at 60 days of age. This increase was postulated to be the result of elevated NGF protein levels in olfactory tissues ipsilateral to the closed naris. In the present study, we used competitive-quantitative reverse transcriptase PCR (rtPCR) to measure levels of NGF mRNA in olfactory mucosae from unilaterally odor-deprived rats. Unilateral naris occlusion (or sham occlusion of controls) was performed on P1, and olfactory epithelial tissue collected on P2, P10, or P30. Results from tissues collected at P30 (N=2) show an average 70% increase in NGF mRNA in epithelium ipsilateral versus contralateral to the occluded naris. Epithelial tissue collected at P2 (N=1), showed a 148% increase in NGF mRNA in tissue ipsilateral versus contralateral to the occlusion. Similar analysis of olfactory bulbs from these animals is in progress. These preliminary findings are consistent with the results of Gómez-Pinilla, et al.

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258.4

COMPARISON OF SALAMANDER OLFACTORY AND VOMERONASAL RECEPTOR CELL DIVISION RATE: VARIATION WITH NATURAL LIFE CYCLE. E.P Dawley*, S. Wagner, C. Stankiewicz, A Fingerlin, W. Sands, H. Kerlin and D. Nibouar. Ursinus College, Collegeville, PA 19426.

Continued mitosis of cells within the olfactory/vomeronasal epithelium to produce new receptors cells is thought to be an adaptation to replace receptors damaged by daily exposure to odorants. We propose that new receptors also may be generated prior to the breeding period of salamanders to enhance chemoreception for courtship activities. Chemoreception may be particularly important for mate recognition and courtship among salamanders, and in previous work we showed that the volume of the vomeronasal epithelium of red-backed salamanders (*Plethodon cinereus*) varies on a yearly cycle. Both males and females have significantly larger vomeronasal organs in the summer pre-breeding period than at any other time of the year. We injected freshly collected salamanders with 5-bromo-2'-deoxyuridine (BRDU), a thymidine analog which is incorporated into the DNA of dividing cells, and used immunocytochemistry to locate cells containing BRDU. Salamanders collected in the summer had significantly more labeled cells than salamanders collected in May or October (ANOVA, single classification). Hormones (e.g., testosterone) may affect receptor cell division rate, and differences in circulating hormones throughout the year may correlate with differences in receptor cell division rate. Testosterone injected into animals or added to olfactory organ cultures, increased numbers of labeled receptor cells over baseline levels.

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258.5

THE EFFECT OF GROWTH FACTORS ON THE DEVELOPMENT OF ADULT OLFACTORY RECEPTOR NEURONS *IN VITRO*. N. Liu, G. Yorke* & E.J. Roisen. Anatomical Sci. & Neurobiol., Univ. of Louisville Med. Sch., Louisville, KY 40292.

Studies in this lab have shown that chemical trauma to adult mouse olfactory epithelium (OE) stimulates mitotic activity *in situ* which continues in culture. Bipolar cells in these cultures are likely to be olfactory receptor neurons (ORN), since they are immuno-positive for 200 kD neurofilament protein, olfactory marker protein, neuron specific, microtubule-associated protein tau, and immuno-negative for GFAP and keratin. BDNF has been shown to promote the survival of embryonic and neonatal ORNs while EGF enhanced mitosis of the basal cells in neonatal OE. Low affinity NGF receptors are expressed on olfactory nerve Schwann cells but not on ORNs, therefore, NGF may be implicated in ORN maturation. The present studies applied BDNF (100 ng/ml), NGF (40 ng/ml), or EGF (4 ng/ml) to cultures of OE to examine their effects on the growth and differentiation of the ORNs. CD1 mice were exposed to an atmosphere containing an atomized solution of 2% $ZnSO_4$ for 30 min; 4 days later OE was removed and trypsinized. The cells were cultured with or without the trophic agents in medium consisting of Minimum Essential Medium supplemented with 10% FBS and gentamicin for 2-3 weeks. The number of ORNs (bipolar cells) and their neurite lengths were determined every 3-4 days; data were analyzed with two factor ANOVA. Under these conditions BDNF increased the bipolar cell number ($p<0.0001$); EGF and NGF did not alter the number of ORNs. None of the agents appeared to affect neurite elongation. Failure of the cells to respond to NGF is consistent with the belief that bipolar cells are ORNs. To determine if the maturation of ORNs could be enhanced, BDNF + NGF + EGF were applied simultaneously. Surprisingly, the average number of ORNs per field was increased ($p<0.01$); however, neuritogenesis remained unaltered. The uniform immunolocalization of MAP2 and tau proteins in both short and long processes of the ORNs suggests that none of the agents enhanced maturation. This study demonstrates that adult ORNs retain their capacity to respond mitogenically to BDNF. Ongoing studies seek to identify agents and conditions which promote ORN differentiation.

Supported by KY Spinal Cord and Head Injury Research Trust.

258.6

INSULIN AND INSULIN-LIKE GROWTH FACTOR I (IGF-I) IN THE OLFACTORY SYSTEM. N. E. Rawson*, C. DellaCorte and H. W. Rees, Monell Chemical Senses Center, Philadelphia PA 19104-3308.

The factors responsible for the unusual regenerative capacity of the olfactory system are not understood. The adult rat olfactory bulb exhibits higher levels of insulin (INS) and IGF-I than any other brain area, yet their functions are not understood. In peripheral nerves and neuronal cultures, INS and IGF-I promote growth and differentiation. To see if they might play a role in the ongoing regeneration of the olfactory epithelium (OE) we are studying the distribution of INS, IGF-I and their receptors using immunohistochemistry and *in situ* hybridization. Immunostaining for IGF-I receptor suggests low levels of expression in normal, adult OE, with substantially more immunostaining in response to reduction of plasma INS and IGF-I by induction of diabetes with streptozotocin. PCR products made using primers designed to selectively amplify IGF-I and its receptor, and the INS receptor from OE cDNA were cloned and sequenced, and were $\geq$99%, homologous to published sequences. Cloned products were used to generate riboprobes for ribonuclease protection assays and *in situ* hybridization. Results suggest local synthesis of IGF-I within the olfactory epithelium, where the initial stages of olfactory neuron replacement occurs. This work funded in part by NIH-NIDCD Grant DC02876-01.

258.7

OLFACTORY RECEPTOR DATABASE: TOOLS FOR SHARING AND ANALYSING PUBLISHED AND UNPUBLISHED SEQUENCES. Matthew D. Healy[1], Jason E. Smith[2], Michael S. Singer[2], Prakash M. Nadkarni[1], Perry L. Miller[1], and Gordon M. Shepherd[2]*. Center for Medical Informatics[1] and Section of Neurobiology[2], Yale School of Medicine, New Haven, CT 06510

Olfactory receptors, believed to be initial sites of odor transduction, form the largest gene family yet identified. Information on these receptors has accumulated from laboratories worldwide. To enhance communication between laboratories and to reduce problems of accessibility, duplication of effort, and scarce links between related data, we have built the Olfactory Receptor DataBase (ORDB). The database, available via the World-Wide-Web (WWW), serves as a common repository for olfactory receptor sequences in both nucleotide and amino acid form. The ORDB is a medium-scale database combining the dedicated features of local databases with the comprehensive information content and broad accessibility of a central server. In addition to sequences, ORDB provides BLAST similarity searches, molecular models, references, e-mail links, and notes from studies of receptor function or distribution. The database includes both published and unpublished data. Unpublished sequence headers are reported by similarity searches, but actual sequences are not disclosed; to maintain data confidentiality, users contact the submitter directly via the e-mail links provided. ORDB has been instrumental in enabling recent correlated mutation analysis and positive selection analysis of olfactory receptor sequences.

ORDB can be accessed at URL: *http://senselab.med.yale.edu/ordb*

Supported by grants to GMS from NIMH, NASA, and NIDCD (Human Brain Project), and to PLM from NLM (IAIMS).

258.8

ENHANCEMENT OF OMP IMMUNOREACTIVITY IN INDIVIDUAL ORNs FOLLOWING OLFACTORY BULBECTOMY IN THE RAT. V.McM.CARR* and A.I. FARBMAN. Dept. of Neurobiology and Physiology, Northwestern University, Evanston, IL 60208-3520.

Olfactory Marker Protein (OMP) is a 19kDa cytoplasmic protein of unknown function found in mature olfactory receptor neurons(ORNs) of the olfactory epithelium (OE). Following olfactory bulbectomy (OB-X), the proportion of ORNs mature enough to express OMP declines greatly. However, we have noticed that the intensity of OMP immunoreactivity (IR) in the few remaining mature ORNs appears enhanced relative to that of mature ORNs in nonbulbectomized OE.

To verify this observation rats were subjected to unilateral OB-X and perfused at 1d-6mos post-OB-X. 10 μm paraffin sections were prepared and reacted with goat α-OMP (1:500-1:50,000; generously provided by Dr. F. Margolis). OMP IR was visualized by immunoperoxidase techniques. Results confirmed our earlier observations: at all post-operative periods and all α-OMP titers examined OMP IR was higher in individual ORNs in operated than unoperated OE. Moreover, at 1:40,000 OMP(+)ORNs were no longer distinguishable in control OE while even at 1:50,000 a few could still be seen ipsilaterally. These observations indicate that ORN OMP levels rise in absence of the ORN bulbar synaptic targets. This may be related to the still unknown function of OMP.

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258.9

ADENOVIRUS COMPONENT SYSTEM MEDIATES GENE DELIVERY TO OLFACTORY NEURONS. M.Pyrski[1], T.Kohout[2], T.B.Rogers[2], A.I.Farbman[3] and F.L.Margolis[1]*. Depts. of Anatomy[1] and Biochem.[2] Univ. Maryland Sch. of Med., Baltimore, MD21201; Dept. Neurobiol.[3], Northwestern Univ., Evanston, IL 60208.

We have utilized an efficient adenovirus-based DNA delivery system to selectively transfect olfactory receptor neurons (ORN) *in vitro*. Organotypic explant cultures of embryonic day 17 rat olfactory mucosa were transfected,after 1 day in culture, with a plasmid containing E.coli *lac*-Z under the control of the CMV promoter. Of several liposomal formulations tested initially, Lipofectamine was most effective. Although its efficiency was below 10%, most of the stained cells showed a specific localization and a typical neuronal shape. Successful transfection was evident after 24 hrs and visualized by incubation of the explants or 10 µm cryosections with x-gal. Immunocytochemistry in tissue sections with anti-OMP and anti-ß-gal confirmed the delivery of functional plasmid to the ORN target cells. To increase the efficiency we used the adenovirus component system that avoids the need to generate recombinant adenovirus constructs. Adenovirus component system mediated gene transfer utilizes an absorbtive complex of replication-defective virus (Ad5dl312), poly-L-lysine and plasmid DNA. This adenovirus system has been reported to successfully transfect 70% of cardiac myocytes in monolayer culture (Rogers et al., 1996). In our olfactory explant cultures, use of this receptor-mediated transfection with at least $1x10^{10}$ complexed virus particles per explant, showed significantly elevated efficiency compared to Lipofectamine. The transfected cells were ORN as demonstrated by their characteristic shape and typical localization in the caudal-dorsal part of the explant. Further, the high specificity of the transfection was indicated by the low numbers of labeled sustentacular cells and chondrocytes and the double labeling (i.e., OMP+, ß-gal+) of ORN. Application of this technique provides opportunities to selectively manipulate ORN phenotype in explant cultures to study the regulation of olfactory function. Future directions include additional characterization of the mechanisms regulating ORN gene expression, phenotypic rescue of ORNs from which genes have been deleted, overexpression of gene products and delivery of anti-sense RNA to create phenotypic gene deletions. (support from Dept. of Anatomy UMAB)

258.10

ADENOVIRUS-MEDIATED GENE TRANSFER IN OLFACTORY NEURONS *IN VIVO*. H. Zhao*[1], J. Otaki[2] and S. Firestein[2]. [1]Interdepartmental Neuroscience Program, Yale University, New Haven, CT 06510, [2]Department of Biological Sciences, Columbia University, New York, NY 10027.

We have developed a technique using adenovirus vector to efficiently transfer exogenous genes into olfactory neurons *in vivo*. A replication incompetent adenovirus (type 5) carrying the reporter gene *lacZ* which codes for the enzyme ß-galactosidase was applied in solution to the olfactory epithelia of rats under anesthesia at a titer of 10^{10} pfu/ml. ß-gal expression was observed one day post infection and was maximal at 3-10 days post infection. Expression could be detected for at least 21 days post infection. Expression patterns were heterogeneous, ranging from a few percent to 25% of the cells in different regions of both turbinate and septal epithelium. Staining was stronger in the olfactory vs. respiratory epithelia. In olfactory epithelium staining was almost entirely restricted to olfactory neurons. ß-gal staining was also observed in the olfactory axons so that nerve bundles could be traced to their targets in the glomerular layer of the olfactory bulb. Intense staining of some glomeruli was evident at 21 days post infection. Another foreign protein, human p75, was similarly expressed in rat olfactory neurons using another adenovirus construct. In neither case was there any evidence of cell loss or tissue damage due to viral infection.

These results demonstrate that recombinant adenovirus can be used for expressing exogenous genes in olfactory neurons. This technique could be used to express foreign proteins or to manipulate expression levels of olfactory specific proteins, including the odor receptor, putative axon guidance and growth molecules, or elements of the transduction cascade.

Supported by NIDCD.

258.11

ELECTRO-OLFACTOGRAMS IN MAN. T. Hummel*, M. Knecht and G. Kobal. Dept. of Pharmacology, Krankenhausstr. 9, Univ. of Erlangen-Nürnberg, 91054 Erlangen, Germany

After chemical stimulation of the human olfactory epithelium it is possible to record negative responses (electro-olfactogram, EOG) which are interpreted as the summated receptor potentials of the olfactory nerve. Stimulation was performed with substances believed to exclusively excite fibers of the olfactory nerve (vanillin 2.1 ppm; hydrogen sulfide 0.8 ppm). Stimulants were presented with stimulus durations ranging between 250 and 1000 ms (interstimulus interval 2-120 s). EOG was recorded by means of tubular electrodes (cutaneous reference contralateral bridge of the nose; impedance $\leq$ 8 kΩ; bandpass DC to 30 Hz, sampling rate 125 Hz). Investigations in more than 60 subjects revealed the following major results:

- EOG amplitudes increased in relation to the subjects' intensity estimates; latencies of EOG onset decreased with increasing stimulus concentrations.
- An increase of stimulus duration produced an increase of EOG amplitudes; in contrast, latencies of EOG onset remained constant.
- When using repetitive stimulation the amplitude produced by the second stimulus was as great as the first responses' amplitude indicating that the peripheral encoding is less subject to desensitization compared to the subjective perception of odorants.
- Only in 2 of 18 trials clear responses to both olfactory stimulants (H2S, vanillin) could be recorded in the same location; in line with immunohistochemical findings the results indicate that odorant receptors are not dispersed homogenously throughout the olfactory epithelium. In addition, endoscopical localization of EOG recording sites indicated that olfactory tissue is located more anteriorly than previously thought.

259.1

AXONAL REGENERATION IN THE CNS OF *APLYSIA* FOLLOWING BILATERAL CEREBRAL-BUCCAL CONNECTIVE LESIONS. S. L. Johnson, M.L. Schroeder, J. A. D. Sánchez, and M. D. Kirk*. Division of Biological Sciences, University of Missouri - Columbia, Columbia, MO 65211.

Following bilateral crushes to the Cerebral-Buccal Connectives (CBCs) of *Aplysia californica,* rhythmic biting is selectively abolished, but recovers within 14 days postlesion (Scott et al. 1995). Recovery of rhythmic biting is mediated by regeneration of axons in the CBCs. To determine the nature and time course of axonal regeneration we used Biocytin backfills to trace fibers that had regenerated across the crush site. Backfills into the buccal ganglia were made at 0, 3, 7, 9, 12, 14, and 17 days postlesion. Secondary labeling of Biocytin was achieved with Streptavidin Lissamine Rhodamine. Labeled axons were then examined with laser confocal microscopy and epifluorescence microscopy. Biocytin provided distinct labeling of regenerating axons, especially at early times postlesion. Regenerating axons were small in caliber, varicose, and occasionally branched. They often traveled a tortuous path through the core of the CBC and usually terminated in a swollen structure, likely to be the growth cone. Regenerating axons grew toward the buccal ganglia at a rate of approximately 125 µm/day and by 9 days postlesion some fibers had entered the ganglionic neuropil. By 12 days postlesion they had traversed the buccal commissure and invaded the contralateral ganglion. These results correlate with physiological evidence that synaptic connections between cerebral and buccal neurons begin to appear at 14 days postlesion. (Supported by NIH grant NS30832 to MDK)

259.2

REGENERATION OF SYNAPTIC CONNECTIONS FOLLOWING BILATERAL LESIONS TO THE CEREBRAL-BUCCAL CONNECTIVES IN *APLYSIA*. J.A.D. Sánchez*, Y. Li, S.L. Johnson, M.L. Scott, and M.D. Kirk. Div. of Biological Sciences, University of Missouri-Columbia, Columbia, MO 65211.

Previous studies in *Aplysia* have demonstrated that rhythmic biting, a component of consummatory feeding behavior, is selectively abolished by bilateral crushes of the Cerebral-Buccal-Connectives (CBCs) and recovers within 14 days postlesion (Scott et. al. 95). In this study we tested for regeneration of specific cerebral-buccal synaptic connections in *Aplysia californica* that may contribute to this functional neural regeneration. Synaptic connections made by Cerebral-Buccal-Interneurons (CBI's) on buccal neurons and their ability to elicit buccal motor programs (BMPs) were tested at various times postlesion. Multiple simultaneous intracellular recordings were made from cerebral (CBI-1, CBI-2) and buccal (B4/5, B31/32, B63) neurons. Following CBC crush, all synaptic connections were initially eliminated. At 14-15 days postlesion, CBI-2 could elicit a single burst of BMP and in 3 of 20 cases produced multiple bursts of BMP, while in only 3 of 5 preparations CBI-2 elicited observable 1:1 epsp's in B31/32. At 14-15 days postlesion CBI-1 did not produce observable epsp's in B4/5, however, in 1 of 4 preparations tested CBI-1 elicited a single cycle of BMP. At 20-24 days postlesion, CBI-2 produced 1:1 epsp's in B31/32 and reliably elicited multiple cycles of BMP. Also 20-24 days postlesion, CBI-1 reliably produced 1:1 epsp's in B4/5; however, these synaptic potentials were small and labile. By 50+ days postlesion, the amplitudes of synaptic inputs to buccal neurons by cerebral neurons approached those observed in control preparations. (Supported by NIH grant RO1 NS30832 to MDK)

259.3

Regeneration of the neuromuscular connections in the locomotory system of the pteropod mollusc *Clione limacina*. Yuri V. Panchin[1,2], Pavel V. Zelenin[1] and Lyudmila B. Popova[1]* [1]A. N. Belozersky Institute of Physico-Chemical Biology, Moscow State University, 119899 Moscow, Russia; [2]Institute of Problems of Information Transmission, Russian Acad Sci., Moscow, Russia.

Neural network for rhythmic wing movements in the swimming mollusc *Clione limacina* is a well studded system. Dorsal (D) and ventral (V) phase motorneurones innervate corresponding wing muscles projecting via ipsilateral wing nerve. When motorneurons axons are crushed in the nerve the regeneration starts and results in a complete restoration of the function in 1-2 months. After axotomy numerous fine neuritis start to grow predominantly in the direction of the dennervated muscles and reach them in 8 -15 days. At this stage motorneurons display no preference to the dorsal or ventral muscles. Motorneurons of the D and V phase have branches that project not only to the corresponding muscles but equally to the antagonistic muscles. Correspondingly muscles fibers display correct, incorrect and mixed innervation. After 2 weeks of regeneration selection starts and a number of wrong neuritis and neuromuscular junctions begins to decrease so, that in 1,5-2 months all the wrong axons are withdrawn and the muscles are innervated only by appropriate motorneurons. Thus, similar to the neuromuscular regeneration in verterbrates, the neuromuscular regeneration in mollusc involves the process of the selection of the motorneuron inputs. This implies the role of the synaptic activity in the choice of the appropriate innervation. Supported by RFBR grant 96-04-49115.

259.4

ANATOMICAL EVIDENCE OF SENSORY AND MOTOR FUNCTION IN EARLY SEROTONERGIC NEURONS FROM *HELISOMA* EMBRYOS. J. I. Goldberg*, R. Koss and T. J. Diefenbach. Dept. of Biological Sciences, Univ. of Alberta, Edmonton, AB Canada T6G 2E9.

In *H.trivolvis* embryos, one of the earliest events in neural development is the differentiation of a single bilateral pair of serotonergic neurons, embryonic neurons C1 (ENC1). The primary neurite of ENC1 projects ventrally towards the pedal band of cilia, where profuse neurite branching occurs in conjunction with the differentiation of ciliary cells. Previously, we provided pharmacological evidence that serotonin released from ENC1 stimulates ciliary activity in the underlying pedal cells, and thereby modulates a cilia-driven locomotory behavior. In the present study, anatomical analyses were carried out to provide further support that ENC1 functions as a motor neuron. Moreover, experiments were conducted to test the novel hypothesis that ENC1 also functions as a sensory neuron. The anatomical methods employed in this study include transmission electron microscopy (TEM), scanning electron microscopy (SEM) and confocal microscopy combined with serotonin immunofluorescence (CM-SI). TEM of the pedal region revealed ENC1 neurites in close association with the basal surface of ciliated epithelial cells. Whereas clusters of synaptic vesicles were found in ENC1 at some sites, postsynaptic specializations were not normally seen. Thus, ENC1 may affect pedal ciliary cells through the paracrine action of serotonin on broadly distributed receptors. In addition to the descending neurite, we observed a large-caliber neurite projecting dorsally from the ENC1 soma that appeared under CM-SI to reach the dorsoanterior surface of the embryo. Both SEM and Normarski DIC microscopy revealed a surface structure at this same location with a ciliated morphology resembling vertebrate olfactory chemoreceptors. TEM analysis confirmed that this specialized surface structure was contiguous with the dorsally-projecting ENC1 neurite. Together, these anatomical data suggest that ENC1 is a sensory-motor neuron that forms a one-cell neural circuit for regulating an early embryonic behavior.

Supported by NSERC of Canada.

259.5

MORPHOLOGY OF THE LARVAL SENSORY NEURONS IN THE IMAGINAL LEG DISK OF THE BLOWFLY. **F. P. Lewis* and G. S. Pollack. Dept. of Biology, McGill University, Montreal, Quebec, Canada, H3A 1B1.**

Five bipolar neurons in the imaginal leg disk of the blowfly *Phormia regina* are associated with a larval sense organ, Keilin's organ. At metamorphosis, this sensory system undergoes extensive rearrangement; three of the neurons undergo programmed cell death, and the remaining two persist and innervate campaniform sensilla of the adult leg (Lakes-Harlan et al., J. Comp. Neurol. 308:188, 1991). We used dye-filling techniques in order to clarify the anatomical and developmental relationships in this system. Single neurons were injected with DiI so as to selectively stain their dendritic terminations, which were imaged, together with cuticular features, using confocal microscopy. The identities of the stained neurons were determined by relative position following retrograde staining of the entire group of neurons with DiO. Central projections were visualized with neurobiotin.

The three neurons that are destined to degenerate at pupation were the ones that innervate the hairs of Keilin's organ; the anterior-most, middle, and posterior-most of the neurons innervate, respectively, the lateral, medial and posterior hairs. The dendrite of the anterior of the two persisting cells terminates medial to the cluster of hairs of Keilin's organ, and the posterior persisting neuron terminates posterior to the hairs. The central projections from the neurons are of two types: a group of three laterally projecting axons and a group of two medially projecting axons. The lateral projections are those of the three non-persisting cells, while the medial projections are those of the persisting neurons. These results suggest that there are two fundamentally different classes of neurons associated with Keilin's organ. Those in the first class are strictly larval neurons that service Keilin's organ and degenerate when no longer required, while the neurons in the second class are retained and possibly remodelled to function in the adult.

(Supported by the Natural Sciences and Engineering Research Council of Canada).

259.6

DAILY AND CIRCADIAN RHYTHMS IN THE MIGRATION OF SCREENING PIGMENT GRANULES IN PHOTORECEPTOR TERMINALS OF THE HOUSEFLY. E. Pyza* and I. A. Meinertzhagen1. Zool. Museum, Inst. Zoology, Jagiellonian University, Ingardena 6, 30-060 Kraków, Poland and 1Life Sciences Centre, Dalhousie University, Halifax, Nova Scotia, Canada B3H 4J1.

Screening pigments in the retinal photoreceptors of insects migrate during the day towards the photoreceptive rhabdomeres and disperse in the cytoplasm during the night. This migration is light-induced and acts as a pupil mechanism. Pigment granules are moreover not only found in the somata but also the synaptic terminals of photoreceptors, in the first optic neuropile, or lamina, where they innervate small groups of lamina interneurons, including monopolar cells L1 and L2. The numbers of synapses established by each terminal exhibit daily and circadian fluctuation. We now examine the numbers of 390-nm diameter ommochrome pigment granules in photoreceptor terminals, during the day and night of day/night (LD) conditions, under constant darkness (DD), and after a 1-h light pulse in DD. Pigment granule profiles were counted at proximal and distal depths in the lamina. Overall, for all conditions examined, there were 55% more pigment granules in receptor terminals at a proximal depth in the lamina than distally. In flies held in LD the number of granules was 47% greater at proximal depths, and 59% greater at distal depths in the lamina, at the beginning of night than it was at the beginning of day. In DD, the number of granules decreased significantly compared with LD, but a circadian rhythm in their number was still detected. The largest number of pigment granules was at the end of subjective day for both proximal and distal lamina levels. A light pulse at the beginning of either the subjective day or the subjective night increased the number of granules in the proximal depth of the lamina, but not distally. Thus the number of photoreceptor terminal pigment granules changes by both direct exposure to light and a circadian mechanism, which during the night is interpreted to move pigment granules out of the soma to more proximal depths, in the lamina.

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259.7

CIRCADIAN RESPONSIVENESS IN THE FLY'S EYE AND EFFECTS OF 5-HT AND PDF. B. Chen, I.A. Meinertzhagen and S.R. Shaw*, Institute for Neuroscience, Dalhousie University, Halifax, Nova Scotia, Canada B3H 4J1.

Two sets of wide-field neurons ramify throughout the optic lobe behind the fly's compound eye. They extend neurites into the first visual neuropile, or lamina, which contains the photoreceptor input synapse. One set of cells is immunoreactive to 5-HT the other to pigment-dispersing factor (PDF), and both are thought to release their contents parasynaptically. Althought their actions are so far unclear, they have been proposed to function as whole-field neuromodulators of vision, possibly involved in the control of rhythmic circadian changes that occur in the sizes of identified lamina monopolar neurons and the numbers of their synapses (Meinertzhagen and Pyza, 1996, TINS *19*, in press). We have re-examined the question of circadian rhythmicity in the visual response, and also tested the effects of these putative neuromodulators after local injections in small (nanoliter) volumes.

Flies (*Calliphora, Musca*) reared on a normal light/dark cycle were transferred to constant darkness for long-term electroretinogram (ERG) recordings of the lamina's *ON*- and *OFF*-transients, generated by the large monopolar cells. Over several days, modest but consistently larger transients could be found during the subjective night phase than during the subjective day. We do not yet know if these changes in amplitude are linked to the neuroanatomical rhythmicity exhibited by the neurons.

The effects on the ERG of 5-HT, PDF and various other candidate transmitters were examined by injecting small quantities into the haemolymph of the head, to target the optic lobe, or, directly into the retina. 5-HT strongly enhanced the *ON*- and *OFF*-transients when injected into the haemolymph around the optic lobe, an action reversed by injected 5-HT antagonists. 5-HT had an opposite action when injected into the retina. The rapidity of onset of the effects may indicate a direct action at different receptor sites, in the lamina and retina. PDF injected into the haemolymph of the head also strongly affected the ERG transients.

Supported by NIH and MRC Canada (IAM) and NSERC Canada (SRS).

259.8

MONOPOLAR CELL AXONS IN THE LAMINA CARTRIDGE OF THE FLIES *DROSOPHILA, MUSCA* AND *CALLIPHORA* EXHIBIT GLUTAMATE-LIKE IMMUNOREACTIVITY. I.A. Meinertzhagen* and X.J. Sun. Life Sciences Centre, Dalhousie University, Halifax, NS, B3H 4J1, Canada.

Glutamate is a well-characterized neurotransmitter at the neuromuscular junction in insects. Whether it is used as a neurotransmitter in the insect's central nervous system is, by contrast, less well documented. The first optic neuropil of the compound eye in insects, the lamina, is possibly the best understood piece of neuropil, with neuron types and synaptic organization that are both structurally well identified. Even though the transmitter phenotype of most of these neurons remains unexplored, several lines of evidence suggest the utilization of glutamate. Using antibodies raised against glutamate and a pre-embedding immuno-electron microscopic technique, we have studied the distribution of the glutamate immunoreactive neurons in the lamina of three species of flies, both at the light microscopic and ultrastructural levels. In white eye mutants of the flies *Musca domestica* and *Calliphora erythrocephala* two of the lamina's monopolar cells, L1 and L2, major relay neurons to the second neuropile or medulla, both show clear immunoreactivity. In addition, these cells' medulla terminals are also immunoreactive, as well as the lamina axon of a third monopolar cell, apparently L3. Generally the lamina axons but not their dendritic spines exhibit immunoreactivity. In white eye *Drosophila melanogaster*, L1 or L2, or both, are immunoreactive. In *Musca*, L2's feedback synapses upon photoreceptor terminals are sites of localized presynaptic immunoreactivity. These findings, taken with other physiological and molecular biological findings, indicate that the monopolar cells in the lamina may store glutamate, which they could use as a neurotransmitter, either in the lamina (in the case of L2) or at their terminals in the medulla.

Supported by grants to I.A.M. (NIH EY-03592, NSERC A 0065).

259.9

VISUAL PIGMENTS AND PHOTORECEPTOR CLASSES OF DEEP-SEA SHRIMPS. T.W. Cronin, J. Kent, T. Frank, E. Widder, J.C. Partridge, P. Herring, and P. Robinson*. Dept. of Biological Sciences, University of Maryland Baltimore County, Baltimore, MD 21228; Harbor Branch Oceanographic Institute, Fort Pierce, FL 34946; School of Biological Sciences, University of Bristol, Bristol BS8 1UG U.K.

The photic environment of the deep sea contains the remains of downwelling sunlight, filtered by the overlying water to a limited waveband centered near 475 nm, together with a diversity of bioluminescent emitters. The visual pigments of deep-sea fishes tend to absorb maximally in the waveband transmitted by the water and emitted by bioluminescent animals, and thus have λ_{max} in the spectral range between 450 and 500 nm. We collected a diversity of deep-sea shrimps using midwater sampling gear capable of maintaining living specimens in total darkness, as well as a benthic trawl sampling at depths near 4500 m. Visual pigments were determined using microspectrophotometry of cryosections from eyes quick-frozen and stored at low temperature immediately after collection. Most species had a single visual pigment ($\lambda_{max} \approx 490$ nm) throughout the retina, but two oplophorid shrimp species (*Systellaspis debilis* and *Oplophorus spinosus*) possessed a second, unexpectedly short-wavelength photoreceptor class peaking near 410 nm. An analysis comparing the spectrum of downwelling light with typical bioluminescent emission spectra suggests that these species may be capable of discriminating bioluminescence from downwelling light, enabling them to counter bioluminescent camouflage systems. Supported by NSF Grant IBN-9413357, NERC Grant GR3/9329 and studentship GT4/93/3/A, and by the Harbor Branch Oceanographic Institute.

259.10

SYNAPTOTAGMIN IMMUNOREACTIVITY: SUBCELLULAR LOCALIZATION *IN VIVO* AND *IN VITRO* IN *DROSOPHILA* PHOTORECEPTOR TERMINALS AND A MARKER FOR DIFFERENTIATING NEUROPILE REGIONS OF THE VISUAL SYSTEM. Chinglu Li*, X-J. Sun and I.A. Meinertzhagen. Life Sciences Centre, Dalhousie University, Halifax, Nova Scotia, Canada B3H 4J1.

The differentiation of photoreceptors in the visual system of *Drosophila melanogaster* includes the early appearance of immunoreactivity to the presumed transmitter, histamine. We have previously reported that synaptotagmin, a synaptic vesicle protein, is expressed in the axons of differentiating photoreceptors that extend from eye-disc fragments *in vitro*. The expression pattern is punctate and occurs independent of contact with target cells; all growth cones are also immunopositive. We have now examined whether the protein is located on synaptic vesicle membranes or whether it exists as protein clusters along the neurites in the cultures. Using DSYT2, an antibody that recognizes the cytosolic domain of the synaptotagmin molecule, we compared the expression of this protein by light- (LM) and electron-microscopy (EM) in both cultured photoreceptors and *in vivo* preparations. Cultures of photoreceptors were initiated from pupae 5h after pupariation. Immuno-EM revealed that synaptotagmin is expressed on synaptic vesicle membrane in cultures maintained for 5 days *in vitro* (DIV). Immuno-positive vesicular structures resembled those found *in vivo* in adult photoreceptor terminals. Prior to this, punctate immunoreactivity to DSYT2 was detected in 3DIV cultures by both LM and EM, but no obvious synaptic vesicles were detected at this stage. Thus prior to the appearance of synaptic vesicles, synaptotagmin may initially exist in cytosolic form.

Synaptotagmin has been implicated in neurite outgrowth, but its role in development is still unresolved. Based on knowledge of the subcellular localization of synaptotagmin in differentiating synaptic terminals, we have used synaptotagmin immunoreactivity to highlight synaptic areas of the differentiating nervous system *in vivo*. The early expression of synaptotagmin in constituent neurites provides a powerful tool to display the early elaboration of neuropile regions. Immunoreactivity was imaged in wholemount preparations using confocal microscopy, and reconstructed image stacks were used to create a 3-dimensional morphogenetic series of the neuropiles of the developing brain.

Supported by grants to I.A.M. (NIH EY-03592, NSERC A 0065)

259.11

EXAMINATION OF ELECTRICAL PROPERTIES OF GLIAL AND EXTRACELLULAR COMPARTMENTS IN FLY FIRST VISUAL NEUROPIL. Kettunen, P., Weckström, M.* and Uusitalo, R. Department of Physiology, University of Oulu, 90220 Oulu, Finland.

In the insect visual systems major signal processing takes place in the first visual neuropil, lamina, in the synapses between photoreceptors and the first visual interneurons, large monopolar cells or LMCs. The synapse is characterised by multiple release sites (up to 200 per presynaptic photoreceptor), tight sealing of the synaptic zones inside glial "cartridges", and graded membrane potentials on both sides of the synapse. The photoreceptor transmitter, histamine, opens fast Cl^- channels in postsynaptic LMCs, thus creating a light-dependent current source potentially able to charge the extracellular space. We examined the non-cellular compartments and lamina glial cells with sharp (R_{el} up to 100 MΩ) glass microelectrodes and varying light stimulation protocols, including prolonged dark and light adaptation. To identify the extracellular space and/or cellular elements we used both markings with Lucifer Yellow and cell membrane permeabilization with intracellular injections of a detergent substance, DMSO. The results show putatively the presence of several extracellular spaces different from the haemolymph. The extracellular compartments exhibit relatively large changes in their voltage, up to 30 mV(compared to haemolymph) in light stimulation, meaning that the normally recorded membrane potentials of neuronal elements should be re-interpreted. The putative glial elements are characterised by very negative dark resting potentials and changes in their input resistance with light stimulation.

259.12

DYNAMICS OF SLOW AND FAST VISUAL TRACKING BY FLIES: NEUROMUSCULAR SUBSTRATE AND SENSORY STIMULATION. C. Gilbert*, P.O. Zanen, R.S. Edgecomb, and A. Pantelias.
Dept. Entomology, Cornell University, Ithaca, N.Y. 14853

Around the yaw axis, flies move their heads to perform slow visual tracking and faster saccades and optokinetic nystagmus (OKN). We studied the neural basis of these differential dynamics of head yaw in the fleshfly (Neobellieria bullata), by combining a newly developed PC-based video-microscopic 3-D measuring method, intra- and extracellular recording of motorneurons and muscle fibers, and histochemical stains of succinic dehydrogenase (SDH) in TH muscles. When viewing a horizontally drifting vertical luminance grating (34° period), stationary flies produce only very slow tracking. After changes in drift direction tracking has a faster velocity which subsequently declines to a steady velocity. Head angular velocity increases with drift velocity for stimulus contrast frequencies from 0.3-1.0Hz then plateaus to 7.0 Hz. Tethered, flying flies produce OKN more often after removal of the halteres. Small amplitude OKN occurs at the end of travel of the slow phase. Electrophysiological properties and differential staining for the oxidative enzyme SDH are correlated in TH muscles, including a newfound TH3 muscle. TH1 stains lightly and has moderate duration psps (0.72±0.2ms at half maximum amplitude). TH2 stains heavily and has significantly faster psps (0.57±0.09ms). TH3 comprises two distinct bundles: lateral, heavily staining fibers with fast psps and medial lightly staining fibers with very slow psps (2.18±0.5ms). We are currently studying differential activation of TH muscles during slow and fast head movements.

Supported in part by a grant from The Whitehall Foundation to CG.

259.13

INTRACELLULAR RECORDING AND STAINING OF VISUAL CELLS IN THE FIRST OPTIC LOBE OF *DROSOPHILA MELANOGASTER*. C.-F. Wu,* Biological Sciences, Univ. of Iowa, Iowa City, IA 52242.

It is important to establish the links between the genes and behavior such that the biochemical defects and corresponding behavioral changes can be understood in neural terms. However, neurons in the *Drosophila* central nervous system are thought to be too small in size for serious physiological investigations. An attempt was initiated two decades ago to study the cell types and their physiological properties in the first optic lobe, lamina, in the brain. Using intracellular microelectrode techniques, electrical potentials in response to light stimuli were recorded and the cells were stained by injection of a fluorescent dye via the same micropipette. The structure of the lamina and the surrounding tissue were also studied by scanning electron microscopy. The results indicated that different cell types, identifiable by intracellular staining, produce either depolarizing or hyperpolarizing potentials of characteristic kinetic properties. By applying currents to shift membrane potential, the reversal potentials of the light responses could be determined. The large number of response types characterized by distinct kinetic properties and reversal potentials observed reflects a rich variety of synaptic processes in the visual pathway.

The data provide the necessary groundwork for further analyses of a large collection of *Drosophila* mutants known to be defective in the development or physiology of the visual system. This work also serves to demonstrate that many neurons in the *Drosophila* brain are accessible to cellular physiological analysis.

I thank Dr. William Pak, in whose lab a large portion of the work was initiated and accomplished, for support and encouragement. Supported by NIH grants to W.L. P. and C.-F. Wu.

259.14

SHAKING-B DISRUPTS THE ELECTRICAL COMPONENT OF A MIXED ELECTRO-CHEMICAL SYNAPSE IN ADULT *DROSOPHILA*. J.R. Trimarchi* and R.K. Murphey, Neuroscience and Behavior Program, Dept. of Biol. Univ. of Massachusetts, Amherst, MA 01003.

We have begun to characterize the synapses between haltere afferents and the first basalar flight motor neuron (B1mn). Our results suggest that these synapses are mixed electro-chemical synapses. Staining haltere afferents with the small intracellular dye neurobiotin results in dye coupling to B1mn. Neurobiotin also passes from B1mn into haltere afferents. A mutation in *shaking-B* that eliminates electrical synapses in the visual giant fiber pathway (Krishnan *et al.*, 1993; Phelan *et al.*, 1996), abolishes the dye coupling between haltere afferents and B1mn.

Stimulation of haltere afferents reliably evokes electromyograms (EMGs) from the B1 muscle that occur at short latencies. In *shaking-B* mutant flies, stimulation of haltere afferents evokes B1 EMGs that occur at abnormally long latencies. Intracellular recordings from B1mn indicate that the *shaking-B* mutation eliminates the electrical component of mixed synapses but does not disrupt the chemical component. Supported by postdoctoral NRSA grant 1 F32 NS09700-01 to J.R.T. and NIH NS-15571 to R.K.M.

CORTEX: HUMAN STUDIES I

260.1

Temporally Resolved Human Brain Activity of Delayed Cued Movements studied by fMRI, W. Richter, S.-G. Kim*, Center for Magnetic Resonance Research, Univ. of Minn., Minneapolis, MN 55455

To examine whether hemodynamic responses (measured by dynamic fMRI) may be used to investigate serial neuronal processes in different regions, a well-known delayed visuo-motor task, studied extensively in monkeys, was used. The task consists of visual presentation, delay period and cued finger movements; delay times were systematically varied between 1 and 9 seconds. fMRI images covering visual cortex, pariatal lobules, and motor areas were acquired using a single-shot EPI technique with a temporal resolution of 200 msec, and movement and reaction times were recorded simultaneously. Motor execution was performed following a GO cue and monitored by a motion sensor. We found that bilateral associative visual cortex and parietal lobules were activated following the visual presentation. Furthermore, the supplementary motor area was activated during the delay time and before the actual motor execution. The primary motor cortex was activated weakly, but significantly, during the delay period, suggesting its involvement in motor preparation. Large activation was seen in the primary motor cortex following finger movements. This finding shows that dynamic fMRI studies can resolve serial neuronal activity during performance of visually-instructed, delayed motor tasks. It is recognized that the plethora of neural processes that operate in a domain of tens of milliseconds will remain beyond the capabilities of fMRI; however, many behavioral tasks involve mental processes lasting from hundreds of milliseconds to several seconds. In this case, fMRI may be able to resolve the processing sequence. (Supported by RR08079 and NS32919).

260.2

FUNCTIONAL MRI ACTIVATION PATTERN OF MOTOR AND LANGUAGE TASKS IN BROCA'S AREA P. Erhard*, T. Kato, P.L. Strick# and K. Ugurbil, Center for Magnetic Resonance Research, Univ. of Minnesota, Minneapolis, MN 55455, # State University of New York, New York, NY

Broca's area located in the inferior frontal lobe and the frontal operculum includes parts of Brodmann's areas (BA)44, BA45 and possibly BA46, is not well defined either anatomically or functionally. Twelve healthy, right handed subjects (4 males) were asked to perform different motor tasks including (I) random tongue movement, (II) toe movement, (III) complex instruction guided finger tapping, (IV) copying of displayed hand shapes and, (V) a language task. We used multislice fMRI with a relatively high resolution and investigated whether areas activated by motor tasks overlap with activation induced by language tasks. The language task showed activation in the lower part of BA6, in BA44, BA45, and in BA46. The toe task, the finger task and the handshape copying task also activated in the inferior part of BA6, in BA44 and to less extend in BA45 and BA46. The more complex motor task (handshape copying) shows relatively larger activation areas in both BA45 and BA46. The tongue-motor task activated in BA44 and the lower part of BA6 but to a much less extend in BA45 and in BA46. Our data shows that Broca's area, in particular the inferior part of BA6, BA44, and the frontal opercular part is involved in several different motor activities related to extremities and facial areas.

260.3

Mental Practice and Motor Skill Acquisition: An fMRI Study, J.E. Deutsch,*[1,3] W.C. Liu[2], J. Maldjian[2], J. DeLuca[3] UMDNJ-SHRP[1], Department of Radiology, UMDNJ-NJMS[2], Newark, NJ 07107, Kessler Institute for Rehabilitation[3]

Physical practice of a complex finger movement sequence was demonstrated to improve motor skill performance with changes in M1 associated with motor learning (Karni et.al, 1995). Imagining and performing movements appear to share the same neural substrates, suggesting that either strategy may be used for motor learning (Jeannerod, 1995). The purpose of this study was to explore the effect of mental practice on the acquisition of a motor skill.

Five subjects were instructed in 2 complex finger movement sequences. Videos of 30 sec. trials served to record baseline speed and accuracy as subjects executed both sequences. They were assigned one sequence to mentally practice for 5-10 min. daily. Speed and accuracy were measured for the trained and control sequences at 3, 7 and 10 days post training. Two of the subjects were imaged, on the 1st and 7th day, with BOLD fMRI using a single slice SPGR technique (24 cm FOV, 5 mm slice thickness, 256x128 matrix) on a conventional GE 1.5 Tesla clinical scanner. The TR/TE/flip angle combination utilized was 40/30/30. The paradigm consisted of cycles of 30" rest/20" activity for a total of 2'20". Functional maps were created using custom software developed in IDL utilizing the fourier transform cross-correlation function. Maximum correlation maps from the fourier transform cross-correlation function were thresholded at a $P < .01$ and a minimum % signal change of 0.5 %. ROI's were drawn and activation volumes compared. To reduce spurious activation a 2-pixel nearest neighbor rule was applied.

After 1 week of practice all subjects improved their trained sequence speed ($F_{(2,8)}=48.7$, $p<.001$). There was no significant activation for the complex motor tasks on day 1. On day 7 activation in the contralateral sensorimotor cortex was significant. Mental practice improved motor function which was represented by a change in sensorimotor cortex activation. *Supported by NIDRR H133P40003

260.4

CORTICAL REPRESENTATION OF HAND AND FINGER MOVEMENTS IN MAN STUDIED BY MAGNETOENCEPHALOGRAPHY. J. Volkmann*, A. Schnitzler, R. Seitz, O. W. Witte and H. J. Freund. Dept. of Neurology, Heinrich-Heine-University, D-40225 Düsseldorf, Germany.

Using whole-head neuromagnetic recordings (Neuromag™ 122-channel MEG) and time-varying multi-dipole modeling we could reliably separate the cortical generators of motor output and reafferent activity for different hand and finger movements (index finger extension, index finger abduction, thumb flexion, little finger abduction and wrist flexion) in 4 right-handed volunteers.

The average distance between dipolar generators for the respective movements within primary motor cortex (M1) was 5.4±1.82 mm in the right and 9.3±3.8 mm in the left hemisphere across subjects. Sources of reafferent activity in primary somatosensory cortex (S1) were usually less separated showing a higher degree of overlap. In all subjects, the size of hand motor area was estimated by the smallest cube containing the dipole solutions for all five movements. The cube volume was consistently larger ($p<0.05$, t-test) in left (mean 433.5±62.8 mm^3) than in right M1 (mean 243.5± 138.4 mm^3). In contrast no significant hemispheric asymmetry was found for reafferent activity in primary sensory cortex. Whether the observed M1 asymmetry is related to handedness remains to be shown.

The observed patterns of movement representation did not correspond to the 'classic' M1 homunculus or semiusculus and were distinct for each subject. This mismatch reflects the difference between the classical approach to define somatotopy on the basis of observing the artificial hand/finger movements evoked by brain stimulation and the study of the relation between cortical activation and voluntary movements. Our results show that even different movements of the same digit are encoded by spatially segregated neuronal populations in primary motor cortex as can now be studied non-invasively in human subjects.

(Supported by the Deutsche Forschungsgemeinschaft, SFB 194, Z2)

260.5

ACTIVATION OF CORTICAL MOTOR AREAS DURING FINGER MOVEMENT SEQUENCES OF DIFFERENT COMPLEXITY. J. Ashe*, P. Dassonville, X.-H. Zhu, K. Ugurbil, S.-G. Kim. Brain Sciences Center, VAMC, and Center for Magnetic Resonance Research, University of Minnesota, Minneapolis, MN 55417.

The exact determinants of functional activation within motor areas of the brain have not been fully elucidated. The complexity of the motor output appears to be one of these determinants. Traditionally, complex motor tasks have been defined in terms of *memorized* sequences of movements. In the present study, we examined the effects of varying the level of complexity within a series of *visually-instructed* finger movement sequences. In this context, the complexity related to the uncertainty of the upcoming visual stimulus. We obtained BOLD-based fMRI multislice axial images at 4 Tesla while subjects performed visually-instructed finger movements with the left and right hands. Subjects were cued to move the appropriate finger by a visual display on which four stimuli, arranged horizontally, were illuminated individually in either a predictable (i.e., simple) or randomized (i.e., complex) sequence at a frequency of 0.75 Hz. After delineating the boundaries of primary motor cortex, supplementary motor area (SMA), preSMA, and premotor (PM) cortex using anatomical landmarks, we compared the levels of activation in each area during the simple and complex sequences. We found the following: (1) In general, all areas showed significant increases in activation during both sequences for ipsilateral and contralateral movements. Exceptions were left primary motor cortex during simple ipsilateral movements and right primary motor cortex during both simple and complex ipsilateral movements. (2) There were consistently greater activations in PM, SMA and preSMA for the complex sequence than for the simple. (3) The trend toward greater activation during complex sequences was more evident during contralateral, rather than ipsilateral, movements. (Supported by NIH grant NS32437.)

260.6

DIFFERENCES IN THE FUNCTIONAL LATERALIZATION OF CORTICAL MOTOR AREAS DURING FINGER MOVEMENTS. P. Dassonville*, X.-H. Zhu, S.-G. Kim, K. Ugurbil, J. Ashe. Brain Sciences Center, VAMC, and Center for Magnetic Resonance Research, University of Minnesota, Minneapolis, MN 55417.

Neurophysiological studies in monkeys have shown that the various cortical motor areas differ in the extent to which the corresponding motor-related activity is lateralized. For example, there are clear differences in the proportion of cells in the supplementary motor area (SMA) and the primary motor cortex that are activated with ipsilateral movements. However, the extent of these differences has not been fully elucidated. To test the functional lateralization of cortical motor areas in humans, we obtained BOLD-based fMRI multislice axial images at 4 Tesla while subjects performed randomized sequences of visually-instructed finger movements with the left and right hands. For individual subjects, we delineated the boundaries of primary motor cortex, SMA, preSMA, and premotor (PM) cortex using anatomical landmarks, and compared the volumes of activated cortex within these areas during ipsilateral and contralateral movements. The principal findings were: (1) All areas were significantly activated during both ipsilateral and contralateral movements, with the exception of right primary motor cortex during ipsilateral movements. (2) The extent of functional lateralization differed among the areas, with primary motor cortex the most strongly lateralized ($p<0.001$), followed by SMA ($p<0.05$) and PM (n.s.). In contrast, preSMA showed no indication of lateralization. (3) There were hemispheric asymmetries in the extent of lateralization: the combined activity of primary motor cortex and SMA was more lateralized in the right than in the left hemisphere. These differences in functional lateralization may account for some of the differences in the severity of deficits following lesions of the various cortical motor areas. (Supported by NIH grant NS32437.)

260.7

SELECTIVE ACTIVATION OF POSTERIOR PARIETAL CORTEX DURING PRISM ADAPTATION. D. M. Clower*, J. Votaw, J. M. Hoffman, G. E. Alexander. Department of Neurology, Emory University School of Medicine, Atlanta, GA 30322.

Adaptation of reaching trajectories to the effects of displacing prisms is a paradigm that has been used extensively in human psychophysical studies to explore the plasticity of internal coordinative mappings between visual input and motor output. Few studies, however, have attempted to localize the cortical regions responsible for such sensorimotor remapping. To address this issue, we have used 3-D PET (positron emission tomography) with bolus injections of ^{15}O-labelled water to measure regional cerebral blood flow in seven male subjects who performed a set of targeted reaching tasks that included: 1) a control task in which subjects reached to visual targets on a touchscreen with no visual distortion; 2) an adaptation task in which subjects reached to targets viewed through laterally displacing prisms; and 3) an error correction task in which the target moved as the subject was reaching, such that a trajectory correction was required to obtain the new target. The prism adaptation task involved both sensorimotor adaptation and trajectory error correction, while the error correction task involved only the latter variable. Using an analysis of variance which effectively subtracted the results of the error correction task from those of the adaptation task, we were able to localize the cortical fields involved in sensorimotor adaptation independent of error correction processes. Our results indicate that a highly focal region of the posterior parietal cortex is active during the sensorimotor remapping that accompanies prism adaptation. *Funded by Emory University.*

260.8

MODALITY RELATED PARCELLATION OF HUMAN PARIETAL CORTEX IN TRAJECTORIAL MOVEMENT CONTROL. F. Binkofski, S. Posse, J.S. Shah, H.W. Müller-Gärtner, A. Kleinschmidt, R.J. Seitz*, H.-J. Freund. Dept. of Neurology, Heinrich-Heine-University, Düsseldorf, Germany, Forschungszentrum Jülich GmbH, Jülich, Germany.

The pattern of activation of the cortical areas involved in trajectorial movement control was examined by fMRI. Five healthy right-handed volunteers (37.4 +/- 3.5 SD years) performed three types of previously practiced movements: (1) self-initiated figure-8 movements of the right index, (2) visual figure-8 tracking with the right index, both performed at 1.5 Hz and (3) blindfolded imagery of figure-8 index movements. An EPI method (TR: 3s, TE: 66ms, α: 30°) utilizing the BOLD effect was employed. Ten slices of 3mm thickness oriented parallel to the AC-PC plane and positioned above the corpus callosum were acquired. The MR protocol consisted of 5 cycles alternating between task (15s) and rest state (15s). After movement correction correlation analysis using a threshold of 0.5 and regional analysis thresholded at z-score of 3 were performed.
Movements were associated with significant activation in contralateral M1, S1 and SMA, with a greater involvement of SMA during the self-initiated movements. All three conditions activated bilateral premotor cortex. The anterior portion of the intraparietal sulcus (AIP) was most strongly activated by task 1, whereas both visual tracking and imagery preferentially activated posterior parietal cortex (PPC). This provides evidence that the same movement trajectory requires involvement of different neuronal aggregates depending on the nature of the sensory-motor transformations: internal kinesthetic representations in AIP and visual representations in PPC.

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260.9

INTERACTION OF INHIBITION AND FACILITATION IN HUMAN MOTOR CORTEX: A TRANSCRANIAL MAGNETIC STIMULATION STUDY. U. Ziemann[1]*, J.C. Rothwell[2], M.C. Ridding[2]. [1] Dept Clin Neurophysiol, Univ. Göttingen, D-37075 Göttingen, Germany. [2] MRC Human Movement & Balance Unit, The Institute of Neurology, London WC1N 3BG, U.K.

Focal paired transcranial magnetic stimulation (TMS) was used in twelve healthy volunteers to study the interaction of intracortical inhibition (ICI) and intracortical facilitation (ICF) in the motor cortex. The effect of one or two subthreshold conditioning stimuli (CS) on the size of the EMG response in the contralateral abductor digiti minimi (ADM) muscle to a suprathreshold test stimulus was evaluated.

A single CS inhibited the test response at interstimulus intervals (ISIs) of 1-4 ms, while the response was facilitated at ISIs of 6-20 ms. These effects probably occur at the cortex level since the same CS had no effect on the size of the H-reflex elicited at appropriate ISIs in the active ADM. ICI and ICF appear to be due to separate mechanisms since (i) ICI had a lower threshold (70% of active motor threshold) than ICF (80%); (ii) ICI was independent of the current direction in the brain induced by the CS, while ICF was maximal with a posterior-anterior, but minimal with a latero-medial current; (iii) When inhibitory and facilitatory CS were given together at ISIs of 3ms and 10ms, the effect on the size of the ADM response is best described by an interaction model, which assumes the linear multiplicative integration of independent inputs to the target cell population (in the form of Y=ICI*ICF).

Our results suggest that subthreshold TMS is capable of activating separate populations of inhibitory and excitatory interneurons which about equipotentially can modulate the excitability of corticospinal neurons in human motor cortex.

260.10

INTRACORTICAL INHIBITION IS INCREASED DURING POST-EXERCISE DEPRESSION OF MOTOR EVOKED POTENTIALS DS Stokić*, WB McKay, L Scott, AM Sherwood, MR Dimitrijevic
Division of Restorative Neurology and Human Neurobiology
Baylor College of Medicine, Houston, Texas 77030

We have previously reported that motor evoked potentials (MEPs) elicited by transcranial magnetic stimulation (TMS) of the motor cortex are depressed following sustained maximal voluntary contraction (MVC). The aim of this study was to determine whether intracortical inhibition, as demonstrated by paired magnetic stimulation, is involved in the post-exercise MEP depression.

In seven healthy subjects MEPs were recorded from both tibialis anterior (TA) muscles. Conditioning and test stimuli were given at 2, 4, 6 and 8 ms interstimulus intervals (ISIs) using a double cone coil centered over the vertex. Conditioning stimuli were 0.9 x individual MEP threshold during 10% MVC of the right ankle dorsiflexors (32 $\pm$ 4% of stimulator output). Test stimuli were 1.25 x individual MEP threshold in relaxation (66 $\pm$ 6%). Control and conditioned stimuli (5 each, 15 s apart) were randomly delivered in relaxation, before and after the MVC of the right ankle dorsiflexors performed for 1 min.

Before MVC, conditioning magnetic stimuli produced a significant inhibition of both left and right TA MEPs at 2 ms ISI (65% of control) with progressive recovery thereafter. After MVC, control MEPs were decreased in the exercised TA (mean $\pm$ SE, 938 $\pm$ 95 μV before and 325 $\pm$ 48 μV after MVC; $p< 0.001$) with no change in the non-exercised TA (433 $\pm$ 48 μV and 484 $\pm$ 54 μV, respectively). Intracortical inhibition was more pronounced after MVC in the exercised TA only (additional MEP decrease of 22% on average, $p<0.001$).

This finding suggests that increased intracortical inhibition is at least in part responsible for post-exercise depression of MEPs.

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260.11

VOLITIONAL MULTIJOINT AND SINGLE JOINT MOVEMENTS EMPLOY DIFFERENT FUNCTIONAL ORGANIZATION OF THE CNS

WB McKay*, DS Stokic, WJ Eaton, AM Sherwood, MR Dimitrijevic
Division of Restorative Neurology and Human Neurobiology, Baylor College of Medicine, Houston, Texas.

We have previously reported that maximal volitional contraction of a muscle focally reduces the responsiveness of the motor cortex to transcranial magnetic stimulation (TMS)(McKay et al, *Exp Br Res*, 105:276-282, 1995). In the current study, we examined motor cortex responsiveness changes when a muscle is activated as part of a multi-muscle, multijoint effort. Six healthy subjects, 32 to 53 years of age, were seated in a chair equipped to measure contraction forces. In the first session, they performed a maximal isometric multijoint contraction consisting of hip flexion and ankle dorsiflexion for 1 minute. The second session required ankle dorsiflexion alone. TMS over the scalp vertex elicited motor evoked potentials (MEPs) recorded through surface electrodes placed over the major muscle groups of both lower limbs. Stimuli were delivered at one minute intervals for five minutes before and after exercise. Both force and EMG amplitude in the exercised TA decreased at a similar rate during the single joint contraction and the multijoint exercise. However, TA MEP amplitudes showed post-exercise depression only following the single joint effort ($p<0.05$). TA MEP amplitudes decreased from $1018 \pm 173\ \mu V$ to $430 \pm 88\ \mu V$ after the single joint effort but were unchanged from $1195 \pm 217\ \mu V$ before and $1088 \pm 192\ \mu V$ following the multijoint exercise. These results suggest that activation of a muscle such as the TA in the context of a multijoint, multi-muscle contraction probably occur as a result of a different functional organization of CNS structures from that of the relatively isolated muscle activation needed for a single joint movement.

Funding provided by the V.L. Smith Foundation for Restorative Neurology and The Kent Waldrep Institute for Paralysis Research.

260.12

THE SILENT PERIOD INDUCED BY TRANSCRANIAL MAGNETIC STIMULATION IN PATIENTS WITH ASTERIXIS

M.A. Pastor*, C. Garcia de Casasola, A. Ibiricu, A. Espí, J.I.Herrero, J.Quiroga, J. Prieto, J. Artieda Dept. of Neurology, and Dept. of Internal Medicine, University Clinic, University of Navarra, 31080 Pamplona, Spain.

The excitatory motor evoked potentials (MEPs) and the inhibitory silent period responses (SPRs) to focal transcranial magnetic stimulation (TMS) were studied in the right extensor carpi radialis (ECR) in 15 normal subjects and 6 patients with asterixis. A Novametrix Magstim device, with a maximal magnetic field strength of 2T, and a loop diameter 7 cm figure of eight coil was used. The stimulus was delivered to a grid of 1 cm sites on the scalp, to locate the maximum duration silent period. The stimuli were delivered at 1.5 individual motor theshold intensity. MEPs and SPRs were recorded from the right ECR via surface electrodes. Patients were tested while relaxed for the MEP, and trained to sustain 50% maximum level of tonic muscle contraction for the SPR. Contraction force was continuously monitored. The recordings were made with a sweep duration of 500 ms and a prestimulus period of 75 ms. The SPR duration was calculated from the stimulus artifact to the first reappearing EMG burst. In all patients the presence of asterixis was checked, recording ECR and FCR EMG, while stretching the arm, through surface electrodes. Motor threshold, expressed in percentage of the maximal stimulator output, was defined as the stimulus level where out of six consecutive stimulus, three responses of at least 30uV were defined on the screen by one of the authors blind to the stimulus intensity. There was a significant difference in magnetic stimulation motor threshold between patients and age-matched controls ($t=4.3$; $p<0.01$). In the control group the mean duration of the SPR in ECR was 164.2, SD 43.6. The mean patients SPR was significantly prolonged 255.6, SD 60.7($T=4.7$;$p< 0.05$) These results suggest that there may be a cortical dysfunction between excitatory and inhibitory mechanisms in patients presenting negative motor phenomena such as asterixis.

260.13

A HIGH FREQUENCY RESONANT CIRCUIT IN HUMAN MOTOR CORTEX

V.E. Amassian*, J.C. Rothwell[1], M. Redding[1], A. Priori[1], and R. Jalinous[2]. Dept. of Physiology, SUNY Health Sci. Ctr. Brooklyn, NY 11203; [1]MRC Human Movement Control & Balance Unit, Inst. of Neurology, London WC1N 3BG, UK; [2]MAGSTIM Co. Dyfed, SA34 OHR, UK.

Indirect (I) corticospinal tract (CT) responses to an electrical stimulus to primate motor cortex are remarkably periodic (~2ms). We sought evidence of a periodic circuit in humans by transcranially stimulating motor cortex with an HF train of magnetic pulses oriented for transynaptic activation of CT neurons. To optimize the need for temporal facilitation, subjects were relaxed and the stimulus reduced so that 2 pulses at , e.g., 1.5-1.7ms interval elicited a near-threshold response in First Dorsal Interosseous (FDI). In 3 of us, 3-4 pulses elicited optimal FDI responses at interpulse intervals of 1.6-1.7ms (Fig. 1); the optimum period in a fourth subject was 1.3ms, but was uhdefinable in the fifth. Our findings probably reflect coincidences of periodic magnetic transynaptic excitation with periodic cortical interneuron chain activity at ~600 Hz, thereby supporting the hypothesis of a clock-like time quantizer in motor cortex (Neurosurgery, 1987, 20: 74-93).

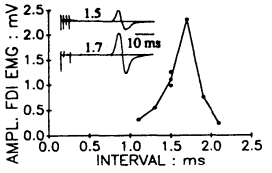

Fig. 1. In the same subject, 10 summed responses are plotted versus intervals; inset, summed responses at 1.5 and 1.7 ms intervals.

260.14

CORTICAL MOTOR OUTPUTS DURING DYNAMIC ISOMETRIC CONTRACTIONS: A SINGLE-JOINT, MAGNETIC STIMULATION STUDY.

O. Soto-Téllez, A. Roy, S. Connolly, H. Abdulhadi, K. H. Chiappa and D. Cros *. Dept. of Neurology, Massachusetts General Hospital, Boston, MA.

Single cell recordings of corticomotoneuronal cells have shown populations with phasic responses relative to the tonic or ramp components of an isometric contraction. This may imply different cortical mechanisms for dynamic versus static contractions. We have previously shown that a mild static background contraction is sufficient to generate a change in the directional response of the twitch evoked with cortical magnetic stimulation, which corresponds closely to that of the background contraction. In these experiment we evaluated cortical outputs evoked with the same methodology, during dynamic linear and circular contractions. 5 normal subjects were studied. The right hand/forearm were immobilized with a cast, except for the second digit. This digit was introduced into a rigid ring which was attached to 2 orthogonally-oriented force transducers, allowing isometric force changes to be recorded in a vertical plane (360 °). The contralateral motor cortex was stimulated using a large circular coil centered around the vertex at a stimulation intensity 10 % above threshold. The evoked twitch was defined by dF/dt plots, and its vectorial attributes (magnitude and direction) compared to those of the immediately preceding contraction (30-50 ms) defined by the same method. 3 paradigms were studied according to the type of background (facilitating) contraction: 1) Static forces in 8 directions of the workspace; 2)Linear dynamic contractions at constant dF/dt in 8 directions; 3) Circular dynamic contractions at constant dF/dt. A tight correlation was found between the direction of facilitation and the direction of the evoked twitch in both the linear static and dynamic contractions ($r2 > 0.87$). During circular trajectories, the direction of the evoked twitch closely matched that of the tangent of the circle defined by the facilitation period ($r2 = 0.88$, slope 0.76 (. 0.04). These results show that the active components (as opposed to postural) of movements are selectively enhanced during dynamic contractions, and suggest a fundamental role of the motor cortex in the specification of immediate trajectory changes. Supported by Clinical Neurophysiology Research Fund, MGH.

260.15

MOTOR CORTEX EXCITABILITY DURING TASK PERFORMANCE IS RELATED TO HAND PREFERENCE IN MAN

M.A. Nordstrom* and J.G. Semmler. Department of Physiology, University of Adelaide, Adelaide 5005, Australia.

Motor unit synchronization is weaker in first dorsal interosseous (FDI) muscle from the dominant hand of right-handed (RH) subjects than the non-dominant hand during index finger abduction (Semmler & Nordstrom, Exp. Brain Res. 104:115-125, 1995). One possible explanation is that corticomotoneuronal (CM) cells controlling FDI are more active when the non-dominant hand is used in this task. We have tested this hypothesis using magnetic (TMS) and electrical (TES) transcortical stimulation, which activate the corticospinal pathway at different sites (Rothwell et al., Exp. Physiol. 76:159-200, 1991). The responses to TMS (but not TES) are significantly influenced by CM cell activity. In eight RH subjects, TMS at passive threshold strength were applied (20/trial, < 0.2 Hz) while the subject performed isometric index finger abduction at various forces (0.5 N to 50% maximal voluntary contraction (MVC)). Motor evoked potentials (MEPs) were recorded from FDI surface EMG. Mean (± SD) passive threshold TMS strength did not differ significantly between hands (dominant *vs.* non-dominant; $41 \pm 3\%$ *vs.* $43 \pm 8\%$; paired t-test, $P>0.05$). MEP areas (normalised to FDI M-wave area) increased with contraction strength, a consequence of increased excitability of cortical and spinal neurons. For the pooled data, normalised MEPs were consistently larger in the non-dominant hand, ranging from 208% of the dominant hand in the weakest contraction (0.5 N) to 123% in the strongest (50% MVC). The protocol was repeated in five of these subjects using passive threshold TES. MEPs following TES were facilitated by muscle contraction to a similar extent in each hand, indicating no lateral differences in spinal excitability. We conclude that during simple index finger abduction, CM cells controlling FDI are more active, and therefore contribute relatively more to the net excitatory command, when the non-dominant hand is used for the task. Supported by NHMRC of Australia.

261.1

VOLTAGE-DEPENDENT OUTWARD CURRENTS IN VESTIBULAR SENSORY NEURONS FROM CHICK EMBRYO BRAIN SLICES. G. Gamkrelidze*, and K.D. Peusner. Dept. of Anat. and Cell Biol., George Washington Univ. Sch. of Med., Washington, DC 20037.

The tangential vestibular nucleus (TN) represents a good model system for studying the development of signal processing in the vestibular system. Previous work in our laboratory has identified two main neuron types in the TN, principal (PC) and elongate cells (EC) based on morphology and electrophysiology. To further characterize these TN neurons at critical developmental ages, we are studying voltage-gated outward currents in 16 day chick embryo brain slices using whole-cell patch-clamp technique. The recording pipette contained 0.1% biocytin that allowed for morphological identification of the recorded neurons (n=5). Neurons were first characterized according to their responses to current steps and vestibular nerve stimulation under current clamp. Then voltage clamp recordings were performed in the presence of 2μM Tetrodotoxin to block inward sodium currents. Initial surveys of membrane currents from a holding potential -60mV utilized voltage steps from -90mV to +30 mV with 5mV increments (n=12). All cells had outwardly rectified I-V curves and peak currents were about 1-1.8 nA at +30mV. PCs generated an outward current in the range of -20 to -30 mV that did not show significant inactivation during a 200 ms voltage step. In contrast, ECs generated a transient outward current, which was followed by an outward current with little inactivation, both of which activated at -40 to -50 mV. The transient and sustained components of the current were further separated using a prestep to -120 mV. The sustained portion was partly blocked by 10mM Tetraethylammonium, while the transient current was fully activated at about -100mV and expressed full steady state inactivation at about -40 mV. This work is the first description of membrane currents in TN neurons. Experiments are currently underway to further separate and characterize the different membrane currents, which could account for differences in excitability reported between PCs and ECs. Supported by NIH grant #DC00970.

261.2

PHYSIOLOGICAL CORRELATES OF VESTIBULAR COMPENSATION IN ISOLATED, IN VITRO WHOLE BRAINS FROM HEMILABYRINTHECTOMIZED GUINEA-PIGS. N. Vibert*, A. Babalian, [1]M. Mühlethaler and P.-P. Vidal. L.P.P.A., CNRS-Collège de France, 15 rue Ecole de Médecine, 75270 Paris cedex 06, France and [1]Dpt. de Physiologie, CMU, 1 rue Michel-Servet, 1211 Geneva 4, Switzerland.

Vestibular compensation following unilateral labyrinthectomy is a model of post-lesional plasticity in the central nervous system. Just after the lesion, the deafferented vestibular neurons (VNs) are silent, whereas the discharge of contralateral VNs is strongly increased. The associated postural and oculomotor disorders disappear in 2 days in the guinea-pig, as a normal activity is restored in both vestibular nuclei. We used data obtained in isolated whole brains of normal guinea-pigs as reference to search for the cellular changes underlying vestibular compensation in brains of hemilabyrinthectomized animals.

In vitro whole brains were obtained from young guinea-pigs 3 to 8 days after labyrinthectomy. During the dissection, vestibular nerves were cut on both the deafferented and intact sides. In vivo, such an acute deafferentation of vestibular neurons on the intact side triggers in compensated animals the Bctcherew's phenomenon: A postural and oculomotor syndrom appears, which is the mirror image of the one induced by the first lesion. Interestingly, this phenomenon was also observed in isolated brains from compensated guinea-pigs. Indeed, extracellular recordings revealed an asymmetry between the resting activities of VNs in both medial vestibular nuclei: their mean discharge was higher on the compensated side than on the newly deafferented side. Furthermore, a spontaneous nystagmus could be recorded from abducens nerves. This result demonstrate that some of the changes underlying vestibular compensation were retained in deafferented brains. In particular, we found that VNs on the compensated side received a stronger than normal excitatory spinal input, whereas the reverse was true for VNs on the intact side. These data fits with the hypothesis that an increased efficiency of cervical proprioceptive inputs to deafferented VNs would be partly responsible for the recovery of their normal discharge during compensation.

This work was supported by the CNRS, the CNES, and the Fondation pour la Recherche Médicale.

261.3

CANAL-SPECIFIC MONOSYNAPTIC EXCITATION AND DISYNAPTIC INHIBITION OF SECOND ORDER VESTIBULAR NEURONS IN THE FROG. H.Straka*, S.Biesdorf and N.Dieringer. Dept. Physiology, Pettenkoferstr. 12, 80336 München, GERMANY.

Second order vestibular neurons (2°VN) receive a monosynaptic, glutamatergic excitation following ipsilateral vestibular nerve stimulation. In addition, most of these 2°VN were disynaptically inhibited by glycine (15 out of 16) or GABA (26 out of 29). Here we specify the pattern of convergence of excitatory and inhibitory inputs on 2°VN from individual semicircular canal nerves of the ipsilateral labyrinth. To that end, we recorded in-vitro synaptic potentials in 2°VN following separate electrical stimulation of the horizontal, anterior or posterior canal nerve before and after bath-application of bicuculline (0.5μM) or strychnine (0.5μM).

Most 2°VN (88 out of 99) received a monosynaptic EPSP from only one of the three semicircular canal nerves. Few neurons (N=10) received a monosynaptic EPSP from two canal nerves and one neuron received a monosynaptic EPSP from all three canal nerves. In 2°VN characterized by a monosynaptic EPSP from one canal nerve, stimulation of one of the other two canal nerves evoked a heterogeneous pattern of synaptic potentials consisting of di- and polysynaptic EPSPs, polysynaptic IPSPs or no response at all. Comparison of the amplitudes of EPSPs recorded before and during the application of glycine or GABA receptor antagonists revealed the presence of disynaptic IPSPs in these 2°VN (14 out of 14). As a rule, the monosynaptic excitation and the disynaptic inhibition of a given 2°VN originated from the same canal nerve (13 out of 14). However, horizontal canal-related 2°VN were not inhibited exclusively by glycinergic nor were vertical canal-related 2°VN inhibited exclusively by GABAergic vestibular interneurons.

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261.4

DYNAMIC RESPONSES OF PIGEON PRIMARY OTOLITH AFFERENTS TO LINEAR ACCELERATION. X. Si, D. E. Angelaki and J. D. Dickman*. Department of Anatomy and Surgery, University of Mississippi Medical Center, Jackson Mississippi 39216.

Otolith afferent responses in pigeons have been studied during linear acceleration. At 2 Hz, the mean maximum gain was 367 spikes/s/g with a range between 65 to 1421 spikes/s/g. Phase leads ranging between 0 and 53 degrees re acceleration were observed. The tuning ratios (minimal /maximal sensitivity) of most afferents (88%) were lower than 0.1 suggesting that these fibers are one-dimensional (cosine-tuned). A response vector (relative to acceleration) was determined for each afferent with 74% of the vectors being directed out of the contralateral ear. Electrical polarization of the labyrinth was used to apply Galvanic currents to the pigeon vestibular primary afferents in order to study the cv (coefficient of variation) and cv* value. Otolith units were then grouped according to their cv* values into regular, intermediate and irregular classes. In addition, the response dynamics were tested on 18 otolith afferents. The gains of the irregular units had greater increases than the intermediate and regular units as stimulus frequency increased between 0.25 Hz to 10 Hz. The phase values remained consistently flat for all three groups across frequency. However, the more irregular firing otolith afferents had larger phase advances than regular firing units. The high gain, flat phase lead and small tuning ratios suggest that pigeons may have a simple one dimensional yet very sensitive otolith afferent system, as required for specialized flight behavior.

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261.5

RESPONSE OF NEURONS IN NUCLEUS FASTIGII IN PHASE-SHIFTED BINAURAL GALVANIC LABYRINTH POLARIZATION. **H.-G. Schlosser, W. Guldin*, O.-J. Grüsser. Freie Universität Berlin, Univ.-Klinik. Benjamin Franklin, Dept. of Physiology, Arnimallee 22, 14195 Berlin, Germany.**

Cells of Nucleus fastigii integrate vestibular stimuli. In anaesthesized Norwegian Rat both labyrinths were stimulated independently by galvanic sinewave currents, while extracellular single-unit recordings were performed on Nucleus fastigii. The independent polarization was performed by sinewave currents between two spheric silverchloride-electrodes (sscE) for each labyrinth. One sscE was implanted epidurally above the occipital cerebral hemisphere, the other was placed by an earbar through the Meatus acusticus externus in front of Membrana tympani. The flowing currents were constantly sinewave shaped by voltage regulation. Glass micropipettes (tip diameter 1.0μm; R = 30MΩ) filled with Pontamine Sky Blue were used for recording and allowed an exact iontophoretic localization.

Most of the cells investigated responded to polarization of each of the labyrinths, some only to ipsilateral or contralateral stimulation. The neuronal response was analyzed with peristimulus time histogram and fast fourier transformation.

Between recordings of 20 periods of stimulation (0.5 Hz) the polarization sinewaves of the labyrinths were phase-shifted between each other in steps of 90 degrees. At a phase-shift (P) of 180 degrees one labyrinth is polarized at the most when the other is at its minimum. Nevertheless this synergistic stimulus of both sides did not provide maximum responses in all cells. Optimum responses of cells were sometimes aroused at P = 90 deg, or even P = 360 deg, i.e., when both labyrinths are polarized in phase.The investigated cells showed an upper frequency-limit between 10.0 and 30.0Hz to a binaural, p = 180 deg stimulation.The minimum current amplitude for cell responses in Nucleus fastigii varied between 52μA and 118μA. It was similar to the threshold of ocular movement in rat.

261.6

LINEAR DYNAMICS AND EYE POSITION SENSITIVITY OF PREPOSITUS NEURONS IN THE ALERT GERBIL. G.D. Kaufman* and A.A. Perachio. Departments of Otolaryngology, Physiology & Biophysics and Anatomy & Neurosciences, Univ. TX Medical Branch, Galveston, TX 77555-1063.

The prepositus hypoglossus nucleus (PrH) has been implicated in horizontal eye velocity storage. This area has reciprocal connections with cells in vestibular, mesencephalic visual, and inferior olivary structures. We have developed an alert gerbil preparation with an eye coil system and extracellular single unit recordings in order to define the signals carried by PrH neurons in the lateral-eyed rodent model. With n>86 PrH cells characterized by pure linear and angular accelerations, a heterologous pattern of unit activity emerges. Dynamic responses were similar to those reported in the primate. Many cells exhibited complex multi-dimensional sensitivity and spatiotemporal convergence, while some had simple 1-D sensitivity to otolith input. Some multi-D cells exhibited vectors of maximum sensitivity with spatiotemporal properties (phase lead re: acceleration of 90 degrees) opposite to that found in medial vestibular cells. Other units responded to canal stimulation only. Many (~30%) of the units burst during the inhibitory portion of the angular rotation cycle, correlating with the quick phase of nystagmus and indicating an eye position component. The findings suggest that the PrH could serve an integrative role in many facets of vestibular function. The gerbil linear VOR response will also be discussed. NIH DC-00385 and DC-00111

261.7

PHYSIOLOGIC DIVERSITY AMONG HORIZONTAL SEMICIRCULAR CANAL AFFERENT NEURONS IN THE CHINCHILLA. L.F. Hoffman*. Div. of Head & Neck Surgery, UCLA School of Medicine, Los Angeles CA 90095.

In this study individual primary afferent neurons innervating the horizontal semicircular canal crista were investigated to determine whether the system dynamic response characteristics exhibit parallel diversity to that exhibited by the single frequency responses [Baird et al., J. Neurophys. 60:182, '88]. Barbiturate-anesthetized chinchillas were prepared for electrophysiologic recording by exposing the superior vestibular nerve approximately 2 mm proximal to the horizontal ampulla. Body temperature was monitored via a rectal probe and closely regulated via a servo-controlled heating system. With the animal restrained in a special head holder on a rotatory turntable, glass micropipettes were used to record spontaneous and stimulus-evoked spike trains from horizontal semicircular canal afferents. Discrete frequency sinusoids were used over a broad stimulus bandwidth (0.0125 - 3.2 Hz) to characterize the system response dynamics of individual afferents. Each afferent's spontaneous discharge was analyzed for coefficient of variation (CV). Response gains and phases relative to stimulus velocity were determined from input-output functions and discrete Fourier analysis of the evoked cycle histograms, respectively. At each frequency examined response phases relative to stimulus velocity exhibited considerable diversity, even for afferents within restricted ranges of spontaneous discharge coefficient of variation. For example, afferents exhibiting CVs greater than 0.2 exhibited ranges of response phases to 0.05Hz sinusoids of 35° (30° - 65°). These same neurons exhibited a similar range of response phases to 0.4Hz sinusoids (0° - 35°). Similar diversity in phase was observed for lower (i.e. 0.0125Hz) and higher (i.e. 1.6Hz) stimulus frequencies. However, the respective phase differences between frequencies were not parallel for each afferent. The data from this study suggest that the system response dynamics for chinchilla horizontal semicircular canal afferents are represented by diversity in their transfer functions, even for afferents with comparable spontaneous discharge CVs.

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261.8

MAXIMUM INFORMATION-PREDICTING MODELS OF BULLFROG SEMICIRCULAR CANAL AFFERENT SPIKE TRAINS. M.G. Paulin* and L.F. Hoffman. Dept. of Zoology, University of Otago, New Zealand & Division of Head and Neck Surgery, UCLA School of Medicine, Los Angeles CA 90095.

This study was undertaken to develop and test new methods of spike train analysis and to apply those methods to investigate the diversity in response dynamics of semicircular canal afferent neurons. We use a class of nonlinear models and a maximum information prediction rate criterion to select an optimal model from this class for each neuron. Bullfrogs were subjected to band-limited (0.05 to 4 or 10 Hz) Gaussian rotational velocity stimuli, with transitions optimized to minimize integrated torque change. Afferent spike times measured with 1msec resolution were converted to spike rates by Gaussian convolution, which optimally preserves timing information while lowering signal bandwidth [Paulin, Biol. Cyber. 66:525, 1992]. Dynamic linear - static nonlinear (ARMA-polynomial) cascade models were fitted to the stimulus-response data. For each neuron we searched over model structures and rate estimation bandwidths. The rate at which each model predicts information about the neuron's responses to novel stimuli was estimated using crossvalidation data collected at the same time as the fitting data. Best information-predicting models had bandwidths comparable to the mean firing rate of the modelled neuron. These models reproduced dynamic features of the semicircular canal afferents studied, predicting spike times in response to novel stimuli as accurately as the neurons themselves do on repeated trials. Some afferent neurons transmit information about the whole head rotational velocity waveform, while others provide enhanced information about specific features of the waveform. Our modelling technique identifies stimulus features associated with particular afferents. These results are congruent with previous reports of functional diversity in semicircular canal afferent responses, but we extend those reports by representing the functional properties of individual neurons in a form naturally suited to analysing the differential roles these neurons may play in collective computation in the CNS.

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261.9

OTOLITH SIGNALS AND CENTRAL PROCESSING IN RAT HORIZONTAL VOR. K.J. Quinn*, S.A. Rude, S.C. Brettler, and J.F. Baker. Northwestern University, Chicago, IL 60611

Otolith participation in vertical vestibulo-ocular reflex (VOR) is widely recognized but a contribution of gravity sense to horizontal VOR during low frequency oscillations has been demonstrated only in cats. To study otolith signals and velocity storage we recorded horizontal VOR in pigmented rats using chronically implanted scleral search coils. Upright horizontal VOR was recorded during sinusoidal vertical axis rotations and velocity steps, with the head pitched 43° down to provide strong horizontal and minimal vertical canal activation. Horizontal VOR was also recorded during yaw in 'on side' (90° rolled) and 'upside down' (180° rolled) head orientations. While canal stimulation was identical in the three conditions, on side rotations provided a dynamic otolith input and upside down rotations altered static otolith signals.

In the upright orientation, low frequency (0.035 - 0.1 Hz) horizontal VOR was phase-advanced compared to cat and monkey (avg = 35° at 0.1 Hz, ± 9.9 sd, n=12). Time constants estimated from 0.05 to 1 Hz sinusoidal rotations averaged 2.5s (n=12) and from 50°/s velocity steps averaged 3.5s ±1.9 sd (n=7). In upside down head orientation, VOR phase was further advanced (73° ± 34° sd at 0.1 Hz, n=12) and both estimates of the time constant were smaller (sine avg 0.9s; step avg 2.8, ± 1.1 sd). This difference suggests either velocity storage when upright or velocity leakage when upside down. In the on side orientation, VOR phases were less advanced than in the other 2 orientations (-1.4° ±15° at 0.1 Hz, n=8), indicating that a dynamic otolith input results in a phase-accurate low frequency horizontal VOR in rats.

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261.10

LOW FREQUENCY DYNAMICS AND OTOLITH SIGNALS ON ANTERIOR AND POSTERIOR CANAL VESTIBULO-OCULAR NEURONS IN ALERT CATS. S.C. Brettler* and J.F. Baker. Northwestern University, Chicago, IL 60611

Accurate low frequency behavior of the vertical vestibulo-ocular reflex (VOR) requires central processing or otolith input to compensate for the advanced phase of the primary canal afferents. We recorded 188 neurons in five alert cats in order to investigate velocity storage and otolith signals on vestibular nucleus neurons. Neurons were classified according to latency of orthodromic responses to ipsilateral labyrinth electrical stimulation and antidromic responses to oculomotor nucleus stimulation.

During horizontal axis sinusoidal pitch rotations, the head velocity signal on vertically sensitive second-order neurons is accurate in the middle frequency range, with a phase of -97°± 17° s.d. (n=98) re acceleration at 0.5 Hz. At lower frequencies however, anterior canal oculomotor-projecting second-order neurons (AC-VON) code velocity more accurately than posterior canal oculomotor-projecting second-order neurons (PC-VON) recorded at 0.05 (AC-VON, -89°±9, n=7; PC-VON, -65°±22, n=8) and 0.02 Hz (AC-VON, -66°±21, n=5; PC-VON, -37°±22,n=6). The AC-VON phase advance only 8°±7 (n=7) from 0.5 Hz to 0.05 Hz while the PC-VON advance 32°±23. Median time constants for PC-VON were 7.7 seconds (n=8) and 19.1 seconds (n=7) for AC-VON. Extension of the velocity code for AC-VON may contribute to longer time constants for upward than downward VOR.

To separate canal responses from otolith responses at low frequencies, 9 second-order vestibular nucleus neurons were recorded during both horizontal and vertical axis (on side) pitch at 0.05 Hz. The AC neurons consistently exhibited large phase advances with the removal of a dynamic otolith input (72°±26, n=4) while the PC neurons were less affected (3°±28, n=5). The otolith input to AC-VON may reflect a greater otolith role in upward than downward VOR. Supported by EY07342.

261.11

GRAVITY DEPENDENCE AND SPATIAL PROPERTIES OF VESTIBULAR REFLEXES IN DORSAL NECK MUSCLES OF THE SQUIRREL MONKEY. J.E. Killian* and J.F. Baker. Northwestern University Institute for Neuroscience, Chicago IL 60611

We recorded electromyographic activity bilaterally using implanted wire electrodes in three dorsal neck muscles of two squirrel monkeys during vertical sinusoidal rotations in total darkness. Thirteen sets of 0.25 Hz, 30° rotations in 16 different vertical planes spaced 22.5° apart were completed, with the monkey initially oriented upright or upside down. In alert but calm animals, muscle activity varies sinusoidally with the rotation. Each muscle is most active for a particular plane of vertical rotation; activity during rotations in other planes is a function of the cosine of the angle between that plane and the plane producing the greatest response. Semispinalis is activated best by rotations near pitch with some roll. Roll rotations excite Splenius best, while Occipitoscapularis's preferred direction lies between those of Semispinalis and Splenius. Compared to corresponding neck muscles previously studied in the cat (Banovetz et al. Exp. Brain Res., 1995), neck muscles in the squirrel monkey are more active in response to roll rotations. This difference in the organization of the spatial properties of the vestibulocollic reflex likely echoes the constraints imposed by the different anatomical arrangement of the head-neck system between the two species.

Responses for all muscles generally peak in phase with maximal angle of tilt. However with the monkey oriented upside down, this peak is consistently 180° opposite to the response when the monkey is rotated about an upright position. Upside down, the monkey reflexively acts to rotations by moving the head even more. The strategy of spatial cooperation among the muscles is unchanged, but the gravitational context dictates a different goal. Thus the vestibulocollic reflexes are under the control of an otolith gravity signal: when upright, vestibulocollic reflexes stabilize the head by compensating for rotations; but when upside down, they are anticompensatory and work with the rotation to right the head with respect to gravity.

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261.12

VESTIBULAR-ONLY (VO) NEURONS AND VELOCITY STORAGE. B. Cohen*, S.B.Yakushin, B. Sheliga and T.Raphan. Depts. Neurol. & CIS, Mt. Sinai Sch. of Med. & Brooklyn College, CUNY, New York, 10029

Activity of VO neurons in rostral MVN was studied in paradigms that elicited velocity storage. Animals were tested during steps of constant angular velocity, during yaw axis optokinetic nystagmus (OKN) and after-nystagmus (OKAN) in upright and on-side positions, and during rotation around axes tilted from the vertical (off-vertical axis rotation, OVAR). Exposure to a stationary surround was used to test effects of light dumping during post-rotatory nystagmus. Visual and oculomotor contributions to unit firing were also determined. In all tests VO neural activity was related to the velocity storage component of eye velocity and not to eye velocity per se. The activity of VO neurons did not depend on the state of alertness, and unit firing was maintained despite loss of eye velocity due to drowsiness. During constant velocity rotation, time constants (Tc's) of VO neurons decreased with increases in stimulus velocity as did eye velocity Tc's, but unit Tc's were always longer. Eye velocity but not VO neural activity changed rapidly during exposure to a stationary surround during light dumping. During OKAN cross-coupling, activity of Type 1 VO neurons was related only to horizontal, not to vertical or roll eye velocity. All VO neurons had a bias component during OVAR and cells receiving otolith information were modulated as a function of head position with regard to gravity. These data demonstrate that VO neurons have a strong link to the horizontal component of velocity storage and have the requisite activity for its realization. The same is not true for other classes of vestibular neurons described earlier.

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261.13

VESTIBULAR-ONLY (VO) NEURONS AND THE SPATIAL ORIENTATION OF VELOCITY STORAGE.

S.B.Yakushin*, B. Sheliga, T.Raphan and B.Cohen. Depts. Neurol. & CIS, Mt. Sinai Sch. of Med. & Brooklyn College, CUNY, New York, 10029

The spatial orientation of VO neurons in rostral MVN of rhesus monkeys was studied and related to the spatial orientation of velocity storage. Axes of maximal sensitivity (AMS), i.e., axes around which rotation gave maximal modulation of firing rate were determined for each unit by sinusoidal rotation around a spatial vertical axis with the animal upright or statically tilted backward or forward, and by pitching about a horizontal axis with different head orientations to the pitching plane. OKN & OKAN were also given in upright and tilted positions. The AMS of most VO units was tilted forward about 20°, indicating input solely from the lateral canals. Some neurons were also modulated when the head was pitched. Based on phase relative to head position with regard to gravity, most Type 1 VO units had convergent information from the otoliths. Other VO neurons had their AMS about the upright position, with the same peak as horizontal eye velocity. These units had input both from a lateral and vertical canal. Type 2 VO neurons received input primarily from the vertical canals. Time constants (Tc's) of VO neurons were reduced along with the horizontal Tc during OKAN in on-side positions. A horizontal Type 2 VO neuron had activity related to the cross-coupled component of vertical eye velocity. Thus, Type 1 & 2 VO neurons receive not only lateral canal, but otolith and vertical canal-related activity and alter their Tc's appropriately during cross-coupling. We propose that an network of VO neurons could implement the spatial orientation of velocity storage.

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261.14

EYE AND HEAD COORDINATION DURING HEAD-FREE GAZE PURSUIT IN THE RHESUS MONKEY: ACTIVITY OF VESTIBULAR NEURONS.

J.E. Roy* and K.E. Cullen. Aerospace Medical Research Unit, Department of Physiology, McGill University, Montreal, Canada.

The activity of neurons in the vestibular nuclei of alert rhesus monkeys were characterized during combined eye-head tracking. Monkeys were trained to 1) pursue a moving visual target and 2) cancel their vestibular ocular reflex by fixating a visual target which moved with the vestibular turntable. Target or table motion consisted of a sine wave (0.5 Hz, 40°/s peak velocity) in the horizontal plane. Neurons were first classified in the head-restrained monkey during spontaneous eye movements, smooth pursuit, cancellation of the VOR (VORc), and VOR in the dark (VORd).

Three classes of neurons are considered below: 1) neurons which were sensitive to ipsilateral head movements (VIs), 2) neurons which were sensitive to ipsilateral head movements and contralateral eye movements (VIEIIs), and 3) neurons whose head and eye velocity sensitivities were in the same direction during smooth pursuit and VORc respectively (EVs). Following the initial characterization of the cells, the monkey's head was released. Although the target remained well within their oculomotor range (± 50°), monkeys made significant use of both head and eye movements to track target motion. During some trials, head motion was momentarily (≈100 ms) halted (by a friction clutch). The activity of VI neurons during combined eye-head pursuit and rapid gaze shifts was well predicted by their head velocity sensitivity during VORc and VORd. In contrast, the activities of VIEII and EV neurons during head-free tracking were not well predicted by a linear model based on head-fixed head and eye movement sensitivities. In particular, the modulation of the activity of VIEII neurons during head-free pursuit was generally less than that predicted by the head-fixed characterization of these neurons, suggesting that the modulation of these pathways was reduced during head-free tracking. Our results indicate that head-fixed models do not adequately describe VIEII and EV vestibular neuron discharges during head-free tracking. (Supported by the Medical Research Council of Canada).

261.15

CENTRAL REPRESENTATIONS OF THE STIMULI THAT DRIVE MOTOR LEARNING IN THE VOR.

Jennifer L. Raymond* and Stephen G. Lisberger, Department of Physiology and W.M. Keck Foundation Center for Integrative Neuroscience, UCSF, San Francisco, CA 94143.

To place constraints on the neural mechanisms for the induction of motor learning in the vestibuloocular reflex (VOR), we examined the responses of Purkinje cells in the floccular complex to stimuli that drive learning. We recorded simple spike (SS) and complex spike (CS) responses in Horizontal Gaze Velocity Purkinje cells (HGVPs) to pulses of head and visual stimulus motion that increased (x2) or decreased (x0) the gain of the VOR. Pulse durations ranged from 80-1000 ms.

The average SS response to ipsiversive head turns was an initial decrease in firing rate for both x0 and x2 stimuli, followed by an increase in firing rate for x0 stimuli or a further decrease in firing rate for x2 stimuli. The differential SS responses to x0 and x2 stimuli were sustained from about 100 ms after the onset of the stimulus to about 100 ms after the offset of the stimulus. For contraversive head turns, responses were in the opposite directions.

The average CS response to ipsiversive head turns was a sustained decrease in firing rate for x0 stimuli and a sustained increase in firing rate for x2 stimuli from about 100 ms after the onset of the stimulus to about 100 ms after the offset of the stimulus. For contraversive head turns, responses were in the opposite directions.

Both the correlation of the sustained CS response with the vestibular stimulus and the correlation of the sustained SS response with the vestibular stimulus contained information about whether the gain of the VOR needed to increase or decrease. However, for short-duration (≤100 ms) stimuli, these responses did not overlap with the vestibular stimulus in time, suggesting that a plasticity mechanism may compare a CS or SS response with a vestibular stimulus that preceded it in time.

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261.16

THE EFFECT OF UVULONODULAR LESION ON NEURONAL PROPERTIES IN THE LATERAL VESTIBULAR NUCLEUS OF RATS DURING NATURAL OTOLITH STIMULATION.

B. Jiang, C.H. Lai and Y.S. Chan*. Department of Physiology, Faculty of Medicine, The University of Hong Kong, Sassoon Road, Hong Kong.

To investigate the contribution of the cerebellar nodulus and uvula on the response characteristics of otolith neurons in the lateral vestibular nucleus, the properties of these neurons to constant velocity off-vertical axis rotations (OVAR), which selectively stimulate the otolith receptors, were studied in adult Sprague-Dawley rats with or without acute ablation of the uvulonodular lobe. All rats were decerebrated under halothane anesthesia. In rats with intact cerebellum, most neurons showed symmetric bidirectional response sensitivity ratio (δ defined as the CW gain over the CCW gain) which remained stable with velocity while 23% of the sampled neurons showed velocity-variable and asymmetric bidirectional response sensitivity. In uvulonodular lesioned rats, however, the proportion of neurons showing asymmetric and velocity-variable δ values increased to 79%. The spread of the δ values over the velocity spectrum was characteristic of each neuron sampled. In uvulonodular lesioned rats, the δ spread of the asymmetric neurons ranged from 0.17 - 2.87 while the corresponding values in control rats was 0.35 - 0.67. Another unique feature observed in uvulonodular lesioned rats was the occurrence of some asymmetric neurons that were responsive only to OVAR of one direction but not to both; this feature was not found in control rats. Our results suggest that the cerebellar nodulus and uvula constitute part of the neural substrate in modulating the gravity-dependent responses of central vestibular neurons during natural otolith stimulation. [Support: HKRGC]

261.17

THE LINEAR VESTIBULO-OCULAR REFLEX (LVOR) FOLLOWING LABYRINTHECTOMY.

G. D. Paige*, L. Telford, S. H. Seidman, and P. Boulos, Dept. of Neurology, Univ. of Rochester, Rochester, NY 14642.

Horizontal and vertical LVOR responses were recorded during inter-aural (IA) and dorso-ventral (DV) translational oscillations (0.5-4 Hz, 0.1-0.5g peak) before and after unilateral labyrinthectomy in squirrel monkeys. Response sensitivities (in °/cm) and phases were analyzed in relation to stimulus characteristics (frequency and motion axis), vergence angle, and recovery time post-lesion. The normal high-pass dynamics of the translational LVOR, and the influence of vergence, remained after labyrinthectomy. However, sensitivities generally decreased, both at dark vergence (1 meter-angle) and as a function of vergence (in °/cm/meter-angle), followed by modest recovery over time. Phase displayed a novel post-lesion lead, even at 4 Hz (roughly 10°). Interestingly, the axis of ocular response shifted after labyrinthectomy. This was most noticeable as a roll of the response axis toward the unlesioned side during IA motion, manifested as the appearance of a small vertical response component along with the larger horizontal response. The axis shift declined over time, consistent with an adaptive shift in response axis toward normal. The phenomenon did not occur in the angular VOR, and may reflect the off-sagittal tilt of the remaining sacculus, operating in the absence of counteracting influences from the opposite endorgan. The two eyes behaved roughly conjugately after labyrinthectomy, indicating that a single utriculus and sacculus can drive the LVOR binocularly, as do canal-driven reflexes. Finally, all angular and linear VORs were abolished following a second labyrinthectomy.

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262.1

TAIL AND EYE MOVEMENTS ELICITED BY ELECTRICAL STIMULATION OF THE OPTIC TECTUM IN GOLDFISH. B. Torres, L. Herrero, F. Rodríguez, C. Salas and P. Nunez-Abades*. Dept. of Animal Physiology and Biology and Dept. of Experimental Psychology. Univ. Sevilla. Spain.

Current studies on the superior colliculus involvement in the motor codification of eye, hear and/or body movements in mammals support two main suggestions: 1) the metric properties of the movement are encoded by both the collicular locus of activity and the amount of such activity, and 2) depending on the specie the colliculus plays a role not only in orienting but also in escape responses. This work was aimed to investigate these two suggestions in the optic tectum of goldfish. Experiments were carried out in restrained goldfish with the tail free to move. Fish tail and eye movements were recorded in the awake animals following the electrical microstimulation of several tectal loci and as function of stimulus parameters. Low intensity (10 - 120 μA) stimulation elicited conjugated contraversive eye movements and the contraction of the axial muscles contralateral to the stimulated tectum. These movements are compatible with the generation of orienting responses. The amplitude and velocity of these orienting movement depended on the tectal stimulated locus and on stimulus parameters. High intensity stimulation (up 120 μA) elicited the contraction of the axial muscles ipsilateral to the stimulated tectum and eye retraction. These movements are compatible with the generation of escape responses. Finally, the threshold to elicited the escape response varies with the tectal zone. Supported by a CICYT PB93-0916 grant.

262.2

NEAR-RESPONSE-RELATED NEURAL ACTIVITY IN THE ROSTRAL SUPERIOR COLLICULUS OF THE CAT. H. Jiang*, D. Guitton and K. E. Cullen. Montreal Neurologocial Institute and McGill University, Montreal, Quebec, Canada, H3A 2B4.

The rostral superior colliculus (SC) has been linked to the neural control of active fixation in both cat and monkey. Cells in the so-called 'fixation zone' are tonically active during fixation and pause during eye saccades (monkey) or head-free gaze shifts (cat). The rostral SC of cats has also been implicated in the control of accommodation, and we report here on the activity of neurons in this area that modulate their discharge in response to stimuli moving towards or away from the animal. Cats were trained to make vergence eye movements in response to approaching or receding visual food targets. Trials were performed either in the light or, when the ambient light was unexpectedly turned off, at a random time either before or after vergence eye movements had been initiated. Thus, in the latter condition, a portion of a vergence movement was made in the dark. Two types of neurons were observed: a) cells that paused during vergence eye movements made in the light or dark but not during conjugate saccadic eye movements; b) cells whose discharge frequency profile resembled vergence velocity but also were not modulated during saccadic eye movements. These near-response-related discharges were recorded from neurons generally located deeper in the rostral SC than fixation cells. Electrical micro-stimulation in this region induced disconjugate eye movements and sometimes pure vergence responses. We conclude that the fixation zone of the cat's SC is involved in changing fixation from far to near (or *vice versa*) targets. This leads to the interesting hypothesis that the SC controls gaze shifts between targets located anywhere in 3-dimensional space.

262.3

DIVERGENT AXON COLLATERALS FROM THE ROSTRAL SC TO THE PRETECTAL ACCOMMODATION-RELATED AREA AND THE OMNIPAUSE NEURON AREA IN THE CAT.
Y. Nagasaka, K. Ohtsuka*, and A. Sato. Dept. of Ophthalmology, Sapporo Medical Univ. Sch. of Med., Sapporo, Hokkaido 060, Japan.

We previously indicated that the circumscribed area in the rostral SC, which corresponds to the area centralis, is involved in the control of lens accommodation in the cat. Recent studies indicated that the area centralis of the SC is also involved in the control of the active fixation (saccade suppression). This area projected to the pretectum, where accommodative responses were evoked by electrical stimulation, and the nucleus raphe interpositus (RIP), which corresponded to the omnipause neuron area. In this study, we investigated collateralization of efferent fibers from the accommodation-related area in the SC to the pretectal accommodation-related area (PA) and the omnipause neuron area in the RIP of the cat using a fluorescent double-labeling technique. This study was conducted in 2 cats, weighing 2.5-3.5 kg. Retrogradely labeled neurons in the ipsilateral SC were examined following injections of fast blue into the RIP and diamidino yellow into the PA, or vice versa. Neurons projecting to the RIP were located in the intermediate layers in the rostral SC, while locations of neurons projecting the PA were scattered in the SC. Double-labeled neurons were located in the circumscribed area in the rostral SC, which corresponded to the area centralis. The presence of double-labeled cells indicated that the accommodation-related area in the rostral SC contains neurons whose axons collateralize to project to both the PA and the ominipause neuron area. These findings suggest that the rostral SC is involved in the control of both accommodation and the active fixation.

262.4

MOVEMENT FIELDS OF THE SUPERIOR COLLICULUS IN TRAINED CATS, Choongkil Lee* and Incheol Kang. Dept. of Psychology, Seoul National Univ., Seoul Korea 151-742.

Pattern of movement fields of the superior colliculus in the cat has been controversial (Guitton et als, 1993; Sparks, 1993; Peck et als, 1994). Since this pattern is critical for understanding collicular mechanism controlling saccadic eye movements, we tried to determine the pattern in trained cats.

Cats, pre-trained to attend to LED's, were trained for water reward to make saccades to briefly-presented visual targets (LED's) spaced 4 deg apart, spanning +/- 30 deg in a tangent array. Neural activities recorded from the superior colliculus were classified into visual (related to stimulus on and off) and motor (related with saccade) components. For unambiguously-classified motor activities, indices of activity level such as number of motor spikes or its density estimate derived by convoluting with a variable kernel, were plotted against horizontal and vertical amplitudes of saccades. To confirm the idea of "moving hill" proposed by Munoz et als (1991), the location of peak density in relation to the onset or offset time of saccades along the "best direction" was also plotted against the vectorial amplitude of saccades.

Saccades with similar amplitude and direction were often associated with highly variable motor activity. When the average trend was considered, the distal border of movement fields were bounded for the "best amplitude" up to 15 deg. The temporal gap between the peak density for motor activity and the movement offset was not fixed across saccades along the "best direction". These results that we have obtained so far are inconsistent with the idea of "moving hill" hypothesis. (Supported by KOSEF grant 91-01-00-04).

262.5

DESCENDING INTRACOLLICULAR PATHWAYS TO THE INTERMEDIATE GRAY LAYER IN THE TREE SHREW.
T. Ha, P. Lee, G. J. Augustine and W. C. Hall* Department of Neurobiology, Duke University, Durham, NC 27710

A visual pathway from the superficial to the intermediate gray layer may play a role in saccade selection. Cells in the deep part of the superficial gray layer receive visual input from both the retina and the cortex and project to the underlying optic layer. The optic layer projects in turn to the intermediate gray layer, which contains most of the efferent cells that project to the gaze centers of the pons. Some intermediate cells have apical dendrites that extend into the optic layer; they may receive visual input directly from superficial gray layer cells or indirectly from optic layer cells. The dendrites of other intermediate cells avoid the optic layer, but may receive visual information from the descending optic layer axons. This intracollicular circuit is highly columnar and may play a role in generating presaccadic responses of a subset of intermediate layer cells. Patch clamp studies cells are now underway to examine the influences of the descending pathway on intermediate layer cells. Supported by NIH grant EY 08233.

262.6

CHARACTERIZATION OF SUPERIOR COLLICULUS RESPONSES TO INTRINSIC STIMULATION. M.A. Meredith* and A.S. Ramoa, Department of Anatomy, Virginia Commonwealth University, Richmond, VA.

While a great deal is known regarding the functional dependence of the superior colliculus (SC) on its various sensory and motor inputs, recent evidence indicates that a rostro-caudally oriented *intrinsic* circuit is critical to SC-mediated functions such as fixation and orientation. The current experiments sought physiological evidence for SC interneurons that might participate in such a circuit. Adult ferrets (n=4) provided para-sagittal slices of the SC that were placed in an interface chamber at 35°C. Routine *in vitro* methods were used to record extracellular action potentials in spontaneously active neurons and their responses to electrical stimulation (concentric bipolar, 37-300 μA, 0.1 ms) presented at rostral and at caudal SC sites. Electrical stimulation was conducted alone or combined with d-APV/CNQX (to block excitatory synaptic transmission).

More than two-thirds (n=23/34) of the SC neurons studied responded to intrinsic SC stimulation: 9 showed brief (avg. duration = 16.6±13.2 ms) increases in firing frequency, 10 revealed relatively long (avg. duration = 55.6±24.5 ms) decreases in spontaneous activity, and 4 responded with both excitation and post-excitatory suppression (avg. duration = 12.5±5 ms; 65.0 ± 10 ms, respectively). An overwhelming number (n=17/23) was affected by rostral stimulation sites, 5 were influenced from caudal stimulation sites, and only one was influenced from both rostral and caudal sites. Fifty μM d-APV/ 200μM CNQX picospritzed between the stimulation and recording site accentuated the suppressive effects of stimulation in 3 of 4 neurons. Adding 25μM d-APV/6 μm CNQX to the bath, eliminated suppressive as well as excitatory responses at the recording site, indicating that the stimulation had excited intrinsic inhibitory interneurons. These data are consistent with the presence of a rostro-caudal intrinsic circuit within the SC and support the hypothesis that SC interneurons can influence SC-mediated behaviors. Supported by NSF grants IBN-9308576 (MAM) and IBN-9421983 (ASR).

262.7

DISTRIBUTION AND TARGETS OF COLLICULAR EFFERENT CELLS: MULTIPLE TRACER STUDIES IN THE CAT AND MACAQUE. E. Olivier and P.J. May*, Lab. of Neurophysiology, U. of Louvain, Brussels, Belgium and Depts. of Anatomy & Neurology, U. of Mississippi Med. Ctr., Jackson, MS 39216.

Intracellular investigations that classify superior colliculus (SC) efferent neurons distinguish T-cells, which project to the contralateral SC, and X-cells, which instead project to the contralateral brainstem and spinal cord. These may subserve eye, and combined head and eye movements, respectively, but their overall population characteristics are unknown. Injections of different fluorescent tracers at separate sites in the cat were used to demonstrate the distribution of tectal neurons projecting to the contralateral SC, and to the contralateral pontine reticular formation and spinal cord. Tectotectal cells (T-cells) were found in the rostral half of the SC and were widely distributed from stratum opticum to the deep gray layer. Only a small portion (~20%) of these T-cells also projected to the contralateral pons. These were constrained to the intermediate gray layer, and made up 15% of the total number of cells projecting in the predorsal bundle (PDB). No T-cells showed spinal projections. The PDB cells without a commissural projection (X-cells) were found from the intermediate to deep gray layers and were distributed evenly within the SC. About half of the tectopontine X-cells also send a projection to the spinal cord. Only a few cells projected exclusively to the spinal cord. In macaque monkeys, T-cells had a much more extensive rostrocaudal distribution than in the cat. The distribution of tectopontine cells was similar to the cat's, but was limited to the intermediate gray layer. Tectospinal neurons were very rare. In view of the monkey's limited direct tectospinal projection, we investigated the tectoreticulo-reticulospinal connection. BDA injected into the SC labeled terminals that contacted HRP labeled reticulospinal neurons primarily in the pontomedullary reticular formation. However, only tectal terminals were found in the reticular formation immediately ventral and rostral to the sixth nucleus. Thus, species specific differences exist in the somatic location and terminal distribution of tectal efferent populations. These data are needed to formulate a coherent model of SC function in eye and head control.

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262.8

THE EFFECT OF EYE POSITION ON THE VISUAL RESPONSES OF SINGLE UNITS IN THE PIGEON OPTIC TECTUM Paul C. Knox, H.C. Whalley. (SPON: Brain Research Association) Centre for Neuroscience, University of Edinburgh, Crichton St, Edinburgh, Scotland.

Many areas of the visual system are known to receive signals indicating the position of the eyes in the orbit. Although the muscles which move the eyes, the extraocular muscles (EOM), contain stretch receptors, and the afferent signals from these are known to reach many visual processing areas, they are not generally considered as a likely source of eye position information. We have found that EOM afferent signals do modify the visual responses of single units in the superficial layers of the pigeon optic tectum (OT) in a manner which suggests that they may signal eye position.

Extracellular visual responses were recorded from the superficial layers of the left OT of paralysed, decerebrate adult pigeons. Visual stimuli were projected to the right eye and the left eye was moved passively by means of a suction contact lens. Visual responses were collected with either the eye held in the central resting position or held deflected by up to 20 in one of four directions (up, down, beak, tail). Sets of eight interleaved PSTHs were compiled online.

The visual responses of many units were clearly modified by holding the left eye at an eccentric position. Approximately 50% of units were affected in a similar manner for all eccentric positions tested. ie holding the eye up or down, to the beak or tail would either decrease or increase the amount of firing in response to the visual stimulus by a comparable amount. Other units were modified only at particular positions. Application of local anaesthetic to the eye carrying the lens did not alter the effects. None of the units responded to mechanical stimulation of the area around the left eye. The receptive field was remapped routinely, and there was no evidence that its position altered in response to deviation of the left eye. Therefore EOM afferents may convey information concerning eye position in the orbit to the OT.

This work was supported by the Wellcome Trust.

262.9

THE PRIMATE SUPERIOR COLLICULUS MAKES USE OF EYE POSITION FOR INITIATING SACCADES. M. Paré* & D.P. Munoz, MRC Group in Sensory-Motor Neuroscience, Queen's University, Kingston, Ontario, Canada K7L 3N6.

Because conspicuous targets can be located anywhere in the visual field and the oculomotor range is limited, it is crucial to keep the eyes centered in the orbit to permit maximal orbital reserve both at the start and end of an impending saccade of undetermined direction. Little is known about the neural mechanisms responsible for recentering the eyes or the related problem of initiating saccades from different eye positions. We have reported previously that saccade latency is: (1) influenced by initial eye position such that the initiation of saccades toward the central orbital position is facilitated (Paré & Munoz, *Soc Neurosci Abstr* 21:1193, 1995); and (2) inversely related to the level of preparatory neuronal activity in the superior colliculus (SC) (Dorris et al., *Soc Neurosci Abstr* 21:1193, 1995). We now report on two experiments designed to test whether SC activity is modulated by eye position. In the first experiment, the activity of neurons with preparatory discharge – buildup neurons – was recorded while monkeys initiated saccades from different eye positions in the gap task. In the second experiment, saccades were evoked electrically using near-threshold stimuli while monkeys fixated (visually or not) a target whose location was varied randomly. When the initial eye position was shifted eccentric in a direction opposite to the direction of the saccades encoded by the SC site under study: (1) the level of SC preparatory activity was enhanced; and (2) saccades were more easily evoked by electrical stimulation. In both experiments, the orbital position effect was potentiated by the release of ocular fixation afforded by the introduction of a gap period. These results suggest that the SC uses initial eye position for initiating saccades and that the eye position sensitivity of SC neurons is gated by fixation-related signals. The motor commands sent to the SC may be either combined with eye position signals in the SC or already modified by eye position.

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262.10

SUPERIOR COLLICULUS ACTIVITY DURING SACCADIC EYE MOVEMENTS PERTURBED BY AIR-PUFF INDUCED BLINKS. H.H.L.M. Goossens, A.J. van Opstal, and C.C.A.M. Gielen*. Univ. of Nijmegen, Dept. Biophysics, Nijmegen, The Netherlands.

Recent findings suggest that the monkey superior colliculus (SC) may be part of the local feedback loop that controls the dynamics of saccadic eye movements, because the instantaneous activity of many saccade-related burst neurons (SRBNs) exhibits a monotonic relation with the current motor error. So far, it has been difficult to substantiate this hypothesis further because of the stereotyped nature of saccades. Recent experiments, aiming at perturbing the saccade trajectory, applied electrical stimulation at crucial stages of saccade execution (omnipause neurons, SC fixation cells), with the possible risk of stimulating adjacent pathways.

In order to circumvent these potential problems, we have perturbed saccadic eye movements noninvasively with saccade-triggered air puffs on the eye. Eye- and eye-lid position were recorded with search coils. Two monkeys were trained to make saccades to flashed targets in darkness. We recorded from SRBNs in the deep SC and compared the activity for perturbed and unperturbed saccades into the movement field.

Reflexive blinks result in a rapid upward-directed eye movement between 5 and 15 deg amplitude. Despite the absence of visual feedback, spatial perturbations of saccade trajectories are consistently compensated. Blink-induced perturbations may result in: (1) a transient decrease in SRBN activity near blink onset, (2) a prolongation of SRBN activity in line with increased saccade duration such that the burst offset remains tightly coupled with saccade offset. These findings are compatible with the presumed role of the SC in the feedback control of saccades.

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262.11

TARGET UNCERTAINTY ALTERS NEURONAL ACTIVITY IN THE MONKEY SUPERIOR COLLICULUS. M. A. Basso* and R. H. Wurtz, Laboratory of Sensorimotor Research, National Eye Institute, NIH. Bethesda, MD 20892.

The primate superior colliculus (SC) contains neurons that are related to events preceding the initiation of saccadic eye movements. Munoz and Wurtz (1995) demonstrated that buildup neurons have a long lead sustained increase in firing frequency prior to the initiation of saccadic eye movements. Moreover, in a gap task with one target and two possible locations, buildup neurons showed a sustained activity prior to the specification of the saccadic target. Glimcher and Sparks (1992) identified prelude burst neurons which also have a long lead increase in activity prior to the onset of saccadic eye movements. Prelude burst neurons begin discharging after the specification of the saccadic target. The present experiment was designed to assess the activity of burst, buildup, and fixation cells of the SC in a task that temporally isolated events leading up to and including saccade initiation.

The onset of a centrally located LED initiated a trial. The monkey was required to remain fixating for a random delay after which, 1, 2, 4, or 8 possible targets were presented. The array remained illuminated for a random period (uncertainty) and then one of the targets dimmed (selection). Another random period preceded the removal of the fixation point which signaled the monkey to initiate a saccade to the dimmed target (initiation).

During the period of uncertainty, buildup neurons showed an increase in activity in the 1 & 2 target conditions. Less activity was present in the 4 & 8 target conditions. In the 4 & 8 target conditions, buildup activity occurred only after saccade target selection. During this selection period, little additional buildup occurred in the 1 & 2 target conditions. Fixation neurons tended to show activity that was the reverse of buildup neurons, ie., increasing activity with increasing target uncertainty in the 4 & 8 conditions. Burst neurons remained inactive until the signal to initiate a saccade was presented irrespective of the number of possible targets. We conclude that buildup activity is dependent upon the predictability of the saccadic target, and that fixation neuron activity is related to the probability of making a saccade. Burst neuron activity is unaffected. Supported by the National Eye Institute.

262.12

IS THE DEVIATION OF SACCADES EVOKED BY COLLICULAR STIMULATION DUE TO A LINEAR RESETTABLE INTEGRATOR? S. Sadeghpour, A. Pouget, J. Schlag* and M. Schlag-Rey, BRI and Dept. of Neurobiology, UCLA, Los Angeles, CA 90095-1763.

When the superior colliculus is stimulated shortly (<40ms) after a visually guided saccade, the resulting eye movement is systematically deviated from the control vector in the direction opposite to the visually guided saccade. Furthermore, the amplitude of the deviation decays exponentially as a function of the intersaccadic interval. These data have been taken as evidence for the existence of a linear resettable integrator hypothesized by several models of saccadic control (Nichols and Sparks, J.N. 1995, 73: 431). This integrator hypothesis predicts that the amplitude of the deviation should be a linear function of the amplitude of the visually guided saccade. We shall present experimental data showing that the relationship between the amplitude of deviation and amplitude of initial saccade is in fact sigmoidal, i.e., it increases linearly with amplitude up to 10-20° and saturates for larger amplitudes. We shall discuss two alternatives to the resettable integrator hypothesis, one involving a damped eye position signal and the other relying on the refractory period of collicular neurons, and compare their respective predictions with our experimental findings.

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262.13

ACTIVITY OF SUPERIOR COLLICULUS BURST NEURONS DURING SACCADE-VERGENCE INTERACTIONS. L. E. Mays*, Dept. of Physiological Optics and Vision Science Research Center, University of Alabama at Birmingham, Birmingham, AL 35294.

The occurrence of a saccade in conjunction with a vergence eye movement results in an increase in peak vergence velocity. The occurrence of convergence in conjunction with a saccade usually results in a lower peak velocity of the saccade. This low saccadic velocity is not due to mechanical effects since the peak firing rate of horizontal medium-lead burst neurons, which control horizontal eye velocity, is also lowered when a saccade occurs during convergence. In other situations which result in low saccadic velocities, such as saccades to remembered target locations, the activity of superior colliculus (SC) burst neurons is lower than usual. The purpose of this experiment was to determine if the activity of SC burst neurons during saccades with convergence is lower than that for comparable saccades alone.

The activity of monkey SC burst neurons with movement fields ranging in amplitude from 2° to 10° was recorded during normal saccades and saccades in association with 6° of convergence. The amplitude and velocity of each saccade were calculated using the average of the left and right horizontal eye position (i.e., cyclopean eye position) and vertical eye position. The locations of the movement fields for SC burst neurons were not significantly altered by convergence. The peak velocity of saccades was reduced by convergence. For most SC burst neurons, both the peak firing rate and number of spikes in the burst were lower for saccades made in association with convergence. The mechanism by which saccades are slowed by convergence is not known. (Supported by NIH grants EY03463 and EY03039 and the McKnight Endowment Fund for Neuroscience).

262.14

GRAVITY INFLUENCES THE COLLICULAR CODE OF SACCADE DIRECTION M.A. Frens, Y. Suzuki, K. Hepp, B. Hess, and V. Henn (SPON: European Neuroscience Association) Neurology Department, University Hospital, CH 8092 ZÜRICH, Switzerland.

We investigated the influence of the direction of gravity on the relation between activity in the deep layers of the Superior Colliculus and oculomotor output. Chronically prepared rhesus monkeys were put in different static roll positions, and eye position was monitored in three dimensions by means of the dual search coil method.

Based on single unit recordings and electrical microstimulation, we found that the saccade vector generated by the Colliculus reorients with respect to a head-fixed coordinate system. Typically the vector rotates in a direction that is opposite to the head roll direction. The amount of reorientation exceeded the amount of ocular counterroll that was observed as a result of rolling the head.

Therefore we conjecture that the Colliculus works in a coordinate system that is neither space-, head- or eye-fixed. It is argued that mechanisms downstream of the Colliculus are responsible for this reorientation.

During visually-guided saccades, the collicular reorientation has no effect on the oculomotor output. In the absence of a target systematic deviations are observed that are in the same direction as the change in collicular output.

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OCULOMOTOR SYSTEM: BRAINSTEM

263.1

CHEMICAL COMPOSITION OF THE PRIMATE ZONA INCERTA (ZI). D. K. Donahoe[1], S. H. Guest[1], and T. P. Ma[1,2]*. Departments of Anatomy (1) and Neurology (2), University of Mississippi Medical Center, Jackson, MS 39216.

We studied the chemical composition of the dorsal (ZId) and ventral (ZIv) laminae of the zona incerta in six cynomolgus monkeys (*Macaca fascicularis*) using immunohistochemical and histochemical techniques at the light microscopic level. Animals were deeply anesthetized and perfused through the heart with aldehyde fixatives. Fifty µm frontal sections were obtained with a freezing microtome. Alternating sections were placed in a primary antibody (GABA, calbindin [CB], parvalbumin [PA], glutamate [glut] or aspartate [asp]) for up to 36 hours, followed by the appropriate secondary, and then visualized using the ABC technique. Other sections were stained for acetylcholinesterase (AChE), cytochrome oxidase (CO), NADPH-diaphorase, Nissl, and/or fiber. Controls eliminating primary, secondary, or ABC had the expected results. There were two populations of GABA-IR cells, small and medium-large cells. Small cells were fairly uniformly distributed, but medium-large cells were primarily located in the ZIv. These small cells likely represent the small local circuit cells and the medium-large cells are the projection neurons. Medium-large PA-IR cells, which had a similar morphology to GABA-IR neurons, were also found in ZIv. While no CB-IR cells were observed, background CB staining was present in ZIv but absent in ZId. A few AChE stained neurons were located in the ZId, but not in ZIv. A small number of lightly stained NADPH cells were found in ZIv. Cytochrome oxidase has been used as a marker to distinguish between ZIv and ZId. There were a few medium-large CO-stained neurons within the ZIv. Results of glutamate and aspartate immunostaining were equivocal. It is our impression that asp-IR neurons are primarily in ZIv whereas glut-IR neurons are located in both. Except for presumed GABAergic local circuit neurons, our results do not show simple relationships between neurotransmitters and incertal cells. However, GABA does appear to be the predominant transmitter in this nucleus. The extent of background staining and the reactivity of the cells lend support to the idea that the zona incerta integrates information from multiple pathways and may provide control signals to its efferent targets such as the superior colliculus.

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263.2

ELECTROPHYSIOLOGICAL PROPERTIES OF NUCLEUS PREPOSITUS HYPOGLOSSI NEURONES IN GUINEA PIG BRAINSTEM SLICES. M. Serafin*, N. Vibert°, C. de Waele°, P.P. Vidal° and M. Mühlethaler. *CMU, 1211 Geneva 4, Switzerland and °LPPA, CNRS-Collège de France, Paris, France.*

The nucleus prepositus hypoglossi (PH) is involved in oculomotor control by its ability to convert a velocity signal arising from the medial vestibular nuclei into a position signal following a process of integration. In the abducens nuclei, this position signal is compounded with a velocity signal arising from the vestibular nuclei in order to generate compensatory eye movements contributing to gaze stabilization. The cellular mechanisms involved in such a transformation are basically unknown. As a first step toward understanding that process, we have investigated the intrinsic properties of PH neurones and found four major cell types within a database of 86 neurones. All of them were endowed, although to a variable extent, with an Ih rectification and subthreshold plateau potentials revealed by brief membrane depolarizations. In addition, the first two types of PH neurones were characterized by rather broad action potentials, a single AHP and an A-like current, whereas the last two types demonstrated a thinner spike, a double AHP and plateau potentials that were particularly prominent (due to the presence of a sodium persistent current). The distinction in between the first (type A, n=15) and second cell type (type A+clusters, n=12) relied on the presence of only a small A-like current in the first type, and subthreshold oscillations and clusters (both of them sensitive to TTX) in the second. The distinction between the third (type B, n=45) and fourth cell type (type B+LTS, n=14) was related to the presence of potent low-threshold calcium spikes in type B+LTS neurones. In addition, type B cells were shown to have long-lasting calcium plateau potentials in presence of either TTX alone, or when potassium conductances were blocked by TEA and 4-AP. Interestingly type A+clusters cells were found in the rostral part of the nucleus and type B+LTS were found only in its ventral part. The location of the B+LTS cells suggests that they could correspond to the burst driving neurones or the vertical bursters. *(Swiss NSF, PIC CNRS/MDRI 292).*

263.3

PHARMACOLOGICAL CHARACTERIZATION OF NUCLEUS PREPOSITUS HYPOGLOSSI NEURONES IN GUINEA PIG BRAINSTEM SLICES. M. Mühlethaler*, N. Vibert°, C. de Waele°, P.P. Vidal° and M. Serafin. *Dept. of Physiology, CMU, 1211 Geneva 4, Switzerland and °LPPA, CNRS-Collège de France, Paris, France.*

In a companion abstract (Serafin et al. 1996, this meeting) we have demonstrated that neurones of the nucleus prepositus hypoglossi (PH) can be subdivided into four major cell types (type A, A+clusters, B and B+LTS) depending on their intrinsic membrane properties. In order to further support this view, we have attempted to determine the responsiveness of the various PH cell types to the major neurotransmitters/neuromodulators which are present in pathways that project to this nucleus. In three of the four cell types (type B+LTS not being yet investigated pharmacologically), brief bath-application of carbachol and histamine had a depolarizing action (with the exception of 2/5 type A+clusters cells which were hyperpolarized by carbachol). The effect of carbachol was in addition shown to persist in TTX, suggesting that it was postsynaptic. In contrast, serotonine and noradrenaline had excitatory or inhibitory effects depending on the cell type. Indeed, both transmitters had depolarizing and excitatory effects on type A and B PH neurones, but hyperpolarizing and inhibitory effects on type A+clusters cells (both effects persisted in TTX). Interestingly, although type B PH cells were always depolarized and excited by serotonine, a transient decrease in firing rate (sometimes accompanied by a transient membrane hyperpolarization) preceding the excitatory phase suggests the presence of a complex response, possibly involving two different serotonergic receptor subtypes (Bobker, J. Neuroscience 1995). Interestingly, in PH type B cells (as in type B cells in the medial vestibular nucleus), long-lasting application of NMDA induced a rhythmic bursting activity (in presence of a DC hyperpolarization). These results indicate that PH neurones are not only heterogeneous in term of their intrinsic membrane properties but also with respect to their neurotransmitter sensitivity. *(Swiss NSF, PIC CNRS/MDRI 292).*

263.4

LONG LEAD BURST NEURONS IN THE OCULOMOTOR BRAINSTEM CARRY SIGNALS APPROPRIATE FOR MOVEMENT PREPARATION. A. Handel & P. W. Glimcher*. Center for Neural Science, New York University, New York, NY 10003.

We studied saccadic long lead burst neurons (LLBNs), a class of brainstem neurons originally defined by Luschei & Fuchs ('72), in two awake-behaving rhesus macaques. A delayed saccade paradigm was used to examine the temporal relationship between the *onset of the high frequency burst response of these units and saccadic onset* (burst lead). Burst lead and burst amplitude were found to vary systematically with the metrics of the saccade. The longest burst leads for each neuron, 55 msec on average, were associated with movements confined to a restricted portion of the oculomotor range.

The activity of LLBNs was also studied while monkeys performed a movement selection task. Subjects initially fixated a central yellow LED for 300-500 msec. Two eccentric yellow LEDs were then co-illuminated for 600-1200 msec, one above and one below the horizontal meridian. The fixation LED then changed color to either red or green. Red indicated that the monkey would be rewarded for aligning gaze with the upper LED (±3-6°) after the offset of the fixation LED 400-1000 msec later; green specified the lower LED as the saccadic goal. The remaining LED served merely as an irrelevant visual distractor. For many LLBNs, firing frequency during the interval after the fixation LED changed color, but before it was turned off, was correlated with the metrics of the future movement. We defined the selectivity of each unit to be *the maximal response for targets minus the maximal response for distractors, divided by their sum.* This index served as a measure of how differently a unit responded to an LED when it served as a saccadic target versus when that same LED was irrelevant to the task.

The population of LLBNs showed a selectivity for targets over distractors that was less than that reported for collicular prelude bursters but similar to the selectivity of Area LIP neurons. Together these data suggest that LLBNs cannot lie between the colliculus and the alpha motoneurons in the colliculo-pontine saccadic circuit.

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263.5

CHANGES OF REMEMBERED SACCADES AND FIXATION DURING TEMPORARY INACTIVATION OF THE MESENCEPHALIC RETICULAR FORMATION (MRF) V. L. Silakov* and D. M. Waitzman. VA Connecticut, 555 Willard Ave, Newington, CT 06111.

Neurons in the MRF burst before and during visually guided (VG) saccades and have a lower level of discharge during the delay period between the brief presentation of a visual stimulus and the execution of a remembered (REM) saccade to that stimulus. Eye movements of three, awake, rhesus monkeys were recorded using a scleral search coil before, during and after the injection of muscimol (1 - 3 µg), a GABA(A) agonist, into the MRF. Injections in the rostral portions of the MRF produced hypometric vertical saccades (both up and down). The primary position of gaze was shifted either up or down depending upon the injection site. The trajectories of vertical and oblique saccades were markedly curved. Injections of muscimol into the caudal MRF produced hypometria of contraversive saccades. Trajectories of both vertical and contraversive horizontal saccades were curved. Injections at the caudal-ventral pole of the MRF produced hypermetric saccades. Following caudal injections macro-saccadic square-wave jerks to the contraversive side were generated during attempted fixation. Evidence of saccade hypometria and reduced saccade velocity suggest that one role of the MRF is to excite portions of the superior colliculus (SC) and/or other supranuclear eye movement regions. The generation of square wave jerks following suppression of the caudal pole of the MRF could be the result of inhibition of omnipause neurons located in the pontine raphe or the fixation zone of the SC. Supported by NIH Research Grant EY 09481 and the Dept. of Veterans Affairs.

263.6

EFFECTS OF IBOTENIC ACID (IBO) LESIONS OF THE MESENCEPHALIC RETICULAR FORMATION (MRF) ON PRIMATE SACCADES D. M. Waitzman* and V. L. Silakov. VA Connecticut, 555 Willard Avenue, Newington, CT 06111.

Temporary inactivation of the MRF using the GABA(A) agonist muscimol produces dysmetric saccades and changes in fixation (see Waitzman and Silakov, *Soc. Neurosci. Abst.*, 1996). We have now explored the effects of activating and then destroying cells using IBO. Eye movements of one awake rhesus monkey were recorded using a scleral search coil before, during and after three separate injections (3 X 7.5µg) of IBO into the MRF. Histologically verified lesions were located at the caudal pole of the MRF where single unit activity related to contraversive saccades had been recorded. Two phases of eye movement changes were found. The initial excitatory phase (first 4 hours) of IBO produced hypermetric ipsi- and contraversive visually guided (VG) and remembered (REM) saccades. The trajectories of saccades of specific amplitudes were curved. During the 2 to 5 days following IBO lesion VG saccades became normometric. Recovery of REM saccades was distinctly different: contra- and ipsiversive saccades remained curved and hypermetric. The changes in ipsiversive remembered saccades were evident for 4 months following the third IBO lesion. Permanent changes in REM rather than VG saccades suggest MRF neurons aid in the retention of the visual activity required to move the eyes to a target. Curved saccade trajectories and increased gain support our idea that MRF neurons participate in the local feedback pathway controlling saccade amplitude. Supported by NIH Research Grant EY 09481 and the Dept. of Veterans Affairs.

263.7

RETROGRADE TRANSNEURONAL LABELLING OF HORIZONTAL EYE MOVEMENT CIRCUITS WITH RABIES VIRUS. N.M. Gerrits[1], W. Graf[2], N. Yatim[2] and G. Ugolini[3]. [1]Dept. of Anatomy, Erasmus Univ. Rotterdam, The Netherlands; [2]CNRS - Collège de France, 75270 Paris Cedex 06, France, [3]Lab. de Génétique des Virus, CNRS, 91198 Gif-Sur-Yvette, France. (SPON: European Neuroscience Association).

Retrograde transneuronal tracing with rabies virus allows a specific labelling of synaptically connected neurons (Ugolini, 1995, J. Comp. Neurol. 356:457). This method was applied to the oculomotor system to test the hypothesis of a modular organization of eye movement circuits. In guinea pigs, rabies virus (CVS strain) was injected unilaterally into the medial rectus (MR) muscle. This muscle was chosen because of its special innervation pattern (ascending tract of Deiters, abducens internuclear pathway). The CNS distribution of the virus was studied immunohistochemically at sequential 12 hr intervals from 1.5 to 5 days post-inoculation (p.i.). Transneuronal transfer was time-dependent. Initially, labelling involved only MR motoneurons (first-order) in the ipsilateral oculomotor nucleus. At 2 days p.i., the onset of transfer visualized abducens internuclear neurons contralaterally. Other cell groups of the horizontal system were labelled transneuronally at 2.5-3 days p.i., i.e., prepositus hypoglossi neurons, medial vestibular nucleus neurons, oculomotor interneurons, reticular formation neurons associated with saccade generation. From 3.5 days p.i., transfer to higher-order neurons revealed a zonal organization in the cerebellar flocculus and ventral paraflocculus, in addition to labelling of other cell groups (e.g., paramedian tract neurons, Scarpa's ganglion, superior and descending vestibular nuclei, Y group, fastigial nucleus and interstitial nucleus of Cajal). Other cortical and subcortical cell groups were labelled at longer survival times. These results suggest a modular organization only at the level of basic neuronal circuits involved in spatial coordination of eye movements. (Supported by CNRS, UPR9053)

263.8

NEUROTRANSMITTERS OF ABDUCENS INTERNUCLEAR AND ASCENDING TRACT OF DEITERS INPUTS TO THE MEDIAL RECTUS MOTONEURONES IN THE CAT OCULOMOTOR NUCLEUS. L.T. Nguyen* and R.F. Spencer. Department of Anatomy, Medical College of Virginia, Richmond, VA 23298.

Abducens internuclear (Abd IN) and ascending tract of Deiters (ATD) inputs are the principal excitatory connections to medial rectus motoneurones that are related to the control of conjugate horizontal eye movements. Previously, we have demonstrated differences in the morphology, mode, pattern and soma-dendritic organization of Abd IN and ATD synaptic endings labelled anterogradely with biocytin. The present study extends these observations to the ultrastructural localization of the excitatory amino acid neurotransmitters, glutamate and aspartate, using a postembedding immunogold procedure combined with the preembedding immunoperoxidase localization of anterogradely transported biocytin from the abducens nucleus and the ventral lateral vestibular nucleus. Consistent with their spheroidal synaptic vesicle content and the asymmetric pre-/postsynaptic membrane profile, both Abd IN and ATD synaptic endings are labelled with glutamate and aspartate, despite their individual morphological differences. The coexistence of glutamate and aspartate, however, may indicate neurotransmitter versus metabolic functions that are associated with these amino acids. Consequently, physiological differences between these two inputs are likely to be related more to the postsynaptic receptor that is associated with each input.

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263.9

ROLE OF NUCLEUS RETICULARIS TEGMENTI PONTIS (NRTP) IN 3-D SACCADE CONTROL. J. Van Opstal[1]*, K. Hepp[2], Y. Suzuki[3], V. Henn[3] [1]Biophysics Dept., Univ. Nijmegen, The Netherlands; [2]Physics Dept., ETH Zürich, Switzerland; [3]Neurology Dept., Univ. Hospital, Zürich, Switzerland.

It is well-documented that voluntary eye movements are constrained by Listing's law (LL), which states that eye position vectors lie in a plane (Listing's plane, LP) throughout the trajectory. In recent experiments we have shown that LL is implemented downstream from the deep layers of the Superior Colliculus (SC), but controversy exists whether LL results from a precise tuning of the 2-D visuomotor input with the mechanical properties of the oculomotor plant, or to a neural control that accounts for the noncommutative kinematics of 3-D rotations. In order to investigate whether the cerebellar pathway is involved in the neural implementation of LL, we recorded from single-units in the NRTP of three rhesus monkeys during rapid eye movements in 3-D, performed electrical microstimulation during spontaneous eye movements in the light, and induced local unilateral NRTP inactivation with small (<700 nl) muscimol injections.

In contrast to the SC, 30% of the NRTP units has a 3-D movement field, with positive and negative torsional components of optimal saccade vectors bilaterally represented. Stimulation produced eye displacements with either a positive or a negative torsional component, depending on the depth within the NRTP, rather than on the laterality of the stimulation site. The first spontaneous saccade following stimulation immediately resets the eye into LP. This torsional reset mechanism is selectively disrupted after a unilateral muscimol injection.

Our results indicate that the NRTP contributes to the stabilization of LP against torsional errors of the saccadic system. We conclude that the saccadic burst generator is 3-D under all conditions (not only during vestibular stimulation), and that LL is implemented by a neural control strategy.

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263.10

COMBINED EYE AND HEAD GAZE-PURSUIT RESPONSES IN MONKEY NUCLEUS RETICULARIS TEGMENTI PONTIS (NRTP). D.A. Suzuki*, K.F. Betelak, and R.D. Yee. Dept. of Ophthalmology, Indiana University, Indianapolis, IN 46202.

A question currently exists concerning whether or not the eye and head movement control systems regulating gaze shifts are independent. NRTP may be a good candidate structure to investigate this question, since it receives inputs from motor cortex and has been shown to contain a population of units that encode smooth-pursuit eye velocity.

Neuronal activity was recorded from NRTP in order to determine if head movement signals also exit in NRTP and if so, to determine whether or not populations of eye and head movement related units are independent. Monkeys were trained to generate combined eye and head gaze-pursuit movements in the horizontal plane. Eye and head positions were monitored with a phase-angle type CNC coil system.

Some NRTP cells did exhibit direction selective responses during gaze-pursuit movements. During some epochs, the monkey switched from an eye-only to a head-only strategy of gaze pursuit. The gaze-pursuit units continued to respond during both the eye-only and head-only portions of the gaze-pursuit movement in the preferred direction. These units appeared to encode a gaze velocity signal, i.e., both eye and head velocity signals. Modulations in unit activity were not observed during spontaneous head movements, suggesting a non-vestibular origin for the head-related activity. Whether the head velocity signal was vestibular in origin or an efference copy signal requires further study.

The results indicate that, at least at the level of NRTP and with respect to gaze-pursuit, signals regulating eye and head movements are not independent. Support: RPB & NIH (EY09082) grants.

263.11

SHIFT AND ROTATION OF LISTING'S PLANE AFTER BILATERAL LESION OF THE ROSTRAL INTERSTITIAL NUCLEUS OF THE MLF.

Y Suzuki*, D Straumann, V Henn. Neurology Department, University Hospital Zurich, CH-8091 Zurich, Switzerland.

Static tilt of the head about the earth-horizontal axis changes tonic otolith input and, subsequently, modifies the orientation of Listing's plane. Head tilt about the roll axis shifts Listing's plane in the opposite torsional direction (counter-roll), and head tilt about the pitch axis counter-rotates the plane about this axis (Haslwanter et al, Vision Res 1992).

In three rhesus monkeys, we bilaterally inactivated neurons in the rostral interstitial nucleus of the MLF (riMLF) with kainic acid. Additional lesions in the nearby interstitial nucleus of Cajal were excluded histologically after the perfusion of the animals. Moreover, there was no phase shift of the three-dimensional vestibulo-ocular reflex due to the lesions, which indicates that the velocity-to-position integrator was unaffected by the kainic acid injections.

Before and after inactivation, monkeys were put in different pitch or roll tilt position from prone to supine or from 90° right ear-down to 90° left-ear-down in 30° step. Spontaneous eye movements were recorded with dual magnetic search coil.

The counter-rolling of Listing's plane was preserved after riMLF-inactivation (Suzuki et al., Exp Brain Res 1995). The counter-rotation of Listing's plane during static head pitch was also observed after riMLF-inactivation, however, its gain was significantly reduced by 17-42%.

Results imply, although basic three-dimensional vestibulo-ocular reflexes are preserved, parameters for static vestibulo-ocular reflexes might be modified by the abolition of burst generator input.

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* perm address YS: Physiology Dept, Hokkaido Univ Med Sch, 060 Sapporo

263.12

Response of saccade related neurons in the primate brainstem during head-free gaze shifts to double target steps. L. Ling, J.O. Phillips*, Y. Iwamoto, S.D. Newlands and A.F. Fuchs Department of Physiology and Biophysics, Univ. of Washington, Seattle, WA 98195

During head-unrestrained tracking of a target which jumps twice in rapid succession during the saccadic latent period, the animal's responses exhibit clear dissociations between the gaze and head movements. The behavioral results suggest that the systems which control eye and head motility process information about the new target step differently. We recorded from cells in the brainstem of primates to examine whether the dissociations are manifested at the level of the saccadic burst generator.

For burst units the strict relationship between number of spikes and saccade amplitude is always maintained. During dissociated responses composed of two head and one eye movements, burst units are not active unless a saccade in the on-direction is observed. Similarly, omni-pause neurons (OPN) pause only if a saccade is present. This suggests that the burst generator is commanded to execute the eye movement which gets expressed.

Usually the eye responses are separated by a minimal inter-saccadic interval of ≈150 ms. However, in some cases gaze shifts follow each other back-to-back. OPNs pause throughout the turn-around. Directional burst units discharge only for the saccadic component in the on-direction. Omni-directional burst units discharge continuously, joining the burst for each movement component. Thus the activity of the burst generator is fully expressed and the saccadic system operates in a strictly sequential fashion.

This work was supported by NIH grants EY00745 and RR00166.

263.13

THE SACCADE BURST-GENERATOR DURING RAPID HEAD-ON-BODY ROTATIONS: GAZE RELATED ACTIVITY OF PRIMATE INHIBITORY BURST NEURONS. K.E. Cullen,* A. Ross and J.E. Roy, Aerospace Medical Research Unit, Department of Physiology, McGill University, Montreal, Canada.

Short lead inhibitory burst neurons (IBNs) located in the pontine reticular formation burst during saccades made in the ipsilateral direction. Previous studies in the head-restrained monkey have shown that the number of action potentials generated in a burst is well correlated with saccade amplitude, and peak firing frequency is well correlated with peak saccadic eye velocity. In the present study, we recorded the activity of IBNs in the rhesus monkey during a paradigm in which the animal's head was passively rotated relative to it's body (PHBR). In control experiments, we recorded the activities of abducens neurons (ABNs) which receive projections from IBNs, and Type I pure vestibular neurons (VNs), during PHBR.

In the PHBR paradigm, the applied head movement typically evoked a compensatory vestibulo-ocular reflex during the first 100-200 ms, after which the gaze trajectory (gaze = eye-in-head + head-in-space) was directed towards the ongoing movement of the head. During these shifts in gaze, the eye's contribution to the ongoing movement was relatively small, and most often poorly correlated with the amplitude of the coincident gaze movement. Accordingly, this paradigm effectively allowed us to uncouple the interdependence between gaze and eye movements that generally occurs during voluntary combined eye-head gaze shifts. As was expected, ABN activity was best correlated with eye movements while VN activity was best correlated with head movements. In contrast, analysis of IBNs, revealed that the activity of these neurons was significantly better correlated with gaze movements than either eye or head movements during PHBR. This observation is in agreement with the results of our previous studies which have characterized the activity of IBNs during active combined eye-head gaze shifts (Cullen et al. 1994). We conclude that that primate brainstem "saccade" burst-generator operates in a coordinate frame which is referenced to the movement of the visual axis in space. (Supported by the Medical Research Council of Canada).

CONTROL OF POSTURE AND MOVEMENT: MOTOR UNITS

264.1

MOTOR UNIT TORQUE IN COMPARTMENTS OF THE LATERAL GASTROCNEMIUS MUSCLE OF THE CAT. A.J. Sokoloff*, A.W. English, T.R. Nichols and T.C. Cope. Depts. of Physiology and Anatomy, Emory Univ., Atlanta, GA 30322.

Many muscles are composed of different 'compartments', each comprised of an unique set of motor units. Although proposed as a functional element of neuromuscular control, the biomechanical features of compartments and their constituent motor units have received little study. Here we report the ankle torques of single motor units in different compartments of the cat lateral gastrocnemius muscle (LG). LG motor units were penetrated intra-axonally in ventral rootlets, stimulated to tetanus, and sagittal (dorsiflexion/plantarflexion) and transverse (abduction/adduction) isometric torques determined with a biaxial transducer attached at the calcaneus. The resultant of each motor unit was expressed as an angle relative to pure plantarflexion (90°) and adduction (0°) torques. Motor unit membership was determined visually or by antidromic stimulation of compartment nerves. Motor units were studied in three of the four LG compartments.

In our preparation, LG motor units produced only adduction and plantarflexion torques. Torque angles for compartment LG1 motor units were 79-83°, for compartment LG2 motor units 78-86°, and for compartment LG3 motor units 84-87°. There was no correlation between motor unit torque angle and torque magnitude. These findings demonstrate that LG motor units produce adduction (off-sagittal) torques in addition to plantarflexion (sagittal) torques. Future studies will determine whether motor units are selected in movements according to compartment membership or motor unit torque.

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264.2

SPIKE TRIGGERED AVERAGING OF MOTOR UNIT ACTIVITY IN PUTATIVE PARTITIONS OF HUMAN BICEPS BRACHII J.F. Prather*, J.L. Denef, T.L. Duncan, J.M. Heinzmann, E.M.C. Smith, R.L. Segal. Div. Physical Ther. and Neuroscience Program, Emory Univ. Sch. of Med., Atlanta, GA 30322.

Partitions within the human biceps brachii (BB) may exist, allowing the central nervous system to differentially activate a segment of the muscle in a task-specific manner. This study examined whether correlated activity exists between putative BB partitions. Twelve subjects performed three trials of five isometric upper limb tasks in which electromyographic (EMG) activity was recorded by pairs of indwelling fine-wire electrodes placed in the BB short head (BBSH) and in the three putative partitions of the BB long head (BBLH). Data from each recording site were analyzed using spike triggered averaging (STA) to look for time-locked activity. A motor unit from the site of interest was time amplitude window discriminated with the acceptance pulse triggering the averaging computer. Typically, 100 sweeps were averaged in each STA trial. Averages from 0 to 10 msec. after the trigger and from 10 to 20 msec. after the trigger were quantified. For each trial, the data from all recording sites were analyzed to examine for time locked activity. Analysis of three subjects has demonstrated that one subject had almost no time locked activity across sites. The second subject had extensive time locked activity, and the third had an intermediate amount. Analyses from all 12 subjects will be coupled with data on the patterns of activation order reported by Segal et al. (Neurosci. Abstr. 1996) to determine whether the putative partitions are differentially activated. Supported by NICHD NCMRR grant HD 32571

264.3

PARTITIONING OF THE HUMAN BICEPS BRACHII R.L. Segal*, J.L. Denef, T.L. Duncan, J.M. Heinzmann, E.M.C. Smith, J.F. Prather. Div. Physical Ther. and Neuroscience Program, Emory Univ. Sch. of Med., Atlanta, GA 30322.

The human biceps brachii (BB) participates in elbow flexion, forearm supination and shoulder flexion. Partitions within the BB may exist, allowing the central nervous system to differentially activate a segment of the muscle in a task-specific manner. This study examined if a relationship exists between the function of the putative BB partitions and the anatomical evidence for partitioning of the BB found in a previous study (Segal, 1992). Twelve subjects performed three trials of five isometric upper extremity tasks in which electromyographic (EMG) activity and force/torque outputs were recorded. Pairs of indwelling fine-wire electrodes were placed in the BB short head (BBSH) and in the three putative partitions of the BB long head (BBLH). Activation order (AO), time of activation (TA) and recruitment threshold (RT) of motor units were determined for each task. AO and TA were studied to determine whether consistent patterns of activation existed between the recording sites. RT was studied to determine the force/torque levels at which activation of motor units in each of the recording sites occurred. Significant differences existed for AO and TA between the BBSH and different combinations of the BBLH partitions. For example, BBSH, regardless of task, was activated after the putative central and lateral partitions of BBLH. TA was significantly different for one task in comparison to the other four tasks. For RT, significant differences were evident between certain tasks. These results suggest that differences exist between the BBLH and BBSH, but may not occur within the BBLH itself. Supported by NICHD NCMRR grant HD 32571

264.4

THUMB POSITION INFLUENCES THE RELATIONSHIP BETWEEN SPIKE-TRIGGERED-AVERAGE FORCE AND RECRUITMENT THRESHOLD OF LOW THRESHOLD MOTOR UNITS. M. Bilodeau*, R.M. Enoka. Dept. Biomedical Engineering, Cleveland Clinic Foundation, Cleveland, OH, 44195 and Physical Therapy Graduate Program, University of Iowa, Iowa City, IA, 52242.

It has been shown that activation of the first dorsal interosseous (FDI) muscle is influenced by whether the thumb is extended or abducted (Laidlaw et al. *Soc Neurosci Abstr* 21: 1920). The purpose of the present study was to evaluate the effect of thumb position on the force exerted by FDI motor units (MUs). The activity of 53 MUs was recorded from the FDI of 7 volunteers using fine wire (25 μm) electrodes. The abduction force exerted by the index finger was recorded at the first interphalangeal joint. The experimental task consisted of a slow ramp increase in the abduction force until a MU became active, and thereafter, sustaining the MU discharge at a minimum rate for 2-3 minutes. The activity of each MU was recorded with the thumb alternately secured in two positions: 1) fully extended in the horizontal plane; and 2) abducted by about 90 degrees in a plane perpendicular to the horizontal. For the abducted and extended thumb positions, respectively, the MU force (mean±SD) was 5.9±5.9 and 3.8±2.7 mN, the recruitment threshold was 413±445 and 225±274 mN, and the average force during the minimum discharge rate task was 504±517 and 444±328 mN. The association between spike-triggered-average force and either recruitment threshold or average force was greater when the thumb was extended (r=0.79 and 0.78 respectively) compared with abducted (r=0.18 and 0.20). These findings indicate that altering the line of pull of FDI by changing thumb position influences the association between the spike-triggered-average force and the recruitment threshold of MUs.
Supported by NIH grant AG 09000 and Fonds de la Recherche en Santé du Québec.

264.5

VARIATION IN THRESHOLD FORCE FOR MOTOR UNIT RECRUITMENT. T.C. Cope* and A.J. Sokoloff. Dept. Physiology, Emory Univ., Atlanta, GA 30322.

Some recent reports stress substantial variation in motor unit force threshold, i.e. the level of whole muscle force at which a unit begins to fire (e.g. Romaiguere et al., *Exp. Brain Res.* 1993). Here we report on the variation of force thresholds for motor units in the medial gastrocnemius (MG) muscle of decerebrate cats. Twenty eight motor units exhibiting a large range in physiological properties (conduction velocity 59-115 m/s, tetanic force 2-29 g, and twitch contraction time 21-95 ms) were recruited in multiple reflex contractions (20-93 trials) evoked by ramp-hold-release stretches of the MG muscle. The mean coefficients of variation in force threshold for individual units were distributed with a median of 11.5% and an upper quartile of 24%. Only 2/28 values exceeded 36% (61% and 82%). Intrinsic variation in unit force threshold may be even lower than these values suggest since multiple regression analysis showed that some of the observed variation in force threshold was related to variation in whole-muscle baseline force, peak force, and/or rate of change in force during the ramp. Force threshold variation was additionally studied in 6 pairs of motor units during ramp stretches in which both units in a pair were recruited (13-49 trials/pair). Few cases of reversal in recruitment order were observed for any of these pairs: reversals in recruitment order occurred in only 3/13 and 3/26 ramps for 2 unit pairs with near thresholds (mean force thresholds differing by less than 10%), and in only 3/117 ramps for the remaining pairs. These findings demonstrate that variation in motor unit force threshold is modest and not accompanied by frequent reversals in motor unit recruitment order.
Supported by NIH grant RO1 21023.

264.6

MOTOR UNIT BEHAVIOR DURING ISOMETRIC CONTRACTIONS OF COOLED MUSCLE. R. Samadani* and W. Z. Rymer, Sensorimotor Performance Program, Rehabilitation Institute of Chicago, Chicago, IL 60611, and Dept. of Biomedical Engineering, Northwestern University, Evanston, IL 60208.

In normal muscle, there exists a precise and orderly relation between the discharge rates of motoneurons and the mechanical properties of the muscle fibers they innervate. In this study, we used cooling of the first dorsal interosseus muscle of the hand to induce slowing of twitch contraction, to determine whether there was a compensatory alteration in the discharge properties of the associated motor units. If such rate alterations were evident, this would support the hypothesis that the CNS uses afferent information about contractile properties of muscle to control motoneuron discharge rate. We recorded surface electromyographic activity and single motor unit firing rates via intramuscular fine wire electrodes, before and after 4-6°C cooling. Subjects performed isometric contractions and ramps to 25% MVC under both normal and cooled conditions. Our findings were that with a 5°C decrease in intramuscular temperature, time to peak twitch increased 65%-70%, and tetanic force decreased during electrical stimulation at 10 Hz-50 Hz. For a given voluntary static force level, increases of 5.8%-31.3% in mean firing rates were observed in cooled muscle, indicating a loss of muscle efficiency (in that higher discharge levels are required to accomplish the same force). In contrast, the mean initial motor unit interpulse intervals during ramping voluntary contractions were not systematically changed. It appears that motoneuron discharge is not modified acutely to match muscle contractile properties. Funding: NRSA (T32HD07418)

264.7

MOTOR UNIT FIRING RATES DECLINE DURING LOW-FORCE SUSTAINED ISOMETRIC CONTRACTIONS IN HUMANS. L. Griffin, S.J. Garland* and T. Ivanova. Dept of Physical Therapy, Univ. of Western Ontario, London, ON, Canada, N6G 1H1

The notion of "muscle wisdom" describes a process whereby a decline in motor unit firing rate accompanies the slowing of muscle contractile properties with fatigue. Previously, using a dynamic submaximal (20% MVC) fatigue protocol, we found that motor unit discharge rates in the triceps brachii muscle were maintained (1). It could be argued that contractions of only 20% MVC may induce low intramuscular pressures and possibly little reflex inhibition from trapped metabolites. We sought to determine whether motor unit discharge rates in triceps brachii muscle decline during low-force sustained isometric contractions. Subjects performed a 10 min sustained isometric elbow extension contraction at 20% MVC to induce the same 30% drop in MVC as in the dynamic protocol. Subcutaneous fine wire electrodes were used to record motor unit activity from the lateral head of triceps brachii muscle. Most motor units that were active from the beginning of the fatigue task demonstrated a reduction in discharge rate. However, newly recruited motor units tended to maintain a constant rate or an increased discharge rate. These findings were similar to that found in biceps brachii muscle (2). Thus, the maintenance of discharge rate found previously during a dynamic fatigue task cannot be attributed to the low force level or to any unique characteristics of the triceps brachii muscle.
This work was supported by NSERC.
References:
1. Miller et al. J Neurophysiol 75: 1629-1636, 1996.
2. Garland et al. J Appl Physiol 76: 2411-2419, 1994

264.8

THE COMMON MODULATION OF MOTOR UNITS DURING SLOW WRIST MOVEMENT IN MAN. N.Kakuda* and M.Nagaoka. Department of Neurology, The National Rehabilitation Center for the Disabled, 4-1 Namiki, Tokorozawa, 359, JAPAN.

It was investigated if slow wrist movement is controlled by discontinuous motor output. In 11 healthy volunteers (4 males and 7 females, 22-45 years old), discharges of two motor units from m. extensor carpi radialis (ECR) as well as joint kinematics were recorded while the subject repeated slow wrist extension movement of 15 degree at 5 deg/sec. The coherence analysis performed on spike trains revealed the common modulation of two motor units between 6-12 Hz in 30 out of total 36 pairs (83 %). It was concluded that an agonist motoneurone pool (ECR) was modulated at 6-12 Hz. The functional significance was indicated in two aspects. First, the common modulation of motor units was significantly correlated to angular acceleration, suggesting that the modulation was so deep as to contribute to kinematics. Second, the common modulation of motor units disappeared during isometric contraction in 23 pairs, implying the task-dependent change.
This work was supported by the Ministry of Health and Welfare of JAPAN.

264.9

DIRECTIONAL TUNING OF SINGLE MOTOR UNITS. U. Herrmann* and M. Flanders Dept. of Neuroscience, University of Minnesota, Minneapolis, MN 55455.

The activation patterns of single motor units (SMUs) during isometric forces in different directions were studied. The main question of interest was whether SMUs of the same muscle show different "best directions" of activation such that different populations within one muscle are activated for forces in different directions.

The activity of SMUs in several human arm muscles was recorded with intramuscular electrodes during force ramps at the wrist in 20 different directions covering the sagittal or frontal plane. The activity of one unit was identified off-line by its characteristic shape and amplitude. For each unit, steady state firing rate and threshold force for recruitment were determined for each direction. A unit's best direction was determined from the frequency data by fitting it with a cosine function and/or from the threshold data with linear regression.

Preliminary results from SMUs (n=20) in biceps of two subjects show that there are significant differences in the directional tuning of SMUs of the same muscle in the same subject for forces in the sagittal plane. While the activation ranges of SMUs overlap to a large extent, their best directions appear to be staggered covering a range of approximately 50 degrees in the upward-backward and upward-forward directions.

(Supported by NINDS Grant NS 27484)

264.10

RESPONSES OF SINGLE MOTOR UNITS IN THE HUMAN FINGER FLEXOR TO ELECTROMAGNETIC BRAIN STIMULATION. S. J. Garland and T. S. Miles*. Department of Physiology, The University of Adelaide, Adelaide, SA 5005, AUSTRALIA

The pattern of responses evoked in single motor units in the human flexor digitorum profundus muscle (FDP) by electromagnetic brain stimulation was measured. Depending on the timing relative to the last spike, weak stimuli evoked spikes at about 20 ms latency in some trials, and at about 50-80 ms in others. Motor unit potentials were evoked at the longer latency only in trials in which no short-latency response was elicited in that unit, and only when the stimulus was given during the first half of the interspike interval. When given during the second half of the ISI, the stimulus evoked a corticospinal response but no second-peak response. The motoneurone fires at either short or longer latency but not both, because if it discharges at corticospinal latency, it will still be hyperpolarised when the Ia afferent volley arrives about 30-60 ms later. The motoneuronal response at second-peak latency was not modified when both FDP and the extensors of the distal finger joint were prevented from pulling on the distal phalanx. Hence, this second-peak response is not secondary to a stretch reflex provoked by stimulus-induced activation of the finger extensors, nor is it the result of a cutaneous signal resulting from stimulus-induced movement of the finger. Its' latency is consistent with the idea that the corticospinal volley evoked by the stimulus activates both alpha and beta motoneurones innervating FDP. The beta motoneurones cause the spindles to twitch, exciting Ia fibres, thus activating the motoneurone reflexly.

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264.11

MOTOR UNIT INTERSPIKE INTERVAL VARIABILITY IN OLDER HUMANS. M.R. Roos, C.L. Rice*, D.M. Connelly, and A.A. Vandervoort. Faculty of Kinesiology, Centre for Activity and Ageing, University of Western Ontario, London, Canada.

Reports about motor unit (MU) interspike interval (ISI) variability in older (≥ 75 yrs) humans are limited, and the results are equivocal. Further, the majority of these studies have been conducted in small hand muscles, and at low force levels. Thus, the main purpose was to quantify age-related changes in MU discharge variability throughout the entire range of voluntary force in the vastus medialis. Five elderly (75-85y) and 4 young (21-30y) male subjects made several visits to the laboratory for MU recordings. MU firing rates were recorded with fine tungsten needle electrodes during contractions at force levels ranging from threshold to MVC. MU sequences were identified with overlayed shape recognition of individual spikes, and all identified MU trains with 5 or more ISIs were analysed for standard deviation (SD) and coefficient of variation (CV) of ISI. Results indicate that at all voluntary force levels, older subjects have higher SDs and CVs of ISI than young. Preliminary data from the tibialis anterior indicate similar results. Increase in MU discharge variability may be the result of MU reorganization and coincident reductions in MU numbers.

Supported by NSERC of Canada

264.12

MULTI-CHANNEL MOTOR UNIT CLASSIFICATION DURING ACTIVE FORCE MODULATION USING A NEURAL NETWORK ALGORITHM. Duke C.C. Du and Gary Kamen. Dept. of Exercise Science, Univ of Massachusetts, Amherst.

We developed a motor unit classification system using the fuzzy Adaptive Resonance Theory (ART) neural network algorithm. The system categorized human motor unit action potentials (MUAPs) solely by their waveform shapes obtained from a 3-channel recording. In this implementation, network resonance (correct identification) was reached when the fuzzy similarities between the top-down expectation and the bottom-up presentation of the input vectors rendered the best likelihood identification of recognized clusters. A vigilance parameter was used to determine maximum dissimilarity of new vectors. The classification system drew nonlinear boundaries; these boundaries also varied throughout the progress of the categorization. The same neural network was used to resolve superpositioned waveforms. The procedure used a half-length waveform of the maximal energy as the top-down expectation vectors. These expectation vectors of all the clusters were employed in the order of their half-length energy levels. Whenever a segment of the composite waveform made the network resonant, a full length non-superpositioned AP from the most adjacent clustered spike was used to subtract the waveform energy from the composite waveform. This iterative classification and subtraction procedure continued until all the clusters were exhausted. In comparison with known data recognized using manual waveform identification, the implementation of this multi-channel fuzzy ART algorithm produces: 1) similar accuracy, 2) elimination of a pre-training procedure, and 3) extraction of superpositions. The results of this classification system includes both firing time information as well as MUAP waveform features.

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264.13

A 20 CHANNEL PERIPHERAL NERVE INTERFACE: RECORDING AND STIMULATION IN MAMMALIAN SCIATIC NERVE. A. Branner, H. Kolb*, R.A. Normann, John A. Moran Laboratory in Applied Vision & Neural Sciences, University of Utah, Salt Lake City, UT 84112

Multichannel interfaces capable of accessing individual fascicles have not been used in peripheral nervous system despite their recent application in the central nervous system. The problem of nerve block and degeneration associated with nerve crush resulting from implantation may have limited this application. We have used a high velocity insertion technique as a possible means to circumvent this problem.

The electrode array has two rows of ten, 1 mm long electrodes that project out from a 1 by 4 mm base. It is made of silicon and is insulated with polyimide. The electrodes are separated by glass at their base and the tips are covered with platinum. Teflon insulated Pt/Ir wires are soldered to the back of the array and provide connection to the data acquisition system. Electrode impedance varied between 50 and 200 MΩ. We implanted the array into the sciatic nerve which was supported by a grooved stainless steel platform.

Acute experiments were made in 13 rats and two cats. Spontaneous signals had amplitudes as large as 370 μV. 50 μV responses were evoked by mechanical stimulation in another preparation. In some experiments we found activity on 40% of the electrodes. As expected, it was relatively easy to stimulate the nerve. For a 0.1 second train of 50 Hz biphasic pulses, each 0.2 msec long, we found a minimal muscle twitch threshold of 4 μA and a mean value of 36 μA. In some animals, stimulation via all electrodes was possible. Recordings from afferent and efferent axons suggested that there was either no or minimal acute nerve injury resulting from the implantation. Following removal of the electrode array and recovery from surgery, no motor deficits in the implanted limb were observed.

These results suggest that the use of high density multichannel arrays in the peripheral nervous system is possible. Long-term applications are being evaluated.

This project is sponsored by a State of Utah Center of Excellence Grant.

264.14

THE EFFECT OF MOTOR UNIT ACTION POTENTIAL SHAPE ON THE EMG: COMPUTER SIMULATIONS BASED ON SINGLE UNIT RECORDINGS. **M. Hulliger* and S.J.Day. Dept. of Clinical Neurosciences, University of Calgary, Calgary, Alberta, Canada T2N 4N1.**

The electromyogram (EMG) is an aggregate representation of the individual action potential (AP) contributions from motor units (MUs) close to the recording electrode. Recently, it has been demonstrated that individual MUAPs summate algebraically (essentially linearly) (Day et al., Proc. Canadian Soc. Biomech. 9, 1996). Therefore, the recorded EMG reflects addition of signal components with the same polarities, and subtraction (cancellation) of components with opposite polarities. The EMG is of considerable interest for the diagnosis of various peripheral neuromuscular disorders. In the present study, sets of recorded MUAP shapes were manipulated to mimic the properties that are observed for different electrode configurations or motoneuron diseases, and subsequently used in a computer simulation of muscle activation to determine the effect of MUAP shapes on the EMG signal.

In acute cat experiments, a substantial proportion of the soleus MU pool was isolated and their MUAP shapes were recorded for two electrode configurations. Data from a few experiments were pooled to form a library of experimentally recorded, realistic and often complex MUAP shapes. Furthermore, for some applications the duration or complexity of MUAPs was systematically altered and input into a computer simulation, which replicated various recruitment and rate modulation strategies, based on the independent activation of appropriate numbers of MUs. The activation patterns of individual MUs were simulated as MUAP trains. The aggregate EMG was estimated by algebraic summation of all MUAP trains.

Systematic alterations of the MUAPs shapes, that were used in simulating isometric muscle activation, had considerable effects on power spectra and the relation between the averaged rectified EMG and the ensemble activation rate. The results from these studies indicate that in future simulations of muscle activation it will be beneficial to use large numbers of experimentally recorded MUAP waveforms, since the wide variety of MUAP shapes obtained for any electrode configuration cannot be represented by small numbers of arbitrarily fabricated waveforms. **Supported by AHFMR and MRC Canada.**

265.1

A NEURAL NETWORK MODEL OF THE SPINAL CORD FOR ARM CONTROL. N. Schweighofer, F. E. Pollick*. Human Information Processing Research Laboratories, ATR, 2-2 Hikaridai, Seika-cho, Soraku-gun, Kyoto 619-02 Japan.

According to the concept of muscle synergy, descending motor commands are transformed into a set of several muscle co-activations. This transformation is an ill-posed problem in the sense that the muscle tensions cannot be uniquely determined from prescribed trajectories and forces. We investigated the acquisition of this transformation using a biologically plausible neural network model of the C3-C4 region of the spinal cord which sends its output to a 2 joint, 6 muscle arm model. Since the ill-posedness of this transformation can be solved by minimizing the sum of the motor command change, we propose that known descending neuro-modulatory pathways provide the spinal network with a reinforcement signal which is related to the motor command change. However, if the spinal cord network is not *a priori* provided with coarse connection pattern, the reinforcement learning does not converge. We mimic the development of the coarse connectivity of the network with a genetic algorithm. When the genetic algorithm converges we select the network which gives the best performance. We then train this network with the reinforcement signal from the neuromodulatory pathway. Our results show that, after learning, coordinated arm movements can be executed with reasonably good accuracy.

Supported by ATR.

265.2

FORELIMB MOTONEURON POOLS ARE CONSERVED IN VERTEBRATES. J.M. Ryan*, J. Cushman, B. Jordan, and C. Baier. Biology Dept., Hobart & William Smith Colleges, Geneva, NY 14456.

A complete description of the anatomical organization of the neurons innervating homologous limb muscles is a prerequisite to any test of the neuromotor conservatism hypothesis. This study used the retrograde neuronal tracer WGA-HRP to selectively label the motorneuron pools of seven homologous forelimb muscles in 30 iguanas (I. iguana), 58 mice (M. musculus), and 42 bats (E. fuscus and M. lucifugus). The muscles studied were Mm. pectoralis, spinodeltoideus, biceps brachii, lateral and long heads of triceps brachii, and the mammalian supraspinatus and infraspinatus, or its homolog, the reptilian supracoracoideus. In vertebrates, motoneurons are arranged in longitudinal columns of cells in the ventral horn of the spinal cord. The number of neurons per pool ranged from 70 - 119 in mice, 40 - 87 in bats, and 58 - 114 neurons in iguanas. In both iguanas and mice the motor pools for the spinodeltoids, biceps, and the supracoracoideus (or its mammalian homologs) lie cranial to the pectoralis and triceps motor pools. In the transverse plane, the pectoralis pool lies medial to those of the other muscles studied. The pools of the biceps and spinodeltoids are located dorsal and lateral to the pectoralis and supracoracoideus (or its homologs in mammals). The resulting motor pool maps demonstrate that the anatomical organization of motoneurons in reptiles has been retained in both quadrapedal and flying mammals and is independent of the anatomical specialization of the forelimb. Funded by a Whitehall Foundation grant GIAM-01 and NSF grant IBN-9318681 to JM Ryan.

265.3

RECRUITMENT ORDER OF RETICULOSPINAL NEURONS FOR THE CONTROL OF SWIM VELOCITY IN LAMPREY. T.Wannier, H.P.Clamann* and H.-R.Lüscher, Dept. of Physiology, University of Bern, Bühlplatz 5, 3012 Bern, Switzerland

In lamprey, the supraspinal control of locomotion is accomplished mainly by the reticulospinal (RS) system. The axons of the RS neurons lie ipsilaterally in the spinal cord where they provide excitatory input to spinal neurons. During locomotion, RS neurons receive phasic inputs, and are presumably rhythmically active with a cycle duration corresponding to the duration of the swim cycle. The temporal and spatial characteristics of the propagation of the population of action potentials along RS axons was investigated using a computer model. The results suggest that if changes of velocity are obtained by recruiting RS neurons independently of their size and conduction velocity, a phasic input from RS neurons will act on segments during the inhibited phase of their cycle. Such an input would probably hinder a smooth rostro-caudal propagation of the contraction wave. In contrast, recruiting RS neurons by size provides a mechanism for stabilizing the propagation of the contraction wave.

To investigate if recruitment of RS neurons by size may occurs in lamprey, the activity of RS neurons was recorded with a surface electrode on the spinal cord, and their recruitment to stimulation of cranial nerves with currents of successively higher intensities was investigated. For this purpose, the in vitro preparation of the brainstem-spinal cord of the lamprey was used. A Vaseline barrier was built between the brain and the spinal cord. The brain was bathed in Ringer and synaptic transmission was blocked in the spinal cord. Under these conditions, the wave of RS action potentials evoked by stimulating a cranial nerve reaches the electrode level with a delay which decreases as the amplitude of the stimulation increases. The data suggest that in lamprey rhythmic activation of RS neurons recruited by size may be the mechanism underlying the supraspinal control of swimming velocity.

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265.4

MODULARITY OF THE FROG SPINAL CORD MOTOR SYSTEM P. Saltiel*, M. Tresch and E. Bizzi, Dept. of Brain and Cognitive Sciences, MIT, Cambridge, MA 02139.

Electrical microstimulation of the lumbar grey with the limb held isometrically is known to produce only a few types of convergent force fields of distinct orientations. In order to verify that non-axonal elements underlie this finding and to delineate their topography, NMDA iontophoresis (-100nA x 30 sec) was applied to the spinal cord at sites where electrical stimulation through the same pipette had elicited a force response. At the responsive sites, forces clustered with the same five orientations (abduction=ABD, rostral flexion=RF, flexion to body=FB, adduction=ADD, caudal extension=CE) than the set of forces obtained with electrical stimulation, confirming earlier results (Soc Neuro Abs 1406,1994). The chemical stimulation seemed focal in that many penetrations had both NMDA-responsive and silent sites, with the distance between the closest active and silent sites being on the average 293μ, and sometimes as little as 100μ.

Out of 72 active sites (33% of tested sites) in 13 frogs, 18 responded with a single tonic force (5 ABD,5 RF,3 ADD,5 CE). These tonic sites defined a coarse topography: CE was obtained from lower cord segment 7 to mid-segment 8 at a depth of 860±189μ, RF from segment 8 at 1098±171μ , ADD from lower segment 8 at 881±121μ, ABD from mid-segment 9 to mid-segment 10 at 1324±142μ.

46 sites responded with a rhythm usually involving two or three types of forces. Not all possible combinations of forces were seen. Each of the most common rhythms (RF,FB; RF,FB,ABD; ADD,CE,FB) could be elicited from two or three regions of the cord. These consisted of regions similar to or close in location to the tonic regions capable of producing those forces that represented components specific to that rhythm, as well as an additional caudal region in segment 10 from which any of these rhythms could be elicited.

These results support the idea of modularity of the motor system within the frog spinal cord as well as the existence of a topographic organization to these modules. They may also provide a framework in which to investigate the hard-wired aspects of the circuitry underlying the genesis of spinal cord rhythms. (Supported by ONR:N00014/90/J/1946).

265.5

REFLEX ORGANIZATION AND CONTROL OF FORCE-FIELD PRIMITIVES IN FROG SPINAL CORD. S.F. Giszter*, M.R.Davies and W. Kargo, Dept Neurobiology and Anatomy , MCPHU, Philadelphia, PA 19129.

In our previous work we explored the hypothesis that the combined effect of motion based reflex feedback and muscle properties lead to multi-joint force-fields that can be simply described as a time-varying scalar modulation of the isometric force-field 'primitive' that was measured in spinal frogs with the limb held immobile. We have now shown that during reflex behaviors the basic orientation of forces following periods of motion closely matches those generated in isometric conditions. However, we also showed that the magnitude of force production could differ significantly. The net effect of this was to alter force field structure and force us to reject the simple hypothesis of uniform scaling of the isometric primitive.

Our current experiments are aimed at understanding the control of force magnitude as a force-field evolves during reflex behavior. We use a 'phantom' haptic interface robot to interact with the frog limb. We are able to present different 3 DOF impedance fields to the limb. We have initially concentrated on the effects of environmental stiffness. Our results suggest that the control of reflex force-fields can be separated into two additive effects. The first control is based on the initial limb configuration and was well captured in early isometric work. This can be summarized as an elastic force-field. The second control is based on the impedance encountered by the limb and force magnitude is uniform across the workspace. This force-field represents a low or zero stiffness force control. These two controls can be summarized as two separate force-fields, one elastic and one of uniform magnitude, which sum to determine the net driving torques and interaction forces. How these controls vary through a trajectory and whether they are adequate to fully explain the free space limb kinematics is our current focus. The results have implications for the notion of motor primitives, for models of motor learning using force-fields, and for equilibrium models of limb motion. Supported by NIH R29-NS34640-01.

265.6

AN *IN VITRO* ADULT MAMMAL WHOLE SPINAL CORD PREPARATION Z. Jiang, R.M. Brownstone* Depts. of Physiology and Surgery, University of Manitoba, Winnipeg MB Canada R3E 0W3

The "classical" preparation to study spinal cord physiology is the *in vivo* cat. Recently, isolated *in vitro* spinal cord preparations have been developed using neonatal rats[1,2,3] or mice[4,5]. These preparations have allowed for the close examination of cellular properties during, for example, locomotor activity. In addition, the hemisected adult mouse spinal cord has been studied *in vitro*[6]. We now report the development of a whole *in vitro* spinal cord preparation in an adult mouse with lumbar roots intact.

Mice up to 21 days of age were decapitated and eviscerated after being anaesthetized with ketamine. In oxygenated artificial CSF a vertebrectomy was performed from cervical spine to sacrum, and the spinal cord with lumbar roots was carefully dissected free. The cord was then secured in a Sylgard-lined dish. Suction electrodes were used to monitor activity in ventral roots and/or white matter funiculi. Suction electrodes may also be used for stimulating dorsal roots or the spinal cord. Neurochemicals including serotonin, NMDA, Noradrenaline, mianserin, and propranolol may then be added to the solution to produce or block activity.

There is a dramatic increase in tonic motor activity following the addition of serotonin to the bath. In addition, there was some evidence of alternating rhythmic activity seen in contralateral ventral roots of the same lumbar segment in response to a combination of serotonin and NMDA.

This demonstrates that this preparation allows for the study of intact adult mammalian spinal cord physiology *in vitro*.

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265.7

DIFFERENTIAL MODULATION OF SPINOCEREBELLAR RESPONSES BY SEROTONIN IN THE CAT

A.M. Rankin, G. Bosco, and R.E. Poppele*. Dept of Physiology and Neuroscience Graduate Program, Univ. of Minnesota, Minneapolis, MN 55455.

We previously examined the firing patterns of neurons of the dorsal spinocerebellar tract (DSCT) during passive movements of the hindlimb. In response to a small amplitude pertubation applied to the limb, the cells exhibit a stereotyped pattern of firing broadly tuned to the direction of movement. They also linearly encode the position and direction of movement of the foot relative to the hip. Raphe stimulation or systemic 5-HTP, a serotonin precursor, produced complex and differential effects on these responses. A common finding was a reduced sensitivity to static foot position, resulting in a nearly uniform firing rate during passive movements of the foot throughout the workspace. However, the overall mean firing rate was essentially unchanged, indicating that the effect is not simply due to a serotonergic inhibition of an excitatory input. Furthermore, the reduction of the position sensitivity could occur even when the neuron retained its sensitivity to movement. These findings suggest that multiple spinal circuits may be involved in the production of DSCT responses and that they may be differentially sensitive to modulation by serotonin. Serotonin may thus provide a useful tool for investigating spinal circuitry responsible for DSCT behavior. (Supported by NIH Grant NS21143)

LIMBIC SYSTEM AND HYPOTHALAMUS II

266.1

PROPERTIES OF MINIATURE IPSCs IN CA1 INTERNEURONS OF THE RAT HIPPOCAMPUS IN VITRO. S. Nurse* and J.-C. Lacaille. Centre de recherche en sciences neurologiques, Département de physiologie, Université de Montréal, Montréal, Canada H3C 3J7.

Hippocampal interneurons form inhibitory synaptic connections with each other, in addition to the contacts made onto principal cells. However, the properties of inhibitory currents at individual synapses in interneurons have not been characterized. Miniature inhibitory postsynaptic currents (mIPSCs) were recorded in CA1 interneurons from several layers (stratum oriens, radiatum and lacunosum-moleculare), and their properties compared to those of pyramidal cells. Interneurons and pyramidal cells were visually identified in hippocampal slices from 3-4 week-old rats, using Nomarski optics and a near-infrared CCD camera. Whole-cell recordings were made using patch pipettes (4-8 MΩ) containing (in mM) 140 KCl, 5 NaCl, 2 $MgCl_2$, 10 HEPES, 2 ATP-tris, 0.4 GTP-tris. Voltage clamp recordings of mIPSCs were made at -80mV during bath application of 50μM AP5, 20μM CNQX and 0.5μM TTX (n=9 interneurons and 4 pyramidal cells). The mean frequency (±sd) of mIPSCs was similar in interneurons and pyramidal cells (0.8±1.0 *vs* 1.1±0.6 Hz, respectively). Mean peak amplitude of averaged mIPSCs was also similar in both cell types (23.6±8 *vs* 35.8±12 pA). Mean rise time of averaged mIPSCs were significantly slower in interneurons than pyramidal cells (2.2±0.6 *vs* 1.1±0.3 ms). The decay of averaged mIPSCs was well fitted by a single exponential function, but the mean decay time constant was faster in interneurons (11±1.9 *vs* 14.2±3.0 ms). In all cells, mIPSCs were blocked by 25μM bicuculline. Thus, mIPSCs in interneurons were $GABA_A$-mediated, as in pyramidal cells, but their kinetics differed. These results suggest that the properties of individual inhibitory synapses may differ on interneurons and pyramidal cells.

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266.2

GAMMA OSCILLATIONS IN CA1 MINI-SLICES. A. M. Borroni* and W. B Levy. Dept. of Neurosurgery, Univ. of Virginia, Charlottesville, VA 22908.

Under certain behavioral conditions, rhythmic events can be observed in the CA1 region of the hippocampus. The most prominent example is the 5-8 Hz oscillation, the theta rhythm. This rhythm reflects the coordinated activation of currents in many hippocampal neurons but is most likely driven by afferents from the septum and the entorhinal cortex. Within a cycle of theta, a faster gamma oscillation (30-60 Hz) can be observed. This rhythm is prominent in other regions as well and is associated with behavioral activation. The importance of understanding the mechanism underlying this gamma rhythm derives from its hypothesized role in short-term memory and perceptual binding. Here we demonstrate that, as with the theta rhythm, gamma oscillations can be generated in the CA1 region in vitro by activating muscarinic receptors. However, unlike theta rhythms in vitro, gamma oscillations occur in CA1 even when this region is isolated from all extrinsic inputs (CA1 mini-slices). Similar to gamma oscillations in vivo, the frequency of the gamma oscillations can be decreased by enhancing $GABA_A$ inhibition. They are also blocked by the $GABA_A$ antagonist bicuculline methiodide. The synchronization of gamma oscillations across CA1 and the recording of these events extracellularly suggest that this rhythm reflects the coordinated activity of many cells rather than a single cell. Thus, gamma oscillations in vitro are likely to reflect inhibitory currents generated at pyramidal cell somata by networks of synchronized inhibitory interneurons. By adding muscarinic agonists, gamma oscillations similar to those seen in vivo can be produced in CA1 thus providing a reduced system for the study of this phenomenon.

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266.3

ASSOCIATIVE LONG-TERM DEPRESSION IN LAYER II OF MEDIAL ENTORHINAL CORTEX. D. X. Zhang*, N. L. Desmond and W. B Levy. Dept. of Neurosurgery, University of Virginia, Charlottesville, VA 22908.

Alonso et al. (1990) reported LTP in the entorhinal cortex (EC). Here we report a LTD that apparently has an associativity requirement for its induction. Horizontal slices from young adult rats were maintained in an interface chamber. Three stimulating and one recording electrodes were placed in each slice. The extracellular recording pipet was placed at the border between layers I and II of the medial EC. Two stimulating electrodes were placed in layer I, with one on the lateral (Lat) and the other on the medial (Med) side of the recording electrode. The third stimulating electrode was placed in layer III medial to the recording pipet. We measured the initial slopes of the responses. Conditioning stimulation of Lat and/or Med at the test intensities consisted of 150 trains (1 train/2 sec). Each train had 4 pulses at an interpulse interval of 300 ms. The total number of conditioning pulses for Med or Lat was 600. During co-conditioning of Lat and Med, each Med pulse was always delayed 150 ms relative to each Lat pulse. Only co-conditioning of the Lat and Med induced statistically significant LTD. The slopes of the Lat and Med responses decreased 18.8±4.6% ($N=16$, $t=4.09$, $p<0.001$) and 21.5±4.7% ($N=16$, $t=4.56$, $p<0.001$), respectively, at 60 min after co-conditioning. Although the responses tend to decrease, there were no significant long-term changes in any responses when either Lat alone or Med alone is conditioned using 600 or 1200 pulses.

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266.4

DISTAL DENDRITIC ACTIVATION MODULATES THE INDUCTION OF LTP OF THE SCHAFFER COLLATERAL RESPONSE IN HIPPOCAMPAL CA1. W.B Levy*, N. L. Desmond and D. X. Zhang. Dept. of Neurosurgery, University of Virginia, Charlottesville, VA 22908.

The existence of laminated inputs along the proximodistal axis of oriented neurons and the possibility of electrical or chemical compartmentation within a neuron have led to the idea of selective interlaminar interactions. Here we investigate an interaction between the distal perforant path (PP) input to hippocampal CA1 and the more proximal Schaffer collateral (Sch) input to s. radiatum. Transverse hippocampal slices from male rats (140-225g) were maintained in an interface chamber. CA3 and DG were dissected after chopping. The PP and Sch stimulating electrodes were placed in CA1 s. lacunosum-moleculare and s. radiatum, respectively. Recording pipets were also placed in these two laminae. The PP stimulating current was chosen to prevent any noticeable overlap between the two inputs. Conditioning consisted of 10 trains (1/200 ms). The Sch and PP trains contained 5 and 10 pulses, respectively, at 100 Hz. The PP train began 2 pulses earlier and ended 3 pulses later than the Sch train. The control conditioning paradigms were Sch alone or PP alone. The data reported here are measurements of the initial slopes of the Sch pEPSPs in s. radiatum. Comparisons between control and paired conditioning are between slices. Sch conditioning alone induces significant LTP (35.4±7.2%; $N=11$, $p<0.001$). However, paired conditioning of the Sch and PP inputs leads to essentially no LTP of the Sch response, i.e. Sch+PP conditioning increases the Sch response only 4.6±4.3% ($N=15$, $p>0.05$). Therefore, we conclude that sufficient inhibition is available in the distal PP input to prevent the induction of homosynaptic LTP in CA1 s. radiatum.

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266.5

SPONTANEOUS, FAST REPLAY OF LEARNED SEQUENCES BY A CA3 NETWORK MODEL. D. A. August* & W. B Levy. Univ. of Virginia Health Sciences Center, Dept. of Neurosurgery, Charlottesville, VA 22908.

Recent results from McNaughton's lab suggest that the hippocampus serves as an intermediate-term memory store that teaches the cerebral cortex. During sleep, the hippocampus replays events that were experienced earlier in the day. This replay is much faster than the originally sensed events as measured by hippocampal cell firing and their correlations. Previously, we presented a model of hippocampal region CA3 that 1) spontaneously replays a learned sequence (Minai & Levy, 1993) and for which 2) the speed of replay in a sequence completion problem is controlled by the level of inhibition (Prepscius & Levy, 1994). These self-modifying networks consisted of sparse, recurrently connected McCulloch-Pitts neurons. Here we report an extension of this CA3 model that uses integrate and fire neurons. When inhibition is decreased from the level used during learning and random activity is added, this modified network spontaneously replays a fast version of a previously learned sequence. We term such speeded up replay temporal compression. Not surprisingly when inhibition is lowered to produce spontaneous replay, neural firing rates increase compared to rates during learning. The amount of temporal compression observed ranged from 20-80 fold. Most interestingly, we were unable to get spontaneous replay at the same speed as a sequence was originally learned. Apparently, the only way to replay the sequence at the speed experienced during learning is to pace the system with external inputs. Supported by NIH MH10702 to DAA. WBL supported by NIH MH00622, MH48161, and Pittsburgh SuperComputing Center Grant BNS950001P.

266.6

CONTROLLING ACTIVITY IN A RECURRENT CA3-LIKE NEURAL NETWORK MODEL. O. V. Yousukhno* and W. B Levy. Univ. of Virginia Health Science Center, Dept. of Neurosurgery, Charlottesville, VA 22908.

We are studying sparse, randomly connected recurrent networks of McCulloch - Pitts neurons as a model of hippocampal region CA3. Neuronal firings in such a randomly connected network appear random. The activity of the network (percentage of neurons that are active) fluctuates under the control of a shunting, fast feedback inhibition, which is proportional to the activity. In this model there are large fluctuations of activity; such large fluctuations impair network performance. Here we show how activity levels can be better controlled if a leak inhibitory conductance is added.

Consider the following: suppose that activity differs from the desired activity value, A. We want, on the average, the activity at the next time step to equal A. What inhibition should the network generate?

Because of the random connectivity of the network, this question is answered by numerically solving probabilistic equations of activity. The resulting relationship between the desired inhibition and preceding activity is well approximated by a linear function in the activity range of interest. In addition to a function that varies proportionally with activity, a constant level of inhibition is needed. Essentially this is equivalent to adding a constant shunting leak conductance. Both constants in this linear relationship depend on the desired level of activity.

Incorporating this modified form of shunting inhibition substantially reduced activity fluctuations in the computational model. In the case of 10% average activity, the fluctuations decreased from a 5-fold span (highest to lowest) in the old model to just 1.3 when the proper leak shunt was added.

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266.7

IDENTIFICATION OF INTERNEURONAL SUBTYPES IN CULTURES OBTAINED FROM POSTNATAL HIPPOCAMPUS. M. Mynlieff*. Dept. of Biology, Marquette University, Milwaukee, WI 53201-1881.

Morphological identification of cell types in short term cultures obtained from the superior region of the hippocampus of postnatal day 7-14 Sprague Dawley rat pups is not possible. Whole cell patch clamp recording in the current clamp mode after 24-96 hours in culture was used to determine if the action potential (AP) duration would be a useful criteria in distinguishing cell types. Single APs could be elicited by a 0.1-0.2 ms 2000 pA depolarizing pulse. The average membrane potential and input resistance were -47.5±1.9 mV (n=27) and 708 MΩ (n=26), respectively. A frequency distribution of the AP duration measured at half-maximal amplitude showed 3 distinct groups of neurons (#1, 1.4±0.1 ms, n=10; #2, 2.1±0.1 ms, n=10; #3, 3.2±0.2 ms, n=7). Based on correlations with studies by Lacaille and coworkers using intracellular recording in identified cells in slices, I propose that group 1 represents basket cells, group 2 represents vertical cells and group 3 represents a combination of stellate cells and pyramidal cells. Further analysis of the fast afterhyperpolarization is needed to distinguish between pyramidal cells and stellate cells. In contrast to the interneurons in a slice preparation, these cells offer good voltage control and environmental control. By waiting a minimum of 24 hours before recording, the cells become more stable than acutely dissociated neurons and will show recovery of functions damaged by enzymatic treatment. Future studies will record from these cells in current clamp mode to quickly characterize the AP before switching to voltage clamp recording to characterize the currents present in the different types of interneurons.

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266.8

INVOLVEMENT OF CHOLINERGIC NEURONS OF THE LATERODORSAL TEGMENTAL NUCLEUS IN THE INHIBITORY RESPONSES IN THE BASAL FOREBRAIN OF THE RAT. L.A. Kadishevitz, S.M. Brudzynski*, Department of Clinical Neurological Sciences, London Health Sciences Centre, University of Western Ontario, London, Ontario, N6A 5A5 Canada.

There is ample evidence that the laterodorsal tegmental nucleus (LDT) contains a major group of cholinergic neurons innervating diverse regions of the rat brain. It had been demonstrated in a preliminary study that electrical stimulation of the LDT had a predominantly inhibitory effect on the mean firing rate of the basal forebrain neurons. The goal of the present study was to demonstrate that these inhibitory responses are mediated by cholinergic transmission. Extracellular single unit responses were recorded from the anterior hypothalamic-preoptic region (AH-POA) before and after single pulse (0.2-2 ms, 200-800 μA) stimulation of the LDT. Stimulation of the LDT produced inhibitory responses in 52 neurons (mean inhibition time 36.7 ± 7.2 ms, SEM for 400 μA), and excitatory responses in 17 neurons in the AH-POA. Iontophoretic ejection of scopolamine into the AH-POA reversed inhibitory responses of 6 neurons to LDT stimulation while the excitatory responses (n = 2) remained unchanged. In 4 rats, the recording sites in the AH-POA, from which inhibitory responses were obtained, were subsequently injected with the membrane lipophilic dye DiA (0.1-0.2 μL). Analysis of fixed tissue preparations using a confocal laser microscope (MRC 600) revealed clusters of retrogradely labelled neurons in the LDT. The present results demonstrate that the cholinergic transmission in the AH-POA is involved in the inhibitory effects of the LDT stimulation.

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266.9

INSULAR AND TEMPORAL CORTICAL EFFERENTS IN THE MONKEY. B. Turner* and M. Knapp. Dept. Anatomy, Howard Univ. Coll. of Med., D.C. 20059.

The anterior temporal and insular cortices are important in the initial stages of learning and memory formation. To determine likely sites of subsequent stages of these functions, we plotted all efferent connections from subdivisions of these regions using Fink-Heimer and autoradiographic series. **RESULTS: Taste** IA: To cortex-heavy in prorhinal and frontal insular cortices. To limbic-heavy in lateral and basal amygdaloid nuclei. To striatum-heavy in ventral putamen. **Audition** TA: To cortex-moderate in frontal areas 10, 11, and 13; lightly in subcallosal cingulate and prorhinal cortex. To limbic-heavy in lateral amygdaloid nucleus. To striatum-lightly in tail of caudate. TGd: To cortex-heavy in subcallosal cingulate. To limbic-heavy in lateral and basal accessory nuclei. To striatum-moderate in tail of caudate. **Vision** TE posterior: To cortex-heavy in area 8 (superior bank of inferior arcuate sulcus). To limbic-lightly in amygdala. To striatum-heavy in ventral putamen and tail of caudate. TE anterior: To cortex-moderate in prorhinal/perirhinal cortex. To limbic-heavy in lateral and basal amygdaloid nuclei. To striatum-heavy in ventral putamen and tail of caudate. TGv: To cortex-heavy in prorhinal/perirhinal cortex, lightly in dorsal bank of principal sulcus. To limbic-heavy in lateral and accessory basal amygdaloid nuclei. To striatum-heavy in ventral striatum, ventral putamen and tail of caudate. **Conclusion:** Temporal and insular projections to other cortical regions are light; those to the tail of caudate and ventral putamen are extremely limited in spatial extent. The amygdala and the hippocampus (prorhinal/perirhinal cortex) have heavy inputs from all temporal and insular sense modalities; and are therefore the best candidates for subsequent stages in learning and memory.

266.10

MEDIAL AND ORBITAL PREFRONTAL CONNECTIONS OF THE MIDBRAIN PERIAQUEDUCTAL GRAY OF THE MACAQUE. X. An, R. Bandler* and J. L. Price. Dept. of Anatomy and Neurobiology, Washington Univ. Sch. of Med., St. Louis, MO 63110; Dept. of Anatomy and Histology, Univ. of Sydney, NSW, Australia 2006

Recent research suggests that distinct, longitudinal columns of midbrain periaqueductal gray (PAG) neurons play important roles in coordinating different emotional coping strategies. A lateral PAG column mediates active emotional coping strategies (fight/flight, hyper-reactivity, hypertension, tachycardia), whereas a ventrolateral PAG column mediates a more passive emotional coping strategy (quiescence, hyporeactivity, hypotension, bradycardia). Previous studies in the rat revealed that a number of specific medial, orbital and lateral cortical fields project densely to terminate as longitudinal afferent columns within specific rostrocaudal portions of the PAG. In the macaque, recent studies indicate that the orbital and medial parts of the prefrontal cortex (PFC) can be subdivided into at least 22 discrete areas. This study, in *Macaca fascicularis*, examined PFC to PAG connections. Retrograde tracer injection (fast blue) centered in ventrolateral PAG resulted in strong bilateral labeling (with an ipsilateral bias) in extensive medial PFC areas (32,25,24,9) and select orbital PFC areas (12o,lai,13a). Anterograde tracer (biotinylated dextran amine, fluororuby) injected in medial and orbital PFC areas (25,12o,13a) verified that these areas project to the ventrolateral PAG. Other studies indicate that these extensive medial and select orbital PFC areas are linked by substantial intracortical connections; receive inputs from the same regions of mediodorsal thalamic and basal amygdaloid nuclei; and project strongly to the ipsilateral hypothalamus. It has been suggested previously that these medial and select orbital PFC areas constitute a "medial" PFC network. By targeting the ventrolateral PAG columnar circuit this "medial" PFC network is organized in a manner to allow it to modulate or trigger the somatic and autonomic reactions coordinated by the ventrolateral PAG column. Other "orbital" PFC areas do not appear to project strongly to the ventrolateral PAG. (Supported by NIH grant DC00093 and Australian NHMRC grant 950143)

266.11

ORBITAL AND MEDIAL PREFRONTAL CORTICAL PROJECTIONS TO THE HYPOTHALAMUS IN MACAQUE MONKEYS. D. Öngür*, X. An and J.L. Price. Dept. of Anatomy & Neurobiol., Washington Univ. Sch. Med., St. Louis, MO 63110

Previous evidence from rats indicates that the orbital and medial prefrontal cortex (OMPFC) modulates autonomic function through projections to the hypothalamus and brainstem. Loss of these connections may underlie many of the behavioral deficits seen with lesions of the OMPFC in humans, but the connections have not been well defined in primates. We investigated the projections from the OMPFC to the hypothalamus in monkeys, in relation to architectonic areas defined from staining patterns with several methods. Two networks can be recognized within the OMPFC based on their cortical and subcortical connections: a medial network that involves medial prefrontal areas and selected orbital areas, and an orbital network that involves other orbital areas.

Five injections of retrograde axonal tracers in the hypothalamus and ventral midbrain of three monkeys (*Macaca fascicularis*) produced labeled cells in selected areas of the ipsilateral OMPFC. An injection in the anterior lateral hypothalamus labeled cells primarily within the medial network, including medial areas 32 and 25 and orbital areas 12o, 14c, 13a and Iai. Another injection centered just lateral to the ventromedial hypothalamic nucleus labeled cells in the same areas and also in other agranular insular areas Iam, Iai, Ial and Iapm on the posterior orbital surface. Many fewer cells were labeled in more rostral prefrontal areas. In other experiments, anterograde tracer injections into several areas in the OMPFC (25, 13a, 12o) labeled extensive axonal projections to lateral and medial parts of the hypothalamus.

In summary, the OMPFC has a substantial projection to the hypothalamus, primarily from areas within the medial network, and from the agranular insular areas. These areas receive substantial inputs from several limbic structures as well as olfactory and visceral afferents, and provide for higher cortical control of autonomic function. Supported by NIH grant DC00093 and by the Division of Biology & Biomedical Sciences of Washington University.

266.12

TOPOGRAPHY OF PROJECTIONS FROM THE HYPOTHALAMUS TO PREFRONTAL CORTICES IN THE RHESUS MONKEY. N.L. Rempel-Clower* and H. Barbas, Dept. Health Sci., Boston Univ., Boston, MA 02215

The hypothalamus has widespread projections to the cortex. However, the topography of hypothalamic projections to most cortical areas in the monkey has not been characterized in detail. We studied hypothalamic projections to prefrontal cortices using injections of retrograde tracers in orbital, medial, and lateral prefrontal areas. All of the prefrontal areas investigated received projections from the hypothalamus, and most retrogradely labeled neurons were found in the posterior hypothalamus. There were differences in the rostrocaudal organization of hypothalamic projection neurons directed to the structurally distinct limbic and eulaminate prefrontal cortices. Specifically, injections in eulaminate prefrontal areas (e.g., areas 46 and 8) primarily labeled neurons in the posterior hypothalamus, and only a few neurons in the tuberal hypothalamus. Injections in limbic prefrontal areas (e.g., PAll, Pro, area 32, area 25) also primarily labeled neurons in the posterior hypothalamus, but in comparison with eulaminate areas, more neurons were labeled in the anterior and tuberal regions of the hypothalamus. There were additional differences in the topography of projection neurons directed to limbic cortices. Labeled neurons projecting to limbic orbitofrontal areas were more prevalent in lateral hypothalamic regions (lateral hypothalamic area, magnocellular tuberomammillary nucleus, paramammillary nucleus) than in medial regions. In contrast, labeled neurons projecting to limbic medial areas were more prevalent in medial hypothalamic regions (posterior hypothalamic area, dorsal hypothalamic area, perifornical nucleus) than in lateral regions. Labeled neurons directed to eulaminate prefrontal areas were distributed equally in the lateral and medial hypothalamus. Results suggest that hypothalamic projections to prefrontal areas originate in areas associated with autonomic function, and there was no evidence of projections from areas with endocrine function (e.g., paraventricular nucleus, supraoptic nucleus). (Supported by grant NS24760.)

266.13

INTRAHYPOTHALAMIC INJECTION OF GABA$_A$ ANTAGONIST REDUCES THE BLOOD CONCENTRATION OF CALCIUM AND POTASSIUM IN RATS.

S. Aou*, K. Shiramine, K. Araishi, and T. Hori, Dept. of Physiology, Fac. of Med., Kyushu Univ., Fukuoka 812, Japan.

The rat hypothalamus has been shown to be sensitive to calciotropic hormones (Matsui et al 1995; Shimizu et al 1986) and regulates calcium metabolism via the vagus nerves (Aou et al 1993; Ma et al 1994). To further elucidate neuronal involvements in blood calcium homeostasis, effects of intrahypothalamic injection of bicuculline methiodide (BM, 5-40 ng/0.2-0.5 μL), a GABA$_A$ antagonist, on the blood concentration of calcium and other electrolytes were investigated in freely moving female rats. BM injection into the lateral hypothalamic area (LHA) elicited decreases in the blood calcium (0.05-0.1 mM) and potassium (0.2-0.5 mM) levels without affecting sodium and chloride ions. BM injection into the paraventricular hypothalamic nucleus (PVN) also induced hypocalcemia but not hypokalemia. The hypocalcemic response to LHA injection of BM was eliminated by a gastric vagotomy, while that to PVN injection was suppressed by a vagotomy of the thyroid/parathyroid branches. Both hypocalcemic and hypokalemic effects of BM injection into the LHA were antagonized by either a peripherally acting β-adrenoceptive antagonist (nadolol, 2 mg/kg, iv) or a histamine H_2 blocker (ranitidine, 5 mg/kg, iv). The results suggest that hypothalamic neurons regulates blood calcium homeostasis via at least two different vagal pathways, the vagus innervating the stomach or the thyroid/parathyroid glands. Histamine H_2 and β–adrenergic receptors are involved in the LHA-mediated hypocalcemia and hypokalemia. Supported by Grants-in Aid (No. 07557008) for Scientific Research from the Ministry of Education, Science and Culture, Japan).

266.14

EFFECTS OF ESTRADIOL AND TAMOXIFEN ON CELL ATP LEVELS IN PREOPTIC AND HYPOTHALAMIC SLICES. J.M.Gray* and K.M. Raley-Susman. Depts. of Psychology and Biology and Program in Biopsychology, Vassar College, Poughkeepsie, NY 12601.

An in situ slice system has been used extensively to study the effects of altered environmental conditions (e.g., ischemia, NMDA) on neural metabolism in hippocampus. Although several investigators have used a hypothalamic slice preparation to examine effects of steroids on electrophysiological properties of neurons, little work has been done using a hypothalamic slice preparation to explore metabolic function in this area. Prior to manupulating conditions, we therefore validated this preparation, comparing results of morphological integrity, protein synthesis and tissue ATP levels to those previously reported for hippocampus. Based on these criteria, we found that 400u slices from the region between the anterior preoptic area and the medial basal hypothalamus from ovariectomized, young adult rats were viable for up to 8 hr, with tissue ATP levels falling between 12-24 hr.

Incubation of slices with 10^{-8} to 10^{-6}M estradiol for 4 hr had no effect on ATP levels. Incubation of the slices for 4 hr with the triphenylethylene antiestrogen, tamoxifen (TAM), however, yielded site-specific and dose-specific changes in ATP levels. Addition of 10^{-8}M TAM significantly increased ATP levels in preoptic area and anterior hypothalamic (POA-AH) slices, while having no effect on medial hypothalamic (mHT) slices. Addition of 10^{-6}M TAM had no effect in POA-AH, but significantly decreased ATP levels in mHT. Incubation with high doses (10^{-6}M) of fluphenazine, a phenothiazine with similar properties to TAM on a variety of calcium sensitive systems (e.g., binding to calmodulin, inhibition of protein kinase C), had effects similar to those found with the lower doses of TAM, that is a selective increase in ATP levels in the POA-AH region.

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266.15

LACTATE INDUCED PANIC RESPONSE IN RATS WITH CHRONIC GABA DYSFUNCTION IN THE DORSOMEDIAL HYPOTHALAMUS (DMH) IS ASSOCIATED WITH INCREASED NOREPINEPHRINE RELEASE IN THE DMH. S.R.Keim*, J.S.Katner and A.Shekhar. Dept. of Psychiatry, Indiana Univ. Med. Center, Indianapolis, Indiana 46202.

Intravenous infusion of sodium lactate elicits a panic-like response in rats with chronic dysfunction of GABA in the dorsomedial hypothalamus (DMH). Increased activity of norepinephrine (NE) pathways is also implicated in panic responses and GABA appears to regulate NE release in the DMH. Therefore, the present study was conducted to test if NE release in the DMH was altered during a lactate infusion using *in vivo* microdialysis. Rats were fitted with femoral arterial and venous catheters and baseline heart rate (HR), blood pressure (BP) responses to lactate infusions (0.5M, 10 ml/Kg i.v. in 20 min) were obtained. Specially constructed loop style microdialysis probes that also had a chronic injection cannulae which was connected to an Alzet infusion pump filled with the GABA synthesis inhibitor l-allylglycine (l-AG; 3.5 nmoles/0.5ul/hr) were implanted into the DMH. After 3 days of recovery, microdialysis probes were infused with artificial cerebrospinal fluid (1.5 ul/min) and baseline dialysates were collected in 20 min fractions. Rats were once again tested with either i.v. lactate or saline infusions while continuing to collect dialysis samples. Infusions of lactate and not saline resulted in significant increases in extracellular NE levels in the DMH. These results suggest that the lactate induced panic responses are associated with an increased release of NE in the DMH. (Supported by MH 52691, AAMHRE and IUMC Biomedical grants)

266.16

ORGANUM VASCULOSUM LAMINA TERMINALIS (OVLT) IS A CRITICAL RELAY SITE FOR THE LACTATE INDUCED PANIC RESPONSE IN RATS WITH CHRONIC GABA DYSFUNCTION IN THE DORSOMEDIAL HYPOTHALAMUS. A.Shekhar* and S.R.Keim. Dept. of Psychiatry, Indiana Univ. Med. Center, Indianapolis, Indiana 46202.

Intravenous infusion of sodium lactate elicits a panic-like response in rats with chronic dysfunction of GABA in the dorsomedial hypothalamus (DMH). The present study was conducted to test if the circumventricular organ OVLT is a potential site that could detect increases in plasma lactate levels and activate the DMH. Rats were fitted with femoral arterial and venous catheters and baseline heart rate (HR), blood pressure (BP) responses to lactate infusions (10 ml/Kg i.v.) were obtained. Unilateral chronic injection cannulae connected to a detachable Alzet infusion pump filled with the GABA synthesis inhibitor l-allylglycine (l-AG; 3.5 nmoles/0.5ul/hr) were implanted into the DMH. Another chronic injection cannula was implanted into the region of the OVLT. After establishing the development of increased baseline anxiety and physiological responses to lactate, rats with DMH GABA dysfunction were once again tested with lactate after injection of either artificial cerebrospinal fluid (a-CSF) or tetrodotoxin (TTX; 100 nl of 10 uM) into the OVLT. Injecting TTX and not a-CSF into the OVLT significantly blocked the lactate induced panic response. Further, direct injections of lactate (100 and 500 nl) into the OVLT elicited a dose-dependent, robust panic-like responses in these rats, suggesting that the OVLT may be the primary afferent site for the lactate induced panic responses in this model. (Supported by MH 52691, AAMHRE and IU Biomedical grants)

266.17

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

Activation of metabotropic glutamate receptors attenuates intrinsic K^+ currents in magnocellular neurons of the supraoptic nucleus (SON) and reduces glutamate and GABA release from presynaptic terminals (Schrader and Tasker, Soc. Neurosci. Abstr. 20: 347). We used the whole-cell patch clamp technique in coronal slices of the rat hypothalamus to determine the metabotropic glutamate receptor subtypes involved in these effects.

L(+)-2-Amino-4-phosphonobutyric acid (L-AP4, 200 μM), specific for mGluR subtypes 4, 6, and 7, decreased the frequency of spontaneous excitatory and inhibitory postsynaptic currents (EPSCs and IPSCs) to 30-50% of that seen in normal medium, and reduced the excitatory postsynaptic current (EPSC) evoked by electrical stimulation to 50-60% of control. L-AP4 also reduced the frequency of miniature EPSCs and IPSCs, suggesting a presynaptic locus for L-AP4 action. Application of (RS)-3,5-Dihydroxyphenylglycine (DHPG, 50-100 μM), an agonist specific for mGluR1 and mGluR5, reduced the transient K^+ current to 40-60% of control values in 5 of 8 neurons. The outward rectifying K^+ currents were also attenuated to 45-70% of control values in 6 of 8 of cells studied. Leak conductances were reduced to 70-90% of control values by DHPG. These studies indicate that glutamate modulates intrinsic K^+ currents in magnocellular neurons by acting at group 1 (mGluR1 and 5) and decreases both glutamate and GABA release from presynaptic terminals with actions at group 3 receptors (mGluR4, 6, and 7). This work was supported by grants from the Louisiana American Heart Association and NINDS.

266.18

ELECTROPHYSIOLOGICAL EVIDENCE FOR LOCAL EXCITATORY INPUTS TO THE PARAVENTRICULAR NUCLEUS IN RAT HYPOTHALAMIC SLICES. **C. Boudaba, K. Szabó and J.G. Tasker*. Dept. of Cell & Molecular Biology, Tulane University, New Orleans, LA 70118.**

Rhythmic bursting patterns in hypothalamic neurosecretory neurons often mediate the pulsatile release of hormones from the pituitary gland. In neurons of the paraventricular nucleus (PVN), patterned electrical activity may depend in part on local excitatory and inhibitory synaptic inputs to neurosecretory cells. A previous study showed evidence for local inhibitory inputs to PVN parvocellular and magnocellular neurons (Boudaba et al., Soc. Neurosci. Abstr. 21:1665, 1995). The aim of the present study was to determine whether PVN neurons receive local excitatory synaptic inputs. Excitatory postsynaptic potentials (EPSPs) were recorded in PVN neurons in coronal, horizontal and parasagittal slices (400-500 μm) using conventional intracellular and patch-clamp recordings. Local neurons were selectively stimulated with glutamate microdrop application (20 mM). During recordings, cells were classified as magnocellular or parvocellular according to electrophysiological criteria (Tasker & Dudek, J.Physiol 434:271, 1991), and were injected with biocytin (0.3-1%) for subsequent immunohistochemical identification with antibodies to neurophysin, oxytocin or vasopressin. In preliminary experiments, glutamate microdrops around the PVN in the three slice planes evoked EPSPs in 12 of 116 (10%) of the PVN neurons tested. Glutamate-evoked EPSPs were recorded in magnocellular as well as in parvocellular neurons. Zones responding to glutamate microstimulation were located in anterior, lateral and dorsomedial hypothalamic areas. Further studies are necessary to determine whether local excitatory inputs to PVN neurons are involved in the patterned electrical activity of these neurons and the pulsatile release of pituitary hormones. This study was supported by NIH NS31187, NSF IBN-9315308 , and a grant from the LA Board of Regents.

266.19

SEVERE REDUCTION OF DEFENSIVE BEHAVIOR BY DISCRETE HYPOTHALAMIC CHEMICAL LESIONS. N.S. Canteras*, S. Chiavegatto, L.E. Ribeiro do Valle and L.W. Swanson†. Dept. of Physiol. & Biophys., Inst. Biomed. Sci., Univ. of São Paulo, SP, 05508-900, Brazil. (†) Dept. of Biological Sciences, Univ. of Southern California, LA, CA, 90089-2520.

In the present study we first determined which hypothalamic regions display altered *Fos* levels when a rat is exposed to a natural predator. Five adult male Wistar rats were placed individually for 10 min. in a closed wire-meshed compartment inside a larger arena containing an adult male cat. One hour later rats were deeply anesthetized and perfused transcardially, and the brains prepared for immunohistochemistry in a standard way. All animals displayed species-specific defensive behavior, and a similar pattern of increased hypothalamic *Fos*-immunoreactivity. The most dramatic increase was localized to the dorsal premammillary nucleus (PMd), and major increases were also observed in the anterior perifornical region, anterior hypothalamic nucleus, and dorsomedial part of the ventromedial nucleus.

To examine a possible role of the PMd in defensive behavior expression, bilateral ibotenic acid lesions were then made, as well as control chemical lesions placed in regions adjacent to the PMd. For this, 10 adult male Wistar rats were exposed initially for 15 min. to an adult male cat (same protocol as for the *Fos* experiments), and their responses were videotaped for behavioral analysis. Several days later ibotenic acid lesions were made, and 10 days after lesion placement the rats were exposed again to a cat for 15 min. In contrast to the control group, bilateral PMd cytotoxic lesions (n=5) almost eliminated the incidence of escape responses (jump directed to the top of the test cage or vigorous running away from the predator) and freezing.

Notably, the *Fos*-responsive cell groups in the medial hypothalamus are interconnected in a distinct neural system, and the present results suggest that the PMd may act as a necessary "final common pathway" for hypothalamic influences on the expression of innate defensive behavior. Supp.FAPESP 93/0019-8.

266.20

A SEX DIFFERENCE IN NEURODEGENERATION OF THE HUMAN HYPOTHALAMUS C. Schultz, H. Braak and E. Braak (SPON: European Neuroscience Association), Zentrum der Morphologie, J. W. Goethe-Universität, D-60590 Frankfurt, Germany.

The human mediobasal hypothalamus (MBH) of 64 individuals (33 males and 31 females [mean age: 77 y. and 78 y.]) was examined for Alzheimer type cytoskeletal pathology. Coronal sections (100 μm) were stained with the Gallyas silver technique for neurofibrillary pathology, the Campbell Switzer technique for amyloid deposits, and antibodies for abnormally phosphorylated tau protein (AT8, Alz-50) and paired helical filaments (PHF-1). A conspicuous pathology was identified in the MBH. It was characterized by axon-like varicose processes, contacting small vessels of the posterior median eminence and the adjacent infundibular (or arcuate) nucleus. This pathology was visualized by the Gallyas technique and by the antibodies AT8, Alz-50 and PHF-1. Alzheimer-type PHF were also present at the ultrastructural level. Remarkably, the cytoskeletal pathology of the MBH exhibited a striking sex-specific prevalence: while it was noted in 24 males (79%), it was identified in only 2 females (6%). Although this sex-specific hypothalamic pathology is demonstrated by techniques which label Alzheimer type cytoskeletal pathology, it also affects non-demented elderly males, devoid of Alzheimer-related neocortical pathology. The MBH pathology thus appears to develop independently of Alzheimer's disease. The cytoskeletal lesions of the male MBH may lead to an impairment of the parvocellular neurosecretory system. Such a decline has been previously suggested for the hypothalamic stimulation of gonadotropin secretion in elderly men.

This study was supported by the Deutsche Forschungsgemeinschaft.

COMPARATIVE NEUROANATOMY: HIGHER VERTEBRATES

267.1

PROLACTIN (PRL) AND GROWTH HORMONE (GH) NEURONS FOUND WITHIN THE AVIAN FOREBRAIN AND DIENCEPHALON IN DIFFERENT REPRODUCTIVE STATES. R. Ramesh[1], W.J. Kuenzel*[1], J.D. Buntin[2], and J.A. Proudman[3], [1]Dept. of Poultry Sci., Univ. of Maryland, College Park, MD 20742, [2]Dept. Biol. Sci., Univ. of Wisconsin, PO Box 413, Milwaukee, WI 53201, [3]USDA, ARS, GGPL, Beltsville, MD 20705.

Avian species, such as turkeys and ring doves, show clear broody behavior which ultimately results in the termination of egg laying and rapid onset of incubation behavior. In our investigations into the neuroendocrine mechanism responsible for the shift in behavior, we observed that specific antibodies for identifying pituicytes also revealed immunostained neurons. The study documents specific forebrain and diencephalic regions where nuclei were identified using antibodies to synthetic avian PRL and turkey GH. Growth hormone immunoreactive (ir) neurons were found in the hippocampus (Hp, turkey only), periventricular hypo. n. (PHN), paraventricular n. (PVN), medial ventromedial hypo. n. (VMN), inferior hypo. n. (IH) and infundibular hypo. n. (IN). Dense GH ir fibers were found in the external zone of the median eminence. Regarding PRL neurons, two reproductive states were analyzed: laying versus incubation states. Plasma prolactin levels for ring doves and turkey hens in the laying and incubation periods were, respectively: 0.27 ± 0.36 and 3.73 ± 0.41; 38.4 ± 8.7 and 164.3 ± 12.5. In the turkey hen, PRL neurons were found in the Hp, amygdala, bed n. of the pallial commissure (nCPa), PHN, bed n. of the stria terminalis (nST), premamillary and lateral mamillary n., dorsal IN and lateral hypo. area. In the ring dove, PRL neurons were identified in the amygdala, septal area, nST and nCPa. A difference was found within the nCPa of both turkey and dove brains between the two reproductive states. In both species PRL neurons were weakly ir and less numerous in laying birds (n=4). Brains sampled from incubating birds (n=4) showed more numerous, darkly ir PRL neurons. Since the nCPa is the major site of gonadotropin-releasing hormones (GnRH) in avian brains, perhaps PRL-like neurons may directly interact (negatively) with GnRH neurons when birds become broody.

267.2

COMPARATIVE LOCALIZATION OF NITRIC OXIDE SYNTHASE AND TYROSINE HYROXYLASE IN THE BRAIN OF THE CHICKEN. G. Brüning*, Dept. of Anatomy, Free University, D-14195 Berlin, Germany

Nitric oxide synthase is known to be expressed in a subpopulation of neurons in the brain of avian species. The distribution pattern reveals remarkable similarities to that of dopaminergic and noradrenergic neurons. Catecholamine- and nitric oxide-synthesizing neurons were detected by double-labeling immunofluorescence microscopy. In addition, nitric oxide synthase was detected by NADPH diaphorase histochemistry.

Tyrosine hydoxylase- and nitric oxide synthase-positive neurons were coextensive in medial thalamic nuclei, mamillary nuclei, area ventralis of Tsai (AVT), substantia grisea centralis (GCt), nucleus tegmenti pedunculopontinus (TPc), locus coeruleus and subcoeruleus. Only a minority of neurons in the AVT, GCt and TPc stained for both enzymes. Nitric oxide synthase-positive neurons did not appear to be a preferred target of catecholaminergic fibers in any part of the brain.

The present results indicate that nitroxergic and catecholaminergic neurons are coextensive but represent mostly separate populations of cells. Nitric oxide may function as an intercellular messenger between dopaminergic and noradrenergic neurons, and other as yet chemically ill-defined neuron populations.

267.3

DISTRIBUTION OF NEUROTRANSMITTERS, RELATED ENZYMES, AND NEUROPEPTIDES WITHIN THE PIGEON CAUDOLATERAL NEOSTRIATUM L.V. Riters *, J. T. Erichsen, J. R. Krebs, V. P. Bingman. Dept. of Psychology, Bowling Green State Univ., Bowling Green, OH 43403; Dept. of Optometry & Vision Sci., Univ. Wales, Cardiff, P.O. Box 905, Cardiff CF 1 3XF, Wales UK; Edward Grey Institute of Field Ornithology, Dept. of Zoology, Oxford OX1 3PS, England.

The caudolateral neostriatum (NCL) has recently been implicated in homing pigeon navigation. Additionally, NCL has been considered a possible homologue to mammalian prefrontal cortex (PFC). Although, NCL has been found to receive dense dopaminergic innervation, little is known about the distribution of other neuroactive substances within this region. The present study was performed to elaborate upon the current understanding of NCL neurochemistry, and to further investigate possible NCL/PFC similarity. The distribution of three neuroactive substances (tyrosine hydroxylase (TH), choline acetyltransferase (ChAT), and serotonin (5HT)) and five neuropeptides (cholecystokinin (CCK), leucine enkephalin (LENK), substance P (SP), vasoactive intestinal polypeptide (VIP), and somatostatin (SS)) was investigated using immunocytochemistry. All substances investigated were found within NCL. TH immunoreactive fibers were found to coil around unlabeled parikarya within NCL. Within TH rich regions of NCL, sparsely distributed smooth ChAT fibers were observed. Sparse fiber and terminal-like 5HT labeling was also concentrated in regions lining the lateral ventricle. CCK terminal-like labeling and sparsely distributed LENK fibers also appeared densest along the ventricle. SS labeled cells were observed throughout NCL. The distribution of labeled VIP fibers was found concentrated in more caudal regions of NCL, while the densest SP labeling was confined to the dorsal half of NCL suggesting regional subdivisions within NCL. Results suggest that the distribution of TH, ChAT, and 5HT within NCL is similar to that observed in rodent PFC.

267.4

AGE-RELATED CHANGES IN CELL PROLIFERATION IN HOMING PIGEONS. V. Simon, R. Strasser,[1] V. Bingman,[1] and D.A. Holtzman*
Neuroscience Program, Oberlin College, Oberlin, OH 44074, & [1]Department of Psychology, Bowling Green State University, Bowling Green, OH 43403.

Relatively few studies have examined whether postnatal neurogenesis changes as a function of age. Two-week old, four-week old, and adult pigeons (n=3, for each group) were injected with the thymidine analog, 5-bromo-3'deoxyuridine (BrdU), and sacrificed 1-2 hours later. Brains were fixed with 4% paraformaldehyde, sectioned at 10 µm, and immunostained for BrdU. The ventricular layer was divided into twelve regions corresponding to adjacent brain structures, and the total number of labeled cells/square mm (cell density) was determined for each region. For two- and four-week old birds, a significant difference was found in the distribution of proliferating cells across ventricular regions ($p<0.05$). In these birds, regions adjacent to the parolfactory lobe (LPO), nucleus accumbens/bed nucleus of stria terminalis (NA/BNST), dorsal hyperstriatum (HD) and paleostriatum (PA) showed higher levels of cell proliferation compared to other ventricular regions. Cell density varied as a function of age in the ventricular regions adjacent to the LPO, NA/BNST. accessory hyperstriatum, neostriatum, ventral hyperstriatum, septum, hippocampus, and dorsolateral cortex, with two-week old birds exhibiting the highest labeled cell densities ($p<0.05$ for all structures). No age-related differences were observed in the ventricular regions adjacent to HD, PA, archistriatum, and pyriform cortex. Regions with highest cell proliferation in the homing pigeon correspond to high proliferation zones seen in other vertebrate taxa. Preliminary data for Hu protein expression, an early neuronal marker, has revealed many labeled cells along the ventricles of young birds. This suggests that many neurons are produced within the first four weeks of postnatal life. Further, double BrdU/Hu-labeled cells have been found within the brain parenchyma in short survival animals. We are currently quantifying these data. Supported by NCRR grant RR08700 to DAH & NIMH grant MH52315 to VB.

267.5

IMMUNOHISTOCHEMICAL CHARACTERIZATION OF THE STRIATAL INPUT TO NEURONS OF THE DORSAL PALLIDUM IN PIGEON, WITH PARTICULAR EMPHASIS ON PALLIDO-THALAMIC NEURONS. L. Medina* and A. Reiner. Dept. Anat. and Neurobiol., College of Medicine, The University of Tennessee, Memphis, TN 38163.

The dorsal pallidum possesses many similar features in birds and mammals. In both vertebrate groups, the principal neuronal type of the dorsal pallidum is a large, fusiform neuron that co-contains GABA, parvalbumin (PV), and the neuropeptide LANT6. Further, in both vertebrate groups the dorsal pallidum receives input from substance-containing (SP+) and enkephalin-containing (ENK+) neurons of the striatum and, in turn, projects to the thalamus and the substantia nigra. In mammals, the dorsal pallidum is subdivided into two segments: a lateral segment receiving mainly ENK+ striatal input, and a medial segment receiving mainly SP+ striatal input. The dorsal pallidum of birds is not subdivided into segments, although the ENK+ and SP+ striatal inputs seem segregated onto separate sets of pallidal neurons. In the present study, we have investigated the pallidal targets of the ENK+ and SP+ striatal input in pigeons, by way of double-label immunofluorescence experiments in the dorsal pallidum to label ENK+ or SP+ fibers of striatal origin and either: (1) PV+ pallidal neurons; or (2) pallido-thalamic projection neurons after injecting a retrograde tracer in the pigeon thalamus. Our results indicate that the perikaryon and proximal dendrites of 54% and 46% of PV+ pallidal neurons, respectively, are covered by either SP+ striatal fibers or ENK+ striatal fibers. About 45% of pallidothalamic neurons are covered by ENK+ striatal fibers, whereas 20% of them are covered by SP+ striatal fibers. The results suggest the existence of separate striato-pallidothalamic pathways in pigeons, adding a new similarity between avian and mammalian basal ganglia. Supported by NS-19620 (A.R.), NS-28721 (A.R.), and NIH Shared Instrumentation Grant PHS-RR08385 (to Dr. Andrea Elberger).

267.6

ANATOMICAL COMPARISONS BETWEEN NEURAL PATHWAYS IN THE BUDGERIGAR AND NEURAL PATHWAYS MODULATING ACOUSTIC STARTLE IN MAMMALS. K. J. Heaton* & K. K. Cookson. UMCP, College Park, MD, 20742.

The modulation of acoustic startle in mammals depends on a complex set of modulatory pathways that include the hippocampus, amygdala, hypothalamus, midbrain central gray, parabrachial region, and pontine reticular region. The present investigation describes pathways tracing studies that reveal several similar pathways in a species of parrot, the budgerigar (Melopsittacus undulatus). For example: (1) the medial achistriatum receives input from the auditory neostriatum and from the parabrachial region, (2) the archistriatum projects directly to the caudal pontine reticular region and projects to the intercollicular midbrain, which in turn projects to the caudal pontine reticular region, and (3) the hippocampus and the medial hypothalamus share afferent and efferent projections with the medial and ventral archistriatum. Furthermore, the modulation of acoustic startle in mammals is related to cholinergic activity in the hippocampus and the caudal pontine reticular region and to dopaminergic activity in the hypothalamus. Immunohistochemical staining for choline acetyltransferase (ChAT) and tyrosine hydroxylase (TH) in the budgerigar shows ChAT-labeled fibers in the hippocampus and pontine reticular region and TH-labeled fibers and somata in the medial hypothalamus. However, despite general similarities in connections and histochemistry, the detailed subregional organization of nuclei in these pathways differs in budgerigars and mammals. Developing an avian model of acoustic startle for comparison with mammals may provide insights into the structure-function relationships of these pathways. Supported by NIH grant MH40698 to S.E.B. and Whitehall grant J91-17 to W.S.H.

267.7

OBSERVATIONS ON THE THALAMUS OF THE BOTTLENOSE DOLPHIN (*TURSIOPS TRUNCATUS*). P. Manger, M. D. Szymanski, M. Sum , M. Sutter*, & L. Krubitzer. Center for Neuroscience and Department of Psychology, University of California, Davis, Davis CA 95616, U.S.A.

Brains of three dolphins were obtained post-mortem and fixed by immersion in 10% formaldehyde for periods of up to 2 years. The thalamus of one of these animals was dissected and sectioned coronally into 50 µm sections, and stained for Nissl and myelin. Detailed analysis indicates that the overall morphology and nuclear subdivisions of the thalamus are similar to other mammals. However, the relative size and detailed architecture of some nuclei differ dramatically. The subdivisions of the ventrobasal nuclear group, including VPm, VPl, and VPi can be clearly defined, and compartmentalization of the VPm nucleus, similar to that reported previously for monkeys, is evident. This may correlate with the dolphin's frequent use of the rostrum in tactile exploratory behaviors, and also with the use of the intricate nasal sac system of the blowhole for sound production. Although the packing density of neurons in VPl is somewhat reduced, this nucleus is large, and is likely to receive inputs necessary for the sensory control of the skin muscles, which may be used to optimize hydrodynamic flow over the surface of the body. Unlike many other mammals, the lateral geniculate nucleus is not laminated. The lack of distinct layers in the LGN is not surprising given the limited penetration of light through the water, and the poor visual acuity of dolphins. Nucleus ellipticus is not found in other mammals, but in the dolphin is extraordinary in both size and appearance. We propose that this nucleus may be related to echolocation abilities described in dolphins. The medial geniculate complex is greatly expanded, particularly the dorsal nucleus. Similar expansions have been observed in other echolocating mammals such as microchiropteran bats. Finally, a large interthalamic fiber tract connecting the two sides of the thalamus extends from the level of the pulvinar to the level of the ventrobasal nuclei. This tract may play a crucial role in subcortical transfer of interhemispheric information, and may compensate for the significantly reduced corpus callosum in these mammals.

267.8

CO-LOCALIZATION OF GALANIN AND TYROSINE HYDROXYLASE IN PURKINJE NEURONS OF THE RAT BUT NOT OF THE MOUSE. A.E. Kresse*, D.M. Jacobowitz and G. Skofitsch. Histopharmacology Unit, Dept. Zool., KFU, A-8010 Graz, AUSTRIA, and Lab. of Clin. Sci., NIMH, Bethesda, MD 20892, U.S.A.

Galanin (GAL) is a widely distributed 29 amino acid long neuropeptide with multiple biological effects in a variety of different subsystems of the central nervous system. We previously reported detailed mappings of the localization of GAL-like immunoreactivity (GAL-LI) and mRNA (Skofitsch and Jacobowitz, 1985, 1990). The coexistence of GAL and tyrosine hydroxylase (TH), the indicator enzyme for catecholaminergic neurons, has been shown before for several regions of the brain (Melander et al., 1986; Sutin and Jacobowitz, 1991). In the present study, however, we demonstrate to our knowledge for the first time the localization of GAL mRNA in Purkinje neurons of distinct regions of the cerebellar cortex of the rat. *In situ* hybridization (ISH) was done using two different radio-labeled synthetic oligonucleotide probes complementary to the nucleotides 152-199 of rat, and to the nucleotides 115-153 of porcine GAL mRNA, respectively. We were able to localize GAL mRNA in male Sprague Dawley rats, however not in C57Bl/6J mice, in Purkinje cells of the flocculus, paraflocculus, the paramedian lobe, and the crus 1 of the ansiform lobe, as well as throughout the lobuli of the vermis and to a lesser extent in the crus2 of the ansiform lobe. Based on the nearly complete match of this distribution pattern with reports of the localization of TH immunoreactivity in the rat and mouse cerebellum, we were able to demonstrate the co-localization of GAL and TH in rat Purkinje cells using ISH for localizing TH and GAL mRNA on adjacent sections and the immunocytochemistry for TH in sections that were subsequently hybridized for the detection of GAL mRNA. Although Purkinje neurons are considered to be GABAergic, the presence of TH in a subpopulation of these cells has so far been shown in the mouse, human and rat. However, the specific biological function of the TH and GAL expression in Purkinje neurons still remains to be elucidated.

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267.9

CONSTRUCTION OF A 42 MICRON 3D RAT BRAIN ATLAS FROM NISSL STAINED SECTIONAL MATERIAL. J. Nissanov[1]*, D. Kozinska[2], C. Gustafson[2] and O. Tretiak[1,2]. [1]Biomed. Eng. & Sci. Inst., [2]Elec. Comp. Eng., Drexel Univ., Philadelphia, PA 19104

The utility of brain atlases is undergoing substantial change from passive guides to active tools for automatic segmentation of neuroanatomical regions and as standard reference sets for multianimal, multimodality fusion of experimental data. In this new role, the need for 3D atlases has become acute. We report on a 3D atlas of intermediate, 42 micron, isotropic resolution. The brain is of a male Sprague-Dawley rat weighing 304g. No fixative was utilized and the fresh brain was rapidly frozen in dry ice cooled isopentane. The tissue was cut on a Leica Polycut cryostat equiped with a blockface imaging system and the sections stained with cresyl violet. Each section was aligned to its corresponding blockface image using rigid body 2D distance-based alignment. This iterative algorithm searches for the transformation which minimizes the distance between the section contour and the outline of the tissue on the blockface. Navigation through this 66MB 3D dataset is possible using Macintosh-based software from the Computer Vision Center for Vertebrate Brain Mapping, a NIH Biomedical Technology Resource located at Drexel University. The software permits rapid resampling through the data along any arbitrary plane of view. The atlas can be used to store and exchange regions of interest with BRAIN, the autoradiographic analysis program from this NIH resource. (Supported by NIH P41RR01638).

267.10

MAPPING THE MIDSAGITTAL CORPUS CALLOSUM IN THE RAT: TOPOGRAPHICAL CORRESPONDENCE OF CALLOSAL REGIONS WITH CORTICAL SUBDIVISIONS.

M. Noonan*, M.A. Sanfilippo, D.J. Chmiel, Jr., M.A. Smith.
Canisius College, Buffalo, New York 14208.

The location in the midsagittal plane of fibers within the corpus callosum of the rat are mapped in correspondence with cortical region. Results are reported from both HRP and silver-staining following localized cortical treatments.

A detailed map is presented delineating the callosal regions corresponding to each Zilles subdivision. Fibers corresponding to most cortical areas are segregated and distinctly bundled in their passage through the callosum at the midline. However, the fibers of some subdivisions overlap and intermingle extensively. In general, there is an anterior-posterior correspondence relating cortical area and callosal locale. Additionally, there is a tendency for fibers associated with medially-located cortical regions to cross in dorsal layers of the callosum and more laterally-located regions to cross in ventral layers. The genu of the callosum consists of fibers corresponding to medial prefrontal cortex (e.g., DPC, IL, MO, VO, Cg3), the trunk includes fibers corresponding to frontal and parietal cortex (e.g., FR1&2 and PAR1&2), and the splenium consists largely of occipital and retrosplenial fibers (e.g., OC2L, RSA, RSG).

The results are expected to guide future callosotomy studies in which only selected regions of the cortex are disconnected.

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267.11

A THREE DIMENSIONAL MULTI-MODALITY BRAIN MAP OF THE NEMESTRINA MONKEY A.W. Toga* and A. F. Cannestra Lab. of Neuro Imaging, Division of Brain Mapping, Department of Neurology, UCLA School of Medicine, Los Angeles, CA 90095

A three dimensional multi-modality computerized map of the nemestrina monkey brain was created with serial sectioning and digital imaging techniques. An adult female Macaca nemestrina weighing 7.2 kg was used in constructing this atlas. CT, PET, and MRI were performed on the monkey before the specimen's head was frozen and cryoplaned. Closely spaced (50 mm) of the specimen blockface images were then digitally acquired and modified to produce whole head and brain only 3D image sets. The resulting data sets were organized into a digital volume and repositioned into a stereotaxic coordinate system defined by Horsley and Clark (1908). From the rotated data sets, orthogonal images were obtained by digitally resampling the volume in order to produce a full set of coronal, sagittal, and horizontal images. Stereotaxic reference grids were applied to each image indicating the A/P, M/L, or H (depending on orientation of cut) position within the digital volume. The amygdala, bony skull, cornea, cortex, eyes, fornix, hippocampus, internal capsule, lateral geniculate, optic nerve, optic radiations, orbit, pons, putamen, septum, substantia nigra, superior colliculus, and ventricles were outlined from the cryosection data set and 3D surface models reconstructed. Structural labels indicating nuclei, tracts, and other neuroanatomical features were incorporated into coronally sliced cryosection images spaced at 500 mm. The CT and MRI data sets were reconstructed into a digital volume and co-registered to the cryosection volume in the Horsley-Clark reference space. CT and MRI co-registration was performed by landmark matching and fiducials. The PET data volume was co-registered to the cryosectioned volume by using a ratio method. All images constructed from this 3D map are available via the Internet using an anonymous file transfer protocol (FTP) and the World Wide Web (*www.loni.ucla.edu*). The foremost advantage of this digital map is an integrated multimodality three dimensional representation which is not possible with traditional atlases. Funding was provided by the Human Brain Project MH/DA52176.

267.12

COMPUTATIONAL NEUROANATOMY OF THE HUMAN INFERIOR PARS OPERCULARIS. Z. Caramanos*, G. Posner, F. Tomaiuolo, D. MacDonald, A.C. Evans and M. Petrides. Montreal Neurological Inst., McGill Univ., Montreal, Canada, H3A 2B4.

Volumetric analysis of the inferior portion of the pars opercularis (**iPO**) was carried out on high-resolution MRIs obtained from 54 healthy volunteers (20 females, 34 males; mean age (sd): 25.5(8.2) yrs). MRIs were acquired on a 1.5 T magnet as datasets of 160 contiguous T_1-weighted, 1-mm thick, sagittal slices and transformed into a standardised stereotaxic space (Talairach & Tournoux, 1988). A single investigator, blind to side, used an automatic segmentation technique to identify grey and white matter in the left and right **iPO**. Grey matter included tissue caudal to the ascending ramus; rostral to the precentral sulcus; dorsal to the sylvian fissure (SF), and ventral to the first protuberance encountered dorsal to the SF. White matter included tissue completely encompassed by **iPO** grey matter. Thus defined, we could consistently identify a region containing part of Broca's Area and Brodmann's area 44. The presence of the diagonal sulcus (**dS**) and its effects on **iPO** volume were also examined.

Repeated measures and *post hoc* analyses of variance examining the effects of SEX, SIDE and TISSUE TYPE on **iPO** volume found: (*i*) a large main effect ($p < .001$) of TISSUE TYPE [Grey Matter > White Matter: mean volume (sd) was 6.2(1.4) vs. 2.2(0.7) cc]; (*ii*) a small interaction ($p = .003$) of SEX by TISSUE TYPE (Female Grey > Male Grey, $p = .04$: 6.7(1.6) vs. 5.9(1.3) cc]; and (*iii*) a small interaction ($p = .03$) of SIDE by TISSUE TYPE [Left White > Right White, $p = .04$: 1.2(0.5) vs. 1.0(0.4) cc]. No main effect of SEX or SIDE, or 3-way interaction was found ($p > .05$). The **dS** was found bilaterally in 14 brains, only on the left in 11, only on the right in 12, and was not present in 17. No interaction of SEX with location or number of **dS** was found (χ^2: $p > .05$). No interaction of **dS** NUMBER with SEX, with TISSUE, or with SIDE was found on **iPO** volume ($p > .05$), but there was a main effect ($p < .001$) of **dS** NUMBER on total **iPO** volume [(1 **dS** = 2 **dS**) > 0 **dS**, $p < .009$: 8.7(1.7) and 9.5(1.7) vs. 7.0(1.8) cc].

In addition to characterising **iPO** grey and white matter in the normal adult human brain, probabilistic maps of the **iPO** were created by averaging individual **iPO** volumes across these 54 brains. Such maps can, for example, be used to localise functional imaging activation peaks in this area with greater certainty than was previously possible.

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267.13

TALAIRACH DAEMON: AUTOMATED MAPPING OF SURFACE ANATOMY ONTO STANDARDIZED COORDINATES. C. S. Freitas, J. L. Summerlin, J. L. Lancaster, P. T. Fox *. Research Imaging Center, University of Texas Health Science Center at San Antonio, San Antonio, TX 78284-6420.

Standardized, 3D coordinates (Talairach & Tournoux, 1988) are used with steadily increasing frequency for analyzing and reporting human neuroimaging research. While 3D coordinates are precise, quantitative descriptions of locations (and location variances), more traditional (but less quantitative) anatomical descriptions, such as surface anatomy (sulci/gyri) and architectonic areas, are needed to allow comparisons between coordinate-based data and those not using standardized coordinates. The T&T (1988) atlas does include Brodmann area (BA) labels, but lacks explicit boundaries between BAs and has many inconsistencies and errors. Consequently, mapping of BAs to standardized coordinates is labor intensive and notoriously inconsistent. For this reason, we have created an electronic tool that uniquely maps BAs and surface-anatomical terms to standardized coordinates.

The T&T (1988) atlas was digitized and manually traced (*Alice*, HPIG) into a quasi-hierarchy of anatomical regions. Hemispheres, lobes, lobules, gyri and nuclei were outlined and labeled. Gray matter, white matter and CSF were also segmented. For cerebral cortex, all BAs were traced as 3D volumes. BA segmentation was based upon the T&T (1988) labels, but with numerous enhancements. All modifications were rule-based and justified by primary publications of Brodmann and others.

The anatomical regions thus defined were entered into a database served by a UNIX Daemon: Talairach Daemon (TD). TD is a multi-threaded, memory-resident, application running on a Sun Sparcstation 20 optimized for high-speed random access by coordinates. TD currently retrieves BA, gyrus, lobe and hemisphere. TD can be accessed from remote applications using TCP/IP sockets. Initial client programs include a Java applet for rapid Internet access. Future access is planned for the BrainMap™ database and for software packages that analyze and display human brain images (e.g., SN and SPM). TD will improve the accuracy and consistency with which coordinate-based studies make reference to traditional anatomical nomenclature. TD's electronic format facilitates emendation. (Funded by P20DA52176-01)

267.14

BRAIN VOLUME AND ITS COMPONENTS IN LIVING APES AND HUMANS. K.Semendeferi*[1], J.Rilling[2], T.R.Insel[3] and H.Damasio[1]. Department of Neurology[1], University of Iowa, IA 52245, Department of Anthropology[2], Emory University, Yerkes Regional Primate Research Center[3].

Here we report preliminary results of an ongoing collaborative effort to use novel neuroimaging techniques for the comparative analysis of the living human and ape brains (bonobo, chimpanzee, orangutan, gibbon, as well as the macaque). Thin, contiguous MR images of the brains were obtained. These were reconstructed in 3D using Brainvox. Volume of the whole brains and some of their major components were estimated.

The two hemispheres were separated and major sulci were identified across species. The volume of the hemispheres, of the cortex and immediately underlying white matter (the white matter core of the gyri), of the frontal lobe and of the temporal lobe were calculated.

The relative size of the cortex and immediately underlying white matter of the hemisphere in relation to the whole brain is 80% in the human, 77.6% in the bonobo, 75.3% in the chimpanzee, 78% in the orangutan, 77.6% in the gibbon, and 82.2% in the macaque. The relative size of the temporal lobe is 18.8% in the human, 18.9% in the bonobo and 15.4% in the chimpanzee. The relative size of the frontal lobe is similar in the human, bonobo and chimpanzee (38.9 %, 38.3 %, and 35.5% respectively).

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268.1

Neuroanatomic substrate of the distinction between real and imagined voices. H. Szechtman*,[1] K. Bowers[1], E. Woody, R. Chirakal, G. Firnau, M. Thompson, E.S. Garnett and C. Nahmias. Depts of Biomedical Sciences and Nuclear Medicine, McMaster Univ, Hamilton, Ontario, L8N 3Z5, and [1]Dept of Psychology, Univ of Waterloo, Waterloo, Ontario, CANADA

Hallucinations, which are imagined voices that are perceived as coming from the external world, raise the question of how the brain "discriminate(s) between imagined events and events in the real world" (Bentall, *Psychol Bulletin,* 107:82-95, 1990). Here we examined brain activity (regional cerebral blood flow, rCBF, measured with $H_2{}^{15}O$ and PET) during 4 separate conditions: at rest and while the subjects were hearing, imagining, and hallucinating voices. The entire procedure was carried out under hypnosis using highly hypnotizable subjects. Analysis using Statistical Parametric Mapping (Friston et al, *JCBFM,* 11:690, 1990) revealed a circumscribed region in the anterior cingulate that was more active during hearing and hallucinating voices than during imagining voices. Thus, this site may tag imaginal events as originating in the external world versus being self-generated. If so, hallucinations may arise from a malfunction of the cingulate.

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268.2

A POSITRON EMISSION TOMOGRAPHY STUDY OF TRANSIENT SLEEP REDUCTION, LIMBIC ACTIVITY AND ATTENTIONAL CONTROL. R. Mahurin, M. Liotti*, H. Mayberg, F. Zamarripa, S. McGinnis, and P. Fox. Research Imaging Center, University of Texas Health Science Center, 7703 Floyd Curl Dr., San Antonio, TX 78284.

We examined the relationship between resting-state ^{15}O-positron emission tomography (PET) regional cerebral blood flow (rCBF), transient sleep reduction, emotional state, and attentional performance in normal subjects. Degree of sleep reduction was calculated by subtracting hours slept on the night preceding the PET scan from self-reported typical hours of sleep. Regression analysis revealed significant positive correlations between reduced sleep and rCBF in paralimbic cortical and limbic subcortical regions, including striatum, anterior cingulate, and mesial-frontal/temporal areas. In contrast, significant negative correlations were found between sleep reduction and rCBF in widespread neocortical regions, including dorsolateral frontal, parietal, occipital, and superior temporal. Sleep reduction was associated with dysphoria, anxiety, and irritability as well as increased false alarms on a continuous response attentional test. In summary, transient sleep reduction appears associated with relative increases in resting-state subcortical-limbic brain activity, decreases in resting-state neocortical activity, release of dysphoric emotions, and impaired response control. The data suggest that sleep reduction disrupts and reverts the normal balance between tonic neocortical inhibitory mechanisms and subcortical limbic influences.

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268.3

PET EVALUATION OF COGNITIVELY RELATED NEUROPHYSIOLOGICAL SEQUELAE ACCOMPANYING INCIDENTAL FINDINGS OF MICROVASCULAR ISCHEMIC DISEASE IN THE ELDERLY: PRELIMINARY RESULTS. G. Esposito*, B.S. Kirkby, J.D. Van Horn, D.R. Weinberger, K.F. Berman. Unit on PET, CBDB/NIMH/NIH. Bethesda, MD, 20892.

In the elderly, MRI evidence of microvascular ischemic disease (MID) has been associated with more pronounced decline in some cognitive abilities. PET studies have suggested that the presence of risk factors for cerebrovasular disease may be associated with greater reduction of regional brain activity with age. To investigate whether cognitive abilities and brain activity during working memory changed in the presence of asymptomatic MID, we used the oxygen-15 water PET method (approximately 42 mCi/scan) with measured arterial input to calculate absolute CBF (ml/100g/min) in three subjects (2W, 1M; mean age 73) in whom the diagnosis of MID was made solely on the basis of incidental findings on MRI scans and in the absence of clinical findings or complaints. The MID patients were compared to eight well-screened controls (2F, 8M; mean age 70) during performance of the Wisconsin Card Sort (WCS) as well as during a sensorimotor control task (WCSc). White matter changes were rated by a neuroradiologist as mild/moderate in subjects 1 and 2 and marked in subject 3. Images were stereotactically normalized and smoothed (20,20,12 mm in the x,y and z dimensions) using the SPM95 package. rCBF data of each of the three MID were compared to those of the controls with Z-statistics (significance threshold $p < 0.01$). Cognitive performance for each MID subject was above the mean for the control group. During both the WCS and the WCSc, we found no significant reductions in absolute global CBF in any of the MID subjects. Higher rCBF was found in some cortical regions adjacent to areas of white matter MRI hyperintensities: in prefrontal and temporal cortex in subject 1, in temporal cortex in subject 2, and in prefrontal cortex in subject 3. rCBF activation (WCS minus WCSc), however, did not differ between any of the MID subjects and the control group. The results in this small cohort suggest that WCS performance and rCBF activation may be unaltered in asymptomatic subjects with MRI-diagnosed MID. Further studies in larger patient populations will be necessary to confirm these preliminary results.

268.4

FRONTAL AND PARIETAL BLOOD FLOW ACTIVATION DURING AN AUDITORY TASK DIFFERENTIATE SCHIZOPHRENIC PATIENTS WITH AND WITHOUT PRIMARY NEGATIVE SYMPTOMS W.T. Carpenter*, A.C. Lahti, H.H. Holcomb, M. Zhao, R.W. Buchanan, C.A. Tamminga. MPRC, University of Maryland School of Medicine, Baltimore, MD 21228.

We previously reported that schizophrenic patients with deficit symptoms (primary negative symptoms) were distinguished from nondeficit patients and normals in a PET/FDG study by reduced metabolism in thalamus and in frontal and parietal cortex (Tamminga, 1992). We now report $H_2{}^{15}O$/PET data on prospectively identified schizophrenic patients with (n=6) and without (n=8) deficit symptoms and normal controls (n=12). PET scans were obtained while patients were neuroleptic-free. During the scan, subjects performed a tone frequency recognition task. This is a graded error related task, where percent accuracy is fixed between groups (80±5%). Four rest, sensory motor control and tone recognition task scans were obtained from each subject. Statistical Parametric Maps (SPM 95) were created to contrast rCBF activity patterns between groups. Deficit schizophrenics showed significant increased activation in superior frontal cortex and reduced activation in right prefrontal cortex compared to normals, while the nondeficit patients showed significant reduced activation of the occipital cortex compared to normals. When deficit and nondeficit schizophrenics were contrasted, the deficit patients showed significant reduced activation in bilateral parietal cortex (R p=0.002, L p=0.023) and right middle frontal cortex (p=0.003) compared to the nondeficit schizophrenics. These data suggest that deficit schizophrenics, in spite of performing the task with similar accuracy, appear to have different "dynamic rules" compared to normals and nondeficit patients.

268.5

CEREBRAL METABOLIC EFFECTS OF TRIIODOTHYRONINE IN ADULTS WITH RESISTANCE TO THYROID HORMONE. **J. A. Matochik*, P. Hauser, A. J. Zametkin, B. D. Weintraub and R. M. Cohen. Lab. of Cerebral Metabolism, NIMH, and Molecular and Cellular Endocrinology Branch, NIDDK, Bethesda, MD 20892.**

Resistance to thyroid hormone (RTH) is caused by mutations in the hormone-binding domain of the hTRß gene encoding the thyroid hormone receptor-ß. The most prominent behavioral manifestation of RTH is deficits in attention. To evaluate the hypothesis that *triiodothyronine* (T3), the active form of thyroid hormone which has sympathomimetic action, may ameliorate the functional abnormalities of RTH, seven adults (4 females, 3 males) received two PET scans to measure cerebral glucose metabolism during the performance of a sustained attention task, one scan while on T3 treatment and the second scan off hormone treatment for at least four weeks. There was no significant change in either global or regional glucose metabolism between the two PET scans. Performance on the attention task was also unaffected by hormone treatment, with the scores on this task being considerably below normal performance levels in both conditions in RTH subjects. Subjects with RTH in the present study had higher metabolic values in the anterior cingulate (on both PET scans) than we have previously observed in unaffected subjects successfully performing our attention task. Despite the relatively low statistical power of the present study, T3 does not appear to have a robust effect on cerebral metabolism or attention.

Supported by the NIH Intramural Research Program.

268.6

A FEASIBILITY STUDY OF THE SAFETY AND EFFICACY OF ALBUMIN MICROBUBBLES IN TRANSCRANIAL DOPPLER. R.E. Stoll*, S.M. Otis, B.W. Chopko. Scripps Clinic And Research Institute , La Jolla, CA, 92037.

Transcranial color and spectral Doppler ultrasonography (TCD) is a dynamic, non-invasive method of examining the intracranial circulation in patients with known or suspected cerebrovascular disease. However, in some patients the clinical utility of TCD examinations is limited by poor tissue penetration and inadequate imaging of vessels, which has driven the search for agents to enhance visualization of the intracranial vasculature. Albumin microbubbles (AM) in the form of Albunex, a commercially available ultrasound contrast agent, is a sterile, nonpyrogenic solution of echogenic microspheres produced by sonication of 5% human albumin aqueous solution. The microspheres reflect ultrasound waves, leading to enhanced signal intensity. We examined the safety and efficacy of AM in TCD in an open-label feasibility study of 10 adult patients. A baseline (noncontrast) TCD examination was conducted just prior to an AM-enhanced study, hence each patient served as his or her own control. AM was administered intravenously in 10 to 30 cc boluses and concurrent TCD was performed. No adverse effects were observed. Three of ten patients showed minimal improvement over the baseline TCD in ability to visualize intracranial vasculature. These three patients had significantly lower systolic blood pressures than those who showed no evidence of improvement. AM have been proven to pass the pulmonary circulation and appear in the left ventricle. We hypothesize that AM may not survive the left ventricular systolic shear forces intact in order to pass into the cranial circulation in sufficient quantity to provide echogenic foci for ultrasound studies. Further studies are indicated to determine the viability of AM in passage through the heart, great vessels, and into the cerebral circulation.

Funded by Mallinckrodt Medical.

268.7

HEPARAN SULFATE AND HEPARIN ATTENUATE REGIONAL CEREBRAL BLOOD FLOW (rCBF) INCREASE IN EXPERIMENTAL BACTERIAL MENINGITIS? J. R. Weber, K. Angstwurm*, D. Freyer, M. Weih, C. Busch, W. Bürger# and U. Dirnagl Depts. of Neurology and Microbiology#, Charité, D- 10098 Berlin, Germany.

Recent reports point to antinflammatory effects of substances which inhibit selectin mediated leukocyte rolling. Heparan sulfat (HS) and Heparin bind to P- and L-selectin and inhibits acute inflammation. We tested the hypothesis whether HS and heparin have an effect in experimental bacterial meningitis on rCBF.

Materials and methods: Male wistar rats were anesthetized and mechanically ventilated. Meningitis was induced by intracisternal injection (i.c.) of pneumococcal cell wall components (PCW). rCBF was measured continuously over 6 hours with laser Doppler flowmetry. pCO_2, PO_2 and pH were measured every 2 hours and mean arterial blood pressure were monitored continuously during the experiment.

LDF % / group	*0h*	*3h*	*6h*	WBC/CSF
controls (NaCL i.c., n=6)	100	103 ± 7	114 ± 11*	7 ± 4*
PCW i.c. (n=6)	100	169 ± 29	234 ± 37	2598 ± 871
PCW + Heparin 86U (n=4)	100	111 ± 16	122 ± 20*	237 ± 95*
PCW + HS 250 µg (n=4)	100	159 ± 27	203 ± 31	1997 ± 708
PCW + HS 500 µg (n=5)	100	131 ± 19	142 ± 23*	436 ± 209
PCW + HS 1250µg (n=6)	100	109 ± 8	120 ± 16*	132 ± 87*

*Statistically significant *(p< .05), ANOVA Duncans test, data mean ± SD compared to untreated and HS in lowest dose.*

Conclusion: Heparansulfat and Heparin attenuate the increase of rCBF and WBC in the CSF. Inhibition of leukocyte rolling may lead to a new adjuvant strategie in meningits.

268.8

THE EFFECTS OF THE CLINICAL IMPROVEMENT OF SCHIZOPHRENIC SYMPTOMS ON BRAIN SPECT. C.J.Shin, I.W.Chung*, S.S.Koong, S.G.Choi. Department of Neuropsychiatry, Chungbuk Nat. Univ. Hosp., Chungju, Chungbuk 360-240.

This study investigated the relationship between regional blood flow changes of brain and clinical improvement of symptoms in 10 maleschizophrenic patients. Single-photon emission computed tomography(SPECT) imaging with 99mTc-HMPAO was performed second and sixth weeks after admission and concurrently psychopathology was examined by Positive and Negative Symptom Scale. All patient were finally categorized into two groups, improvement or no change. We examined the difference of the changes in two functional images between groups. Prefrontal cortical activity showed no significant difference between two groups and clinical improvement had no effect on that. However, second SPECT images of no change group showed lower left temporal lobe activity than that of improvement group. Our results probably suggest that temporal lobe activiy have relation to underlying schizophrenic symptoms.

By Grant of Chungbuk National University Hospital.

268.9

RELATIONSHIP BETWEEN REGIONAL BRAIN TEMPERATURE AND BLOOD FLOW BEFORE AND AFTER DEEP HYPOTHERMIC CIRCULATORY ARREST, JW Kuluz*, B Gelman, R Perryman, D Mulvaney, N Wang, CL Schleien. Dept of Pediatrics and Surgery, Univ of Miami, Miami, FL 33101.

Deep hypothermic circulatory arrest (DHCA) and cardiopulmonary bypass (CPB) for repair of congenital heart disease in children are associated with neurologic sequelae including seizures and cerebral infarction. This may be caused by flow-metabolism imbalance secondary to temperature effects on vascular resistance during the cooling and rewarming periods associated with bypass.

Methods: 5 piglets (10 ± 2 kg) were anesthetized and catheterized. Temperature probes were placed in 6 brain regions: 2 anterior and 1 posterior superficial cortex, diencephalon, and both caudate nuclei. Deep hypothermia (15-18 °C) was achieved on CPB gradually over 40 min followed by DHCA for 45 min and slow rewarming over 40-80 min. Regional CBF (microspheres) was measured at baseline, and at 4 time points during rewarming. Group 1 piglets (n=2) received pH-stat (corrected for temperature) and group 2, alpha-stat (n=3) blood gas management.

Results: The mean coefficient of variation of regional brain temperature increased during both cooling (7.0%) and rewarming (4.5%) compared to baseline (0.8%). Deep structures rewarmed at a faster rate than superficial cortex. Large variation in regional CBF was observed. CBF remained very low in cortex in 4 of 5 animals, whereas more complete restoration of CBF was attained in deep structures. Severe hypoperfusion was more common in gp 2, especially in superficial structures. After complete rewarming, global CBF was greater in gp 1 (71% vs 36% of baseline).

Conclusion: Cooling of the brain during CPB and rewarming after DHCA resulted in heterogenous changes in regional brain temperature and blood flow. pH-stat management resulted in higher flow and may protect against ischemic injury. Regional differences in the effectiveness of cooling and rewarming may explain unexplained postoperative regional ischemia and clinical neurologic sequelae.

268.10

UNILATERAL LESION OF HIPPOCAMPAL CA1 SUBFIELD DUE TO TRANSIENT FOREBRAIN ISCHEMIA PLUS UNILATERAL HYPERTHERMIA, AND ITS DYNAMICS OF CBF AND BRAIN TEMPERATURE. E. TABUCHI[1]*, R. TAMURA[1], H. HAGINO[2], H. KURIMOTO[1], M. KURACHI[2], T. ONO[1].
[1]Depts. of Physiology & [2]Neuropsychiatry, Toyama Medical & Pharmaceutical Univ., Toyama 930-01, Japan.

It is known that the hippocampal CA1 subfield (CA1) is particularly vulnerable to ischemia and neurons are susceptible to hyperthermia during ischemia. We produced an unilateral lesion of CA1 due to transient forebrain ischemia plus unilateral hyperthermia, and bilaterally monitored cerebral blood flow (CBF) in CA1 and brain temperature (BT) to know its hemodynamics, using rats. They were subjected to 10 min ischemia by bilaterally occluding the common carotid arteries one day after permanent occlusion of the vertebral arteries. After the experiment, the brains were perfused and fixed, and the number of normal neurons in CA1 and some other brain regions were histologically investigated. The number of normal neurons significantly decreased only at heated side of CA1. Mean CBF decreased during ischemia to about 25 % and increased transiently after ischemia to 110 % at both unheated and heated sides, compared with the pre-ischemic level. There was no change in CBF between sides. BT decreased immediately after start of ischemia at both sides and BT at heated side started increasing 5 to 10 min after start of ischemia compared with unheated side, but BT did not change between sides after reperfusion of blood. In addition, there was a positive correlation between the post-ischemic hyperemia and the degree of decrease in CBF during ischemia.

268.11

EXERCISE-INDUCED SPECIFIC BRAIN COOLING EFFECT, S. Yeo*, M. Scarbough. School of Nursing, University of Michigan, Ann Arbor, MI 48109-0482

Acute exercise increases body temperature, but usually does not involve the hypothalamic regulatory mechanisms of fever. Thus, the setpoint at the PO/AH area remains unchanged. Some argue that in order for the setpoint to remain unchanged, the brain has to be cooled aggressively during the heat production period. This theory, specific brain cooling, has been proven with various mammalian species.

The purpose of the study was to assess the effect of exercise-induced hyperthermia on brain and deep trunk temperature. Eight women, age 18 to 50 years old (30.9 ± 12.6; M ± S.D.), participated in the study. Subjects performed their regular aerobic exercise in a temperature controlled environment while body temperature (ear and rectal) and heart rate (HR) were measured simultaneously and repeatedly before, during, and after exercise. Glass mercury rectal thermometers (Baxter Healthcare Corporation, Deerfield, IL) were used for measurement of deep trunk temperature, an infrared tympanic membrane thermometer (Thermoscan Pro-1, Thermoscan Inc., San Diego, CA) for measurement of brain temperature, and a portable heart rate monitor (Polar Pacer, Polar Electro Inc., Port Washington, NY) for monitoring heart rate.

Rectal temperature was higher than brain temperature for all but one of the forty pairs observed. Resting mean temperatures at the two sites indicated .6 °C difference (36.8 ± .14 °C at the brain and 37.4 ± .12 °C at deep trunk; M ± S.E) and this mean difference gradually increased. The mean difference at 30 minutes into exercise (36.6 ± .20 °C at the brain and 37.9 ± .11 °C at deep trunk; mean ± S.E) was the largest (1.3 °C), then gradually becoming smaller. The time pattern varied for the two modes of temperature ($F=9.67$; $p<.001$). Rectal temperature changed over time ($F=7.86$; $p<.002$), and brain temperature did not ($F=1.5$; $p=.25$), indicating that brain temperature did not respond to exercise. While rectal temperature was strongly correlated with HR ($r=.60$), brain temperature did not correlate either with rectal temperature ($r=.02$) or with HR ($r=.08$). Thus deep trunk temperature responds to exercise at moderate levels. On the other hand, brain temperature does not increase due to exercise. Supported by NIH (K07 NR00047).

269.1

HIPPOCAMPUS, TIME, AND MEMORY, REVISITED. A. Dietrich*, J. D. Allen, B. N. Bunnell. Dept. of Psychology, University of Georgia, Athens, GA 30605.

Rats with lesions to the hippocampus proper and the subiculum were tested for timing behavior and temporal memory. Using the peak procedure, they were trained to discriminate a 40 s interval and a retention gap tested the memory for time. Results were interpreted within the theoretical framework of the internal clock and with respect to current theories on hippocampal function. Timing behavior was unaffected by either lesions and no shifts in the temporal discrimination functions were observed. The lesions also failed to show a deficit in the memory for temporal events. For all groups, the retention gap increased the mean peak time by the time of the gap. This indicated that all rats used the stop rule which required the use of working memory. Thus, it was concluded that the hippocampus is neither necessary for accurate timing behavior nor for the memory of temporal events.

269.2

TRANSFER OF DISCRIMINATION LEARNING ACROSS DIFFERENT TEMPORAL RANGES. S.P Clarke*, R.B. Ivry. Dept. of Psychology. University of California, Berkeley. Berkeley, CA 94720

If separate neural systems are involved in representing temporal information across different ranges, then animals trained to discriminate between stimuli of short duration should show no transfer of learning to a discrimination between stimuli of a longer duration, and vice-versa. To test this hypothesis, rates of learning were assessed for each of two groups of 12 Fisher 344 rats. One group was trained on a short range discrimination (SR; 200 vs. 800 msec), the other on a long range discrimination (LR; 20 vs 80 sec). Both groups were then transferred to a medium range discrimination (MR; 2 vs. 8 sec) with the lever mapping reversed for half the animals in each group. The results of this experiment suggest that the animals trained on the SR task showed some transfer to the MR task while those in trained on the LR task did not. One curious finding is that the animals in the SR to MR group with the lever mapping reversed showed better performance after the transfer than all other groups. A second experiment will control for contextual cues and seek confirmation that the animals attended to the relevant stimulus dimension. Groups of animals will be trained on the SR, MR or LR temporal discrimination or an intensity discrimination. The intensity task will provide a baseline to assess the effects of contextual cues after transfer to the temporal discrimination. The other groups will be transferred to stimulus durations within the original training range. The lever mapping manipulation will be effected in half of each of these groups to assess how well the animals attended to the relevant stimulus dimension. This work was supported under a grant from the Whitehall Foundation and NINDS grant #NS30256.

269.3

AN ANATOMICALLY GROUNDED THEORY OF RODENT NAVIGATION. A.D. Redish*, D.S. Touretzky. School of Computer Science and Center for the Neural Basis of Cognition, Carnegie Mellon University, Pittsburgh PA 15213.

Drawing on proposals of several other research groups concerning the neural implementation of path integration (McNaughton et al., 1996), place field formation by competitive learning (Sharp, 1991), pattern separation and completion in hippocampus (Marr, 1969), and maintenance of an internal head direction representation (Skaggs et al. 1995, Elga et al., 1996), we synthesize a comprehensive theory of rodent navigation grounded in anatomy and tested in computer simulations. In doing so, we have adapted the proposals to each other, demonstrated that the resulting system accounts for a broad spectrum of data, and revealed interactions between the components that lead to explicit, testable predictions.

Sensory cues enter the place code via deep Entorhinal Cortex and are refined by competitive learning in the deep-to-superficial EC connections. The path integrator's representation is maintained as a hill of activity in a 2D array of cells in subiculum, and *path integration* is accomplished by shifting this hill in a loop that includes parasubiculum and superficial EC. A representation of *head direction* is maintained by an analogous hill of activation in a 1D array of cells in postsubiculum, and is updated similarly by a loop including the anterior thalamic nuclei. Rodents represent their position within a coordinate system that is goal or task dependent; selection of the appropriate *reference frame* occurs by pattern separation and completion in the dentate gyrus and CA3/CA1 subsystems.

While reference frame information (critical for episodic memory) is represented in CA1, path integrator coordinates (critical for navigation) are represented in subiculum, suggesting an anatomical separation between navigation (fornix, subcortical projections) and episodic memory (deep EC, cortical projections).

Funded by the Center for the Neural Basis of Cognition.

269.4

Long Term Depression and Navigation: a simulation model. S. L. Morgan, J. Spruill, G. Yuen*, T. J. Teyler. Dept. Neurobiology, NE Ohio College of Medicine, Rootstown, OH & Tenn State Univ. Nashville, TN.

Animals have the ability to navigate through extremely complex environments with relative ease. The hippocampus has been implicated as a spatial map of the environment, as evidenced by the well documented existence of place cells and an ensemble code for space (O'Keefe and Nadel, 1978; Wilson and McNaughton, 1993). We have attempted to modify an original model by Neil Burgess (1994), based on hippocampal anatomy and physiology, in an effort to enhance its navigational capabilities. The modified model consists of five layers and one thousand neurons with a feedforward architecture and a simple population code for space. The model calls for a random connectivity of the place cells at the start of the trial followed by an experience dependent long term potentiation (LTP) like mechanism that can increase synaptic weights in the place cell layer. Initial experiments indicated that the model allowed for good navigation in a controlled environment but the initial random connectivity provided conflicting navigational signals at times. If initial random connections were weighted heavily they could act as extraneous "attractants" that prohibited the rat from navigating to the goal correctly. We decided to implement a form of long term depression (LTD) in order to trim out some of the extraneous initial connectivity. Our preliminary data indicates that the implementation of LTD has, by trimming initial random connections, enhanced the ability of the rat to navigate to the goal. (Supported by ONR Nooo14-92-J1372)

269.5

US DEVALUATION DOES NOT ATTENUATE WIN-STAY RESPONDING IN RATS ON THE RADIAL MAZE. J. R. Sage* and B. J. Knowlton. Dept. of Psychology, University of California, Los Angeles, CA 90095.

Previous studies have implicated the caudate nucleus in stimulus-response (S-R) habit learning. However, the content of this learning has not been examined using traditional S-R versus stimulus-stimulus (S-S) differentiation procedures. In order to characterize the nature of the association learned by rats in the caudate-dependent win-stay task on the radial maze, we employed a US devaluation procedure using conditioned taste aversion (CTA). Rats were trained to approach baited arms on the maze which were signaled by a light. When the animals reached a high level of accuracy, the food reinforcer used on the lighted arms was paired with LiCl injections for the experimental group. When performance was then reassessed on the maze, the experimental group continued to approach the lighted arms with similar accuracy and speed as compared to the control groups and to pre-CTA performance. The results suggest that the rats are not recalling the food reinforcer in order to mediate their response to the light. As previously suggested, an S-R, rather than S-S, association may be characteristic of win-stay and caudate dependent learning. Supported by the UCLA Academic Senate.

269.6

SPATIAL LEARNING BY HIPPOCAMPAL RATS IN THE MORRIS WATER TASK. T. Schallert*, L. B. Day, M. Weisend, & R J. Sutherland Dept. of Psychology, University of Texas at Austin, Austin, TX 78712 and University of New Mexico, Albuquerque, NM 87131.

Day and Schallert (*Behav. Neurosci*, 1996) showed that impairments caused by anticholinergic drugs in standard water maze task studies may be the result of deficits in ability to inhibit non-efficient escape strategies. Special training procedures designed to promote the use of spatial search strategies and to prevent non-spatial strategies revealed that drugged rats and controls did not differ in acquisition or in probe trials in which the hidden platform was moved. In the present study, rats with kainate-colchicine hippocampal lesions and controls were tested in the Morris Water Task using similar training procedures. That is, instead of using a small platform for all trials, an oversized hidden platform that occupied nearly the entire pool was used during the early trials and was effectively "shrunk" into one quadrant of the pool by substituting smaller platforms over subsequent trials. Rats were released from the 4 compass points. During the probe trial, hippocampal animals showed increases in distance and latency to arrive at the new platform location comparable to controls, and swam preferentially in the previously-correct quadrant. Although the nature of the information that was controlling the trajectories in the hippocampal rats appeared to differ from that of control rats, it appears that compared to traditional training, our procedure enables rats with hippocampal damage to adopt a strategy supporting superior spatial localization performance. Funded by NS 23964.

269.7

DIFFERENCES IN THE SPATIAL CODING PATTERNS OF VENTRAL SUBICULAR NEURONS IN SIMPLE AND COMPLEX ENVIRONMENTS. R.G. Phillips*, H. Tanila[1] & H. Eichenbaum. Ctr. Behav. Neurosci., SUNY Stony Brook, NY 11794 and [1]Dept. of Neurology, Univ. of Kuopio, 70211 Kuopio Finland.

Neurons in hippocampal fields CA1-3 encode spatial representations of intramaze cues, extramaze cues and usually combinations of these and other fixed cues. Because the subiculum is the main target of hippocampal output, an attempt was made to determine if cells in this area code information in the same way as hippocampal neurons. Rats were chronically implanted with two movable tetrodes for unit recording and trained to explore a four-arm radial maze, receiving lateral hypothalamic stimulation as a reward at the end of each arm. Recordings were made in two conditions: (1) a complex environment of intramaze cues consisting of distinctive visual, tactile, and olfactory stimuli, and distal cues consisting of visual patterns attached to a curtain surrounding the maze; and (2) a simple environment in which all arms were blank and a single visual cue remained on the curtain.

In the complex environment no ventral subicular neurons showed identifiable location, cue, or reward related firing patterns, but instead tended to fire equally throughout the maze. However, when the number of cues was reduced to create the simple environment, some ventral subicular cells showed distinct place fields. These place fields did not follow the remaining extramaze cue when it was rotated, but instead changed their firing patterns qualitatively or disappeared. The original place fields were recovered when the cue was returned to its original position. These findings suggest that cells in the ventral subiculum encode spatial relations between a simple cue and the fixed environmental stimuli, but in a manner different from that of the hippocampus proper. Supported by NINDS, NIMH.

269.8

PERFORMANCE ON A CONDITIONAL ASSOCIATIVE LEARNING TASK BY RATS WITH LESIONS OF THE FORNIX OR DORSAL HIPPOCAMPUS. V. Sziklas*, S. Lebel, and M. Petrides. McGill University, Montreal, Canada.

Rats with lesions of the fornix (F), the dorsal hippocampus (H), or a control operation (OC) were trained on a spatial conditional associative learning task (CAL) in which they had to learn to associate particular scenes with specific visual stimuli. They were subsequently trained on a spatial working memory task, the eight-arm radial maze. Animals with damage of the fornix were able to learn the CAL task at a rate comparable to that of the OC animals. Performance of the H group, however, was significantly impaired in comparison with both the OC and the F groups. Both the F and H animals were impaired on the radial maze. The present finding of a dissociation between the effects of F and H lesions on the CAL task suggests that the spatial learning deficit following damage of the fornix may be specific to remembering places the animal has visited on a given trial while leaving intact the ability to learn associations between specific scenes and arbitrary visual stimuli located within them. By contrast, lesions of the hippocampus appear to lead to a more general spatial learning impairment.

This work was supported by a grant from NSERC.

269.9

THE HOMING PIGEON HIPPOCAMPAL FORMATION AND LEARNING STRATEGIES FOR GOAL RECOGNITION. R. Strasser* & V. P. Bingman. Psychology Dept., Bowling Green State University. Bowling Green, Ohio 43403.

The role of the hippocampus may vary depending on the behavior displayed and context. To better understand these differences, learning strategies and the role of the hippocampal formation (HF) were examined in an indoor food-site and an outdoor loft recognition task. In Experiment 1, pigeons with lesions to HF and control pigeons were trained to find food hidden in one of four uniquely colored bowls, located next to a landmark, in a constant location in a room. HF lesioned pigeons learned to choose the correct bowl faster than controls ($p=0.0197$). Subsequently, on four unrewarded test trial types, two cues were placed in conflict to each other and the third cue was removed. Controls chose randomly in all test trials except when the correct color bowl was placed in conflict with the landmark. In contrast, in all test trials, pigeons with HF lesions made appropriate responses to stimuli associated with food reward during training trials. If the correct color was an option, it was preferentially chosen by the HF lesioned birds. The results suggest control pigeons encode a holistic representation of the goal that is sensitive to environmental manipulations. Pigeons with HF lesions, however, appear to use a relatively simple, associative learning strategy and therefore, choose appropriately even with changes in the relationship among the stimuli. The role of HF in goal recognition was then examined in a different context involving home loft recognition. Control and HF lesioned homing pigeons were raised in either a loft facing west with red boards or a loft facing east with green boards. Pigeons were later tested with the boards switched between the two lofts. Control pigeons returned to the loft that occupied the correct location but the wrong color and HF lesioned pigeons chose both lofts equally. In this task, HF lesioned pigeons do not appear to respond preferentially to color. Although there are behavioral and contextual differences between these two tasks, together they suggest that pigeons with HF lesions can still identify goals, however, the learning strategies used to identify a goal are much different than those used by control pigeons.
Funding by: Sigma Xi Grant

269.10

THE EFFECTS OF IBOTENIC ACID CA1 HIPPOCAMPAL LESIONS, OR PERFORANT PATHWAY KNIFE TRANSECTIONS, ON SPATIAL MEMORY IN THE RAT. L. Stubley-Weatherly*, J.W. Harding, and J.W. Wright. Dept. of Psychology, Washington St. Univ., Pullman, WA 99164-4820

Although a substantial body of literature exists documenting the role of the hippocampus in learning and memory, there is less consensus concerning the behavioral involvement of discrete hippocampal regions. The present experiments examine specific hippocampal neurocircuitry in relation to spatial memory. Male Sprague-Dawley rats served as subjects, with rats in experiment 1 receiving either bilateral knife transections of the Perforant Pathway (PP) or cortex control knife cuts. Animals in experiment 2 received either ibotenic acid-induced bilateral lesions (IBO) of the hippocampal CA1 region, or aCSF vehicle control infusions. Upon recovery, subjects were tested on a motor skills task, later followed by a spatial learning and memory paradigm utilizing the water maze. Acquisition of the spatial task, using a hidden platform within the water maze, was conducted for 8 consecutive days. A Probe trial and Visual platform cue test were also conducted on days 8 and 9, respectively. Preliminary data suggests that animals with PP transections demonstrate the greatest performance deficit in this memory task. The IBO animals also show impaired performance, but to a lesser extent than PP rats. Both groups performed significantly worse than their respective controls. Additionally, preliminary studies indicate that the icv delivery of an angiotensin-IV analog facilitates performance in animals with hippocampal neurocircuitry damage.

269.11

BEHAVIORAL EFFECTS OF QUANTITATIVE ISCHEMIC DAMAGE TO HIPPOCAMPAL CA1 IN ADULT FEMALE RATS. T.L. Briones* and B.A. Therrien. The University of Michigan, Ann Arbor, MI 48109-0482

CA1 neurons in the hippocampus (HPC) are selectively vulnerable to effects of cerebral ischemia. Evidence exists that damage to CA1 neurons result in spatial learning deficits and that gender difference exists. However, findings relating the extent of CA1 damage and behavioral deficits are conflicting and no studies have been done using female animals. The purposes of this study were to determine: 1) the duration of ISCH in female rats that would result in confined damage to CA1; and 2) the effects of CA1 cell loss on the degree of spatial learning impairment. Adult female Wistar rats were randomly assigned to receive either 5 min ISCH (n=3), 10 min ISCH (n=5) or sham surgery (n=7). The 4-vessel occlusion method was utilized to induce global cerebral ischemia. After a 3-day recovery period, rats were tested for 6 days in the Morris water maze. Behavioral testing revealed that rats with ischemic damage were markedly impaired compared to shams in both directional heading error (DHE) and swim time ($p=.01$ and $p=.05$, respectively). Histological analysis showed moderate damage to CA1 (10% to 18%) and CA3 (8% to 15%) with minimal damage to CA2 (<10%) in the 5 min ISCH group. In comparison, significant ($p<.05$) damage was seen in the 10 min ISCH group in CA1 (52% to 65%), CA2 (18% to 30%), CA3 (20% to 32%), and subiculum (<15%). No damage to other brain regions was seen in either groups. Animals with greater CA1 cell loss have longer mean swim time (sham = 8.15 ± 0.9 sec, 5 min ISCH = 12.45 ± 2.8 sec, and 10 min ISCH = 16.29 ± 3.3 sec; $p=.00$) and showed increased DHE (sham = 25.40 ± 2.64 degrees, 5 min ISCH = 34.90 ± 5.88 degrees, and 10 min ISCH = 41.44 ± 5.46 degrees; $p=.01$) in the spatial learning task. We conclude that cell loss in both 5 and 10 min ISCH was not confined to CA1; and that greater extent of CA1 cell loss results in persistent spatial learning deficits in female rats with cerebral ischemic damage.
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269.12

THE EFFECTS OF BRIEF BRAIN ISCHEMIA ON STAGES OF MESSAGE PROCESSING AND THEIR CELLULAR MECHANISM Z. Y. Yang, J. X. Cai.* Kunming Institute of Zoology, Chinese Academy of Sciences. China 650223.

In this study,we examined which stages of message processing were affected by brief ischemia in mongolian gerbils. Occluding the common carotid arteries for 10 min induced brief ischemia.The step-through apparatus was used for memory test,the training or error numbers and the latency were recorded as memory grade. The cell numbers per unit length of CA1 region were counted. All results were analyzed with unpaired t-test. I. To study the effect of ischemia on message acquirement, the operation was done 5 days previous to training, the criterion of successful train-ing was that the animals do not enter the dark room in 5 min. The training numbers was used to measure the ability of message acquirement, and in sham group,it was 2.778 ± 0.278(n=9) while in ischemia group was 5.9 ± 0.924 (n=10) ($0.0005<P<0.005$); II.To study message consolidation,naive gerbils recieved operation immediately after one-trial training, 5 days later, the animals were tested.The error numbers of sham group(Es) was 2 ± 0.5(n=8),that of ischemia group(Ei) was 5.375 ± 0.706(n=8) ($0.0005<P<0.005$);the latency of sham group(Ls) was 78.375 ± 41.490, that of ischemia group (Li) was 2.375 ± 0.625 ($0.025<P<0.05$); III.To study message represent,naive gerbils were trained one-trial, then retrained every day for 5 min until they did not enter dark room for three consecutive days,then operation was given. After 5 days relaxation, the animals were tested. $Es=0.333\pm0.142$ (n=12), $Ei=0.636\pm0.310$ (n=11) ($0.1<P<0.375$); $Ls = 235.917\pm29.862$, $Li = 221.455 \pm34.722$ ($0.375<P<0.4$); In histological check,the cells of CA1 region of sham group was 205.586 ± 1.788/mm (n=29),that of ischemia group was 109.690 ± 5.664/mm (n=29) ($P<0.0005$). These results indicated that brief ischemia resulted in the impairment of the acquirement and consolidation of message,but not the represent of message, and the impairment was directly related to the delayed neuron death in CA1 region of hippocampus. (This work was supported by Chinese Academy of Sciences KY-85-05.)

269.13

IMPAIRMENTS ON A MOVEMENT INTEGRATION TASK IN RATS WITH FIMBRIA-FORNIX LESIONS. I.Q. Whishaw* and J. Tomie, Department of Psychology, University of Lethbridge, Lethbridge, Alberta,

The ability of animals to monitor their movements to subsequently produce shorter paths is referred to as movement integration. It is displayed most dramatically by animals taking short direct homeward paths after circuitous outbound excursions. We have developed a test of movement integration and have compared the performance of control and fimbria-fornix (FF) rats. The task takes advantage of the tendency of rats to carry large pieces of food to a shelter rather than eat in the open. Thus, rats were placed in a burrow that they leave in search of food. Once they learned to return directly to the burrow with food, the location of the burrow was changed. Both control and FF rats searched for food and carried it to the burrow but the FF rats were slower in learning to return to the correct burrow than were control rats. The results provide evidence of a definitive impairment in path integration in rats with FF lesions and the results are discussed in relation to the hypothesized role of the hippocampal formation in spatial behavior. Supp. NSERC of Canada

269.14

HIPPOCAMPAL INVOLVEMENT IN RADIAL MAZE LEARNING IN INBRED MICE: RELATIVE IMPORTANCE OF INTRA- AND EXTRA-MAZE CUES. W. E. Crusio[1] and H. Schwegler[2] **(SPON: European Brain and Behaviour Society).** [1]Génétique, Neurogénétique et Comportement, URA 1294 CNRS, UFR Biomédicale, Université René Descartes (Paris V), 45 rue des Saints-Pères, 75270 Paris Cedex 06, France. [2]Institut für Anatomie, Universität Magdeburg, Magdeburg, Germany.

Male mice from nine different inbred strains were tested at the age of three months in an 8-arm radial maze with only four out of eight arms containing food rewards, permitting simultaneous assessment of working (WM) and reference memory (RM). In the first experiment, the maze was always placed in the same way (allowing the use of intra-maze cues). In the second one, the maze was turned by 45° between trials (prohibiting the use of intra-maze cues). Other animals from the same strains were processed histologically to estimate the strain-specific extents of the hippocampal intra- and infrapyramidal mossy fiber projections (IIPMF). Turning of the maze significantly decreased performance in a strain-dependent manner. The extents of the IIPMF correlated strongly with both WM and RM if the maze was turned between trials. Similar correlations were only found in the early phases of learning in the other condition. We conclude that the IIPMF are involved in spatial learning and that non-spatial within-maze cues may influence learning performance in some inbred strains.

Supported by the Centre National de la Recherche Scientifique (URA 1294).

269.15

A SELECTIVE ANTAGONIST OF ALPHA-2 ADRENOCEPTORS FACILITATES THE EXCITABILITY OF THE DENTATE GYRUS AND IMPROVES THE RETENTION OF THE RADIAL ARM MAZE TASK. A. Ylinen[1, 2*], M. Pitkänen[1], R. Pussinen[2], A. Haapalinna[3], E. Koivisto[1], R. Lappalainen[2], J. Sirviö[2] and P. Riekkinen[1, 2]. [1]Dept. of Neurology and [2]A. I. Virtanen Institute, University of Kuopio, Kuopio, Finland, [3]Orion Corporation, Orion-Farmos Pharmaceuticals, Turku, Finland

A specific and selective alpha-2 antagonist, atipamezole, which is known to increase the release of noradrenaline, was found to dose-dependently enhance the excitability of granule cells to the perforant path stimulation in awake rats. Atipamezole, by the dose of 300 μg/kg but not by the dose of 1000 μg/kg, increased the size of population spike while it did not affect the slope of excitatory postsynaptic potentials. The administration of alpha-2 antagonist failed to enhance the potentiation of synaptic responses when measured 24 hours after (when its effect on synaptic transmission was disappeared) high frequency tetanus in the dentate gyrus. However, behavioral data indicated that 300 μg/kg atipamezole improved the retention (6 hours) of radial arm maze task (increased number of correct choices before firs error) possibly reflecting an enhanced memory encoding. Although the facilitation of the excitability of granule cells and enhanced intermediate-term memory may not be causally related to each other, those findings are in line with the content that noradrenergic innervation in the dentate gyrus modulate coming information to the hippocampus which facilitates 'noticing'.

269.16

THE EFFECTS OF HIPPOCAMPAL CCK_B RECEPTOR ACTIVATION ON PLACE LEARNING IN THE MORRIS WATER MAZE IN RATS . **R.J. McDonald[1]*, K.A. Cappell[1], F.J. Vaccarino[1,2] and N.J. DeSousa[1].** [1]Dept. of Psych., Univ. of Toronto, Toronto, Canada, *M5S 1A1*; and [2]Clarke Inst. Psychiatry, Toronto, Canada, *M5T 1R8*.

The present experiment examined the effects of intra-hippocampal infusions of pentagastrin, a CCK_B agonist, on performance in the Morris water maze, a paradigm involving hippocampal based memory.

Nine male Wistar rats implanted with bilateral cannulae into the hippocampus were randomly assigned to either vehicle or pentagastrin (10 nM) treatment groups (n= 4 and 5, respectively). Behavioral testing in the water maze was divided into two phases: a visible platform phase (days 1, 2, 4, 5, 7, and 8) during which a small platform protruded above the level of the opaque water; and an invisible platform phase (days 3, 6, and 9) in which the platform was submerged below the surface of the water. In both cases, the location of the platform remained constant and the task was to swim from four different start locations to the platform over eight daily trials. Animals were pretreated with their respective treatment on visible platform days only and tested drug-free on the invisible platform days.

Results showed that on the visible platform days treatment groups did not differ significantly across several measures including latency to platform, swim path distance, swim speed or percentage of time spent in the correct quadrant. However, on the invisible platform days pentagastrin treated rats spent a significantly greater percentage of time navigating in the correct quadrant than their controls, reaching maximal performance on their first day.

These preliminary data demonstrate that activation of hippocampal CCK_B receptors facilitates place learning and that this effect is not due to changes in motivational, perceptual, or motoric processes. Funding: Medical Research Council of Canada grant to FJV; and Connaught Fund grant to RJM.

269.17

DYNAMIC REWARD AND SPATIAL CODES OF CAUDATE NUCLEUS NEURONS. S. J. Y. Mizumori*, K. E. Unick & B. G. Cooper, Department of Psychology, University of Utah, Salt Lake City, UT 84112

In addition to the nucleus accumbens (Lavoie & Mizumori, 1994), the caudate nucleus may contribute dynamic spatial and reward information to guide navigation (Mizumori & Cooper, 1995). 104 caudate neurons were recorded as 6 rats performed multiple spatial memory trials on a radial maze. Alternating maze arms contained large or small amounts of reward. Consistent with our previous report, a variety of spatial correlates were observed, such as head direction and/or location, and directional movement relative to maze geometry or to 180° turns at the arm ends. Here, performance in darkness often resulted in elevated overall firing rates accompanied by a change in the location component of the spatial correlate (59 cells tested). The directional component of these same cells was rarely altered during dark trials. Thus, *single caudate neurons contribute to dead reckoning and landmark-based navigation, suggesting an involvement in both response and environmental spatial learning.*

During asymptote maze performance, 6 cells were sensitive to different magnitudes of reward: they increased or decreased firing just prior to encounters with large or small reward (but not both), or differentially discharged during consumption of these rewards. One of these cells was tested during acquisition of the maze task. A shift in the temporal relationship between cell firing and reward consumption was observed. On Day 1 of training, the cell preferentially fired during consumption of large rewards with no consistent location bias. By Day 7, the same cell anticipated encounters of large rewards by about 500 msec with a location bias. Thus, *individual caudate neuron reward and spatial codes appear to be experience-dependent.* [Supported by AG09299 and IBN 9514880]

270.1

IBOTENIC LESIONS OF THE NUCLEUS ACCUMBENS IMPROVE DETECTION OF SPATIAL NOVELTY AND RADIAL MAZE PERFORMANCE IN DBA/2 MICE CHARACTERIZED BY A GENETIC HIPPOCAMPAL DYSFUNCTION. M. Ammassari-Teule* A. Mele and P. Roullet. Ist. di Psicobiologia e Psicofarmacologia, C.N.R., Dip. Genetica e Biologia Molecolare, Università di Roma "La Sapienza" Rome, Italy and Inst. Neurosciences, CNRS-URA 1488, Université Paris VI, France.

C57BL/6 (C57) and DBA/2 (DBA) inbred mice with either bilateral ibotenic lesions of the nucleus accumbens or sham lesions were repeatedly placed in an open field containing five objects. After habituation to the object configuration, their reactivity to the displacement (spatial change) or to the substitution (non spatial change) of some of these objects was examined. When sham-lesioned mice were considered, strain differences concerning the reactivity to spatial change were found. C57 fairly reacted to the new configuration by exploring more the displaced than the non displaced objects while DBA did not show any reaction. Both strains, however, equally reacted to nonspatial change by spending more time in contact with the new object than with the familiar ones. When lesioned mice were considered, DBA with nucleus accumbens lesions showed a clear reaction to spatial change that was not present in sham-lesioned animals. This lesion, however, did not affect the reactivity to spatial change in C57 and leaved the reactivity to non spatial change of both strains intact. Subsequently, when tested in a radial eight-arm maze, C57 performed better than DBA. Spatial learning performance was similar in lesioned and sham-lesioned C57 mice. Conversely, DBA lesioned mice made more errorless trials and displayed a higher proportion of 45° angle turns than DBA sham-lesioned mice. The behavioural improvement observed in lesioned DBA mice supports the view that, in that strain, the nucleus accumbens may exert an inhibitory control on limbic structures and cortical areas more directly involved in the modulation of spatial learning. The fact that inbred mice represent extreme but still normal models of neural and behavioral functioning suggests that poor spatial learning performance recorded in individuals belonging to natural populations may depend on such an inhibitory mechanism.

270.2

NEONATAL 5,7-DHT LESIONS ALTER PERFORMANCE OF PASSIVE AVOIDANCE AND A NOVEL ODOR DISCRIMINATION TASK IN ADULT MICE M. Libbey[1], J. Berger-Sweeney[1], C. F. Hohmann[2]*. [1]Department of Biological Sciences, Wellesley College, Wellesley, MA 02181; [2]Department of Biology, Morgan State University, Baltimore, MD 21239.

We have shown previously that electrolytic lesions to the basal forebrain (BF) on postnatal day (PND) 1 interrupt cholinergic innervation of cortex and produce profound alterations in behavior and cortical morphology that persist into adulthood (*Int. J. Develop. Neurosci.* 12:239-253). In our electrolytic BF lesion model, the cholinergic neurons, of interest, are destroyed but so are the monoaminergic fibers en route to cortex. To determine the contribution of the monoaminergic system to the behavioral impairments, we performed lesions using the monoaminergic toxin 5,7-DHT (DHT). Unilateral (UNI) and bilateral (BILAT) lesions to the BF were performed on PND1 in 25 BALB/cByJ mice; 13 littermates were controls. The mice developed normally to adulthood when locomotor activity, passive avoidance retention, and odor discrimination with delayed non-match-to-sample learning were assessed. The BILAT males exhibited a significant passive avoidance retention deficit relative to the other groups. Both the UNI and BILAT groups (both sexes) performed a novel odor discrimination with delayed non-match-to-sample task significantly more accurately and more quickly than controls. The female BILAT group was slightly, but significantly, hypoactive relative to the other groups. The results indicate that the subcortical monoaminergic projections to cortex that pass through the BF influence adult behavior and probably contribute to some of the deficits noted previously in the electrolytic BF lesion model. We are currently testing electrolytic lesion groups on this novel odor discrimination task.
(Supported by NSF IBN 9222283)

270.3

SELECTIVE CHOLINERGIC IMMUNOLESIONS OF THE MEDIAL SEPTAL AREA DO NOT IMPAIR SPATIAL WORKING MEMORY. R.W. McMahan[1], T.J. Sobel[1], M. Gallagher[1,2*], and M.G. Baxter[2]. [1]Department of Psychology and [2]Curriculum in Neurobiology, Univ. North Carolina, Chapel Hill, NC, 27599.

Based on non-selective lesions, the septo-hippocampal pathway has traditionally been thought of as essential for spatial memory. The immunotoxin 192 IgG-saporin was injected into the medial septal area of male Long-Evans rats to remove selectively cholinergic innervation of hippocampus. Control (vehicle) and lesioned rats were tested post-operatively on a water version of a radial arm maze task. A water version of this task was implemented to exclude food restriction motivation and local intramaze cues, while also increasing the spatial memory load compared to the standard Morris water maze task. Rats were placed in the center of the maze, facing the junction of two arms (varied randomly), and allowed to swim down the maze arms to escape on a hidden platform located 1cm below the surface of opaque water. Rats were able to escape from each of the eight arms once during a session, because each platform was removed following a successful escape. After initial training, a delay was interposed. In the pre-delay component, four of the arms were blocked from entry. In the post-delay component, all arms were available for escape. The delays were 60 sec, 1 hr, 3 hr, and 6 hr. Errors that occurred after the delay were scored in 2 categories: retroactive, in which the rat searched an arm previously chosen for escape before the delay, and proactive, in which the rat searched an arm that had already been chosen for escape after the delay. Retroactive errors increased with longer delays, but were unaffected by the lesion. Proactive errors, which did not vary with delay, were also unaffected by the lesion. Results indicate that normal spatial working memory is possible without the septo-hippocampal cholinergic projection. (Supported by NIH grants K05-MH01149 and P01-AG09973 to MG)

270.4

COMBINED SELECTIVE LESIONS OF BOTH SEPTOHIPPOCAMPAL AND CORTICOPETAL BASAL FOREBRAIN CHOLINERGIC PROJECTIONS DISRUPT ATTENTIONAL PROCESSING BUT NOT SPATIAL LEARNING. M. G. Baxter[1]*, D. J. Bucci[1], P. C. Holland[3], and M. Gallagher[2]. [1]Curriculum Neurobiol. and [2]Dept. Psychol., Univ. North Carolina, Chapel Hill, NC 27599; [3]Dept. Psychol., Duke Univ., Durham, NC 27708.

The immunotoxin 192 IgG-saporin can be used to produce selective lesions of basal forebrain cholinergic neurons, permitting direct assessment of the role of these neurons in cognitive function. Most studies with this toxin have shown that selective cholinergic lesions of individual basal forebrain nuclei disrupt attention but do not impair learning and memory. Simultaneous damage of cholinergic neurons throughout the basal forebrain cholinergic system (BFCS) produced by intracerebroventicular infusions of 192 IgG-saporin produces deficits in learning and memory, but these lesions also result in cerebellar Purkinje cell loss and motoric impairments that complicate the interpretation of the behavioral results. We examined both spatial learning and attentional processing in rats given injections of 192 IgG-saporin stereotaxically placed in both the MS/VDB and nBM/SI, in an attempt to produce near-complete BFCS damage without cerebellar damage. These lesions produced an average of 78% depletion of hippocampal choline acetyltransferase (ChAT) activity and 64% depletion of cortical ChAT activity without affecting striatal ChAT activity. Spatial learning (assessed in the Morris water maze) was entirely normal in lesioned rats. Attentional processing was assessed in an associative learning task in which relationships between conditioned stimuli (CSs) are manipulated to alter attention to the CSs. Control rats showed a modulation of attention that was reflected in improved associative learning; this effect was not present in the lesioned rats. These results provide further support for the hypothesis that the BFCS is involved in attention, but not memory processes. Supported by NIH grants P01-AG09973 and K05-MH01149 to MG, and R01-MH53667 to PCH.

270.5

EFFECTS OF SELECTIVE CHOLINERGIC OR ELECTROLYTIC LESIONS OF THE MEDIAL SEPTAL AREA ON SPATIAL DELAYED ALTERNATION IN WEANLING RATS. C. S. Carter[1]*, M. G. Baxter[2], and M. E. Stanton[1,3]. [1]Dept. Psychol. and [2]Curriculum Neurobiol., Univ. North Carolina, Chapel Hill, NC 27599; [3]Neurotoxicology Division, US EPA, Research Triangle Park, NC 27711.

The hippocampus is important for cognitive development, and structures associated with it, such as cholinergic afferents from the medial septum and vertical limb of the diagonal band (MS/VDB), may contribute to the ontogeny of behaviors that rely on the hippocampus for their expression. Fornix transection disrupts the development of performance in weanling rats in an appetitive T-maze task: spatial delayed alternation (Freeman and Stanton, *Behav. Neurosci.*, 105:386-395, 1991). The present study was designed to examine more specifically the role of the cholinergic septal projections to the hippocampus in this effect. Rat pups were given MS/VDB lesions on postnatal day (PND) 17, either by electrolytically destroying the whole structure or by specifically targeting cholinergic neurons with the immunotoxin 192 IgG-saporin, and were tested behaviorally on PND26. Sham-operated control rats acquired the task rapidly. Both the electrolytic- and saporin-lesioned rats were impaired on the first block of training but improved across blocks of training. Averaged across blocks, electrolytic-lesioned rats were more impaired than saporin-lesioned rats relative to controls. AChE histochemistry revealed a loss of cholinergic fibers to the hippocampus in both lesioned groups. Electrolytic-lesioned rats had damage throughout the MS/VDB whereas saporin-lesioned rats showed only loss of cholinergic neurons, with GABAergic neurons intact. Hence, both cholinergic and noncholinergic projections from the MS/VDB to the hippocampus may contribute to the ontogeny of spatial delayed alternation. Based on the relative lack of effect of specific cholinergic lesions of the MS/VDB on other spatial learning and memory tasks in adults, this may also reflect a specific developmental role for the septohippocampal cholinergic projection. Supported by NIMH grant 1-F31-MH11292-01 and the US EPA.

270.6

MEDIAL FRONTAL CORTEX LESIONS: EFFECTS ON BEHAVIORAL PLASTICITY IN THE USE OF SPATIAL AND KINESTHETIC CUES IN RATS. W. L. Isaac*, S. A. Williams, and S. V. Isaac. Psychology. East Tennessee State University, Johnson City, TN 37614.

The role of medial frontal cortex (MF) of rats in using spatial and kinesthetic cues on a plus-maze was examined. Contradictory reports of performance changes in maze tasks following MF lesions suggests the need to better characterize the cues utilized during behavioral recovery of function. Using a Place versus Response task provides an opportunity to this end. Male Long-Evans rats (50 days old at surgery) were used. It was a 2x2x5 (Surgery x Task x Blocks of Days) mixed groups design with a repeated measure. ANOVAs were used to assess behavioral measures. All subjects acquired their tasks although controls met criterion in fewer days than MFs. Reversal within the Place or Response task for the rats demonstrated marked differences in behavioral plasticity between groups. Controls reversed the Place habit more easily than MFs, and MFs reversed the Response habit more easily than Controls. This lends support to the idea that MF lesions reduce behavioral plasticity on spatial tasks. It also suggests that MF lesions increase reliance on kinesthetic cues without impairing the use of such cues. (ETSU Grant-in-Aid)

270.7

COMPARISONS OF RIGHT VS. LEFT VENTROLATERAL ORBITAL CORTEX LESIONS ON ALLOCENTRIC SPATIAL LEARNING IN RATS. K.J. Burcham*, B. Haskins and J.V. Corwin. Psych. Dept., Northern Illinois Univ., DeKalb, IL 60115.

Two interconnected regions of rodent neocortex, the ventrolateral orbital cortex (VLO) and posterior parietal cortex (PPC) have been found to be involved in the learning of allocentric spatial tasks. Further, the effects of unilateral PPC lesions have indicated a lateralized effect: right but not left PPC lesions produce a significant deficit in allocentric spatial tasks. In the present study, we examine the effects of unilateral VLO lesions to determine if the VLO is also lateralized for allocentric spatial processing.

Four groups of subjects received either: unilateral right or left VLO lesions, bilateral VLO lesions, or sham control lesions. Subjects were tested for allocentric spatial learning in a Cheeseboard-maze. Subjects must learn to locate a food reward that is maintained in a constant location relative to extramaze cues, when the placement of the subject is varied. All subjects received 5 days of acquisition training followed by 5 days of reversal training.

The results indicated that during acquisition bilateral VLO operates were impaired relative to shams and right VLO operates (p's $< .05$). Left VLO operates were impaired relative to right VLO operates only on Day 1 of acquisition ($p < 05$). During reversal learning, the bilateral VLO group was impaired relative to the right VLO group ($p < .05$). The results provide limited support for the contention that the VLO is asymmetrically organized for allocentric spatial processing.

Supported by funds from the NIU Psychology Department

270.8

Medial Prefrontal Cortex or Nucleus Basalis Magnocellularis Lesions and T-Maze Learning in the Rat. Salazar, R.A.* Adams State College, Dept. Of Psychology, Alamosa, Colorado 81102. In this experiment, rats were tested on conditional and simple instrumental tactile or visual discrimination in a T-maze to test the hypothesis that the cholinergic projection system from the nucleus basalis magnocellularis (NBM) to the medial prefrontal cortex (MPF) is necessary for configural but not simple association learning. Six lesion groups (n=5/group) were included in this experiment: 1) bilateral MPF-ablation (MPF-A), 2) bilateral MPF-quisqualate (2ul/min) lesion group (MPF-Q), 3)bilateral NBM quisqualate (.8ul/min) lesion group (NBM-Q), 4) combined cross quisqualate lesion group)a unilateral NBM-Q lesion on one side and a unilateral MPF-Q lesion, contralateral to the NBM-Q lesion; CONTRA), 5) ipsilateral combined quisqualate lesion group (a unilateral NBM-Q lesion and a unilateral MPF-Q lesion on the same side; IPSI), and 6) sham operate or control group (CTL). All surgeries were performed prior to behavioral testing. Results from this experiment suggest that there is a dissociation among groups on both simple and conditional discrimination. The MPF-A rats were significantly slower at acquiring the tactile discrimination than the rest of the groups. NBM-Q and MPF-Q lesion groups did not reach criterion on the conditional (visual-tactile) discrimination task. The NBM may be important for this particular type of configural association task.

270.9

DORSAL INSULAR CORTEX LESIONS IMPAIR THE RECALL, BUT NOT THE ACQUISITION OF THE MORRIS WATER MAZE. C.E. Ormsby*, C.L. González-Rodríguez, and F. Bermúdez-Rattoni. *Dpto. Neurociencias, Instituto de Fisiología Celular, México, D.F. 04510, México.*

The insular cortex (IC) of the rat is a structure that has been characterized as mediating several aversive conditionings. Lesions of the IC impair the acquisition and retention of conditioned taste aversions, Morris water maze (MWM), and Inhibitory avoidance (IA), suggesting this is an associative area for aversively motivated and visceral learning. The present experiments were made in order to examine the role that different portions of the IC have on the Morris water maze (MWM) and inhibitory avoidance (IA). Wistar rats were bilaterally lesioned in one of three portions of the IC. One was lesioned in the dorsal (external) IC (IC-DOR group), another one was lesioned ventraly (internal) to the IC (IC-VEN), and another was lesioned in the adyascent parieto-temporal cortex (PTC group). Results showed that the IC-VEN group had impaired acquisition of the IA and MWM tasks, whereas the IC-DOR group were able to learn the IA task and showed normal acquisition of the MWM, but had impaired recall on a test trial made 24 hrs later. The PTC group did not show impairment in any of the tasks. Histologies showed that the IC-VEN group had additional lesions in the ventral caudate, claustrum and endopiriform nucleus; whereas the IC-DOR lesions only involved cortical tissue.

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270.10

THE EFFECTS OF TOTAL AND PARTIAL PARIETAL CORTEX LESIONS ON MEMORY FOR AN OBJECT/SPATIAL LOCATION PAIRED ASSOCIATE TASK IN RATS. J.M. Long*, J. Mellen, R.P. Kesner, Dept. of Psychology, University of Utah, Salt Lake City, UT 84112.

Previous research has suggested that large lesions of rodent parietal cortex (PC) impairs performance on an object/spatial location paired associate (O/SL-PA) task (Long and Kesner, 1995, Soc. Neuro. Abst. p.1215). The present experiment was conducted in order to test the hypothesis that more restricted lesions of the PPC would result in O/SL-PA deficits. Long-Evans rats were either given a dorsal, ventrolateral, or dorsal plus ventrolateral PC lesion, or a control surgery under Nembutal anesthesia. After a one-week recovery period the rats were tested either on an object or a spatial location go/no-go successive discrimination task. After reaching criterion (a minimum of a 5 sec. difference between reward and non-reward trials) they were trained on the other discrimination. After reaching criterion on the second discrimination all rats were trained on a successive discrimination, go/no-go task in which they had to remember which object/spatial location pairs had been associated with reward. Two objects and two spatial locations served as stimuli. Ten reward and ten non-reward trials were given daily until they reached criterion (same as above) or for 600 trials. Preliminary data indicate that compared to controls none of the PC lesions impaired object or spatial location discrimination. In the O/SL-PA task, data indicate that dorsal plus ventrolateral and dorsal PC lesioned , but not ventrolateral PC lesioned rats, were impaired relative to controls. These results suggest that dorsal PC is not involved in either object or spatial location discrimination , but rather is involved in discrimination of the combination of object and spatial location information .

NSF # IBN 95 11635

270.11

IMPAIRED SPATIAL MEMORY AND BEHAVIORAL ANOMALIES IN SEGMENTAL TRISOMIC Ts65Dn MICE. P.J. Yarowsky[■], G.E. Demas[■], S.L. Klein[■], L.J. Kriegsfeld[■], B.K. Krueger[#], and R.J. Nelson[■]. Depts. of Pharmacology [■] and Physiology[#], Univ. of Maryland Sch. of Med., Baltimore, MD 21201 and Dept. of Psychology, Behavioral Neuroendocrinology Group[■], The Johns Hopkins Univ., Baltimore, MD 21218.

Ts65Dn mice have a triplicated portion of chromosome 16 syntenic to the distal end of human chromosome 21. In order to determine their suitability as an animal model of Down syndrome (DS), we have investigated their spatial memory, anxiety, aggression, and reproductive behavior in Ts65Dn mice. Six month old Ts65Dn mice displayed levels of spontaneous alternation in a T-maze comparable to controls, demonstrating that simple spatial memory was not impaired. However, on 8 of 10 daily trials in a 12-arm radial maze (RAM), controls performed significantly above chance on the initial 12 choices, while Ts65Dn mice performed only near chance levels, indicating a deficit in spatial working memory. On trials 9 and 10, Ts65Dn mice performed as well as controls but required a greater number of choices to complete the RAM. After a 50-day retention period, control mice performed above chance levels when retested, while the improved performance of Ts65Dn mice was lost, suggesting that long-term memory was also defective. In an elevated plus maze, Ts65Dn mice did not display higher anxiety levels which could affect their performance in the RAM; they visited open arms more frequently and spent more time in open arms than did controls. In tests of aggression, Ts65Dn displayed increased offensive, but decreased defensive, aggression relative to controls. Ts65Dn mice also displayed reproductive anomalies, attempting fewer intromissions with an estrous female compared to controls. Ts65Dn mice behaved normally in several sensorimotor tests. Thus, many of the behavioral characteristics of Ts65Dn mice are similar to those of DS.

SRIS Univ. of Maryland, NIH HD2220, AG10686.

270.12

ALPHA2C-ADRENOCEPTOR OVEREXPRESSION IMPAIRS DEVELOPMENT OF NORMAL WATER MAZE SEARCH STRATEGY IN MICE. M.G.Björklund*[1], M.Riekkinen[1], J.Puoliväli[1], P.Santtila[1], J.Sirviö[2], J. Sallinen[3], M. Scheinin[3], A.Haapalinna[4] and P.Riekkinen Jr[1]. [1] Department of Neurology, University of Kuopio, P.O. Box 1627, FIN-70211 Kuopio, Finland, [2] A.I.V-institute, Kuopio, [3] Department of Pharmacology, University of Turku, [4] Orion-Farmos Ltd, Turku.

We investigated the role of α2C-adrenoceptors (α2C-AR) in the regulation of cognitive functions by studying the behavior of female FVDA mice that overexpress α2C-adrenoceptors (OE). We evaluated non-spatial (visible platform) and spatial (hidden platform) reference memory in a water maze (WM) test. The distribution of swimming during a free swim trial was similar in OE and control (C) mice. On the contary, the OE mice were impaired compared with the C mice in developing normal search strategy during the visible and hidden platform stages: OE mice swum more in the peripheral incorrect annulus than C mice. Both at the visible and hidden platform stages DEX 5 ug/kg further increased searching from the incorrect peripheral annulus only in OE mice. Daily treatment with ATI before the training trial normalized search strategy of OE mice. However if ATI treatment was discontinued OE mice exhibited again the abnormal search strategy. The effect of α2C OE on the cortical EEG activity was analysed to study if α2C-AR mediate the sedative effect of α2-agonists and are important for cortical EEG arousal. No differencies were observed on cortical EEG activity between the OE and C mice and DEX as effectively increased slow waves in OE and C mice. Passive avoidance training and testing trial behavior and dose responses of ATI and DEX treatments were identical in the OE and C mice. Our results suggests that α2C-AR OE impairs execution of navigation strategies, but does not affect arousal or anxiety. (Sponsors: Univ. of Kuopio, Finnish Academy of Sciences and Orion-Farmos Ltd)

270.13

THE EFFECTS OF REPEATED DISORIENTATION IN THE RAT: I. BEHAVIORAL IMPAIRMENTS.

J.P. Goodridge*, P. Dudchenko, and J.S. Taube. Department of Psychology, Dartmouth College, Hanover, NH 03755.

This study examined the effects of disorientation on the acquisition of two reference memory, spatial tasks. The first task was an 8-arm radial maze where one arm was consistently baited with water. The second task was a Morris water maze containing a submerged platform. Female, Long-Evans rats were assigned to one of three groups. One group (CLEAR) was transported to the maze room in a clear container with an open top and resided in this container during the intertrial intervals. A second group (OPAQUE) was identical to the first, except that an opaque container was used. The third group (OPAQUE+DISORIENTATION) was gently rotated back-and-forth in an opaque container in an attempt to produce disorientation. Across 45 acquisition days on a radial maze, 7/8 animals in the CLEAR group, 2/8 OPAQUE animals, and 0/8 OPAQUE+DISORIENTATION animals acquired the task. In the Morris water maze, however, no group differences in acquisition were observed.

To explore the dissociation between the two tasks further, partitions were placed into the water maze to create a swimmable version of the 8-arm radial maze. At the end of one of these arms was a submerged platform. Results revealed that disoriented animals were able to learn this task in 14 days and did not appear to differ from animals placed in a clear container. These findings suggest that the effects of disorientation may depend on the motivational system demanded by the task.

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270.14

THE EFFECTS OF REPEATED DISORIENTATION IN THE RAT: II. HEAD-DIRECTION AND PLACE CELL STABILITY.

P. Dudchenko*, J.P. Goodridge, and J.S. Taube. Department of Psychology, Dartmouth College, Hanover, NH 03755.

A previous study demonstrated that repeated "disorientation" of a rat may interfere with the stimulus control exerted by salient visual cues over the spatial orientation of place and head direction (HD) cells (Knierim et al., 1995). Recent work in our lab, described in a separate abstract (Goodridge et al., 1996), has also shown that disorientation appears to impair acquisition of an appetitively-motivated radial arm maze (RAM) task. In the current study, we were interested in whether the HD and place cells in the animals that displayed this spatial deficit would subsequently exhibit a lack of orientation to salient visual cues in the environment following disorientation procedures.

Long-evan rats, with recording electrodes in either the anterior thalamic nucleus or the CA1 hippocampus, were screened for cells in a cylindrical apparatus containing a white cue card. Before and after each recording session, the rats were rotated back-and-forth in an opaque container. Identified HD or place cells were recorded following rotation of the white cue card in the cylinder, and when possible, following rotation of a white cue curtain on the RAM. A total of 21 cue card rotation sessions in the cylinder have been conducted with 8 HD cells and 2 place cells. In 20/21 sessions, rotations of the cue led to a shift in the cell's preferred direction or firing field. On the RAM, rotation of the white cue curtain resulted in a corresponding shift in the HD cells preferred directions in all instances (8/8), though on one occasion a reorientation to the cue curtain was observed across recording days. These results suggest that the acquisition impairment attributed to disorientation in our previous study may not be due to an impaired perception of landmark stability per se, but rather to the animals' inability to utilize this landmark information to guide spatial behavior.

Supported by NIMH Grants MH 10843, MH 48924, MH 01286.

270.15

IMPAIRMENT OF LONG-TERM BUT NOT SHORT-TERM MEMORY FOR A SOCIALLY TRANSMITTED FOOD PREFERENCE TASK IN TRANSGENIC MICE WITH A SELECTIVE LOSS OF LTP IN THE THETA FREQUENCY RANGE.

R. Bourtchouladze* and M. Mayford. Ctr. Neurobiol. & Behav., Columbia Univ., New York, NY 10032.

The standard behavioral paradigms used to analyze the structural, physiological, and molecular contributions to learning and memory generally do not assess ecologically relevant learning and memory used by animals in their everyday behavior. We have adapted and developed the social transmission of food preferences paradigm, originally used for rats (Galef and Wigmore, 1983) for studying memory in genetically modified mice. Our pilot studies on C57/BL6 and DBA/2J mice showed that during social interaction olfactory cues pass from a recently fed mouse (a demonstrator) to a naive conspecific (observer) influencing that observer's subsequent diet selection. The observer exhibits enhanced preference for the diet its demonstrator ate. Using this paradigm we assessed learning and memory in CaMKII-Asp-286 transgenic mice. CaMKII-Asp-286 transgenic mice have a selective loss of hippocampal LTP in the range of the theta frequency and an impairment of spatial memory (Mayford et al., 1995). In the present study, memory for food preference was tested immediately (short-term memory) or 24 h after social training (long-term memory). Both transgenics and wild-type controls acquired the food preference normally. However, transgenic mice displayed significant loss of memory compared with controls when tested 24 h after social learning. These findings indicate that hippocampal LTP in the range of the theta frequency might be selectively involved in a long-term, but not short-term retention of natural, ecologically important memory. Supported by NIMH and NYSPI.

MOTIVATION AND EMOTION: BRAIN STIMULATION

271.1

CHLORISONDAMINE FAILS TO ATTENUATE LATERAL HYPOTHALAMIC BRAIN STIMULATION BUT BLOCKS ITS POTENTIATION BY NICOTINE.

P. Bauco*, R.A. Wise. Ctr. Stud. Behav. Neurobiol. and Dept. of Psychol., Concordia Univ., Montréal, Que., Canada, H3G 1M8.

A cholinergic projection from the pedunculo-pontine tegmental nucleus to the ventral tegmental area is thought to play an important role in the rewarding effects of lateral hypothalamic (LH) brain stimulation. Ventral tegmental dopamine neurons have both muscarinic and nicotinic receptors; thus we determined the effects of nicotinic blockade on LH self-stimulation. Chlorisondamine (CHL), a nicotinic antagonist, induces long term blockade of the locomotor stimulant and rewarding effects of nicotine. We assessed the ability of CHL to block self-stimulation and to block nicotine's ability to potentiate self-stimulation. Rats were first trained to lever press for LH stimulation using the rate-frequency curve-shift paradigm and then treated with CHL (10mg/kg, s.c.; n=7) or saline (n=6). Two days after treatment all animals were tested drug-free and then tested following nicotine (0.4mg/kg s.c.). Drug-free self-stimulation thresholds were normal but were not decreased by nicotine in CHL pretreated animals whereas they were decreased by an average 30% in animals not treated with CHL. Thus nicotinic receptors were effectively blocked by the treatment and do not normally play a significant role in LH self-stimulation.

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271.2

LATERAL HYPOTHALAMIC SELF-STIMULATION RELEASES ACETYLCHOLINE IN THE VENTRAL TEGMENTAL AREA (VTA).

P.V. Rada, G. Gibbs, J. S. Yeomans*, B. G. Hoebel. Dept. of Psychology, Princeton University, Princeton, NJ 08544-1010, USA

Research suggests that the behavior reinforcement circuit from the lateral hypothalamus (LH) to the mesolimbic dopamine system includes a descending pathway to the midbrain and pons where cholinergic cells project to dopamine cells in the VTA and from there to forebrain dopamine terminal regions (Yeomans et. al. 1993). To find additional evidence for this cholinergic link, acetylcholine (ACh) was sampled by microdialysis in the VTA every 20 min for 1 hr before, during and after rats performed electrical self-stimulation of the LH with implanted electrodes. The result was a 100% increase in VTA extracellular ACh during the self-stimulation period in 5 out of 5 animals. At a lower current, just above threshold for self-stimulation, ACh increased 40%. Similar results were obtained with automatic, non-contingent LH stimulation.

Atropine infused into the VTA by reverse dialysis in the same rats inhibited self-stimulation completely. These two experiments that (1) measure ACh and (2) block ACh receptors suggest that a cholinergic input to the VTA is important for hypothalamic self-stimulation. Apparently ACh neurons project from the midbrain and pons to the VTA carrying information necessary for the reinforcement of instrumental behavior as seen in the self-stimulation phenomenon. Supported by USPHS grant NS30697 to BGH

Reference: Yeomans, J.S. et. al., *Behav. Neurosci. 107*:1077-1087, 1993

271.3

EFFECTS OF ADENOSINE A2 RECEPTOR-SELECTIVE LIGANDS ON BRAIN STIMULATION REWARD THRESHOLDS IN THE RAT B.A. Baldo* and G.F. Koob. Dept. of Neuropharmacology, The Scripps Research Institute, La Jolla, CA 92037.

The nucleoside adenosine exerts inhibitory effects upon neural function throughout the brain. Several cell-surface G protein-linked adenosine receptors have been cloned which differ from one another in their pharmacologic binding profiles, regional distributions, and purported roles in behavioral regulation. The high-affinity adenosine A2a receptor exhibits the highest degree of specificity in its anatomical distribution within the brain, being highly expressed in nucleus accumbens, olfactory tubercle, and striatum. Within striatum, A2a receptors are co-localized with D2 receptors on striatal output neurons, where they are thought exert a negative modulatory influence upon dopaminergic function. In accordance with that hypothesis, agonists selective for A2 receptors exhibit dopamine antagonist-like effects in several behavioral assays. In order to assess the role of adenosine A2 receptors in modulating reward-related behavioral performance, the effect of a selective A2 receptor agonist, CGS 21680, and a selective A2 receptor antagonist, 8-(3-chlorostyryl)caffeine (CSC), on brain stimulation reward (BSR) thresholds was assessed. In accordance with the hypothesis that A2 agonists exert dopamine antagonist-like effects, CGS 21680 (0.1-1.0 mg/kg) significantly elevated BSR thresholds at doses which did not affect response latency or extra responses. In contrast, CSC (0.01-10.0 mg/kg) did not have any significant effects on BSR thresholds, extra responses, or response latency. These results suggest that stimulation of A2 receptors modulates central reward processes in the rat. This effect may be due to the interaction of the adenosine system with dopaminergic function. Supported by: DA04398, NSF Predoctoral Fellowship (BAB)

271.4

ENHANCED REWARD OF VENTRAL TEGMENTAL AREA SELF-STIMULATION OBSERVED WITH AMPHETAMINE INJECTIONS INTO THE CORE, BUT NOT THE SHELL, OF THE NUCLEUS ACCUMBENS. K. L. Sweet and D. B. Neill*. Department of Psychology, Emory University, Atlanta, GA 30322.

Amphetamine injections into the nucleus accumbens have been shown to increase the number of responses in intracranial self-stimulation (ICSS) of the ventral tegmental area (VTA). We used a rate independent method of ICSS -- autotitration self-stimulation, to examine this phenomenon. In our version of autotitration, the intensity of the stimulation dropped 3 μa every fifth lever press. A single response on a separate lever reset the intensity back to an individually determined maximum. Sprague-Dawley rats bearing electrodes in the VTA were trained on this procedure. Intra-accumbens injections of amphetamine sulfate (2.5, 5, and 10 μg in 1 μl) were made just prior to VTA ICSS sessions. Our findings showed that amphetamine injections into the core region of the nucleus accumbens, but not the shell, lowered the mean reset intensity. This was interpreted as reward enhancement.

Supported by grant IBN-9412703 from the National Science Foundation.

271.5

ALTERATIONS OF REWARDING BRAIN STIMULATION AND CENTRAL MONOAMINES ASSOCIATED WITH CYTOKINE ADMINISTRATION. L. Kokkinidis*, C. Song, Z. Merali & H. Anisman. Dept of Psychology, Univ of Saskatchewan, Saskatoon, Canada, School of Psychology, University of Ottawa, Ottawa, Canada, Institute of Neuroscience, Carleton University, Ottawa, Canada.

Cytokines released from activated immune cells may serve as messengers between the immune and central nervous system. Indeed, cytokines have been shown to influence endocrine and transmitter functioning, and may thus come to induce behavioral alterations. In the present investigation we demonstrate that interleukin (IL)-2 provokes a relatively sustained disruption of responding for rewarding brain stimulation from the medial forebrain bundle. Systemic IL-1β administration likewise induced a disruption of responding, but this effect was less marked than that induced by IL-2, and was relatively transient. In contrast to these treatments, IL-6 was without effect on responding for rewarding brain stimulation. Since, the rewarding value of lateral hypothalamic brain stimulation may involve DA activity within the nucleus accumbens, DA release was assessed in freely moving rats following cytokine treatment. Both IL-2 and IL-6 provoked marked inhibition of DA release from the accumbens, while IL-1 had little effect in this respect. Administration of IL-1 and IL-6 both were found to increase interstitial 5-HIAA, whereas IL-2 had no effect. Evidently, although cytokines have marked central amine effects, the alterations of responding for rewarding stimulation cannot simply be attributed to variations of accumbal DA activity.

Supported by the Medical Research Council of Canada

271.6

PEDUNCULOPONTINE TEGMENTAL LESIONS AND MEDIAL FOREBRAIN BUNDLE SELF-STIMULATION M. Waraczynski* and M. Perkins Dept. of Psychology, Univ. of Wisconsin-Whitewater, Whitewater, WI 53190.

Many studies of the neuroanatomical substrate supporting medial forebrain bundle (MFB) self-stimulation have involved lesioning forebrain targets suspected of contributing to that substrate. These lesions have had mixed success in revealing brain areas important to conducting the reward signal generated by MFB stimulation. This study investigated the role of a midbrain region, the pedunculopontine tegmentum (PPT). Monopolar stimulating electrodes were implanted in the lateral hypothalamus (LH) and ventral tegmental area (VTA) of male rats. At the same time, a lesioning electrode was implanted in the PPT area. The rats were trained to self-stimulate at both the LH and VTA sites, where possible, and baseline rate-frequency data were collected at three stimulating currents (typically 200, 400, and 800 uA) on a daily basis, until the frequency required to maintain half-maximal responding stabilized around some central value. The PPT lesion was then made by passing 300 uA of anodal current through the lesioning electrode for 60 seconds. In some cases these lesions resulted in modest but stable upward shifts in the frequency required to maintain half-maximal responding, at both the LH and VTA stimulation sites. This suggests that PPT lesions remove some part of the substrate supporting the rewarding effect of MFB stimulation.

This research is supported by FIRST grant #MH52588 from the National Institute of Mental Health to the first author.

271.7

SELF-INFUSION OF D1 AND D2 AGONISTS INTO THE NUCLEUS ACCUMBENS OF RATS. S. Ikemoto*, B.S. Glazier, J.M. Murphy, and W.J. McBride. Inst. of Psychiatric Res., Dept. of Psychiatry, Indiana Univ. Sch. of Med. and Dept. of Psychology, Purdue Sch. of Sci., IUPUI, Indianapolis, IN 46202.

The dopamine (DA) system within the nucleus accumbens (ACB) has been strongly implicated in brain reward processes. Rats self-administer amphetamine directly into the ACB and exhibit conditioned place preference following microinjection of DAergic agonists into the ACB. The objective of the present study was to examine the involvement of D1 and D2 receptors within the ACB in rewarding processes using the intracerebral self-administration paradigm. Rats (n = 7) were given the opportunity to self-infuse one of the following into the shell region of the ACB (100 nl/infusion): artificial CSF, the D1 agonist SKF 38393 (SKF, 0.5 and 1.0 mM), the D2 agonist quinpirole (QUIN, 0.5 mM), or the combination of 0.5 mM SKF and 0.5 mM QUIN. During the 3 hour session, the mean self-infusions (±SEM) were 11.9 (±1.5) for artificial CSF, 14.6 (±2.7) for 0.5 mM SKF, 14.3 (±3.1) for 1.0 mM SKF, 11.5 (±3.1) for 0.5 mM QUIN, and 29.4 (±3.4) for 0.5 mM SKF plus 0.5 mM QUIN; rats obtained statistically more infusions of the SKF/QUIN mixture than any of the other solutions, $p < 0.01$. Six out of 7 rats, when receiving the SKF/QUIN mixture, responded more on the lever that produced infusions than on the control lever that had no consequence. These results suggest that simultaneous activation of D1 and D2 receptors within the ACB shell produces a more potent rewarding effect than the activation of either receptor subtype alone. (Supported by the NIAAA, AA09619)

271.8

TOWARDS A DOSE-RESPONSE ANALYSIS OF INTRACRANIAL DRUG SELF-ADMINISTRATION. J.R. Stellar*, J. Chevrette, & G. W. Hesse Department of Psychology, Northeastern University, Boston, MA, 02115.

In an advance over the intracranial drug self-administration (ICSA) pump previously reported (Stellar & Lindenfeld Neurosci. Absts 1994), we have developed a new pump based on a small stepper motor that drives a threaded plunger into a Delrin block to expel 10 NL of fluid through PE tubing. The tubing is connected to a 30g stainless steel injector tip that is inserted through a guide cannulae into the accumbens of the rat brain. A Plastic One lead provides a head anchor for and mechanical support to the drug containing tubing.

Amphetamine (1.66 ug/injection) is self-infused by pressing a trans-illuminated response key on a VI-3 second schedule. After a drug injection is earned a 3 minute black out period occurs during which the key light is out and responding is ineffective. When the key light is again illuminated, the first ICSA response starts up the VI. In 30 or 60 minute sessions, rats are observed to respond up to 300 responses per hour during the VI schedule time, some 10 times what had been previously reported. When the amphetamine was discontinued, ICSA behavior underwent extinction followed by re-acquisition when the drug was reinstated.

Having established high rates of ICSA responding, we are now attempting to create an effective dose-response curve allowing an ED-50 analysis of drug reward potency that is much like that seen with electrical self-stimulation in the standard rate-frequency curve.

(Supported by University overhead-return funds from past NIDA grant.)

271.9

VENTRAL PALLIDUM SELF-STIMULATION (VP-SS) FOLLOWING MORPHINE INJECTED EITHER SYSTEMICLY OR INTO THE MESOLIMBIC DA SYSTEM. G. Panagis,* A. Kastellakis and C. Spyraki. Lab. Pharmacology, Dept. Basic Sciences, School of Medicine, University of Crete, Heraklion 71110, Greece.

The dopaminergic and enkephalinergic systems have been known to modulate brain stimulation reward. Recently we reported that VP-SS is influenced by pharmacological manipulations of the DA neurotransmission (Panagis and Spyraki, 1996, Psychopharmacology, 123: 280-288). We have also shown that enkephalinergic-DAergic interactions exist between the pallidum and the NAC (Anagnostakis and Spyraki, 1994, Brain Res. Bull., 34: 275-282). In the present study, the influence of morphine on VP-SS was assessed using the curve-shift paradigm. Rats received treatment with morphine administered either systemicly (1.25; 2.5 mg/kg, i.p.) or into the VTA (1.25; 2.5; 5.0 µg/0.5 µl/side) or the NAC (0.625; 1.25; 2.5; 5.0; 10.0 µg/0.5 µl/side). The effects of the different doses of the drug on threshold and asymptotic rate were determined immediately or one hour after administration and for a period of 1-3 hours. We observed that systemic morphine -at only one dose (1.25mg/kg, i.p.)- significantly increased (25-30%) the VP-SS threshold but not the bar-pressing rate. Morphine injections into the VTA did not influence VP-SS threshold or performance. A tendency for lower VP-SS thresholds was noticed after some doses of morphine (1.25-5.0µg) injected into the NAC. The results with systemic morphine were unexpected and point to an antagonistic effect of the drug on VP-SS reward. Furthermore the results with the intacerebral injections show that enkephalinergic manipulations within the mesolimbic DA system have a marginal effect, if any, on VP-SS reward. The results suggest that morphine lacks a facilitatory role in VP-SS. Due to the known excitatory effect of morphine on DA neurons, the results also raise questions about the involvement of DA mesolimbic system in the mediation of VP-SS reward.

Supported by PENED 665 (C.S.) and a U.C. predoctoral fellowship (G.P.)

271.10

EXAMINATION OF AMINOGLUTETHIMIDE INDUCED SENSITIZATION OF LATERAL HYPOTHALAMIC SELF-STIMULATION (LHSS) IN FOOD RESTRICTED RATS, G.C. Abrahamsen*, M. Kandawire and K.D. Carr, Dept. of Psychiatry, NYU Med. Ctr., New York, NY 10016.

A number of recent studies have suggested that corticosterone may play an important role in stress induced sensitization of drug effects. We have recently shown that acute decreases in circulating corticosterone have no effect on the sensitization of LHSS observed in food restricted rats. Furthermore, the steroid synthesis inhibitor aminoglutethimide further sensitizes LHSS in food restricted rats two hours following administration when blood corticosterone is back to pre-injection levels. Two experiments were conducted to examine the possible mechanisms underlying this effect. Experiment 1 demonstrated that aminoglutethimide (50 mg/kg) sensitized LHSS in food restricted rats following systemic, but not central, administration. Systemic aminoglutethimide also produced elevated serum levels of the steroid precursor pregnenolone in food restricted rats. Given that prior studies have shown that adrenocortical precursors of corticosterone can alter brain $GABA_A$ receptor activity, a second study was conducted to assess the effects of pregnenolone and progesterone on LHSS in food restricted and control rats. Systemic administration of pregnenolone and progesterone (.1, 1 and 10 mg/kg each) each failed to affect LHSS in food restricted or control subjects. Current studies examining the opioid and GABAergic contribution to aminoglutethimide sensitization of LHSS in food restricted rats will be discussed.

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271.11

FOS EXPRESSION IN THE CAUDAL DIENCEPHALON AND BRAINSTEM FOLLOWING LATERAL HYPOTHALAMIC SELF-STIMULATION. A. Arvanitogiannis*, C. Flores, and P. Shizgal. CSBN, Concordia University, Montréal, Québec, Canada H3G 1M8.

Expression of immediate early genes provides a means of visualizing activated neurons. Previously, we employed Fos immunohistochemistry to stain forebrain neurons driven by rewarding stimulation of the lateral hypothalamus (LH). In the present study, we assessed the effect of such stimulation on Fos-like immunoreactivity (FLIR) in the caudal diencephalon, midbrain and hindbrain. During a 1 h test session, rats worked for 0.5 s stimulation trains composed of 0.1 msec, 800-1000 µA pulses, at the minimal stimulation frequency required to maintain asymptotic responding. The rats were perfused 15 min later, and their brains were then removed and processed for FLIR. Among the areas where FLIR was greater ipsilateral to the stimulation electrode are the posterior LH, arcuate nucleus, dorsomedial hypothalamus (DMH), central gray, ventral tegmental area (VTA), pedunculopontine area (PPTg), pontine nuclei, locus coeruleus, and parabrachial nucleus (PBN); cells in the dorsal and median raphé nuclei were stained bilaterally. It has been proposed that transsynaptically activated neurons in the VTA and PPTg contribute to the rewarding effect of MFB stimulation. Although lesion results fail to confirm a role for DMH or PBN neurons in MFB self-stimulation, the role of many of the other groups of Fos-positive cells has not been evaluated by means of modern lesion methods.

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271.12

REWARDING EFFECTS OF INTRA-ACCUMBENS INJECTIONS OF TESTOSTERONE A. Cornell, M. G. Packard, G. M. Alexander, & B. King* Dept. of Psychology, University of New Orleans, N.O., LA, 70148.

Peripheral injections of testosterone have rewarding effects in male rats as measured in a conditioned place preference paradigm, a technique commonly used to study the affective properties of drugs. The present study examined the neuroanatomical bases of the rewarding properties of testosterone by administering the hormone directly into the nucleus accumbens of male rats. On alternating days, rats implanted with bilateral cannulae in the nucleus accumbens received intracerebral injections of testosterone in a water-soluble cyclodextrin inclusion complex (0.125, 0.25, or 0.5 ug/0.5 ul), or saline immediately prior to being confined for 30 minutes to one of two compartments of a place preference apparatus. All rats received 8 days of pairings (4 hormone parings, 4 saline parings). On day 9 the rats were given a 20 minute test session during which they had access to all compartments of the apparatus. No hormone was injected prior to the test session. On the test day, rats spent significantly more time in the compartment previously paired with bilateral intra-accumbens injections of testosterone (0.25 and 0.5 ug/0.5 ul) than in the compartment previously paired with saline injections. The findings indicate that intra-accumbens injections of testosterone are *sufficient* to produce reward. The rewarding affective properties of testosterone may, in part, mediate the role of the hormone in enhancing sexual motivation. The findings also add tentative support to the hypothesis that androgenic anabolic steroids may possess addictive properties in humans.

271.12

THE EFFECTS OF CHOLECYSTOKININ-8 ON FEEDING AND REWARD THRESHOLDS INDUCED BY ELECTRICAL STIMULATION OF THE LATERAL HYPOTHALAMUS, S.L. Kubelka* and C.H. Bielajew. School of Psychology, Univ. of Ottawa, Ottawa, Ontario, Canada K1N 6N5.

We had demonstrated (Society for Neuroscience Abstracts, 1991) that bombesin, a putative satiety peptide, increased thresholds for stimulation-induced feeding (SIF) without influencing self-stimulation (SS) thresholds elicited from the same electrode, suggesting that the neural pathways mediating feeding and reward are pharmacologically distinct. The present study evaluates whether cholecystokinin octapeptide (CCK-8), a related satiety peptide, has the same effect. Two groups of animals were used; those that exhibited both SIF and SS from the same lateral hypothalamic electrode, and a second group from which only SS was observed; this second group was included in order to investigate the effects of CCK-8 in a SS site that did not support feeding. The frequency thresholds associated with each current were assessed following 0, 3, 6, and 9 µg/kg intraperitoneal injections of CCK-8. The results of a randomized block anova showed no significant effect of current level or dose of CCK-8 on the frequency thresholds of either behaviour when compared to the saline condition; likewise, the SS rates varied negligibly throughout the course of the study. Others studying CCK-8 have reported a dose-dependent reduction when food intake rather than feeding threshold is measured. Unlike our earlier findings with bombesin, SIF thresholds appear to be less sensitive to the peripheral effects of CCK-8; we are currently evaluating central CCK's effects using more resolved SIF measures. [This research was funded by a Natural Sciences and Engineering Research Council of Canada grant.]

271.14

TREATMENT EFFECTS OF PAROXETINE ON REWARDING MEDIAL FOREBRAIN BUNDLE STIMULATION IN RATS. A.T.M. Konkle and C.H. Bielajew*. School of Psychology, Univ. of Ottawa, Ont., K1N 6N5, Canada.

In order to assess the self-stimulation paradigm as a potential animal model of depression in which to study the effects of the selective serotonergic re-uptake inhibitor, Paroxetine, the acute and chronic treatment effects on the thresholds obtained from rewarding medial forebrain bundle stimulation were determined. Male rats were implanted with a monopolar stimulating electrode in either the lateral hypothalamus or ventral tegmental area, and trained to self-administer rewarding stimulation. Animals received daily systemic injections of one of three doses of paroxetine, either with or without stimulation, and the third group received the same number of vehicle injections with stimulation. Threshold values were collected over a period of six hours on day 1 (acute phase); no marked difference in the thresholds were observed. The animals were tested every third day thereafter (chronic phase), for a total of ten sessions. Paroxetine produced a delayed facilitation in self-stimulation similar to the time course of the effects observed clinically. In addition, the animals' weight and food intake were monitored daily. A marked difference between the control and stimulated groups in % efficiency of food utilization, measured by calculating the ratio of weight change to food intake, was observed during drug treatment. Interestingly, this effect persisted only in animals not receiving stimulation, in that they continued to demonstrate a gradual weight loss throughout the study, while animals receiving stimulation recovered and in fact, showed a weight gain. This suggests that stimulation counteracts the anorectic effects of chronic Paroxetine treatment.

[This work was supported by a Natural Sciences and Engineering Research Council of Canada grant]

271.15

DISCREPANCY BETWEEN RESPONSE RATE AND TIME ALLOCATION AS MEASURES OF RESPONSE STRENGTH: IMPLICATIONS FOR THE MEASUREMENT OF REWARD MAGNITUDE. K. Conover*, S. Fulton, R. D'Abramo, and P. Shizgal. CSBN, Concordia University, Montréal, Québec, Canada H3G 1M8.

The single-operant matching law relates response strength to the relative value of an experimenter-controlled reinforcer and competing sources of reinforcement not under experimental control. In deriving these relationships from first principles, Heyman assumed that response rate and time allocation are equivalent measures of response strength. To test this assumption, we examined the distribution of inter-response times (IRTs) in rats working for brain stimulation reward (BSR). If the rats divide their time between working for BSR and other activities (e.g. exploring or grooming), responses for BSR should occur in bursts, and a log-survivor analysis should reveal two populations of IRTs, a "work" distribution with a shorter mean and a "leisure" distribution with a longer mean. As the value of the experimenter-controlled reinforcer is increased, so should the proportion of IRTs belonging to the work distribution. These predictions were confirmed. However, contrary to Heyman's assumption, the mean of the work distribution increased linearly with the variable interval (VI), and response rates changed dramatically over a range of VIs where time allocation was asymptotic. These results have implications for matching-based analyses of the effects of drugs, lesions, hormones and physiological manipulations on response strength. Time allocation may prove superior to response rate as an index of the magnitude of reinforcement and the sensitivity of the neural circuitry that computes this value.

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271.16

REWARDING BRAIN STIMULATION INDUCES ONLY SPARSE C-FOS IMMUNOREACTIVITY IN DOPAMINERGIC NEURONS

Iain S. McGregor* and Glenn E. Hunt Departments of Psychology and Psychiatry, University of Sydney, NSW 2006, Australia

FOS immunohistochemistry was used to map brain areas activated by rewarding brain stimulation. Rats were implanted with monopolar electrodes aimed at the lateral hypothalamus. Prior to their first and only one-hour test session, the rats were randomly divided into three groups: self-stimulators (n=4), yoked controls (n=4) and unstimulated controls (n=6). For the self-stimulators, a nose-poke response triggered delivery of 0.5 sec pulses of brain stimulation to themselves as well as to their yoked partner in an adjacent chamber. Nose pokes did not initiate the stimulus for the yoked-controls or in unstimulated controls. In the test session, self-stimulating rats completed 1000 responses in 40 minutes (or less) and were perfused 1hr later.

A high density of FOS immunoreactivity was seen in both self-stimulators and yoked animals, but not in unstimulated controls. FOS was particularly prominent in the lateral septum, nucleus accumbens (shell), and the paraventricular, dorsomedial and lateral hypothalamus. Other less densely activated areas included the medial prefrontal cortex, the medial and lateral preoptic areas, the bed nucleus of the stria terminalis, the anterior ventral tegmental area and the locus coeruleus. Co-staining of FOS and tyrosine hydroxylase (TH) was seen in only a small proportion of A10 dopamine neurons in the VTA. FOS was also present in the retrorubial fields but not in TH positive neurons. In contrast, the FOS expressed in the locus coeruleus was co-labeled with TH indicating that almost all of these A6 neurons were activated after electrical stimulation. Other TH positive neurons that were FOS positive included many of the A5 and A7 noradrenergic neurons located in the brain stem, and a few A12 dopamine neurons in the hypothalamus. Double labelling was not present in the intra-hypothalamic dopaminergic cells (A11, A13 or A14 cells).

Thus when neuronal activation is indexed by FOS immunoreactivity, rewarding brain stimulation does not appear to preferentially activate dopaminergic neurons.

National Health and Medical Research Council of Australia

271.17

PERFORMANCE FOR BRAIN STIMULATION REWARD AS A FUNCTION OF THE RATE AND MAGNITUDE OF REINFORCEMENT. P. Shizgal*, K. Conover, and A. Arvanitogiannis. CSBN, Concordia University, Montréal, Québec, Canada H3G 1M8.

The curve-shift paradigm has been used to distinguish whether drugs and lesions alter performance for brain stimulation reward (BSR) by changing the magnitude of the BSR or the performance capacity of the subject. We show that in principle, such a distinction cannot be drawn unambiguously because identical changes in the function that maps stimulation strength into response strength can be produced either by altering the rate of reinforcement or by simultaneously altering both the magnitude of BSR and the performance ceiling. These effects can be disambiguated by measuring performance as a function of both reinforcement rate *and* stimulation strength. We dub the resulting three-dimensional (3D) structure "the reinforcement mountain." Analysis of how the mountain shifts in the 3D space can reveal whether lesions, drugs, or physiological manipulations alter BSR before or after the output of the ("reward-growth") function that translates the electrically-induced impulse flow into the magnitude of reinforcement. Among the questions that can be addressed by means of this 3D analysis are whether basal forebrain neurons with descending medial forebrain bundle (MFB) projections constitute an early stage in the circuit subserving MFB self-stimulation and whether dopamine neurons contribute to BSR before or after the output of the reward-growth function.

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271.18

REWARDING ELECTRICAL STIMULATION OF THE VENTRAL TEGMENTAL AREA ATTENUATES PAIN DURING THE FORMALIN TEST. R.M. Anderson* & P.-P. Rompré. Psychiatry Department, University of Montréal(Québec), Canada, H3C 3J7.

Anderson and colleagues (1995) have shown that rewarding electrical stimulation of the ventral tegmental area (VTA) abolished the aversion produced by stimulation of the nucleus reticularis gigantocellularis (Gi). Given that the Gi is involved in pain, they hypothesized that VTA electrical stimulation-induced analgesia, and the present study was designed to test this hypothesis. Male rats were implanted with a right VTA stimulating electrode and were separated into three groups: VTA-self-stimulation, VTA-trained, VTA-sham (never stimulated). Phasic and tonic pain were induced by a 0.05 ml injection of 1.5% formalin into the left or right rear paw and pain scores were rated according to the scale of Dubuisson & Dennis (1979) in each group of rats for one hour. Results show that pain scores were significantly lower in the VTA-self-stimulation compared to VTA-trained and VTA-sham rats, and the level of analgesia was not dependent upon the side of formalin injection. This analgesic effect, however, was observed only at stimulation frequencies that sustained asymptotic rates of responding. Moreover, reward thresholds were not significantly altered either during the formalin test, or the following day. These results provide evidence that rewarding electrical stimulation of the VTA has analgesic properties, a finding that may have clinical implications for the relief of chronic pain.

Supported by a grant from NSERC to P.P.R.

BIOLOGICAL RHYTHMS AND SLEEP: CIRCADIAN RHYTHMS AND SLEEP

272.1

SLEEP DEPRIVATION AND STATE COLLEGE STUDENTS. L. Amini-Sereshki* and J. Allard. Biology and Psychology Departments, Worcester State College, Worcester, MA 01602.

This study was conducted to examine the effect of amount of sleep on the academic performance of randomly selected state college students. Sixty seven students were included in this study. Twenty-two and a half% reported that during weekdays they received 8-9 hours of sleep per night, 76.1% received 5-7 hours of sleep, and 1.5% less than 4 hours of sleep. On weekends, however, 28 of the 57 students (49%) received 8-9 hours of sleep per night, another 49% received 5-7, and the remaining 2% received only one to four hours of sleep per night. Sleep time was described by 62.7 % as insufficient. There was a significant relationship between the frequency of difficulty in concentrating in the classroom and insufficient sleep time. Thirty five % reported that sleepiness interferes with their concentration in class at least twice per week and 6% reported that they often had difficulty concentrating in class because they were sleepy. Thirty four and a half % of the students experienced a poor examination performance due to sleepiness more than once during the academic year. Seventy % of the students worked in addition to attending school. Twenty one % worked less than 10 hours/week, 46% 10-20 hrs/wk, 29% 21-40 hrs/wk, and 4% more than 40 hrs/wk. Working was not related to whether students felt they were getting enough sleep, but the number of hours they worked was related to whether they felt they were getting enough sleep. This preliminary study suggested that insufficient sleep interferes with the students academic performance.

This work was in part supported by a WSC grant to L.A.

272.2

3D SPECTRAL REPRESENTATION SOFTWARE. APPLICATIONS IN HUMAN SLEEP STUDIES OF NORMAL AND TEMPORAL LOBE EPILEPTIC SUBJECTS.

R. Fernández-Mas, A. Martínez-Cervantes, A. Valdés-Cruz, V.M. Magdaleno-Madrigal and R. Salceda*. Div. De Invest. En Neurociencias, Inst. Mex. Psiquiatría, México, 14370 D.F.

Automatic sleep evaluation and scoring algorithms do not provide 100% reliability yet. In our sleep recordings, we need very high temporal resolution to allow the cuantification of small micro-stage changes in the EEG. This is done by means of visual comparation of the power spectra distribution of each EEG channel. This method consists of a main tridimensional (3D) power spectra graph, and adjacent sub-bands indicating the absolute or relative power in the elecrooculogram (EOG) and the electromyiogram (EMG) channels, using false color spectral display. This graphical approach to the solution of the cuantification of sleep, of course, depends on the operator's experience on sleep scoring, but offers more data in terms of the architecture of the Fourier coefficients as a function of time, and can be used to calibrate the band select criteria of automatic sleep scoring programs.

We used this method in normal and epileptic subjects to compare the visual manual scoring criteria with online cuantification, and to measure the micro frequency-shifting of the Alpha rhythm, sleep spindles and other subtle changes in the EEG embedded rythms. The system was designed using a SUN workstation for the graphical display and a parallel machine using DSP chips devoted to Fourier estimations in real time. This software was written using C++ SPARC compiler and the graphical generation using the X window environment.

We conclude that the on-line cuantification of sleep looses some relevant data on the frequency domain. This software can be used as an aid, where high resolution in the frequency domain evolution is needed in a multi channel real time recording.

Work partially supported by IMP grant #3410 and CONACyT #3605N, DGAPA-UNAM IN-202894 and PUIS-UNAM.

272.3

THE RELATIONSHIP OF AUDITORY STIMULATION TO THE K-COMPLEX. M.L. Brazas*, Psychology Department, University of South Florida, Tampa, FL. 33620.

Previous studies of the K-complex of stage 2 sleep in humans have failed to exclude the possibility that the K-complex is an auditory evoked response. Past work has been confounded by the ability to the subjects to hear sounds, thus raising the possibility that all K-complexes produced were the result of auditory stimulation.

This study tested the hypothesis that spontaneous K-complexes occur in the absence of auditory stimulation.

The EEG of four congenitally deaf subjects (2 male and 2 female) were recorded overnight using an ambulatory EEG recorder. An age and sex matched control group was also recorded. Both spontaneous and evoked-type K-complexes were found in all records. Sleep staging demonstrated sleep parameters consistent with a first night of lab recording. The perimental group, however, had slightly higher REM sleep percentages and shorter REM latencies than the control group. These results were also different from normative data collected. The presence of K-complexes in a group of subjects incapable of processing sounds clearly demonstrates that the K-complex cannot be an auditory evoked event.

This study was sponsored in part by Oxford Instruments, INC., Clearwater, FL.

272.4

EFFECTS OF THE GENERAL ANESTHETIC ISOFLURANE ON GAMMA RHYTHMS AND CONSCIOUSNESS IN HUMANS.
G. Plourde, C. Villemure, P. Fiset, A. Achim, S. Backman* Dept. of Anesthesia, Royal Victoria Hospital and McGill University; LNC, UQAM, Montreal (Quebec) Canada H3A 1A1.

Whether gamma rhythms reflect physiological processes linked to consciousness is controversial. We examined the effects of isoflurane (ISO) on consciousness and gamma rhythms, using the 40 Hz auditory steady-state response (ASSR) in 10 volunteers (18-35 yrs). Stimuli were 500 Hz tonebursts (10 ms: 82 dB PeSPL; right ear; 35-45/sec). Consciousness was defined as responsiveness to verbal commands. EEG was recorded from Cz-M2 (0.1-100 Hz). Responses to 18,000 stimuli were averaged for each period. The periods were: baseline; ISO 0.50; ISO 0.38; ISO 0.26 (end-tidal vol% concentration with 15 mins equilibration) and recovery. ASSR amplitude (measured by FFT) (μV) was [mean (SD)] 0.31 (0.26) during baseline; 0.04 (0.03) for ISO 0.50; 0.09 (0.05) for ISO 0.38; 0.28 (0.26) for ISO 0.26 and 0.29 (0.33) during recovery. Significant differences ($P<0.05$ - ANOVA/Tukey's HSD) were: baseline and recovery > ISO 0.38 and ISO 0.50; ISO 0.26 > ISO 0.38 > ISO 0.50. The number of unconscious subjects was 0, 4 and 9 for ISO 0.26, 0.38 and 0.50 respectively. We conclude that the concentration ranges for suppression of consciousness and maximal attenuation of the ASSR by isoflurane are similar.

Supported by FRSQ, Canadian Anaesthetists' Society and Ohmeda (Pharmac. Div.).

272.5

VIGILANCE STAGES DURING NOCTURNAL HYPOTHERMIA IN WELL-FASTED PIGEONS. M.E. Rashotte*[1], E.L. Polyakov[2], R.P. Henderson[1] and Yu.F. Pastukhov[2]. [1] Program in Neuroscience, Dept. of Psychology, Florida State Univ., Tallahassee, FL 32306-1051; [2] Sechenov Institute of Evolutionary Physiology & Biochemistry, St. Petersburg, Russia.

Vigilance stages in feeding and in fasted pigeons have been reported to be similar, unlike in other birds studied (doves, geese and penguins). Fasting was more severe in the latter cases, however, and we now report that deeper fasting results in changed nocturnal vigilance stages in pigeons also. Cortical EEG, EOG, neck and pectoral muscle EMG, EKG, abdominal body temperature (Tb), and behavior were recorded for 24h periods when food was ad lib and after fasting had induced ≈ 20% loss in body weight (LD 12h:12h; Ta = 21 °C). Six pigeons (≈ 350g original body weight) were studied. Dark-phase electrophysiological data were scored in 1s epochs for vigilance stage: wakefulness (W), transitional stages (T), slow wave sleep (SWS), and paradoxical sleep (PS). Fasting induced about 3.6 °C decrease in nocturnal Tb (36.3 °C vs. 39.9 °C). Total sleep time/night (SWS + PS) was higher in fasting (86%) than in feeding (75.5%), and the PS/SWS ratio was lower in fasting (0.18 vs. 0.22). In fasting, W and T decreased by 75% and 20%, respectively, and SWS increased by 15%. SWS episode duration increased by 30%. The only significant change in PS occurred in one bird (a decrease from 11.4% to 8.6% per night); the other birds showed small increases or decreases. Detailed examination of Tb, vigilance stages, EKG, and pectoral EMG in different parts of the dark phase suggests interactions which might relate to energy conservation in shallow daily nocturnal hypothermia in pigeons, and also to the criteria for identifying PS in birds. The pigeon may be an interesting model system in which to study the relationship between vigilance stages and shallow torpor, and to examine the possibility that experimentally-induced larger changes in sleep stages would result in deeper torpor. *Supported by NSF Grants IBN-9421924 and IBN-9222369.*

272.6

MATERNAL DEPRIVATION ALTERS A REM SLEEP ASSOCIATED BEHAVIOR C.M. Anderson[1]*, A.J. Mandell[2], K. A. Selz[2], L.M. Terry[3], S.L. Andersen[1] & M.H. Teicher[1] [1]Dept. of Psychiatry, Harvard Med. Sch. & Developmental Biopsychiatry Research Program, McLean Hospital, Belmont, MA 02178. [2]Dept. of Psychiatry & Behavioral Sciences, Emory Univ. Sch. of Med., Atlanta, GA 30322. [3]Dept. of Psychology, College of Liberal Arts, Florida Atlantic Univ., Davie, FL. 33314.

Periods of maternal deprivation (MD) in 2 to 10-day-old neonatal rats resulted in acute changes in spontaneous sequences of nuchal muscle atoina (NA) associated with REM sleep. We analyzed these highly variable recordings (surface EMG sampled for 2 hours @ 300 Hz) with Hurst's rescaled range analysis (Range normalized by S.D.; R/S), which affords comparisons between time series of fluctuations with non-convergent statistical moments. This analysis indicated that spontaneous fluctuations in NA in neonatal rats over short time scales was statistically similar to longer time scales (i.e., fractal in time). NA sequences in neonatal rats, which are in a REM sleep-like state > 50% of the time, were also found to be described by long-tailed convolutionally stable self-similar Lévy distributions. When neonates were subjected to 2 hours of MD, an increase of the mean length of NA and a decrease in episode number was observed over and above normal maturation changes compared with 0 hours of MD. Also a significant increase in the Hurst exponent $F(1,24) = 5.692$, $p<0.05$ and a decrease in the Lévy α exponents were found (e.g., animals with 2 hours of MD collapsed across age were found to be significantly different from 0 hours of MD, $F(1,4) = 415.028$, $p<0.0001$) suggesting that MD changes the nature of the underlying probability density distributions resulting in more clustered behavior in time. Rats MD and cold stressed 4 hrs/day from P2 to P20, demonstrated the same differences from controls at P50 in spontaneous pauses in locomotor activity. These findings suggest that mathematical tools sensitive to subtle changes in self-similar clustering of spontaneous highly variable behaviors over multiple time scales may have utility for studing children or adults who suffered early stress, abuse or neglect. (Supported by MH-43743-07, MH-53636-01A1 the FAU foundation and ONR Biological Intelligence Div.)

272.7

THE EFFECTS OF TIME OF DAY AND DECIBEL ON THE ACOUSTIC STARTLE RESPONSE OF THE EGYPTIAN SPINY MOUSE, *ACOMYS CAHIRINUS*. C.C. Chabot*, S.R. Boulé, and J.P. DelPrete. Department of Natural Sciences, Plymouth State College, Plymouth, NH. 03264.

Previous studies with rats have demonstrated a daily modulation of the acoustic startle response (ASR) amplitude. Until now this modulation had yet to be demonstrated in any other mammal. We report here similar findings in the Egyptian spiny mouse. ASR amplitude of male and female Egyptian spiny mice (*Acomys cahirinus*, n=11 and 5 respectively; 72-79 days old) was measured over four sessions in a twenty-four hour period. The animals were housed in an LD 12:12 cycle, 4/cage in a light-tight chamber with food available ad libitum. During testing, mice were placed individually in a 5 x 5 x 8 cm wire cage in a sound attenuated chamber where they were allowed to acclimate for ten minutes before each session. Each session consisted of 10 trials each at 80, 90, 100, 110, and 120 db (42 msec white noise) with an interstimulus interval of 22 sec. Differences between means were assessed using repeated measures ANOVA ($p<.05$) and pre-planned mean contrasts. We found a significant effect of time of day and db on response amplitude. Pre-planned contrasts indicated significantly increased responses during D versus L. There was a significant effect of db, but not time of day on latency. There were no significant effects of sex on either response amplitude or latency. These results are essentially identical to those seen previously in rats and thus extends these findings to another mammalian species. Funded by the Department of Natural Sciences, Plymouth State College.

272.8

LIGHT-DARK CYCLE MASKING OF WHEEL-RUNNING IS REDUCED IN AGED MICE. M.J. Bradbury*, M.J.H. Kas, W.C. Dement and D. M. Edgar. Stanford Sleep Research Center, Stanford University School of Medicine, Stanford CA, 94305.

Aging leads to dampened amplitudes of entrainable circadian rhythms in rodents, possibly due to impaired photic transduction mechanisms within the SCN. While light entrains the SCN, modulation of behaviors by light-dark (LD) cycles in SCN-lesioned mammals demonstrates that neurons outside of the SCN can generate masking signals . It is not known if the transduction of LD masking is also attenuated with age. Therefore, we employed a short-day photoperiod regime to assess age-differences in light-dark masking of wheel-running activity in male, C57BL6 mice. In LD 12:12 cycle (30 lux), 8 young (3 months of age) and 7 old (26 months of age) mice ran more during the dark phase ($p=0.0008$ and 0.02, respectively). However, old mice ran less than young mice during the dark phase ($p=0.0002$). Twenty-four hours of a LD 1:1 cycle, a photoperiod outside of the range of entrainment, (30 lux) started at former ZT 13. Young, but not old mice ran more during the former ZT 12-23 than former ZT 0-11 (young, $p=0.0006$). Data from this 24 hour trial were divided into quartiles based on the former LD cycle. Running during dark pulses and light pulses was expressed as the proportion of total running in each quartile. Masking, as assessed by the inhibition of wheel-running during light, was greater in young mice than in old. Young rats ran less during the light pulses than during dark pulses in all quartiles (former ZTs: 12-17, $p=0.0001$; 18-23, $p=0.0002$; 0-5, $p=0.01$; 6-11, $p=0.04$). Old mice ran less during light pulses than during the dark pulses corresponding to the former ZT hours of 18-23 ($p=0.001$) and 6-11 ($p=0.04$) only. Thus, LD 1:1 fragments the waveform of wheel-running in aged mice more than in young mice. In addition, inhibition of running during 1 hour light pulses was attenuated in aged mice. Supported by AG06490.

272.9

THE EFFECT OF TIME OF TESTING ON THE BEHAVIOR OF RATS AS DETERMINED IN A DRUG DISCRIMINATION PARADIGM, E. Fisher* and B.L. Stringer, Pharmaceutical Sciences. SW OK State Univ., School of Pharmacy, Weatherford, OK 73096

It is well known that a large number of drug effects are influenced by circadian rhythms, and that rats can be trained to discriminate phencyclidine from saline. The research performed was designed to determine if the time of day that testing occurred would effect the learning of rats trained in a drug discriminative stimulus paradigm. Twelve rats were divided into two equal groups designated as AM and PM. The AM group was injected at the beginning of a 12 hour light cycle; the PM group at the end of this cycle. Following a double alternation schedule, rats were injected with 2 mg/Kg of phencyclidine or physiological saline. Rats underwent daily 15 minute training sessions Monday through Friday in a modified Skinner box containing two levers. Reinforcements were obtained on a fixed-ratio schedule of 32 with only the phencyclidine appropriate, or the saline appropriate lever activated. Training continued for a total of 100 sessions. Upon statistical analyses, the PM group showed a statistically higher response rate (P=0.028), and a higher percent correct lever responding (P=0.001) when injected with saline than did the AM group. The AM group showed a statistically higher response rate (P=0.005), and a higher percent correct lever responding (P=0.020) when injected with phencyclidine than did the PM group. These results indicate a definite circadian influence on the effects of phencyclidine as measured in this drug discrimination paradigm.

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272.10

EFFECTS OF ACUTE STRESS ON THE CIRCADIAN CYCLE OF VIGILANCE IN THE RAT: AN INTER-INDIVIDUAL ANALYSIS. J.J. Bouyer *, J.M. Deminière, W. Mayo, H. Simon and M. Le Moal. INSERM U.259, Université de Bordeaux II, 33077 Bordeaux Cedex, France.

Acute stress is given to modify circadian rhythmicity and stress hormones such as glucocorticoids and melatonin are involved in vigilance cycles. Sleep-wakefulness parameters are submitted to inter-individual differences and they have been used to characterize individuals -animals and humans- into "early vs. late subjects" and "short vs. long sleepers", their combination defined for a given individual a relatively stable structure which define a stable relation between the parameters across time. The aim of the present study was to verify the existence of such structure in the laboratory rat, to test the effect of acute stress and to examine whether after stress the individual was able to recover its pre-stress parameters. Intra-individual stability and inter-individual variability were assessed through four circadian parameters: 24 hr mean quantity of wakefulness (25.3 ± 0.6 min/h), amplitude of cycle (15.2 ± 0.9 min/h), beginning time of active period (19:18 ± 12 min) and its duration (8 hr 49 min ± 27 min). The parameters remained unchanged during three recordings and even if environmental factors such as an immobilization stress (IS) transiently modified them, they returned to baseline values some days later. IS induced a phase advance of beginning time of the active period (17:30 ± 42 min, $p<0.05$) and increased its duration (10 hr 05 min ± 27 min, $p<0.05$). Inter-individual differences between short and long sleepers ($p<0.001$) was faded after IS (ns): short sleepers slept more, long sleepers slept less. These modifications were attributed to the stress, without any implication of the sleep deprivation inherent to immobilization. To sum up, in all subjects, stress induced phase advance and lengthened the active period. However, inter-individual analysis evidenced a stress effect on the wakefulness quantity different according to subjects.

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272.11

CIRCADIAN ACTIVITY IN THE MONGOLIAN GERBIL: BOTH NOCTURNAL AND DIURNAL RHYTHMS. J. Hallonquist*, P. Gray-Allan, T. Lao and R. Wong. Dept. Psychology, Univ. British Columbia, Vancouver, B.C., Canada V6T 1Z4.

Because reports of circadian rhythms in Meriones unguiculatus conflict, we monitored wheel-running in LD 12:12 -- first in 34 gerbils from Tumblebrook Farms. The last of 6 wks indicated 21 to be nocturnal (N), 5 to be diurnal (D), and 8 to be "questionable" ("?") due to inactivity or unstable phase, thus confirming existence of both night- and day-active gerbils of each sex.

Second, after 10 wks in LD 12:12, 15N & 10D gerbils were exposed to an 8-hr delay of the LD cycle. Except for 2N gerbils reclassified as "?", all maintained phase positions re. to the shifted LD cycle for 7 wks, often with delaying transients. Thus N & D rhythms reflect circadian differences rather than mere inverse masking.

Finally, we monitored another 10N & 6D gerbils for 22 wks. Phase positions remained stable, despite restriction from running during wks 18 & 19 in 6N & 4D animals. Only 2 (1N, 1D) changed -- to "?" before wheel restriction. Thus it appears unlikely that differences in feedback from activity to the circadian oscillator are alone responsible for N and D rhythms in gerbils.

Studies of heredity/environment interaction and physiological mechanisms determing phase position in gerbils may increase understanding of circadian regulation and its dysfunction. Support: MRC of Canada grant to JH.

BIOLOGICAL RHYTHMS AND SLEEP: SLEEP III

273.1

TOPOGRAPHICAL DISTRIBUTION OF THETA WAVES AND DESYNCHRONIZATION ON CORTICAL SURFACE IN DESYNCHRONIZED SLEEP OF THE RAT. [1]L.V.Venturini, [1,2]G.Ballester, [1]K.Sameshima and [1]C.Timo-laria*. [1]Univ. São Paulo School of Medicine; [2]Fed. Univ. São Paulo Medical School (EPM). 01246-903 São Paulo SP, Brazil.

The theta waves and the desynchronization occur in the alert wakefulness and in the dreaming activity, where the spectra of these rhythms change in relation to the behavior type and in relation to the different cortical areas. This study aimed at realizing the electro-oscillographic mapping of the cortical surface during wake and dreaming activity to develop the hypothesis of that each rhythm is associated with a phylogenetic age of the specific cortical areas and represent different degrees of attention. The electro-oscillograms are obtained by intra-muscle electrodes, for the monitoring of the behavior type, and cortical electrodes, arranged as matrices and implanted by stereotaxy on the cortical surface of Wistar rats. In the phylogenetically older areas, theta waves are recorded during wake and dreaming activity, with frequency and extent of the theta relating with the intensity and the type of attentive behavior. In the frontal cortex (area 10), that is a more recent phylogenetic acquisition than posterior areas, a desynchronization is recorded. Areas of electro-oscillographic transition are observed in the posterior portion of the area 10, area 6 and frontal portion of the areas 3 and 4, where subtle change in the spectral pattern occurs at neighboring coordinates belonging to the same cytoarchitectonic area. It is concluded that the desynchronization is a command signal phylogenetically more recent than the theta rhythm, and that both mobilize specific neuronal groups. The increase of the theta rhythm frequency tending to the desynchronization, in transitional areas, reflects the functional affinity of these areas with the frontal cortex and shows the relation of the desynchronization with higher degrees of attention.

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273.2

CHANGES IN PROTEIN PHOSPHORYLATION PATTERNS IN THE BRAIN DURING THE SLEEP-WAKING CYCLE. C. Cirelli* and G. Tononi. *The Neurosciences Institute, San Diego, CA 92121*

Sleep and waking are associated with strikingly different behavioral and electroencephalographic patterns. Little is known, however, concerning differences between these two states at the cellular level. Cellular responses to many extracellular signals occur through changes in phosphorylation of intracellular proteins, which depend on a precise equilibrium between the activity of protein kinases and phosphatases. In this study, we used antibodies suited to the immunocytochemical detection of proteins phosphorylated on serine, threonine (Zymed), and tyrosine (Transduction Lab) residues to determine whether changes in cellular phosphorylation patterns accompany the electrophysiological changes occurring during the sleep-waking cycle. WKY rats implanted for chronic polysomnographic recordings were sacrificed during the light period after either 3h of sleep (S-L, n=6) or 3h of gentle sleep deprivation (W-L, n=6). In all cases, the levels of protein phosphorylation on both serine and threonine residues were markedly higher in rats sacrificed after 3h of waking than in rats sacrificed after 3h of sleep, particularly in the cerebral cortex. In two animals (W-L and S-L), the calcium chelator BAPTA AM (Calbiochem) was added during the perfusion in order to block phosphatase 2B activity, but the difference in serine and threonine phosphorylation levels between waking and sleep was still present. With the anti-phosphotyrosine antibodies used in this study (a polyclonal and a monoclonal), the staining of microglial cells masked that of neurons, and changes in neuronal tyrosine phosphorylation were difficult to determine. In another series of experiments, we asked whether the increase in protein phosphorylation during waking was due to changes in the activity of neuromodulatory systems with diffuse projections, such as the noradrenergic locus coeruleus (LC), which are highly active during waking and less active during sleep. Unilateral lesions of LC were produced by local injection of 6-hydroxydopamine (see Tononi et al., this meeting). In animals sacrificed after 3h of spontaneous or forced waking (n=6), phosphoserine and phosphothreonine staining in the cerebral cortex was high on the side in which noradrenergic fibers were still present, and low on the lesioned side. These results suggest that the release of norepinephrine, which is high during waking and low during sleep, is a major factor determining the increase of protein phosphorylation that is observed during waking. (Supported by Neurosciences Research Foundation).

273.3

CHANGES IN GENE EXPRESSION BETWEEN WAKEFULNESS AND SLEEP REVEALED BY mRNA DIFFERENTIAL DISPLAY. M. Pompeiano*, C. Cirelli, and G. Tononi. *The Neurosciences Institute, San Diego, CA 92121*

The expression of the immediate early genes c-fos and NGFI-A is powerfully modulated during the sleep-waking cycle. The protein products of these genes can act as transcription factors, suggesting that the expression of other genes may also be modulated by sleep and waking. In this study we employed a modified version of mRNA differential display (DD) to systematically investigate changes in gene expression between sleep and waking. WKY rats were implanted for chronic polysomnographic recordings. To distinguish among genes whose expression is related to sleep and waking *per se*, and genes expressed in relation to circadian factors or stress, 3 groups of rats were considered. A first group (S-L, n=7) was sacrificed after 3h of sleep during the light period. A second group was sacrificed after 3h of sleep deprivation during the same circadian period (W-L; n=7). A third group (W-D, n=6) was sacrificed after 3h of spontaneous waking during the dark period. Total RNA was isolated from the cerebral cortex and reverse transcribed. cDNAs of each individual animal were amplified by PCR in the presence of [α-33P]dATP (Dupont), by using one out of three 3' composite anchor primers E1T12M (E1=5'CGGAATTCGG, M=A,C, or G) and one out of twenty-seven 20mers 5' composite arbitrary primers. PCR products for each animal were run in duplicate on denaturating polyacrylamide gels. Dried gels were exposed to X-ray film and autoradiographs were scanned using a phosphorimager. So far, 20 combinations of 3' and 5' primers have been examined, and 13 differentially expressed bands have been identified. Eight bands were expressed more in W-L and W-D rats than in S-L rats, 4 bands were expressed more in S-L rats than in W-L and W-D rats, and 1 band was expressed more in S-L and W-L rats than in W-D rats. Six out of the 13 bands have been subcloned into the pCRII vector (Invitrogen). In situ hybridization to confirm the cloned bands and nucleotide sequence analysis are being performed. These results indicate that the expression of several genes is modulated by the physiological sleep-waking cycle. The identification of these genes may help elucidate the functional consequences of sleep and waking. (Supported by Neurosciences Research Foundation).

273.4

CORRELATION OF SLEEP STATES WITH EXPRESSION LEVELS OF IMMEDIATE EARLY GENES IN THE RAT CEREBRAL CORTEX.
C. Pavlides*, S. Ribeiro and C. Mello. The Rockefeller University, New York, NY, 10021.

It is known that sleep deprivation leads to increased levels of the immediate early gene (IEG) NGFI-A in various brain regions, whereas long periods of sleep decrease NGFI-A levels. However, the specific contributions of slow-wave sleep (SWS) and rapid-eye movement-sleep (REM) are unknown. Our primary goal was to compare the expression patterns of the plasticity-related IEGs NGFI-A, NGFI-B and Krox-20 in the rat brain after wakefulness (WK), SWS or REM. Adult male rats were chronically implanted with electrodes in the dentate gyri for EEG recording. After a 5-day recovery period, each rat was placed inside an isolation box where behavior was observed and EEG recorded in order to identify WK, SWS or REM. Thirty minutes after engaging in one of these states animals were decapitated and their brains processed for *in situ* hybridization. We found a decrease in cortical expression of all three genes in the REM group when compared to WK animals, while SWS rats exhibit intermediate levels. The results show a correlation between REM sleep and low expression levels of early genes thought to be involved in neuronal modification. We speculate that this gene expression pattern may represent a generalized and transient decrease of plasticity in the cortex during REM. To test whether this phenomenon is related to sleep-mediated memory processing, rats exposed to enriched environment prior to sleep are presently being studied.
(*Work supported by Whitehall Foundation, NIH and CNPq*).

273.5

NEURONS WITH REM-SELECTIVE DISCHARGE ARE PRESENT IN THE TRAPEZOID BODY IN FREELY BEHAVING CATS. Mahesh M. Thakkar*, Priyattam J. Shiromani, Donald G. Rainnie and Robert W. McCarley. Dept. of Psychiatry, Harvard Medical School, Brockton VA Med. Ctr., Brockton, MA 02401, USA.

Auditory stimulation is known to enhance REM sleep. The trapezoid body has been implicated to have a crucial role in brainstem auditory processing. However, no existing studies have looked at the firing properties of neurons in the trapezoid body across behavioral states. Hence, we recorded single units from this region across behavioral states, in freely behaving, naturally sleeping cats. Male adult cats, anesthetized with an intravenous injection of sodium pentobarbital (35 mg/kg), were implanted with chronic sleep recording electrodes and a mechanical microdrive containing two cannulae in which two bundles of seven insulated flexible stainless steel microwires (32 μm) were inserted. Seven to ten days were allowed for the animals to recover from surgery before the recordings were started.The microwires were advanced in steps of 25-50 μm until a single neuron spike with a signal to noise ratio of at least 3:1 was encountered. The unit activity was continuously monitored to rule out artifacts. Recording sites were histologically verified. Polygraphic records were classified into five different states: active wake (AW), quiet wake (QW), slow wave sleep (SWS), REM sleep without eye movements (REM -) and REM sleep with eye movements (REM +). The firing pattern of all the neurons were then categorized in relation to these behavioral states. A total of 43 neurons were recorded. Preliminary results indicate that all the neurons recorded showed alterations in their firing pattern with behavioral states. Out of 39 neurons analyzed 18 (46%) showed increased firing during REM sleep, 19 (49%) showed increased firing during both waking and REM sleep and 2 (5%) neurons showed decreased firing during REM sleep.
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273.6

State-dependent discharge of neurons in the rat posterior hypothalamus.
T. L. Steininger, Md. N. Alam, R. Szymusiak and D. McGinty *V.A. Med Ctr., Sepulveda, CA 91343; Depts. of Med. and Psych., UCLA, Los Angeles, CA.*

The posterior hypothalamus (PH) has long been thought to be important for the maintenance of waking, since Von Economo described neuropathology in this region resulting in a profound and persistent somnolence. The role of this region in the control of EEG arousal is supported by lesion studies in several species. The histaminergic tuberomammillary nucleus (TM), in the ventral PH has diffuse projections throughout the brain. Histamine has potent effects on arousal. Studies in the unanesthetized cat have shown that TM neurons fire in relation to waking, i.e. they are nearly silent in non-rapid eye-movement (NREM) and REM sleep, whereas PH neurons fire in correlation with complex movements, and fire at peak rates during both waking and REM sleep. To date, these findings have not been replicated in the rat.

In the present study, chronic single-unit recording techniques in the freely-moving rat were utilized to investigate the state-dependent firing of PH and TM neurons. Our results agree with findings in the cat, i.e. histaminergic TM neurons fired at a tonic rate during waking, ceased firing prior to the onset of NREM sleep (and EEG synchronization), and did not fire during REM sleep. Several classes of PH neurons were observed. The majority were tonically active at low rates during waking (0.5-2 Hz), fired at even lower rates during NREM, and fired at waking rates during REM (36% of cells). Another class of PH neurons fired at higher rates (10-15 Hz) in relation to waking movements, slowed their firing during NREM sleep, and increased their firing rates during REM sleep (24% of cells). Other cells recorded in the region dorsal to TM had irregular activity which was either a combination of tonic and movement related activity, or unrelated to behavioral state. (Supported by Dept. Veterans Affairs, and HHS MH47480)

273.7

THE EFFECTS OF 192 IgG-SAPORIN LESIONS OF THE BASAL FOREBRAIN CHOLINERGIC NEURONS ON THE CORTICAL EEG DURING DIFFERENT BEHAVIOURAL STATES IN RAT. W.J. Fortin* and K. Semba, Dept. of Anat. & Neurobiol, Dalhousie Univ., Halifax, N.S. B3H4H7 Canada.

Basal forebrain cholinergic neurons are thought to play an important role in cortical EEG desynchronization. Noradrenergic and serotonergic neurons are also implicated for a similar role during waking; however, these neurons are quiescent during REM sleep and therefore cannot account for EEG desynchronization in that state. We examined whether selective lesions of the cholinergic basal forebrain neurons differentially affect the frequency content of the EEG in REM sleep and waking in freely behaving rats. Rats were microinjected with 192 IgG-saporin (100ng/0.2μl) into the right basal forebrain and were chronically implanted with EEG and EMG electrodes. The animal's activity and behavioural state were observed and noted during recording sessions. The FFT power spectra were computed between DC and 100Hz for 16s EEG epochs. AChE histochemistry revealed almost complete depletion throughout the ipsilateral cortex. Inspection of EEG records together with the FFT indicates the following preliminary results. Large amplitude slow wave bursts (3-6Hz; 2-8s duration) were present intermittently during active exploration. Similar bursts were occasionally seen during REM sleep, although they were much less prevalent. In general no obvious changes were seen in the delta component of the EEG during REM sleep; however, there was an apparent reduction in power in the beta and gamma ranges. These reductions were not seen during active exploration. These data suggest that selective lesions of the basal forebrain cholinergic neurons reduce the high frequency components of the cortical EEG in REM sleep and induce intermittent slow activities in the waking cortical EEG. The lack of dramatic slowing of the EEG in REM sleep suggests that even in the absence of cholinergic, noradrenergic and serotonergic inputs, mechanisms exist to induce cortical EEG desynchronization. Supported by the MRC of Canada and The Scottish Rite Charitable Foundation.

273.8

CARBACHOL INFUSION INTO THE MEDIAL PONTINE RETICULAR FORMATION OF RATS ENHANCES REM SLEEP. G. A. Marks* and C. G. Birabil. Dept. of Psychiatry, Univ. of Texas Southwestern Med. Center, Dallas, TX 75235

Micro injection of the cholinergic agonist carbachol into the medial pontine reticular formation of cats produces long lasting and dramatic increases in the time spent in REM sleep. Similar studies utilizing rat have thus far failed to reach a consensus as to the most efficacious site of infusion or dose infused. Carbachol effects on REM sleep, when found, also are typically much less dramatic than that reported for cat. Preliminary to an investigation of the mechanisms of cholinergic induction of REM sleep in the rat, we sought to determine whether manipulating time of day and bilateral versus unilateral infusions might lead to an increase in the magnitude of the carbachol effect.

Under anesthesia, Long-Evans Hooded rats were surgically prepared for chronic sleep recording and additionally implanted with bilaterally symmetric guide cannulae. Cannula-pairs were aimed at medial sites through the anterior-posterior extent of the pontine reticular formation. After one week recovery from surgery, animals were adapted to the unrestrictive, recording environment were they remained tethered for the duration of the experiment. Starting during adaptation, each animal was handled for at least five minutes every day at the time of day of drug infusion. A 60 nl volume of carbachol (0.5μg, 50ng, or 10ng) and/or saline were bilaterally administered in every combination in counter-balanced order across animals. The six hour electrographic recordings were conventionally scored in 15 sec. epochs into wake, slow wave and REM sleep.

We find that bilaterally injected carbachol typically does not increase REM sleep beyond that of either left or right unilateral injections and, most often, leads to less of an increase or to a decrease compared to saline control values. Time of day is a variable influencing the effect of carbachol on REM sleep. Diminished effects were found with mid-light period infusions. Increases in REM sleep were greater when infusions occurred at one hour before lights-out and tended to be even greater at lights-on but accompanied by increased variability.
This work was supported in part by NIH grant MH49364

273.9

STEREOTYPED PATTERN OF SUPPRESSION OF UPPER AIRWAY MOTOR TONE DURING THE CARBACHOL-INDUCED, REM SLEEP-LIKE ATONIA. L. Kubin, V. Fenik, A.I. Pack and R.O. Davies*. University of Pennsylvania, Philadelphia, PA 19104.

We have previously shown that microinjections of carbachol into the rapid eye movement (REM) sleep-triggering region of the dorsal pontine tegmentum of decerebrate cats produce, in addition to postural atonia, a stereotyped suppression of respiratory motor output to respiratory pump muscles (diaphragm, and inspiratory/expiratory intercostals), and a profound suppression of hypoglossal (XII) nerve activity (Kimura et al., J. Appl. Physiol., 68: 1435, 1990). EMG recordings in humans and chronically instrumented animals suggest that the magnitudes of suppression present in distinct upper airway muscle groups during natural REM sleep also follow a stereotyped pattern. To assess this, we recorded the efferent activities of the vagus, recurrent laryngeal (RL), pharyngeal branch of the vagus, genioglossal branch of XII nerve, phrenic and a C4 branch innervating postural neck muscles in decerebrate, paralyzed, vagotomized and artificially ventilated cats. Pontine injections of carbachol were performed to produce postural atonia and respiratory depression and atropine injections to reverse the carbachol effects. There was a differential pattern of the carbachol-induced suppression among the upper airway nerves studied (4 cats, 7-11 measurements of the change in activity of different nerves). The inspiratory activity of the XII nerve was suppressed the most (to about 12% of the control), followed by the pharyngeal expiratory activity (about 24%), inspiratory RL nerve activity (about 56%) and phrenic (about 66%). This pattern, albeit exaggerated, is consistent with that observed during natural REM sleep. (HL42236)

273.10

PREMOTOR TRIGEMINAL INTERNEURONS ACTIVATED DURING CARBACHOL-INDUCED ACTIVE SLEEP. F.R.Morales, S.Sampogna, J.Yamuy, K.Kohlmeier and M.H.Chase*. Department of Physiology and the Brain Research Institute, UCLA School of Medicine, Los Angeles, CA 90024.

The present report is an anatomical description of medullary interneurons that innervate trigeminal motor nuclei; these neurons are also those that express *c-fos* during the state of active sleep induced by carbachol microinjection in the pontine reticular formation (AS-Carbachol). Premotor neurons that project to the trigeminal motor nuclei were identified by retrograde labeling following the microinjection of the b subunit of Cholera toxin (CTb) into the trigeminal motor pool. In each cat, two weeks after CTb injection, an AS-Carbachol state of approximately two hours length was induced. The cats were then perfused for immunohistochemistry. A block of tissue that included the brainstem was frozen and cut in 15 µm serial sections. Neuronal activation during AS-Carbachol was determined by *c-fos* expression (Yamuy et al., J. of Neurosci., 1993, 13: 2703-2718). A population of double-labeled neurons was observed in the ventromedial medulla ipsilateral to the recording site. This region is located medially to the posterior third of the facial nucleus and extends caudally to the rostral pole of the inferior olive. Between 600 and 800 doubled-labeled neurons were contained in this region. Most of these neurons were relatively large-sized, multipolar cells (40-50 µm, soma longest diameter). Others were medium-to-large-sized neurons with a fusiform cell body (30-50 µm, soma longest diameter). Based upon the fact that this is a unique population of *premotor* interneurons that express *c-fos* during the AS-Carbachol state, we postulate that they are the interneurons responsible for the postsynatpic inhibition of trigeminal motoneurons during active sleep. Supported by USPHS grants MH 43362, NS 23426 and NS 09999.

273.11

THE EFFECTS OF TEMPERATURE AND SYNAPTIC BLOCKADE *IN VITRO* ON NEURONS OF THE HORIZONTAL LIMB OF THE DIAGONAL BAND OF BROCA IN THE RAT BASAL FOREBRAIN. T. Hays*, S. Moraitty, R. Szymusiak and D. McGinty. Neurophysiology Lab, V.A.M.C. (151A3), Sepulveda, California 91343

Since sleep and thermoregulation appear to be coupled in the preoptic area/anterior hypothalamus (POAH) and because the horizontal limb of the diagonal band of Broca (HDB) has sleep regulatory functions similar to those in the POAH, we examined the thermosensitivity of HDB neurons. Using a microwire electrode (15 µm wire, 2 MΩ impedance), we studied the thermosensitivity of rat HDB neurons *in vitro* under normal conditions and under conditions of a low calcium-high magnesium synaptic blockade (SB). Of 52 HDB neurons tested, thirty-four neurons (65%) were warm sensitive (WS), three (6%) were cold sensitive (CS), eleven (21%) were temperature insensitive (TI) and four (8%) were both warm and cold sensitive (WS/CS). Of 34 neurons tested for thermosensitivity under SB, 11 were WS, 4 were CS and 19 were TI. Nearly half (48%) of the WS neurons maintained warm sensitivity under SB, 43% became TI and 9% became CS. Baseline firing rates of neurons significantly decreased during SB and then increased during recovery from SB. In addition, a distinct anatomical distribution of thermosensitive neurons was found in the HDB. The most ventral aspect of the HDB (interaural +0.9-1.3 mm) had proportionally fewer temperature sensitive neurons than areas more dorsal (interaural +1.3-1.7 mm), and only one of seven ventral HDB neurons remained thermosensitive during SB. In the dorsal HDB, over half of the neurons (65%) maintained thermosensitivity during SB. These results demonstrate that the HDB contains inherently thermosensitive neurons in the HDB, and may be a site at which thermosensitive neurons interact with sleep-regulatory mechanisms.

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273.12

EXTRACELLULAR LABELING OF SINGLE NEURONS BY BIOTIN-CONTAINING COMPOPUNDS SHOWS BROAD-SPIKE NEURONS IN THE LATERODPRSAL TEGMENTAL NUCLEUS TO BE CHOLINERGIC. Y. Koyama[1], M. Kusakabe[1], Y. Kayama*[1], T. Honda[2] and Y. Sugiura[2], Depts. of Physiol.[1] and Anat.[2], Fukushima Med. Coll., Fukushima Japan 960-12.

The neurons encountered in and around the laterodorsal tegmental nucleus (LDT) can be divided into two populations by the shape of their action potentials; one generates action potentials with longer duration (broad-spike neurons), while the other has those with shorter duration (brief-spike neurons). After anatomical and physiological studies, we have proposed that the broad-spike neurons are cholinergic, although no direct evidence has not been obtained. Using glass pipette electrode, we recorded activity of single neurons extracellulary in and around the LDT of urethane anesthetized rat. Biocytin (3-6 %) or Neurobiotin (4 %) was ejected iontophoretically from the recording electrode; they were uptaken in a single neuron. The frontal sections of 50 μ thickness were made and the compounds were revealed fluoresently with Texas red. The sections were then processed for NADPH-diaphorase (NADPH-d) histochemistry or anti-choline acetyltransferase. In the LDT, we have succeeded to recover Biocytin or Neurobiotin in 13 neurons with spike width (positive component of action potential) longer than 0.75 m sec and found that 12 of them were NADPH-d positive. All of 22 neurons which had spike width shorter than 0.7 m sec were NADPH-d negative. The quality of labeling was similar in two tracers. The results support the proposal that the majority of broad-spike neurons in the LDT are cholinergic, while brief-spike neurons are non cholinergic.

273.13

HUMAN BRAIN ACTIVATION DURING REM SLEEP DETECTED BY fMRI. J. P. Sutton*, H. C. Breiter, J. B. Caplan, F. R. Huang-Hellinger, K. K. Kwong, J. A. Hobson and B. R. Rosen. Neural Systems Group and NMR Center, Massachusetts General Hospital, Harvard Medical School, Bldg. 149, 13th St., Charlestown, MA 02129.

We have studied ten subjects during sleep using simultaneous functional magnetic resonance imaging (fMRI) and electrophysiological measurements, including the EEG, EOG and EMG. Here, we report on visual and medial temporal cortical activation of a subject during REM sleep. The subject (m, 36 yrs) was studied for four hours using a head coil and asymmetric spin echo instascan sequence at 1.5 T (TE=20, TR=20,000). Cz (EEG), both orbits laterally (EOG) and the submental region (EMG), all referenced to a mastoid lead, were recorded. Ten contiguous 7 mm axial slices were examined, including most of the neocortex and the brainstem, and the data were motion corrected. Following two hours of sleep, an eight minute segment of REM sleep was identified, in part, by rapid eye movements on the EOG and significant eye motion relative to the head based on imaging data. Physiological motion precluded a quantitative comparison of absolute fMRI signal changes across states. However, significant increases in correlated activity within V1, and between V1 and the medial temporal cortex, were observed in REM sleep relative to NREM sleep ($p<.0001$). These findings are consistent with state-dependent differences in human brain activation, including V1.

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273.14

SLEEP PROMOTION BY THE ROSTRAL HYPOTHALAMUS: A POTENTIAL MECHANISM. J.E. Sherin*, J. K. Elmquist, and C. B. Saper. Dept. of Neurology, Harvard Medical School/Beth Israel Hospital, Boston, MA 02115.

We recently identified a discrete population of sleep-activated ventrolateral preoptic neurons that project to the histaminergic tuberomammillary nucleus, a caudal hypothalamic cell group thought to promote behavioral arousal. As an intact rostral hypothalamus has long been known to be necessary for the expression of normal sleep, this sleep-activated circuit was hypothesized to use inhibitory neurotransmitters and thereby subserve a sleep-promoting mechanism. The present study was conducted to determine the neurotransmitter(s) used in this pathway. Using retrograde tracing in combination with immunocytochemistry, we report here that GABAergic and galaninergic markers colocalize in the vast majority of ventrolateral preoptic neurons which innervate the tuberomammillary nucleus. Both of these neurotransmitters have been shown to inhibit post-synaptic targets, and, in this pathway, may promote sleep by decreasing the activity of histaminergic neurons. Further, galaninergic markers may serve to identify a population of sleep-activated neurons in the preoptic area.

This work was supported in part by USPHS grants NS33987, NSO9474, and MH10832.

274.1

THE SONGBIRD HIPPOCAMPUS IS A SITE OF HIGH AROMATASE ACTIVITY. C.J. Saldanha*, V.T. Cam, & B.A. Schlinger. Dept. of Physiological Science and the Laboratory of Neuroendocrinology-BRI, UCLA, Los Angeles, CA 90095.

In songbirds, the hippocampus (HP) plays a fundamental role in memory-intensive behaviors such as the storage and retrieval of food. Estrogens (E) influence the morphology and physiology of neurons in the vertebrate HP. We have found that aromatase (estrogen-synthetase) mRNA is expressed at high levels in the zebra finch (ZF) HP. Thus, aromatase may provide local E to regulate steroid-dependent neural function. We asked whether aromatase activity was detectable in the HP of developing and adult ZF. We also compared aromatase activity in the HP of several songbird species and in a non-songbird. In a 15 min incubation with ^{3}H-androgen and measurement of formed E, aromatase was found to be high in HP-enriched primary cultures of 3-6 day old ZF (1.07±0.225 pmolesE/mg protein) and similar to telencephalic cultures (1.73±0.98 pmolesE/mg protein). Additionally, there is no apparent influence of sex on aromatase in the HP since HP-enriched cultures from female and male birds show similar levels of activity (4.70±0.99 and 3.65±0.30 fmolesE/well respectively). Likewise, in adults, aromatase activity is similar in homogenates of female and male HP (3.06±0.77 and 2.34±0.63 pmolesE/mg protein respectively). Hippocampal aromatase seems unique to songbirds since we have found it to be high in ZF, canaries, and house sparrows, but undetectable in a non-songbird (budgerigar). Thus, songbirds may be excellent animal models for the study of estrogenic modulation of hippocampal function. Supported by CA Dept of Health Services 95-23342.

274.2

IMMUNOCYTOCHEMICAL LOCALIZATION OF AROMATASE-IMMUNOREACTIVE CELLS AND THE DEFINITION OF PREOPTIC NUCLEI IN THE ZEBRA FINCH BRAIN. P. Absil*, G.F. Ball, N. Harada and J. Balthazart, Lab. Biochemistry, Univ. Liège, Belgium; Dept Psychol., Johns Hopkins Univ., Baltimore, MD 21218, and Molec. Genetics, Fujita Univ., Toyoake, Japan.

A polyclonal antibody raised against quail recombinant aromatase was used to localize by immunocytochemistry cells immunoreactive for the enzyme aromatase (ARO-ir) in the forebrain of male zebra finches. Staining was enhanced in some birds by the administration of the non-steroidal aromatase inhibitor, R76713 (racemic Vorozole) prior to the perfusion of the birds as previously described in Japanese quail. Large numbers of ARO-ir cells were found in nuclei in the preoptic region and in the tuberal hypothalamus. A nucleus was identified in the preoptic region based on the high density of ARO-ir cells within its boundaries that appears to be homologous to the preoptic medial nucleus (POM) described previously in Japanese quail. In several birds alternate sections were stained for immunoreactive vasotocin, a marker of the paraventricular nucleus (PVN). These data facilitated the clear separation of the POM in zebra finches from nuclei that are adjacent to the POM in the preoptic area-hypothalamus, such as the PVN and the ventromedial nucleus of the hypothalamus. ARO-ir cells were also detected widely throughout the telencephalon. They were discerned in medial parts of the ventral hyperstriatum and neostriatum near the lateral ventricle and in dorsal and medial parts of the hippocampus. They were most abundant in the caudal neostriatum where they clustered in the dorso-medial neostriatum, and as a band of cells coursing along the dorsal edge of the lamina archistriatalis dorsalis. They were also present in high numbers in the ventro-lateral aspect of the neostriatum and in the nucleus taeniae. None of the telencephalic vocal control nuclei had appreciable numbers of cells immunoreactive for aromatase within their boundaries with the possible exception of a group of cells that may correspond to the medial part of magnocellular nucleus of the neostriatum. The distribution of immunoreactive aromatase cells in the zebra finch brain is in excellent agreement with the distribution of cells expressing the mRNA for aromatase that has recently been described in this species. This widespread telencephalic distribution of cells immunoreactive for aromatase has not been described in non-song bird species such as the Japanese quail, the ring dove and the domestic fowl.

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274.3

THE ROLE OF GONADAL HORMONES AND ENVIRONMENTAL CONDITIONS ON THE DISTRIBUTION OF ARGININE VASOTOCIN IMMUNOREACTIVITY (AVTir) IN MALE ZEBRA FINCH BRAINS. C.F. Harding* & S.R. Barclay, Biopsychology Program, Hunter College, CUNY, New York, NY 10021.

Arginine vasotocin is the primary hormone regulating water and electrolyte balance in birds, allowing them to adapt to changes in water availability. In zebra finches, reproduction is cued by water availability rather than changes in photoperiod. It therefore seemed possible that AVT might be involved in the regulation of reproductive behavior in this species. We examined the effects of hormonal status and water availability on the distribution of AVTir in male finch brains.

Two groups of males (castrated and intact) were housed for five weeks in conditions which typically stimulate high levels of reproduction: unlimited water, high humidity, fresh greens. The third group (intact) was housed in nonstimulatory conditions: limited water, low humidity, no greens.

AVTir was distributed in many areas involved in the regulation of male reproductive behavior, including vocal control and auditory nuclei, the bed nucleus of the stria terminalis, and the lateral septum. Castration strongly reduced staining in many of these areas, suggesting AVT plays a role in coordinating reproductive behavior in zebra finches. Decreased water availability did not cause such striking changes in AVTir.

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274.4

EFFECTS OF DSP-4 ON DISTRIBUTION OF DOPAMINE-BETA-HYDROXYLASE IMMUNOREACTIVITY (DBHir) IN MALE ZEBRA FINCH BRAINS. S.A.Waterman* & C.F. Harding, Biopsychology Program, Hunter College, CUNY, New York, NY 10021

Systemic or central administration of the neurotoxin DSP-4 decreases courtship singing in male zebra finches. As expected, DSP-4 treatment decreased norepinephrine (NE) levels in telencephalic vocal control nuclei, but it also reduced NE levels in hypothalamic nuclei in these birds. In mammals, DSP-4 preferentially affects noradrenergic function in cortical areas leaving hypothalamic areas relatively unaffected. To assess the extent of DSP-4's effects on the noradrenergic innervation of the finch brain and to allow more direct comparison with mammalian studies, we used immunocytochemical techniques to determine how DSP-4 treatment affected the distribution of DBHir in male zebra finch brains. Males were pretreated twice with zimelidine (20mg/kg) to protect serotonergic neurons and then given either DSP-4 (50mg/kg) or saline ip. Birds were perfused 10 days after the second treatment. DSP-4 treatment significantly reduced innervation by DBHir fibers throughout the finch brain. Specifically, DBHir staining decreased in the cerebellum, optic tectum, telencephalic and hypothalamic nuclei compared to controls. In areas where DBHir processes were seen in DSP-4 treated animals, they often appeared swollen and shortened. In zebra finches, DSP-4 is not highly selective for neurons which project to the telencephalon but also affects those which project to the hypothalamus. *Funded by PSC CUNY Grants 664280 & 665221 and NIH Grant RR08176*

274.5

REPRESENTATION OF SONG COMPONENTS IN THE AUDITORY FOREBRAIN OF CANARIES. S. Ribeiro*, G. Cecchi, M. Magnasco and C. Mello. The Rockefeller University, New York, NY, 10021.

Song playbacks induce expression of the immediate early gene ZENK in the caudo-medial neostriatum (NCM), a portion of the auditory telencephalon of songbirds. We have taken advantage of this phenomenon to address in detail how song, a complex and natural auditory stimulus, is represented in NCM. Adult canaries were presented with playbacks of various types of song stimuli, and ZENK protein expression revealed by immunocytochemistry. Maps of labeled cells in NCM were reconstructed and analysed on a computer workstation equipped for image processing. Whole canary songs resulted in labeling of cells throughout NCM, whereas isolated syllables revealed more discrete domains of activation. For example, syllables resembling pure tones resulted in labeled cells mostly restricted to the rostral third of NCM. Each tone-like syllable activated a distinct domain, with lowest frequencies mapping dorsally and highest frequencies ventrally, in a graded fashion. Thus, rostral NCM appears to be tonotopically organized. When tone-like syllables were presented simultaneously or in close sequence, labeled cells were also seen in more posterior regions, where presumably combination-sensitive neurons are present. Finally, the caudal third of NCM appears to be mainly sensitive to rapid frequency modulations. These results indicate that frequencies are represented in the dorso-ventral axis and stimulus complexity in the rostro-caudal axis. A computer program for tridimensional reconstruction of ZENK activation patterns in NCM is currently being developed. We believe that an understanding of the birdsong processing rules will give some key insights into how complex external objects are represented in the vertebrate brain. (*Work supported by NIH, CNPq and Mathers Foundation*).

274.6

EXPRESSION OF c-FOS PROTEIN IN ADULT ZEBRA FINCH HVc AND RA IS INDUCED BY THE MOTOR ACT OF SINGING R.R. Kimpo* and A.J. Doupe, Neuroscience Graduate Program, Departments of Psychiatry and Physiology, UCSF, San Francisco, CA 94143.

Bird song is a complex learned behavior involving auditory and vocal motor systems. We investigated both auditory and motor aspects of song by measuring the induction of the protein product of *c-fos* (FOS), an immediate early gene (IEG), in brains of awake behaving male adult zebra finches. IEG expression is a useful marker for neuronal activation; previous studies in the songbird brain have shown that the IEG ZENK is expressed in auditory regions, including areas surrounding song nuclei, in response to auditory stimulation, especially with conspecific song (Mello et al, 1992,1994). In this study we show that FOS immunoreactivity is evident within song nuclei; however, FOS was not induced by auditory stimulation but by the motor act of singing.

Birds were presented with tapes of conspecific songs for 50 min, and only those which countersang during stimulation had FOS immunoreactive (IR) cells in HVc and RA, two song nuclei known to have premotor activity. To help determine whether FOS expression was due to the motor act of singing and/or the resulting auditory feedback, we deafened birds by bilateral cochlear removal. Singing was induced in deaf and hearing birds by exposing them to zebra finch female(s) for 50 min. Birds that did not sing had no FOS-IR cells in the two song nuclei while both deaf and hearing birds that sang had numerous FOS-IR cells in HVc and RA. There was a positive correlation between singing time and FOS-IR cell density in these song nuclei.

These experiments demonstrate strong IEG induction due to the motor act of singing and independent of auditory feedback in song nuclei HVc and RA. Since juveniles undergoing song learning are more dependent on auditory feedback than adults for normal song production, this method may reveal differences in neuronal activation of song nuclei at different stages of song learning.

(Supported by a UCSF Opportunity Fellowship and the Klingenstein Fund)

274.7

RETINOIC ACID IN THE BRAIN OF ADULT SONGBIRDS: A POTENTIAL ROLE FOR ALDEHYDE DEHYDROGENASE EXPRESSION IN THE SONG SYSTEM. N. Denisenko*, F. Nottebohm and C. V. Mello.
The Rockefeller University, New York, N.Y., 10021

We have previously identified an mRNA that is highly-enriched in three song control nuclei - HVC, lMAN and RA - using PCR-based mRNA differential display. We have now isolated a full-length clone from a zebra finch forebrain cDNA library. Sequence analysis revealed homology to class 1 (cytosolic) aldehyde dehydrogenase (ALDH) of different species. ALDH1 expression in HVC and lMAN, as analysed by in situ hybridization, was high and comparable at all ages tested (20, 38, 50, 60 day-old juveniles and adults). Expression in RA appears to be age dependent: it peaked at day 38, declined thereafter, and disapeared by day 60. Thus, ALDH1 expression in RA appears to correlate with the duration of the "sensitive period" for song acquisition in zebra finches. In other systems, expression of class 1 ALDH has been implicated in retinoic acid production. To test whether retinoic acid can also be detected at sites of ALDH1 expression in the brain of adult zebra finches, we used a retinoic acid sensitive cell line. These cells contain an exogenous ß-galactosidase gene under the control of a retinoic acid sensitive element. Coculturing HVC explants with these cells resulted in a blue color reaction, indicating the presence of retinoic acid in the HVC explants. Explants from the "shelf" - a region immediately adjacent to HVC that does not express ALDH1 - did not induce any color reaction in these cells. Pretreatment of HVC explants with 50 µM disulfiram (a specific inhibitor of class 1 ALDH) prior to the coculture prevented the induction of the color reaction. These results suggest that ALDH1 expression in song nuclei of adult songbirds is related to retinoic acid production. We are currently investigating the possible role of retinoic acid in these brain regions. *(NIH grant # MH 18343)*

274.8

CYTOCHEMICAL STAINING FOR PROTEIN KINASE C IN THE SONG CONTROL NUCLEI OF ZEBRA FINCH DURING SONG LEARNING. H. Sakaguchi[1,2]*. Dept. of Physiology, Dokkyo Univ. Sch. of Med.[1], PRESTO, Research Development Corporation of Japan[2], Mibu Tochigi 321-02 Japan.

Bird song learning during the sensitive period is closely related to synaptic plasticity in the song control nuclei. Protein kinase C (PKC) has been used as a molecular marker for synaptic plasticity in memory and learning. In order to visualize the most plastic neurons, the distribution of PKC was studied in the song control nuclei during the sensitive period of song learning.

The male zebra finch was studied at three developmental ages: 30 and 50 days after hatching and in adults. Cytochemical staining was performed using rhodamine-conjugated bisindolylmaleinide (Rim-1), a fluorescent derivative that inhibits PKC. Rim-1 stained the soma of some neurons in the song control nuclei, high vocal center (HVc), robust nucleus of the archistriatum (RA), lateral magnocellular nucleus of the archistriatum (LMAN) and area X at all three developmental ages. Furthermore, PKC was strongly expressed in the soma and dendrites of RA neurons at day 50. The results suggest that PKC may be a specific substance related to the synaptic plasticity of RA neurons involved with song learning in the zebra finch.

274.9

SINGING INDUCES GENE EXPRESSION IN SELECTED NUCLEI OF THE AVIAN SONG SYSTEM. E. D. Jarvis* & F. Nottebohm, Lab. of Animal Behavior, The Rockefeller University, NY, NY 10021.

The direct motor pathway from HVC to RA is involved in the acquisition and production of learned song in songbirds; the recursive loop from HVC to Area X to DLM to lMAN is thought to be involved just with song learning. This study explored the relation between singing and expression of the immediate early gene ZENK, which has been implicated in the formation of song memories in the auditory forebrain. Male canaries were stimulated to sing by exposing them to tape recorded playbacks of conspecific song. After 30 min. from the onset of singing, the birds were killed, their brains removed and ZENK expression was analyzed by in-situ hybridization. We found that birds who sang within the 30 min. period had increased levels of ZENK mRNA in 6 forebrain song nuclei: Area X, medial and lateral MAN, HVC, RA, and Av (avalanche). ZENK levels were higher (up to 10 fold) the more the bird sang and was not detectable in birds that did not sing. ZENK expression peaked 30-40 min. after the 30 min period of singing and declined to non-detectable levels 1-2 hrs after the end of singing and expression was lower if the bird continued to sing thereafter. Cutting the syringeal nerve or deafening did not prevent ZENK induction even though the song produced was aberrant or could not be heard. Zebra finches also showed ZENK expression in their song nuclei after singing. Our results raise basic questions about the role gene expression plays in song production and in particular in nuclei, such as Area X and lMAN which are not needed for the maintenance of learned song. Perhaps a little bit of learning occurs every time a bird sings or perhaps ZENK expression may be needed to replenish proteins used during the act of singing -- it could be both. Reasearch supported by a training grant to E. Jarvis (NIH 2T32MH 15125-17A1)

274.10

THE SONG SYSTEM OF EUROPEAN STARLINGS EXHIBITS DEVELOPMENTAL CHANGES IN THE DISTRIBUTION OF NMDA RECEPTORS DURING THE FIRST YEAR OF LIFE
J. M. Casto*, A. Pahlawanjan, and G.F. Ball, Dept of Psychology, Behavioral Neuroendocrinology Group, Johns Hopkins University, Baltimore, MD 21218, USA

In adult European starlings (*Sturnus vulgaris*), 3 song control nuclei can be discerned from the surrounding neural tissue based on N-Methyl-D-Aspartate (NMDA) receptor density. HVC and lMAN exhibit low NMDA receptor density relative to surrounding structures. In contrast, area X is defined by high receptor density relative to the surrounding lobus parolfactorious (LPO). We used *in vitro* receptor autoradiography for NMDA receptors (defined by MK-801 binding) in order to assess the distribution of NMDA receptors at days 20, 50, 100, 150, 210, and 345 in HVC, lMAN, and area X of young starlings.

No sex differences in NMDA receptor density exist in lMAN, or HVC, between days 20 and 210. NMDA receptor density in lMAN decreases between days 20, 50, and 100. Receptor density then remains constant between days 100 and 210. In contrast, NMDA receptor density in HVC remains constant between days 20 and 210. Between days 210 and 345, males, but not females, exhibit decreases in lMAN and HVC receptor density. These sex-specific decreases in NMDA receptor density in both HVC and lMAN may be related to sex differences in the hormonal mileau associated with the onset of reproduction.

Unlike adults, 20 and 50 day old starlings exhibit low NMDA receptor density in area X relative to the LPO. Throughout development area X exhibits decreases in NMDA receptor density, however, receptor density in LPO decreases more rapidly, so that by day 210 area X can be defined by high receptor density relative to the LPO. These developmental patterns of NMDA receptor distribution may be related to song system sexual differentiation and the process of song learning.

Supported by NIH grant MH50388

274.11

DEVELOPMENTAL REGULATION OF mRNA FOR THE NMDAR1 SUBUNIT IN ZEBRA FINCHES M.E. Basham*, E.J. Nordeen, S.N. Haber and K.W. Nordeen, Brain and Cognitive Sciences and Neuroscience Program, Univ. of Rochester, Rochester, NY, 14627

Activation of NMDA receptors is important for many forms of neural and behavioral plasticity including song learning in zebra finches. Males of this species learn to sing during a restricted ontogenetic period, and several discrete neural regions are necessary for normal song development. In one of these regions, the lMAN, the density of NMDA receptors declines as song behavior develops. In the present study, *in situ* hybridization methods revealed that the mRNA for the constitutive subunit of the NMDA receptor (NMDAR1) is regulated developmentally in both the lMAN and another song control region, Area X.

An oligonucleotide probe designed to recognize all splice variants of the NMDAR1 subunit mRNA labeled many structures throughout the forebrain and brainstem of juvenile and adult male zebra finches. As predicted by the earlier receptor binding studies, the levels of mRNA for the NMDAR1 within the lMAN declined between 30 days of age and adulthood. In contrast, within Area X, mRNA levels for the NMDAR1 subunit increased during this same period. At 30 days of age, the hybridization signal within Area X was not different from that in the surrounding tissue. However, in older birds, Area X was distinct due to elevated mRNA expression. The pattern and intensity of mRNA expression in birds raised as isolates until 60 days of age did not differ significantly from age-matched controls or normal adults. Thus, during vocal development, the NMDAR1 subunit is transcriptionally regulated within several song control regions, but this regulation is not dependent upon exposure to a song model. NIMH MH 45096.

274.12

DEVELOPMENTAL REGULATION OF NMDA RECEPTOR MODULATORY SUBUNITS IN MALE ZEBRA FINCH K.W. Nordeen*, M.E. Basham and E.J. Nordeen, Brain and Cog. Sci. and Neurosci. Program, U. of Rochester, NY

Song memorization in zebra finches normally occurs between 20-60 days of age, and requires activation of NMDA receptors. Within the lMAN, a brain region necessary for normal song learning, ontogenetic changes in the binding properties of NMDA receptors suggest regulation of receptor function. Since different modulatory subunits (NR2A-NR2D) confer different functional properties to the NMDA receptor, we explored whether NR2 subunits are developmentally regulated within regions implicated in song learning.

A polyclonal antibody that recognizes the NR2A/2B subunits in rats also recognizes protein in male zebra finches that is the expected weight of these subunits. However, unlike the widespread immunolabeling seen in rats, labeling in the zebra finch brain was restricted largely to the LPO. In all 10 day and some 20 day old males, dark immunopositive cells were present throughout the LPO including the song-control region Area X. In older males, Area X was distinct from the surrounding LPO due to more darkly labeled neuropil, and immunostaining that outlined rather than filled cell bodies.

Because the immunoreactivity was highly restricted compared both to that seen in rat, and to the known distribution of NMDA receptors in zebra finches, we suspect that this antibody does not recognize both NR2A and NR2B in zebra finches. Preliminary *in situ* hybridization studies show that the mRNA for the NR2B subunit is distributed widely throughout the telencephalon at all ages examined (30days-adult). Low levels of expression are evident within the lMAN, another area implicated in song learning, and no developmental changes in the expression of this message have been detected. We are determining if developmental changes in NR2A mRNA might underlie the pattern of results seen in the ICC. NIMH MH 45096.

274.13

NADPH-d NEURONS IN VOCAL CONTROL NUCLEI OF THE BUDGERIGAR. Steven E. Brauth# Wenru Liang and Stuart K. Amateau. Department of Psychology, Univ. of MD., College Park, MD. 20742.

We identified neurons staining for nicotinamide adenine dinucleotide phosphatase diaphorase (NADPH-d) in these 5 telencephalic vocal control nuclei in both adult and nestling (1-4 weeks posthatch) budgerigars (*Melopsittacus undulatus*): the central nucleus of the anterior archistriatum (**AAc**), central nucleus of the lateral neostriatum (**NLc**), oval nucleus of the hyperstriatum ventrale (**HVo**), medial oval nucleus of the anterior neostriatum (**NAom**) and magnicellular nucleus of the lobus parolfactorius (**LPOm**).

All 5 vocal nuclei contain labeled neurons in adults including most of the large projection neurons in both the dorsal and ventral subdivisions of AAc (AAcd and AAcv). Both HVo and NAom contain many labeled neurons, although the density of these appears less than that of AAc. Prominent, large stained neurons in LPOm are scattered throughout the nucleus; many also exhibit prominent dendritic shafts. NLc contains a lower density of labeled neurons in adults compared to other vocal nuclei and surrounding fields.

In nestlings, AAcd, AAcv, NLc and LPOm contain large numbers of NADPH-d+ cells at 1 week posthatching. All nuclei except NAom exhibit higher densities of labeled cells at 2-4 weeks posthatch than in adults. Differences in NADPH-d expression in nestlings and adults may reflect developmental changes related to either pathway formation and/or the emergence of vocal learning functions. Supported by NIH grant MH40698 to S.E.B.

274.14

EVIDENCE FOR A SENSITIVE PERIOD FOR SENSORIMOTOR INTEGRATION DURING SONG DEVELOPMENT IN THE ZEBRA FINCH, C. Pytte* and R. A. Suthers. Dept. of Biol. and Sch. of Med., Indiana University, Bloomington, IN 47405

Zebra finches develop song in reference to an auditory song model acquired during a sensitive period of song learning. Song development also appears to require subsong and plastic song production during which motor commands are thought to be matched with auditory feedback which is compared with the memorized song model. To determine whether or not subsong and plastic song production must occur within a sensitive developmental period in order for normal song development, we reversibly paralyzed bilateral ventral syringeal muscles using local injections of botulinum toxin (Botox) at various times during song development. Botox blocks acetylcholine release thereby preventing synaptic transmission at the neuromuscular junction. The effects of syringeal Botox injections on the acoustic structure of song at the doses used in this study (3.0 μm) were reversed in adults by 3-4 weeks. Juveniles injected prior to, or early in the period of subsong (20-25 days) developed normal songs which crystallized at about day 90. However, birds injected with Botox immediately prior to the usual period of late plastic song and song crystallization (days 80-90) did not develop normal songs, although the abnormal songs did become stable and highly stereotyped. These results suggest that auditory matching of a bird's own song with that of the model is not necessary for crystallization to occur since experimental birds crystallized songs which had not been heard as juveniles. In addition, there appears to be a sensitive period for sensorimotor integration in the zebra finch, after which the bird does not continue to change motor commands necessary to copy his tutor's song. Supported by grants NIH NS 29467 and NSF IBN 9411191 to R.S. and an NIH NIDCD Research Training Grant DC-00012 to Indiana University.

274.15

DEVELOPMENTAL EFFECTS OF SONG DEPRIVATION IN ZEBRA FINCHES. C.E. Collins*, D. Airey, J. Guzman, V. Lisi, N-Y. Nam, K. Tremper and T. J. DeVoogd. Department of Psychology, Developmental Neuroscience Group, Cornell University, Ithaca, NY 14853.

Song learning in zebra finches (*Taeniopygia guttata*) takes place during a juvenile sensitive period. Environmental manipulation deprived one group of zebra finches of the opportunity for song learning. Individuals in this group untutored condition (GU) were raised by their female parent only in the presence of siblings, while individuals raised in the normal social condition were raised in an aviary with both parents and siblings. Normal socially reared zebra finches and GU finches were studied for possible neuroanatomical, behavioral and hormonal differences. Longitudinal behavioral data, collected between 25 days of age and 120 days of age, indicate no significant differences in the development of or time of onset of 6 species-typical behaviors, including song. Birds in both conditions begin to produce plastic song around 38 days of age. Preliminary analysis of song structure between the two groups of birds indicates a highly significant difference in a measure of note rate, i.e. number of note types produced per unit of time ($p<.01$). The time of song crystallization in the two groups is currently being studied as well as other details of song structure. Measures of testosterone (T) titers throughout development demonstrate significantly higher levels of T in the GU condition males and females compared to their normally reared counterparts ($p<.02$). The divergence in T levels begins around 60 days of age and declines around 110 days of age. Anatomical measures in song structure HVC reveal significant differences in synaptic density in 55d old zebra finch males, with no between group differences in HVC volume or cell density. RA volume differs significantly between the two groups at 55d of age and may reflect a trend toward a higher song rate in normally raised male zebra finches compared to GU males.

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274.16

LONG-TERM EFFECTS OF SONG PERTURBATION ON SONG PRODUCTION IN ZEBRA FINCHES. G.E. Hough II* and S.F. Volman. Dept. of Zoology, Ohio State Univ., Columbus, OH 43210.

When adult zebra finches (*Taeniopygia gutatta*) are deafened by cochlear removal, their learned songs become profoundly degraded within 16 weeks (Nordeen & Nordeen, 1992). Cochlear removal prevents auditory feedback from song, but it also may induce neuronal changes in brain areas necessary for song production. To ask whether correct auditory feedback alone is necessary for song maintenance, we used a reversible technique that distorts vocal output and does not damage the auditory system.

Birds were implanted with a flexible bead made from a dental impression material (Permlastic, Kerr Inc.). The 1-2 mm diameter beads were inserted between the two internal tympaniform membranes of the syrinx, altering the biomechanics so that song was distorted. Aberrations in syllable structure included low volume, upward frequency shifts, missing harmonics, or more complex effects. Four out of five birds tested thus far also deleted at least one, syllable from the ends of their motifs within four weeks after bead insertion. We removed the beads after 16 weeks. Birds healed from surgery within one week, and by that time all distorted syllables had returned to their pre-bead phonology. In no case, however, did deleted syllables reappear.

The quick recovery of syllable fine structure following bead removal indicates that auditory feedback is not necessary to maintain syllable morphology. However, the permanent deletion of song syllables suggests that auditory feedback and/or continual song production is required to preserve the complete song pattern.

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274.17

BENGALESE FINCHES DEPEND UPON AUDITORY FEEDBACK FOR MAINTENANCE OF STABLE ADULT SONG. S. M. N. Woolley and E. W Rubel*. Virginia Merrill Bloedel Hearing Research Center, University of Washington, Seattle, WA 98195

The Bengalese finch, *Lonchura Striata*, is an age-limited song learning species; once song has crystallized by the age of sexual maturity, it normally does not change over time. In this regard, song learning in Bengalese finches is quite similar to that of the closely related Zebra Finch. Although both species depend on auditory feedback for song learning, the role of auditory feedback in adult song is less clear. Classical studies suggest that once song is crystallized in age-limited learners, auditory feedback is not necessary for maintenance of stable song. We now know, however, that Zebra Finches show significant changes in song 8-16 weeks after deafening (Nordeen & Nordeen, *Behav. & Neural Biol.* 57: 58, 1992). To determine whether Bengalese finches also depend on auditory feedback for maintenance of crystallized song, we deafened adult males.

We recorded song from 12 adult (>160d) male Bengalese finches monthly for 3 months. We then deafened 6 of the 12 birds by surgical cochlear removal. Song from all 12 birds was recorded weekly for 6 weeks after surgery and then again at 12 weeks. The 6 control birds continued to sing stereotyped and stable song over months. Deafened finches, however, showed rapid and marked changes in song. As early as 1 week, we observed degradation of syllable order, truncation and compression of syllables, and the appearance of new or unrecognizable syllables. This degradation became more exaggerated over time. Our results demonstrate that Bengalese finches depend more heavily upon auditory feedback to maintain a stable motor pattern for song than has been reported for other age-limited song learning species. Supported by NIDCD grant DC00395 & GM10708.

274.18

EFFECTS OF HVC LESION ON SONG PREFERENCES IN FEMALE ZEBRA FINCHES. S. MacDougall-Shackleton, S. H. Hulse* & G. F. Ball. Behavioral Neuroendocrinology Group, Dept. of Psychology, Johns Hopkins Univ., Baltimore, MD 21218.

The oscine song control nucleus HVC (high vocal center) has been demonstrated to function in song production. However, neurons in HVC also exhibit auditory properties, and HVC has been shown to be important for conspecific song discrimination by female canaries. We tested preferences of female zebra finches (Taeniopygia guttata) for conspecific and heterospecific song both prior to and following lesion of HVC. We also tested female preferences for complex songs (containing 12 different syllables) over simpler songs (containing 3 different syllables). Birds were implanted with Silastic tubes containing 17-ß estradiol and were isolated in sound attenuating chambers for at least 10 days. Next birds were presented with 4 minutes of each type of song interspersed with 4 minutes of silence. Response of the birds was videotaped and the number of copulation solicitation displays were scored. Following response, we made bilateral electrolytic lesions to HVC (experimental group) or elsewhere in the telencephalon (control group). Prior to lesion, female zebra finches performed a greater number of solicitation displays to conspecific song than to heterospecific song. Females also tended to perform more displays to 12-syllable songs than to 3-syllable songs. Following lesion, control-lesion birds maintained these preferences. Preliminary data indicate that HVC-lesion females, however, displayed equally to all song stimuli, including heterospecific song.

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274.19

EARLY VOCAL AND NEURAL DEVELOPMENT IN THE BUDGERIGAR. W.S. Hall*, K.K. Cookson, J.T.Heaton, T. Roberts, S.Shea, and S.E. Brauth. Dept. of Psychology, Univ.MD., College Park, MD, 20742-4411.

We investigated the early development of vocal behavior and general cytoarchitecture of vocal nuclei in budgerigars (*Melopsittacus undulatus*). The duration and internal acoustic organization of vocalizations were analyzed from hatching to six weeks of age. Vocal development during this period exhibits five transitions: (1) innate foodbegs with relatively short durations and few repeated segments at week 0; (2) more mature foodbegs with slightly longer durations and more repeated segments at 1-2 weeks; (3) immature transitional calls with very long durations and peak numbers of repeated segments at 3-4 weeks; (4) more mature transitional calls with shorter durations and fewer repeated segments at 5-6 weeks; and (5) socially learned contact calls at 6+ weeks. Observation of general cytoarchitectural organization suggests asynchronous maturation of vocal nuclei. The nXII of the hindbrain has an adult-like appearance by 1 week posthatching. The dorsomedial nucleus of the intercollicular midbrain (DM) has an adult-like appearance by 2 weeks posthatching. The central nucleus of the archistriatum (AAc), the central nucleus of the neostriatum (NLc), the oval nucleus of the neostriatum (NAo), and the oval nucleus of the hyperstriatum ventrale (HVo) have an adult-like appearance by 2-4 weeks posthatching. Transitions in budgerigar vocal development are similar to transitions in human vocal development from birth to single word utterances. These similarities may be due to similar patterns of neural implementation and asynchronous maturation of vocal centers.

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274.20

EFFECTS OF VOCAL ARCHISTRIATAL LESIONS AND EARLY DEAFENING ON VOCAL DEVELOPMENT IN THE BUDGERIGAR. J. T. Heaton* & S. E. Brauth. UMCP, College Park, MD 20742.

The production of socially learned contact calls in budgerigars depends upon descending projections from the central nucleus of the anterior archistriatum (AAc) to brainstem syringeal and respiratory nuclei. In order to examine the role of this pathway in vocal development we lesioned the vocal archistriatum in nestlings aged 5, 9, 13, 22, 26, and 33 days posthatch. In addition, we deafened 5 nestlings at 9-10 days to assess the role of auditory feedback in call development.

Call development in budgerigars can be described as a series of 5 periods or transitions (Hall et al., this session) from simple patterned food begging calls (Periods 1 & 2 - up to 2 weeks posthatch), to longer, harsh-sounding calls (Period 3 - around week 3), to complex patterned begs (Period 4 - weeks 4-5) which decrease in duration near the time of fledging and form the first contact calls (period 5). Lesioned and deafened nestlings both demonstrated nearly normal period 2-3 calls, however these birds continued to produce noisy, harsh-sounding calls similar to period 3 calls into adulthood, thus failing to develop both complex begs and contact calls. Call durations for AAc-lesioned birds remained very long compared to controls, while those of deafened birds were only slightly longer than controls.

Pre-grammatical vocal development in humans can also be described in terms of 5 periods. Like that of budgerigars, human vocal development also exhibits dependence on auditory feedback at period 4, which may mark the initiation of forebrain vocal control in both humans and budgerigars.

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INVERTEBRATE LEARNING AND BEHAVIOR II

275.1

A STUDY OF HABITUATION OF eat-4, A PROSPECTIVE LEARNING MUTANT IN C. ELEGANS. S.R. Wicks and C.H. Rankin*. Dept. of Psychol., U. of British Columbia, Vancouver, BC, CANADA V6T 1Z4.

We have demonstrated that the tap withdrawal reflex of *C. elegans* displays habituation, dishabituation and sensitization. The genetic analysis of learning in our lab takes the form of a mutant screen for animals that show disrupted non-associative learning. As a compliment to this screen we have been assessing habituation in a variety of mutants isolated by the *C. elegans* community. One such mutant, eat-4, isolated by Leon Avery's lab, appears to possess many of the characteristics of a true learning mutant—the withdrawal reflex is intact; the modulation of the reflex by experience is exclusively altered—and may be used to illustrate what types of changes a habituation screen should be sensitive to.

Habituation of eat-4 animals is very rapid compared to wild-type worms. Controls trained at a 10-s interstimulus interval (ISI) generally reach an asymptotic level of responding after about 8 stimuli. By contrast, eat-4 animals appear to be unresponsive after just 2 or 3 stimuli. The asymptotic phase of habituation is also affected by the mutation. The eat-4 worms rarely respond after habituation to asymptote, while control worms generally continue to respond, albeit at depressed levels, even after prolonged stimulation. Recovery from habituation in eat-4 animals is also delayed. Habituation training at short (2-s and 10-s) ISIs leads to a relatively rapid recovery in intact animals; wild-type worms show significant recovery by a 30-s time point and are fully recovered by a 5 min time point. eat-4 worms show retarded recovery from both 2-s and 10-s ISIs

The eat-4 gene is thought to encode a transporter required for glutamate neurotransmission. Our results suggest that, although the initial levels of responding are normal in eat-4 animals, habituation of the tap withdrawal reflex is broadly affected. This work was funded by NSERC and HFSP grants.

275.2

HABITUATION STUDIED USING AN *APLYSIA* PREPARATION WITH TWO SETS OF INDEPENDENT SENSORY INPUTS L. Guan* Y. Tsau and J.-Y. Wu. Department of Neurology and Institute for Cognitive and Computational Sciences, Georgetown University Medical Center, Washington DC, 20007

During habituation of the gill withdrawal reflex, a large number of neurons in the *Aplysia* abdominal ganglion underwent a non-uniform reduction in their response to siphon stimulation (Falk et al J. Neurosci. 13:4072, 1993). Part of this change can be assigned to the homo-synaptic depression of synapses from sensory neurons to motor neurons and interneurons. However, there may be other components of habituation related to higher level of processing and are not directely related to the sensory neurons. In order to distinguish these two kinds of habituation, we have modified the reduced siphon-ganglion-gill preparation. In our modified preparation there were two isolated parts of siphon skin attached to the abdominal ganglion via separate branches of siphon nerve. When the parts of the siphon were placed into two separate chambers and bathed with high Mg^{++} and low Ca^{++} saline while ganglion and gill were in normal sea water, two independent sets of sensory inputs to the abdominal circuitry were established. A light touch to either part of siphon elicited a normal gill withdrawal reflex. Habituation could be induced by repeated (1/min) touches to either part of the skin. There were no noticeable changes in the reflex elicited from the control side after a complete habituation induced by touching the other part of the siphon. We are presently analyzing the spiking data optically recorded form a large number of abdominal neurons.

(Supported by NIH NS 31425).

275.3

PRESYNAPTIC RECEPTORS FOR NEUROMODULATORS EVOKING SHORT-TERM HETEROSYNAPTIC PLASTICITY IN *APLYSIA* ARE DISTRIBUTED DIFFERENTIALLY. Z.-Y. Sun*, B. Kauderer and S. Schacher. Cntr Neurobiol.& Behav., Columbia Univ., Coll. of P & S, NYSPI, New York, NY 10032.

The efficacy of *Aplysia* sensorimotor connections is modulated up and down by 5-HT and FMRFamide. Previous *in vivo* studies indicated that short-term changes in efficacy of these connections require the actions of the neuromodulators at or near the sensory neuron (SN) terminals. Other changes evoked by 5-HT appear to require the actions of 5-HT at or near the SN cell body. To explore the regional distribution of 5-HT and FMRFamide receptors mediating changes in synaptic efficacy and excitability, we combined methods using dissociated cell culture of SN-L7 connections, epifluorescent light microscopy to visualize sites of synaptic contacts, electrophysiology, and focal applications of neuromodulators to four sites on the SN (cell body, axon, SN varicosities contacting L7, and processes contacting L7 but without varicosities). We found that short-term changes in synaptic efficacy were evoked only when the neuromodulators were applied to regions containing SN varicosities. Applications to neighboring regions that had the very same SN neurites, but no varicosities, failed to evoke a change in synapse efficacy. The two neuromodulators differed in the location of receptors mediating changes in excitability. Whereas an excitability increase was evoked only when 5-HT was applied to the cell body, an excitability decrease was evoked only when FMRFamide was applied to regions containing SN varicosities. The functional distribution of these receptors on the SNs *in vitro* parallels the distribution of varicosities that are immunoreactive for the respective transmitters *in vivo*. These results suggest that receptors for neuromodulators evoking short-term changes in the properties of SNs are distributed and expressed differentially along the surface of the SN as it interacts and forms connections with the motor cell. Subsequent studies will examine whether the same sites enriched for receptors are also critical for the expression of long-term synaptic plasticity.

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275.4

THRESHOLD REVERSAL FOR SYNAPTIC FACILITATION AND INCREASED EXCITABILITY WITH SEROTONIN AND TAIL NERVE STIMULATION IN *APLYSIA* SENSORY NEURONS. S.A. Bunge[1], J. Mauelshagen[2], T.J. Carew.*[1,2] Yale University, Departments of Biology[1] and Psychology[2], New Haven, CT, 06520.

Tail shock induces sensitization of the tail and siphon withdrawal reflexes in *Aplysia*. Tail shock also induces a number of modulatory effects in the tail sensory neurons (SNs), including increased excitability and facilitation of synaptic transmission from tail SNs to motor neurons. Both of these modulatory effects are mimicked by exogenous application of serotonin (5HT) (Walters et al., 1983).

Recently we have shown that the different modulatory effects induced by 5HT in the SNs have different activation thresholds (Stark et al., 1996, Emptage et al., 1996). To extend these observations, in the present study we examined the activation thresholds for increased excitability and synaptic facilitation, induced either by 5HT (n=7) or by electrical stimulation of the tail nerve P9 (n=15). Using exogenous 5HT, we found that the threshold concentration (2 μM) sufficient to produce significant excitability increases ($p<.02$) produced no significant synaptic facilitation. Conversely, the intensity of nerve stimulation sufficient to produce significant synaptic facilitation (median increase = 209% of baseline, $p<.001$) produced no excitability changes. We subsequently observed significant synaptic facilitation with 30-60 μM 5HT (median increase = 166%, $p<.05$) and significant increased excitability with higher intensity nerve stimulation ($p<.005$), indicating that both 5HT and nerve stimulation were capable of inducing both forms of modulation. The reversal of relative thresholds for these modulatory effects may reflect the differential access of exogenous 5HT and endogenous 5HT (released by tail nerve stimulation) to the SN cell body and synaptic terminals respectively.

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275.5

POSTTETANIC POTENTIATION AND ACTIVITY-DEPENDENT FACILITATION AT *APLYSIA* CULTURED SENSORY-MOTOR NEURON SYNAPSES INVOLVE BOTH PRE- AND POSTSYNAPTIC MECHANISMS. J.-X. Bao* and R.D. Hawkins. Ctr. Neurobiol. & Behavior, Columbia Univ., NY, NY 10032.

Both posttetanic potentiation (PTP) and activity-dependent facilitation (ADF) induced by pairing tetanic stimulation and the facilitatory transmitter serotonin (5-HT) at *Aplysia* sensory-motor neuron synapses have been thought to be entirely presynaptic. We have further studied their cellular mechanisms at pleural sensory-L7 and sensory-LFS synapses in isolated cell culture. Tetanic stimulation (20 Hz for 2 s) of the sensory neuron or a single puff of 5-HT (50 μM) produced a short-lasting potentiation of evoked release, while pairing tetanus with 5-HT induced a larger and longer-lasting facilitation. Consistent with previous results (Eliot et al., 1994a,b), PTP and ADF of evoked release were accompanied by a large increase in the frequency but not the amplitude of spontaneous release. In addition, PTP was almost completely blocked by presynaptic injection of EGTA, a Ca^{2+} chelator with slow kinetics, and ADF was significantly reduced by presynaptic injection of a peptide inhibitor of protein kinase A. However, PTP and ADF were also significantly reduced by postsynaptic injection of BAPTA, a Ca^{2+} chelator with fast kinetics, or strong postsynaptic hyperpolarization during the tetanus (Bao & Hawkins, 1995). By contrast, postsynaptic hyperpolarization had no effect on the increase in the frequency of spontaneous release during PTP or ADF. These results suggest that during PTP and ADF at *Aplysia* sensory-motor neuron synapses, a postsynaptic increase in intracellular Ca^{2+} may lead to formation of a retrograde messenger which interacts in some way with presynaptic residual Ca^{2+} (PTP) or cAMP (ADF) to enhance evoked (synchronized) release. Supported by NIMH and Human Frontier Science Program Foundation.

275.6

GROWTH FACTOR-INDUCED LONG-TERM SYNAPTIC FACILITATION IN TAIL SENSORIMOTOR SYNAPSES OF *APLYSIA*. S.E. McKay* and T.J. Carew, Dept. Psychology, Yale University, New Haven, CT

Neurotrophic factors have long been known to mediate events in the developing nervous system of vertebrates, including proliferation, differentiation, neurite outgrowth, and cell survival. Recently it has been suggested that neurotrophins may play a role in both developmental and adult forms of synaptic plasticity in both vertebrates and invertebrates. To explore this possiblity we have begun to investigate neurotrophin effects in the mollusc *Aplysia*. Monosynaptic connections between tail sensory neurons (SNs) motoneurons (MNs) in the intact ganglia show both short-term (minutes) and long-term (days) facilitation in response to serotonin. In our studies, 63% of synapses (n=8) showed long-term, but not short-term, facilitation following 30-60 minute treatments with 30 ng/ml brain-derived neurotrophic factor (BDNF). Among the synapses showing a response to BDNF (n=5) the x̄% increase in EPSP amplitude was 112%, while controls, treated with 30 ng/ml cytochrome c (n=2) showed an average 17% reduction in EPSP amplitude (U=0.00, p=0.047). Treatment with BDNF resulted in no change in either the membrane resistance or resting potential of SNs and MNs, or in the threshold for spike initiation among the SNs. These results suggest that BDNF may alter synaptic efficacy by producing long term molecular or structural changes in either the SNs or the MNs. Further studies with other neurotrophins, including NGF, NT-3, EGF, and CNTF are currently underway; preliminary results suggest that only a specific set of growth factors may be capable of mediating the changes associated with synaptic plasticity.

We are grateful to Amgen for providing the BDNF and NT-3 used in these studies. Supported by NIH grant R01-MH-14-1083.

275.7

SYNAPTIC FACILITATION IS INDEPENDENT OF SPIKE DURATION IN SENSORY NEURONS OF JUVENILE *APLYSIA*. L.L. Stark* and T.J. Carew. Interdepartmental Neuroscience Program and Department of Psychology, Yale University, New Haven, CT. 06520.

In adult *Aplysia*, serotonin (5HT) activates two forms of presynaptic facilitation at the synapse between tail sensory neurons (SNs) and tail motor neurons (MNs). One form, associated with major (>15%) SN spike broadening (SB), is predominantly expressed in non-depressed synapses. A second, spike duration independent (SDI) form is predominantly expressed in depressed synapses. During the last stage of juvenile development, 5HT-induced SB is not fully mature; major SB is often absent (Marcus & Carew, 1990, 1992). Thus, we could examine the impact of minor and major SB on non-depressed synapses at this immature stage. We found that 5HT-induced minor SB (x̄=8.03% increase) was associated with significant synaptic facilitation (x̄=130.60% increase; p=.05, N=7). However, major SB (x̄=43.68%) produced no significant facilitation (x̄= -10.79%, N=7) Moreover, minor SB was associated with significantly greater synaptic facilitation than major SB (p<.04). In adults the reverse was observed: major SB produced significantly more facilitation than did minor SB (p<.04). Therefore, even in non-depressed synapses, SDI facilitation is the predominant, if not exclusive, form of synaptic facilitation in juveniles. This difference is not due to the inability of the juvenile SN to show synaptic facilitation associated with SB, since directly broadening the SN action potential with 25μM 4-AP resulted in significant SB and synaptic facilitation, with no significant difference between juveniles and adults.

In adults, 5HT increases cAMP in the SNs, leading to increased excitability and minor SB. In juveniles, we found that the cAMP analog 8-br-cAMP (x̄=5.9mM, N=10) significantly increased excitability (x̄=156.60%, p<.001) and induced minor SB (x̄=11.38%, p<.03). However, preliminary evidence indicates that 8-br-cAMP does not facilitate synaptic transmission, suggesting that another second messenger underlies SDI facilitation in juvenile *Aplysia*. Supported by NIH grant 5-F31-MH10673 to LLS & NSF, IBN9221117 to TJC.

275.8

ALTERNATIVE SPLICE VARIANT OF THE VOLTAGE-GATED NA^+ CHANNEL OF *APLYSIA* IS MISSING PKA CONSENSUS SITES.

[1,2]W.L. Johnston, [2]V.F. Castellucci and [1]R.J. Dunn*, [1]Centre for Research in Neuroscience, McGill University, Montreal General Hospital Research Institute, Montreal, Canada; [2]Institut de Recherches Cliniques de Montréal and Université de Montréal, Montréal, Canada.

We recently cloned the voltage-gated Na^+ channel from the marine invertebrate, *Aplysia*. RNase protection analysis, using a probe derived from the first intracellular loop, demonstrates that this channel is specific to the nervous system. We also used RT-PCR to amplify the first intracellular loop, which contains all four of the channel's PKA consensus sites. In addition to the previously cloned version, we also identified a splice variant in which all four PKA sites are missing. Sequence analysis demonstrated that splicing results in a shorter version in which a 450 base pair segment is missing, beginning in the middle of the first PKA consensus site and ending 140 base pairs downstream of the forth PKA consensus site. It is predicted that this channel should be insensitive to phosphorylation by PKA. We are actively investigating the physiological roles of these two isoforms of the Na^+ channel and their possible contribution to short- and long-term facilitation in the *Aplysia* nervous system.

Research supported by the MRC, Canada and the NIH.

275.9

ROLE OF PKC IN INTERMEDIATE FORMS OF MEMORY; MODULATION OF SYNAPTIC TRANSMISSION AND EXCITABILITY. F. Manseau, W. S. Sossin and V.F. Castellucci *. Lab. de Neurobiologie, IRCM, Département de Physiologie, Univ. de Mtl., Montréal, H2W 1R7, Canada.

Previous studies indicate that at least two second messenger activated kinases (PKA and PKC) contribute to short-term facilitation of sensorimotor synapses by 5-HT in Aplysia withdrawal reflex. Little is known about the contribution of these systems in the newly described intermediate-term facilitation (Ghirardi *et al.* 1995). On the other hand, biochemical observations (Sossin *et al.* 1994; Yanow and Sossin 1996) suggest that PKC could be implicated. Using a dissociated cell culture preparation, we have compared the strength of sensorimotor connections before and at 3 hours after pharmacological activation of PKC (PDBU 100 nM, 90 min.). We found a significant increase of EPSP amplitude: + 160 % (n = 14) as opposed to: - 8 % (n = 12) for control synapses (inactive isomer 4-α-PDBU). These results are consistent with those recently published by Wu *et al.* (1995). While performing these experiments we noticed a change in the excitability of sensory neurons at the 3 hour time-point after PDBU treatment. Excitability was measured as the number of action potentials discharged over a specific range of depolarizing currents before and after treatment with PDBU or 4-α-PDBU (100 nM, 90 min.). At 3 hours post-treatment, PDBU (100 nM, 90 min.) caused a significant increase of excitability (+ 109 %; n = 28 p<0.001) which was still observed after 24 hours (+ 83 %; n = 16 p<0.05). Inactive isomer had no effect at either time points (+ 4 %; n = 25 n.s. and + 3 %; n = 22 n.s.). These intermediate- and long-term effects were only partially blocked by a protein synthesis inhibitor, and could also be induced by a short (5 min.) exposure to PDBU. The increase in excitability was not associated with a change in cell resistance. We propose that PKC might be implicated in an intermediate form of memory and that one component is independent of protein synthesis. Sources of funding: FCAR and MRC of Canada.

275.10

Cloning of Conditioning-Associated Protein cp20, a Calcium- and GTP-binding Protein which Enhances Membrane Excitability

T.J. Nelson*, C.-L. Yi, S. Cavallaro, D. McPhie, B. G. Schreurs, A. Favit, and Daniel L. Alkon, Laboratory of Adaptive Systems, NINDS, National Institutes of Health, Bethesda MD 20892 and Harvard Medical School, McLean Hospital, 115 Mill St., Belmont MA 02178 USA.

Cp20, a low-MW learning-specific GTP-binding protein that has also been implicated in Alzheimer's disease, was cloned from squid optic lobe and expressed. In addition to binding GTP, the cloned protein bound 2 molecules of Ca^{2+} , with a Ks of ≈400 nM. The cDNA sequence of cp20 bore a close homology with the sarcoplasmic calcium-binding protein scp1 as well as low-MW GTP-binding proteins such as adenosine ribosylation factor (ARF). Cp20 was also a high-affinity substrate (Km=5nM) for the α-isoform of protein kinase C. Phosphorylation of cp20 was markedly inhibited by GTP. Cloned cp20 was microinjected into *Hermissenda* photoreceptor neurons and rabbit Purkinje cells. In both cases, cp20 was highly effective at enhancing membrane excitability; *e.g.*, purkinje cell excitability was increased by 25%. This effect is consistent with cp20's known reduction of both i_A and i_{Ca-K+} potassium currents and with our previous demonstration of correlations of cp20 phosphorylation with memory storage. The unprecedented combination of calcium- and GTP-binding activity exhibited by cp20 suggests that it may have a pivotal role as a point of convergence between these two critical signalling pathways.

275.11

A NOVEL NEUROACTIVE PEPTIDE FROM THE ATRIAL GLAND OF *APLYSIA*. Samuel K. Moore, Frederick W. Kauer, Valerie L. Begnoche, and Earl Mayeri*. Dept. of Physiology, University of California, San Francisco, CA 94143-0444 USA.

The atrial gland is an exocrine gland located in the reproductive tract of the marine mollusk *Aplysia*. It expresses large amounts of biologically active peptides of the egg-laying hormone (ELH) gene family, which are thought to play important roles in regulation of reproductive physiology and behavior. Using HPLC purification methods we have isolated and partially characterized a novel peptide from the gland. Amino acid composition, partial sequence analysis and mass spectrometry indicate that the peptide has 31 amino acids, is about 3400 Da and is unrelated to the ELH gene family or other biologically active peptides. Application of the peptide at 1-5 μM concentration to identified cells in the abdominal or cerebral ganglia produces prolonged excitation, in which a silent cell is depolarized by a few millivolts and fires action potentials at a steady rate for 30 min or more. Many other cells in the CNS are unaffected by the peptide, and it has no obvious effects on reproduction or behavior when injected into intact animals. The peptide is present in the gland in very high amounts, approximately 120 µg per 446 gm animal, suggesting that it is important for reproductive control.

(Supported by NIH grant NS16490.)

275.12

NERVE INJURY, BUT NOT 5HT TREATMENT, RAPIDLY INACTIVATES THE TRANSCRIPTION FACTOR NF-κB IN APLYSIA AXONS. M. Povelones, K. Tran, D. Thanos, R. T. Ambron*. Depts. of Anatomy and Cell Biology and Biochemistry and Molecular Biophysics, Columbia University, New York, NY 10032.

There is considerable evidence for a convergence of intracellular signaling pathways for axon regeneration and 5HT-induced learning. Divergent pathways must exist also, since regeneration, but not learning, requires extensive growth and pathfinding. The transcription factor NF-κB normally resides in the cytoplasm via an association with an inhibitory subunit, IκB, which masks its nuclear localization signal (NLS). In response to extracellular signals, IκB is destroyed and NF-κB is imported into the nucleus. We have identified a protein in axoplasm from Aplysia (ap) that has the properties of NF-κB. The apNF-κB binds to κB oligonucleotide probes and the binding is inhibited by mammalian IκB-α. Constitutively active and latent apNF-κB were present and, like its vertebrate counterpart, the latent form was activated by deoxycholate. Since axoplasmic proteins that contain an NLS can be retrogradely transported to the nucleus, active apNF-κB could signal the nucleus about events in the axon periphery. To see how apNF-κB responds to injury, peripheral nerves to the body wall were crushed; surprisingly, there was a loss of apNF-κB binding at the crush site and, within 15 min, as far as 2.5 cm along the axon. By 45 min there was a reduction in the soma. In contrast, exposure of either the intact animal or the nervous system *in situ* to levels of 5HT that induce learning had no affect on apNF-κB activity. This indicates that the events regulated by apNF-κB are incompatible with regeneration and that they do not influence events induced by 5HT. Supported by the NIH

275.13

TEMPORAL ASPECTS OF ACTIVATION OF ADENYLYL CYCLASE BY Ca^{2+} AND NEUROTRANSMITTER: POSSIBLE MECHANISM FOR CS-US SEQUENCE PREFERENCE DURING CONDITIONING. Depts. of Pharmacology & Anesthesiology, University of MD School of Medicine, Baltimore, MD 21201. C. Onyike and T.W. Abrams*.

During classical conditioning of the defensive withdrawal reflex in *Aplysia*, within the sensory neurons of the conditioned stimulus pathway, the dually-regulated Ca^{2+}/CaM-sensitive adenylyl cyclase may serve as an associative integrator for the cellular signals from the conditioned and unconditioned stimuli (CS & US). Conditioning is more effective when CS onset precedes US onset. If adenylyl cyclase is a critical molecular integrator, it should be more effectively activated when Ca^{2+} (the signal from the CS) precedes 5HT (the signal from the US) than when stimulus onset is simultaneous. We previously observed that a Ca^{2+} pulse prior to a 5HT pulse produces better activation of *Aplysia* neural cyclase than the backwards sequence. Comparison of the temporal activation profiles of *Aplysia* cyclase has now indicated that the Ca^{2+}/CaM response onset is slower than the 5HT response onset. More detailed studies of the activation profile of recombinant mammalian brain type I adenylyl cyclase revealed that stimulation by a pulse of Ca^{2+} actually rose after, and persisted after, free Ca^{2+} declined, providing a possible brief memory of neuronal activity. Why does cyclase stimulation by CaM persist seconds after a Ca^{2+} pulse? By activating CaM, and then lowering free Ca^{2+} <1 sec before a CaM pulse reached the cyclase-containing membranes, we found CaM in solution had no memory for Ca^{2+}. Using a photoactivatable linker attached to CaM, we are characterizing the time course of persistent CaM-to-cyclase coupling once free Ca^{2+} levels have dropped. Supported by RO1 MH55880-02 from NIMH.

275.14

RECIPROCAL OCCLUSION OF EXCITABILITY CHANGES IN Hermissenda TYPE B PHOTORECEPTORS BY PHORBOL ESTER AND ASSOCIATIVE TRAINING *R. McEwen, M. Smith, I. Jin, and J. Farley*. Program in Neural Science, Indiana University, Bloomington, IN 47405.*

Previous research has implicated activation of protein kinase C (PKC) in the induction and maintenance of memory-related changes in Hermissenda Type B photoreceptors. Here, we asked if the acute effects of phorbol ester stimulation on type B cell excitability were attenuated if animals were first exposed to associative training, viz. repeated pairings of light and rotation. Synaptically-intact Type B cells (n = 6) from untrained animals responded to 10^{-7} M phorbol 12-myristate 13-acetate (PMA) stimulation with an average (mean ± sem) steady-state light response enhancement of +4.83 ± 0.73 mV. In contrast, B cells (n = 5) from associatively-trained animals responded to PMA stimulation with a decrease in their steady-state light response (-3.20 ± 0.94 mV). In parallel experiments, we also examined the ability of phorbol ester stimulation to occlude short-term excitability changes in type B cells produced by in vitro conditioning. In control preparations (n = 8), five pairings of light and intracellular current stimulation of a statocyst caudal hair cell produced an average 8.43 ± 0.31 mV of cumulative depolarization. In contrast, B cells (n = 5) from preparations pretreated with PMA showed only 3.50 ± 0.92 mV of depolarization, a significant reduction of ~58% Collectively, our results provide additional support for the involvement of PKC in both short- and long-term changes in associatively-produced changes in B cell excitability. The failure of PMA to completely occlude in vitro conditioning-produced changes in B cell excitability implies the involvement of additional signal transduction pathways, probably involving a tyrosine kinase (see Jin & Farley abstract, this volume). Supported by grants from NIH.

275.15

PROTEIN PHOSPHATASE INVOLVEMENT IN LEARNING-PRODUCED CHANGES IN *Hermissenda* TYPE B CELLS H. Huang* and J. Farley. Programs in Neural Science, Indiana University, Bloomington, IN 47405.

The possible involvement of protein phosphatases in the production of memory-related changes in type B cell excitability was assessed by exposing isolated nervous systems to five pairings of light and electrical stimulation of statocyst hair cells (*in vitro* conditioning), in the presence or absence of calyculin A, an inhibitor of PP1 & PP2A. Type B cells from control preparations ($n = 5$) depolarized by 7.2 ± 0.84 mV (mean ± sem). In contrast, preparations exposed to a 20 nM bath-concentration of calyculin depolarized by only 1.75 ± 2.47 mV. However, these preparations had depolarized by 12.5 ± 2.12 mV *before* the start of *in vitro* conditioning. In separate experiments, leakage of calyculin A from the recording electrode into the B cell prior to conditioning reduced the subsequent development of cumulative depolarization by at least 75%, after correction for the effects of leakage. Thus, calyculin A appears to occlude the depolarization of B cells that is produced by conditioning. Comparison of the effects of bath-applied calyculin upon synaptically-intact *vs.* -isolated B cells from untrained specimens, over a 20 min time period, revealed that the rate of depolarization was ~10-fold greater in the former case (~1.65 mV/min vs 0.15 mV/min) but input resistance increases were less pronounced. These results suggest that in addition to inhibition of B cell phosphatases, bath application of calyculin A also changes B cell excitability through its effects upon cells presynaptic to B cells. Collectively, our results suggest that basal PP1 and/or PP2A-activity counteract the effects of basal, as well as conditioning-produced, protein kinase activity upon B cell excitability. Supported by grants from NIH.

275.16

REGULATION OF TRANSLATION BY PKC AND PKA DURING INTERMEDIATE PHASES OF FACILITATION IN *APLYSIA*. S. K. Yanow and W. S. Sossin*. Montreal Neurological Institute, McGill Univ., Montreal, QC H3A 2B4.

Long-term facilitation in *Aplysia* provides a model in which to study cellular mechanisms involved in long-term memory. In *Aplysia*, serotonin (5-HT) induces an intermediate phase of facilitation that is translation-dependent yet independent of transcription (Ghirardi M, Montarolo PG, Kandel ER (1995) Neuron 14: 413-20). In clusters of sensory neurons, 5-HT stimulates an initial decrease in translation followed by a transient increase in protein synthesis that are both independent of transcription (Barzilai A, Kennedy TE, Sweatt JD, Kandel ER (1989) Neuron 2: 1577-86). This regulation of translation may be important for the induction of intermediate memory. We found that 5-HT induced identical changes in translation when whole pleural ganglia were used. In this system, we asked which protein kinases activated by 5-HT were important in mediating the changes in translation. Inhibition of PKC with Chelerythrine (10uM) blocked both the initial decrease and the transient increase in translation. Blocking PKA activation with RpcAMP (500uM) only blocked the transient increase. This suggests a model where PKC mediates the decrease in translation, but both PKC and PKA activation are required for the transient increase.These conclusions are further supported by experiments where addition of phorbol esters (200nM) is sufficient to induce the initial decrease in translation, but does not stimulate the transient increase. These results suggest that both PKC and PKA are required for the induction of intermediate memory. This work is supported by grants from the EJLB Foundation and the MRC of Canada.

275.17

Molecular Cloning of a DCO like Catalytic Subunit of the Protein Kinase A from Honeybee Neuronal Tissue

H. Rosenboom, P. Braun and U Müller*,
Inst. f. Neurobiologie, Freie Universität Berlin, 14195 Berlin, Germany

Using PCR with degenerated primers against a conserved sequence of catalytic subunit of the protein kinase A (PKA) we obtained a cDNA fragment from a honeybee brain cDNA library. Screening the same library with the fragment gave a full length clone. The protein encoded by the open reading frame has at least 90% aminoacid identity compared to drosophila DCO PKA catalytic subunit. As in drosophila, this isoform seems to be prominent in the brain, since 25 isolated clones from the honeybee library tested so far, coded for the DCO like isoform.

This honeybee PKA catalytic subunit sequence information is the basis for an antisense approach to reduce PKA activity in cultured neurones from honeybee brain.

Two assay systems for testing the PKA activity and concentration in cultured honeybee neurones were established. Kinase activity is examined with a phosphorylation assay. The concentration is measured with an ELISA using an antiserum against the drosophila DCO isoform.

Employing these assays, several antisense oligonucleotides are screened on their PKA reducing activity. First results will be presented.

HORMONAL CONTROL OF REPRODUCTIVE BEHAVIOR II

276.1

USING THE DIFFERENTIAL DISPLAY POLYMERASE CHAIN REACTION TO IDENTIFY PROGESTERONE RESPONSIVE mRNA IN THE RAT HYPOTHALAMUS

C. J. Krebs*, E. D. Jarvis, R. E. M. Scott and D. W. Pfaff. Laboratory of Neurobiology and Behavior, Rockefeller University, New York, NY 10021.

The sexual behavior of the female rat, termed lordosis, is known to be facilitated by the effects of progesterone (P) on the hypothalamus. P is the ligand for the progesterone receptor (PR), a member of the steriod hormone receptor superfamily that modulates gene expression in response to steroids in the brain and throughout the body. One event thought to contribute to lordosis is a PR mediated change in gene expression within the ventromedial nucleus (VMN) of the hypothalamus. We are applying the differential display (DD) technique of Liang and Pardee to identify mRNAs that are altered in response to P in the female rat hypothalamus. Total RNA was isolated from the hypothalami of ovariectomized, estradiol primed rats treated with either P or vehicle for 3 hours. DD-PCR reactions were performed on each RNA sample, independently. To date, we have screened 1/8th of the mRNA populations and have identified six differentially regulated bands. To verify the regulation of these mRNAs, we are screening their corresponding cDNAs by slot blot with probes generated from the mRNAs of P treated and vehicle treated animals. We will further characterize these mRNAs by sequencing and in situ hybridization. These studies will identify the genetic targets of the PR within the rat hypothalamus and should contribute to our understanding of lordosis behavior at the molecular level.

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276.2

Sex Difference and Lateral Asymmetry in Rat Hippocampus: Relation to Androgen Receptor Activity.

Murali N. Naidu, Neil V. Watson & S. Marc Breedlove*, Dept. Psychology, Univ. California, Berkeley, CA 94720.

Sex differences in spatial learning ability have been reported in both rats and humans and a variety of studies have implicated the hippocampal formation as important in such learning. Thus there are reports of sex differences in the size of various hippocampal components, including the granule cell layer of the dentate in rats (Roof & Havens, 1992).

Sprague-Dawley rats, 117-123 days old, were sacrificed and perfused. The brains were removed, postfixed at least a week in paraformaldehyde, frozen sectioned coronally in 50 µm thickness. Every 4th section was mounted and stereologically examined using a microprojector by an observer blind to group membership. We found that the volume of the hippocampal formation was larger in males than in females ($p<.05$, t-test, N=6 per group). There was also lateral asymmetry in hippocampal volume in males only ($p<.05$, matched-pairs t-test), such that the sex difference in volume was significant on the right but not on the left.

	Left Hip.	Right Hip.	Total Hippocampus
Males	43.7±0.97	46.2±1.52	89.9±2.45 (mm^3)
Females	38.9±2.22	40.2±1.69	79.1±3.87

We will examine the hippocampus of androgen-insensitive males and of perinatally androgenized females. Supported by NS28421.

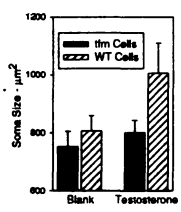
276.3

NEURONAL SIZE IN THE SPINAL NUCLEUS OF THE BULBOCAVERNOSUS (SNB): DIRECT MODULATION BY ANDROGEN IN ANIMALS WITH MOSAIC GENETIC ANDROGEN INSENSITIVITY.

Neil V. Watson*[1], Louise M. Freeman[2] and S. Marc Breedlove[2], *Dept. Psychology, [1]Simon Fraser University, Burnaby, BC, V5A 1S2, [2]University of California, Berkeley, CA, 94720.*

Motoneurons of the SNB innervate the perineal muscles bulbocavernosus and levator ani (BC/LA). Both the SNB and BC/LA express androgen receptors (AR), and display profound androgen-dependent sexual dimorphism, being virtually absent in untreated females. Interestingly, the developmental SNB dimorphism arises indirectly, through the action of androgen on the SNB's target muscles. Androgen prevents perinatal regression of the BC/LA , which thereafter inhibits SNB degeneration. Androgens also affect the adult SNB: castration reduces mean soma sizes, while androgen treatment increases them. However, it is unknown whether this effect is mediated directly via the SNB cells' AR, or indirectly via androgen's effects on the BC/LA.

We generated rats that were genetically mosaic for the X-linked *tfm* mutation, with roughly 50% of SNB cells expressing the normal, androgen-sensitive wild-type (WT) AR, and 50% expressing the defective *tfm* AR. We provided mosaics with either testosterone (T) or blank implants, and subsequently used AR immunocytochemistry to identify the two cell types within each animal's SNB. As shown here, testosterone increased soma size only in WT SNB cells. If the effects of androgen on cell size were mediated indirectly, the presence or absence of AR in the SNB cells would not matter and the effect on cell size would be equivalent between the cell types. Instead, these data provide strong evidence that androgens act directly on SNB motoneurons, via their AR receptors, to alter soma size in adulthood. *(Supported by NIH NS28421)*



276.4

EFFECTS OF NEONATAL TESTOSTERONE ON THE DEVELOPMENT OF THE SENSORY PUDENDAL NERVE IN RATS.

S. E. Gans*, R. H. White, and C. L. Moore, Dept. of Psychology, Univ. Massachusetts, Boston, MA 02125.

The sensory pudendal nerve is larger in males than in females, and its axons are larger, more thickly myelinated, and more numerous in males. To determine whether these differences can be reversed with testosterone, female neonates were treated with 500 µg testosterone propionate ($n = 4$) on the day of birth and compared with neonatally oil-treated males ($n = 4$) and females ($n = 5$) at 21 days of age. Treatment significantly increased the cross-sectional area of the nerve, the size of myelinated axons, and the number of both myelinated and unmyelinated axons (all p values < 0.05). A comparison of the 21-day-olds in this study with adults from a previous study (Moore & White, Neuroscience Abstracts, 1994) indicated no change with puberty in the number of myelinated axons. However, these axons were significantly larger in adults ($p < 0.0001$), particularly among males ($p < 0.06$). The cross-sectional area of the nerve was also larger in adults than in juveniles of the same sex ($p < 0.0001$), but growth was significantly greater in males (sex x age interaction, $p < 0.0001$). The results suggest that myelinated axon number in this nerve is established by 21 days, partially in response to neonatal testosterone. However, the full extent of sex difference in axonal size and myelination is not established before puberty (Supported by HD07772 to SEG and NSF IBN 91-21238 to CLM.).

276.5

EFFECT OF SEX AND ADULT TESTOSTERONE TREATMENT ON FOS INDUCTION IN FERRET OLFACTORY BULB AND FOREBRAIN BY ODORS FROM ESTROUS FEMALES K.R Kelliher*, S.R. Wersinger and M.J. Baum. Department of Biology, Boston University, Boston, MA 02215

Chemosensory cues from conspecifics may contribute to sexual partner preference and other aspects of reproductive success in breeding ferrets. Using nuclear Fos protein immunoreactivity (Fos-IR) as a marker, we made a sex comparison of neuronal activation in the main and accessory olfactory bulbs (OB) and in forebrain regions which receive olfactory inputs after exposure to odors from estrous female ferrets. We also assessed the ability of testosterone propionate (TP) to modulate the effects of olfactory cues on neuronal Fos-IR in males and females. Adult ferrets were gonadectomized and given TP or oil vehicle for 5 weeks. Subjects were isolated for two days, then placed either in a clean cage or in a cage containing bedding from an estrous female for 90 min. and perfused with 4% paraformaldehyde; brains were later processed for Fos-IR. The number of Fos-IR neurons was significantly greater in the granular layer of the main and accessory OB and in the medial amygdala (MA) of both males and females exposed to estrous bedding. In female subjects exposed to estrous bedding there were also significantly more Fos-IR neurons in the medial preoptic area/anterior hypothalamus (mPOA/AH) and in the ventro-lateral aspect of the ventromedial nucleus of the hypothalamus (VLH); no such effect occurred in males. Adult treatment with TP generally augmented regional Fos responses to chemosensory cues when they occurred in either sex; this effect of TP attained statistical significance in the main OB and mPOA/AH of females. Neuronal responses to estrous bedding were similar in the peripheral stages (i.e., OB; MA) of the olfactory projection pathway, suggesting that the detection and initial processing of chemosensory stimuli is similar in the two sexes. By contrast, the subsequent processing of chemosensory inputs by more central forebrain neurons (i.e., mPOA/AH; VLH) is sexually dimorphic. (Supported by HD 21094; MH 00392)

276.6

SEXUALLY DIMORPHIC PROCESSING OF SOMATOSENSORY AND CHEMOSENSORY INPUTS TO FOREBRAIN LHRH NEURONS IN MATED FERRETS S.R. Wersinger* and M.J. Baum. Department of Biology, Boston University, Boston, MA 02215

Mating induces a preovulatory LH surge in the estrous female ferret but significantly inhibits LH secretion in the breeding male. This dimorphism is reflected in a sex difference in Fos-IR in both LHRH and non-LHRH neurons after mating. We used the immunocytochemical localization of Fos and LHRH to ask whether the sex dimorphism occurs in the initial transmission or in the central processing of sensory stimuli? We also assessed the contribution of mating-associated sensory stimuli vs chemosensory cues alone to the neuronal Fos responses. Breeding male and female ferrets were allowed to intromit for up to 90 minutes. Additional chemosensory-stimulated subjects were placed in a cage in which an opposite-sex breeding-condition ferret had been housed for 48h. Unpaired control ferrets were placed alone in a clean testing cage. All subjects were perfused 90 min after the beginning of the stimulation period. In both sexes mating augmented Fos-IR in the granular layer of the olfactory bulbs, in the midbrain central tegmental field (CTF), and the medial amygdala (MA), regions situated early in the input pathway to the hypothalamic LHRH neurons. Other than the CTF, these same forebrain regions were activated in both sexes after exposure to chemosensory cues alone. However, more central forebrain regions (the preoptic area (POA), the bed nucleus of the stria terminalis (BNST), the ventro-lateral portion of the ventromedial hypothalamus(VLH)) as well as the LHRH neurons were activated by mating only in the female. In females, but not in males, chemosensory stimuli alone augmented Fos-IR in the medial POA and the VLH but not in the BNST or LHRH neurons. These results suggest that the sex dimorphism in mating-induced LH secretion reflects a sex difference in central processing, since chemosensory and genital-somatosensory stimuli activate forebrain regions early in the input pathway to the LHRH neurons equivalently in both sexes. Chemosensory cues are also processed in a dimorphic fashion; however, even in females, these cues are insufficient to activate fully the input pathway or LHRH neurons. (Supported by HD 210942; MH 00392)

276.7

DIFFERENTIAL EFFECTS OF PREOPTIC/ANTERIOR HYPOTHALAMIC LESIONS ON SEXUAL PARTNER PREFERENCE AND MATING IN MALE FERRETS H.A. Kindon*, R.G. Paredes and M.J. Baum Dept. Of Biology, Boston University, Boston, MA 02215

In a previous study (J. Neurosci. 15:6619-6630, 1995) we found that bilateral excitotoxic lesions of the medial preoptic area/ anterior hypothalamus (mPOA/AH), which included but were not restricted to the sexually dimorphic male nucleus (Mn) of the mPOA/AH, caused male ferrets to approach and interact sexually with a stud male as opposed to an estrous female in T-maze tests given while subjects were gonadectomized and treated daily with estradiol benzoate (EB). In the present study electrolytic lesions, which caused extensive bilateral damage to the mPOA/AH including the Mn-POA/AH, caused males to shift their mean preference from an estrous female to a sexually active stud male. The partner preference of males with extensive mPOA/AH damage more closely resembled that of sham-operated females than that of sham-operated males or that of males with either partial bilateral POA/AH damage, including only unilateral Mn-POA/AH destruction, or with lesions outside the mPOA/AH. Males with extensive bilateral mPOA/AH lesions showed an even stronger preference to approach the stud male in tests in which subjects could see, hear and smell, but not physically contact the stimulus animals. When given testosterone propionate, males with either extensive or partial bilateral damage to the mPOA/AH showed significant reductions in their coital performance, regardless of whether they were tested with a sexually receptive male or female. Heterosexual partner preference in male ferrets depends on the functional integrity of sexually dimorphic mPOA/AH neurons. When these neurons are either destroyed (males with extensive bilateral mPOA/AH damage) or absent (sham-operated females), subjects' preference is apparently maintained by non-behavioral, distal incentive cues from the stud male. Supported by HD21094 and MH00392.

276.8

BEHAVIORAL MASCULINIZATION IN ESTROGEN RECEPTOR GENE DEFICIENT TRANSGENIC FEMALE MICE. Sonoko Ogawa[1]*, J. Taylor[2], D. B. Lubahn[2], K. S. Korach[3], & D.W. Pfaff[1] [1]:Lab. Neurobiol. & Behav., The Rockefeller University, NY, NY10021, [2]:Dept. Biochem. & Child Health, Univ. of Missouri-Columbia, Columbia, MO 65211 & [3]:Lab. Reprod. & Develop. Toxicol., NIEHS, Research Triangle Park, NC 27709

Previously, we have reported that estrogen receptor (ER) gene disruption reduced specific sexual and aggressive behaviors and feminized open-field behaviors in male mice. In the present study, behavioral consequences of loss of functional ER were determined in female estrogen receptor deficient transgenic (ERKO) mice. Gonadally intact ERKO and age-matched wild-type (WT) female mice were individually housed and tested for sexual, aggressive, maternal and open-field behaviors at the age of 17-30weeks. In sexual behavior tests, in the home cage of individually housed stud Swiss Webster (SW) male mice, all ERKO females (6/6) were vigorously attacked instead of being mounted by resident males with short latencies. The same males showed attempted mounts to diestrus WT females and mounts and intromissions to estrus WT females. When introduced into the home cage of individually housed SW female mice, ERKO females (3/6) showed male-type offensive attacks toward resident female mice. In pup-retrieving tests, only 2 out of 6 ERKO females retrieved pups (3-6days old) to their nest and crouched over them while all 6 WT females showed both retrieving and crouching. Moreover, it was found that ERKO females used significantly less nest materials and made poor nests compared to WT females. Finally, in contrast to male ERKO mice which were more active than WT males, ERKO females tended to be less active in open-field tests (i.e., showed lower number of line crossings and entered less frequently to the center area) compared to WT female mice. These findings collectively suggest that ER gene disruption produced partial masculinization of behavioral phenotype in female mice. (Supported partially by Harry Frank Guggenheim Research Grant to SO.)

276.9

ADULT SEX REVERSAL IS ASSOCIATED WITH CHANGES IN AVT MRNA EXPRESSION IN THE PREOPTIC AREA. J. Godwin, R. Sawby[τ], R.R. Warner#, D. Crews, and M. S. Grober[τ]*. Dept. of Zool., Univ. of Texas, Austin, TX 78712; Dept. of Biol. Sci., [τ]Univ. of Idaho, Moscow, ID, 83844 and #Dept. of Eco. Evo. & Mar. Bio., UCSB, Santa Barbara, CA, 93106.

Arginine vasotocin (AVT) is an important regulator of social and reproductive behaviors in a many vertebrates. The sex-changing Caribbean bluehead wrasse, *Thalassoma bifasciatum*, provides a useful model for examining behavioral actions of AVT between and within sexes. This species is a diandric protogynous hermaphrodite. Individuals begin life in the initial phase (IP) as either females or males. Dependent upon specific social stimuli, either IP sex may transform into a terminal phase (TP) male. All three sexual phases exhibit unique combinations of secondary sexual characters, courtship behavior, and territorial aggression, which change rapidly (on the order of seconds to minutes) in response to social cues. Using *in situ* hybridization, we estimated relative preoptic area AVT-mRNA abundance in wild caught females, the two alternate male morphs, and females undergoing sex change induced through removal of large males from reefs. Unmanipulated females showed the lowest abundance of AVT mRNA. Both IP and TP males exhibited significantly greater abundance than females (220% and 360% of female values respectively), but the male morphs did not differ significantly from each other. Sex changing females showed all sexual and aggressive behaviors characteristic of TP males and exhibited hybridization signals not different from IP or TP males (398% of unmanipulated female values). The assumption of a dominant male behavioral phenotype, via sex or role change, is associated with increased abundance of AVT-mRNA in the preoptic area, suggesting AVT may play a role in generating within- and between-sex behavioral differences in this species. Supported by grant NIH NS-09219 (JG), NSF-DEB 91-17379 and NSF-OCE 92-01320 (RRW), NIMH-41770 (DC), and NSF-IBN 9309555 and NIH-1P20RR09181-01 (MSG).

276.10

SEXUAL ALLOMORPHISM: INDIVIDUAL VARIATION BETWEEN THE SEXES. T. O. Fox*[1], S. A. Tobet[1] and M. J. Baum[2]. Program in Neuroscience, Harvard Med. Sch., Boston MA 02115[1], and Department of Biology, Boston Univ., Boston MA 02215[2]

Sex differences develop in response to the actions of gonadal steroid hormones interacting with experiential factors. Traits can be sexually dimorphic, with one type expressed in females and another in males (e.g., gonadal structure; certain brain structures). For many other traits, some individuals of either sex may be "sex-typical" or "sex-atypical." Such traits are sexually allomorphic; i.e., mean values differ between the sexes, but significant between-sex overlap and within-sex gradation exist (e.g., many brain structures; neuronal phenotypes; behaviors; cognitive styles).

One specific example of a sexually allomorphic trait is sexual partner preference in ferrets. When adult ferrets were gonadectomized, injected daily with estradiol benzoate, and tested in a T-maze, on average, females preferred to approach and interact with a sexually active stud male whereas males preferred to approach and interact with a receptive estrous female. There was considerable variation, however, in the distribution of preference scores, such that 26% of the 57 male subjects tested preferred to approach the stud male. By contrast, only 6% of the 53 female subjects tested preferred to approach the estrous female. These data represent control ferrets from several experiments conducted over a decade.

Previously we would have referred to any sex difference as a sexually dimorphic trait, sometimes accompanied by the qualifier that some animals were atypical. By describing certain traits as sexually allomorphic, we emphasize that individual variations are typical in the population and that "female" and "male" are not stereotypical and bipolar conditions. Supported by NIH and NSF grants.

276.11

DEVELOPMENTAL EXPRESSION OF NEURAL ANDROGEN RECEPTOR IN MOUSE BRAIN: SEX DIFFERENCES. S. Lu and N.G. Simon*. Dept.of Biological Sciences, Lehigh University, Bethlehem, PA 18015.

The presence of androgen receptor (AR) in neural target tissue is critical for the production of androgen-mediated effects. During sexual differentiation of the brain these effects include, for example, masculinization of structures regulating gonadotrophin secretion and sensitization of neural substrates underlying male-typical reproductive and aggressive behaviors. The expression of AR was assessed in male and female mice at several time points from birth to puberty to assess whether sex differences in AR were associated with the androgen-dependent ontogeny of these dimorphic characteristics. All maintenance and care procedures of CF-1 mice fully complied with Federal guidelines. Brain extracts from male and female mice aged day 1, 4, 7, 14, 21, 28, and 35 were prepared from limbic tissue blocks that included regions that express high levels of AR. AR protein levels were determined by Western blot- ECL using an anti-AR antibody, PG-21 (a gift from Dr. G. Prins). Scanning densitometry of the resulting blots showed a specific 97 kD band that presumably represents AR in both sexes. Over the period studied, there were significant sex differences in the expression of this protein, with males exhibiting higher densities and a more rapid increase in AR expression and in age-related increase in AR expression in both sexes. This profile of AR expression in neural tissue provides additional insights into the relationship between receptor density and the development of neural target tissue sensitivity to androgen.

Supported by NIH (1R15HD 31696) and NSF (IBN-9512015).

276.12

ANDROGEN METABOLISM IN THE BRAIN OF THE GREEN ANOLE LIZARD. J. Wade*. Department of Psychology and Program in Neuroscience, Michigan State University, East Lansing, MI 48824.

Both androgenic and estrogenic metabolites of testosterone (T) are important for activating sexual behavior in the green anole lizard, *Anolis carolinensis*. For example, male sexual behavior is abolished by castration and can be elicited in either males or females by treatment with T or 5α-dihydrotestosterone (DHT). Ovariectomy inhibits receptivity in females, and the behavior can be reinstated with either estradiol (E) or T treatment. In an effort to begin to directly test the role of neural steroid metabolism, assays to measure the activity of aromatase (which catalyzes the conversion of T to E) and 5α-reductase (which catalyzes the conversion of T to DHT) were conducted in whole homogenates and subcellular preparations of brain tissue from male and female anoles. Assays were done during the breeding season using ^{3}H-T as substrate. In incubations using tissue pooled from males and females, production of ^{3}H-DHT was substantially higher than formation of ^{3}H-E. These results suggest that 5α-reductase is more active than aromatase. In addition, preliminary evidence suggests that incubation temperature affects the two enzymes differently. Further characterization of the enzymes and an investigation of potential sex differences in their activity should help elucidate the role of androgen metabolism in the activation of sexual behaviors in the green anole lizard. Supported by MH54985.

DRUGS OF ABUSE: ETHANOL, BARBITURATES, AND BENZODIAZEPINES II

277.1

COGNITIVE AND MOTOR DEFICITS ARE ASSOCIATED WITH CHRONIC ETHANOL CONSUMPTION IN C57BL/6J MICE. B. Koller, C. Taylor, D.E. Krug*, R. Reynolds, H. Lal, and M.J. Forster. Department of Pharmacology, University of North Texas Health Science Center, Fort Worth, TX 76107-2699.

Chronic alcohol abuse is associated with impaired performance on sensorimotor and visuospatial tasks. The current studies investigated the extent to which analogous deficits result from chronic ethanol exposure in mice. C57BL/6J mice were given a liquid diet including alcohol (0, 5, 10, or 20 mg/kg/day) or a mouse chow diet for either 5 or 7 months. Pair fed control mice were maintained at caloric equivalence with sucrose/dextrin. Four weeks after the diet was discontinued, the mice were evaluated on cognitive, motor, and sensorimotor tasks. In a water maze task, the 5 month alcohol group had a general decrease in spatial performance. Other general effects of chronic alcohol included impaired ability to traverse an elevated path, an impaired ability to tread or remain hanging from a horizontal wire, and a reduction of total locomotion, rears, and stereotypy counts. In several tasks, the 7 month-alcohol mice were less impaired than the 5 month-alcohol mice including both the spatial memory and active avoidance tasks. The current findings suggest that mice can provide a useful model of chronic alcohol-induced brain dysfunction. [Supported by NIH grant AA09622]

277.2

POSTNATAL ETHANOL EXPOSURE ALTERS SEIZURE SUSCEPTIBILITY IN YOUNG RATS R. Sircar*, D. Kim. Dept. Psychiatry and Neurology, Albert Einstein Coll. of Med., Bronx, NY 10461.

Timing of exposure to ethanol during development is important for the long-term detrimental effects of ethanol (Thomas et al., Neurosci. Abs. Vol 21 (3) 824.1, 1995). Majority of studies have focused on the effects of ethanol during the prenatal period or the early neonatal period. NMDA receptors have been implicated as neurochemical correlates underlying the behavioral effects of ethanol. Ontogenic studies of the NMDA receptor suggest that it becomes functional during the second postnatal week. To test the hypothesis that exposure to ethanol during late postnatal period, i.e., period of NMDA receptor maturation, has long-term behavioral effects, we exposed rats to ethanol (2.5 mg/kg/day; intragastric) from postnatal day 10 till day 20. Control rats received equivalent volumes of water. Rats were tested behaviorally in the pentylenetetrazol-induced seizure paradigm between postnatal days 29 and 31. The frequency of and latency to reach each seizure stage was measured (twitches, clonic and generalized tonic-clonic seizure). All control rats exhibited generalized tonic-clonic seizure whereas a significantly lower percentage of ethanol-exposed rats had generalized tonic-clonic seizure. Also, the latency to reach generalized tonic-clonic seizure was significantly longer in ethanol-treated rats compared to controls. The latency to clonic seizure or twitches did not differ between the two groups and there was no difference in the frequency to these seizure stages between control and experimental rats. Postnatal ethanol-treated rats were less susceptible to PTZ-induced seizures compared to controls. These data indicate that the developing glutamatergic system may play an important role in the behavioral effects of ethanol exposure during late postnatal period.

277.3

ETHANOL PLACE-PREFERENCE CONDITIONING IN C57BL/6 MICE: A PRELIMINARY STUDY. C. Nocjar*, M. Tavernatti and L.D. Middaugh. Dept. of Psychiatry, CDAP, Med. Univ. of SC, Charleston, SC 29425.

Recently, it was reported that ethanol preferring rats (P rats) do not show a conditioned place preference (CPP) for ethanol (Stewart et al., 1996). The present study assessed whether C57BL/6 mice, which consume amounts of ethanol comparable to P rats, would show ethanol CPP. Male and female mice were given a non-drug pretest to determine baseline preferences for the black and white sides of the CPP apparatus with free-access to the entire chamber. Mice then received 8 pairings of 1.75 g/kg ethanol (ip) in their non-preferred side and 8 pairings of saline in their preferred side of the chamber. Drug conditioning sessions (10 min) began immediately post injection to include drug onset effects. Motor activity was assessed during conditioning sessions. Chamber preferences were then re-assessed during a non-drug posttest where animals once again had free-access to both sides of the CPP apparatus. Ethanol stimulated motor activity and produced a CPP (15-20% increase in the amount of time spent in the ethanol paired chamber when compared to pretest times). Gender did not influence these effects. The ethanol drug-seeking behavior evidenced in this study suggests that the C57BL/6 mouse may be a good model for the study of alcohol addiction since this mouse strain will not only self-administer large quantities of the drug but also seek out its reinforcing effects. (AA10761)

277.4

THE EFFECTS OF ETHANOL OF THREE TESTS OF IMPULSIVE DECISION MAKING IN THE RAT John Evenden* Dept of Behavioural and Biochemical Pharmacology, CNS Research and Development, Astra Arcus, S-151 85 Södertälje, Sweden.

Ethanol has popularly been supposed to increase impulsive behaviour in man. This is generally assumed to be manifested in the form of impulsive aggressive behaviour. However, many of the effects of ethanol appear to depend upon expectations which are culturally and environmentally determined. In the present study objective tests of appetitively motivated decision making in rats were designed emphasising different aspects of impulsivity previously studied in man. These reflect the stages of preparation to make the decision, execution of the action decided upon, and assessment of the expected outcome. These were assessed by versions of an uncertain visual discrimination test, fixed consecutive number tests and the delay of reinforcement procedure, respectively. The same motivation (food deprivation), reinforcer (food pellets) and response (lever pressing) were employed in all three tests. Ethanol (0.1 - 1.0 g/kg i.p.) had no effect on reaction time nor the number of correct choices in the uncertain visual discrimination test. Ethanol (0.3 -3.0 g/kg p.o.) had no effect on the chain length or local measures of response rate in the fixed consecutive number test. However at doses of 0.1 - 1.0 g/kg i.p., ethanol produced a significant shift in the preference for immediate reinforcement rather than delayed reinforcement in the delayed reinforcement procedure. Recent published data indicated that rats showing a preference for immediate reinforcers ("impulsive" rats) also show an increased preference for consuming ethanol. In combination with the data presented here, these suggest that ethanol consumption can lead to a circle of increased preference for immediate reinforcers, leading to increased ethanol consumption, which leads to a further preference for immediate reinforcers. Since preparation and execution of behaviour were unaffected by the drug at the same doses, the behavioural substrate for maintaining this vicious circle remain intact until ethanol consumption reaches motorically disabling levels.

277.5

INTERACTIONS OF ETHANOL AND HYPOGLYCEMIA ON RAT MOTOR ACTIVITY. P. Duncan*. Psychology Dept. Old Dominion Univ., Norfolk, VA 23529.

Hypoglycemia (HG) produces mental confusion and anxiety in humans, and reduces motor activity in rats. Ethanol (ETH) also produces dose-related decreases in rat locomotion. The primary purpose of this experiment was to determine the effects of ETH at 0, 300, 600, and 1000 mg/kg on locomotor activity in hypoglycemic, compared to euglycemic rats. Acute ETH effect on blood-glucose (BG) level was also of interest. Insulin (2 units/kg) or saline was injected IP 30 min prior to a BG measurement, which was followed immediately by an IP injection of ETH or saline, and then a 60-min activity-recording period. A within-subjects factorial design was used, with each of nine rats receiving all eight combinations of insulin and ETH treatments. Insulin produced marked HG at both 30 min (70 vs 48 mean mg/deciliter) and 90 min (68 vs 28) post-injection. ETH in the euglycemic state significantly lowered motor activity only at the highest dose (0=962, ETH 1000=403 mean counts/60 min). Activity was decreased by insulin treatment, which also shifted the ETH dose-response to the left, with a significant difference seen at the lowest dose (0=639, 300=121, 600=177, 1000=138). ETH slightly lowered BG (71 vs 60), and ETH 1000 in combination with insulin tended to further decrease BG (mean at 90 min post insulin=22). These results show that a state of moderate to severe HG greatly increases the behavioral depressant effects of ETH. Since HG was only slightly increased by the highest ETH dose, the behavioral potentiation probably results from the summation of two separate types of CNS impairment.

SUPPORTED BY OLD DOMINION UNIVERSITY

277.6

ETHANOL-INDUCED ATAXIA IN MICE LACKING SEROTONIN 1B RECEPTORS. G.L. Schafer and J.C. Crabbe*. Dept. of Veterans Affairs and Oregon Health Sciences University, Portland, OR 97201

Serotonergic pathways have been implicated in a variety of ethanol (EtOH) related effects. Recently, knockout mice have been developed that lack the serotonin-1b (5-HT1b) receptors (Saudou et al, 1994). These mice present a useful tool for investigating the role of these receptors in mediating CNS sensitivity to EtOH. We have begun to test these mutant mice (5-HT1b$^-$) and their wild-type (wt) counterparts for various EtOH-induced behaviors, including ataxia. Previous studies from our lab have indicated that wt mice are more sensitive to grid-test ataxia (Crabbe, et al, submitted). Other studies in our lab have demonstrated that ataxia is not a genetically uniform trait. Therefore we decided to test 5-HT1b$^-$ and wt mice using other measures of EtOH-induced ataxia. 5-HT1b$^-$ and wt mice were tested for sensitivity to EtOH-induced ataxia using a fixed-speed rotarod. Initially, mice were given practice on an accelerating rotarod until a stable performance level was achieved. The results from these practice sessions indicated that 5-HT1b$^-$ mice took longer to achieve the same performance level than the wt mice. Twenty-four hours the last practice test, mice were tested on the fixed-speed rotarod immediately after an injection of 2.5 g/kg EtOH (i.p.). Latency to fall and brain EtOH concentration (BrEC) at time of fall were measured. The results indicated that 5-HT1b$^-$ mice were more sensitive to EtOH than wt mice as indicated by a lower BrEC (1.30 ± .11 mg/kg vs. 1.65 ± .09 mg/kg, respectively). It is unclear why opposite results from grid test were observed. However, it is probable that these tasks may reflect different physiological components of ataxia. Studies are currently in progress to assess differences in acute functional tolerance to EtOH in these lines of mice.

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277.7

CHRONIC ETHANOL NORMALIZES TONIC GRASP OF NRTP-DAMAGED RATS AT DOSES THAT DO NOT AFFECT GRASP OF NORMAL RATS. R.M. Chesire* & B.E. Digman, Col. Health Sci. & Psych. Dept., Univ. Hawaii-Manoa, Honolulu, HI 96822.

Changes in the ability to cling to a horizontal rod with the forepaws have been used to indicate drug- or neural damage-induced dysfunction in rats. Normal rats cling briefly in such positions, but rats made cataleptic by dopamine deficiency (e.g., systemically administered haloperidol, electrolytic lesions of the lateral hypothalamus) show exaggerated tonic grasp and can cling for extended periods of time. Rats given lesions of the nucleus reticularis tegmenti pontis (NRTP) show a variety of symptoms, including rapid forward locomotion and excessive tonic grasp. We have demonstrated that alcohol does not impair and can actually improve some motor functions of NRTP-damaged rats. We report here that tonic grasp is among these. The number of seconds spent clinging to a microspatula were measured in normal and NRTP-dmaged rats administered tap water (TW), TW+25% sucrose (SU) or TW+SU+5%-30% ethanol (ETOH) in their water bottles over a period of ≈ 85 days. Increases in ETOH doses (1% per unit time) were determined by performance on the tilting plane test. Unoperated rats showed no differences in any condition, but did cling significantly less than NRTP-damaged rats (F=14.8;p<0.01). NRTP-damaged rats showed significantly reduced length of tonic grasp at doses from 10%-26% ETOH (F=4.44; p<0.036). The results provide further support for the idea that, in the presence NRTP dysregulation, normalization of motor function creates an appetitive stimulus for substance abuse.

277.8

ONTOGENY OF ETHANOL SLEEP TIME: AGE-RELATED ALTERATIONS IN ETHANOL SENSITIVITY AND ACUTE TOLERANCE. M.M. Silveri and L.P. Spear*. Center for Developmental Psychology, Binghamton University, Binghamton, NY 13902-6000.

The purpose of the present study was to examine the ontogeny of acute tolerance and sensitivity to ethanol in terms of ethanol-induced sleep time. Male and female Sprague-Dawley rats at postnatal days P16, 26, 36, 46, 56, and 96 received intraperitoneal injections of 3.5, 4.0, 4.5, or 5.0 g/kg ethanol. Sleep time (interval between loss and regain of righting reflex) was determined, animals were sacrificed immediately following regain of the righting reflex, and trunk blood samples were collected. Blood samples were analyzed via gas chromatography for blood alcohol content (BAC) at time of awakening. EtOH sleep time increased with age, with a gender effect (males sleeping longer than females) evident at P56 and 96. In addition, BAC levels at time of awakening significantly decreased across age with P96 animals exhibiting the lowest BAC. Acute tolerance (indexed by the magnitude of the slope of the linear regression obtained from plotting dose of ethanol vs. BAC at the time of awakening) also decreased during ontogeny, with little evidence of acute tolerance for this measure being seen in P46 and older animals. Initial sensitivity to ethanol was shown to increase with age; these estimates of initial brain sensitivity to ethanol at each age were derived by plotting sleep time versus BAC at awakening, with the extrapolated point at zero sleep-time serving as an index of initial brain sensitivity. These findings suggest that increases in ethanol sleep time seen during development may be related both to an ontogenetic increase in brain sensitivity to ethanol as well as an ontogenetic decrease in the development of acute tolerance during the ethanol-induced sleep. Supported by NIAAA grant R01 AA10228.

277.9

INDUCTION AND MAINTENANCE OF MODERATE BLOOD ETHANOL LEVELS BY ATRAUMATIC PUSATILE GASTRIC INFUSIONS IN "EXPERIENCED" RATS: EFFECTS ON BRAIN OPIOMELANOCORTINERGIC ACTIVITY. Rasmussen,D.D.*, Bryant,C.A., Boldt,B.M., Colasurdo,E.A., Wilkinson,C.W., V.A. Medical Center and Univ. Of Washington, Seattle, WA 98195

Forebrain neurosecretion of pro-opiomelanocortin (POMC)-derived peptides, especially the potent opioid ß-endorphin (ßEND), appears to mediate some responses to ethanol administration. However, attempts to characterize ethanol-induced changes in activity of this opiomelanocortinergic system have yielded variable results, perhaps in part due to confounding non-specific stress responses. We have developed a model in which "experienced" rats (previously introduced to ethanol administration, so the subjective response is not a novel stimulus) receive pulsatile intragastric ethanol infusions during the dark (active) photophase to produce and sustain (for 3 h) moderate (0, 50, 90-100, or 120-150 mg/dl) blood alcohol levels (BALs). BALs of approximately 50 mg/dl did not significantly alter ßEND concentrations in any brain area examined at 30 min after initiation of infusion, whereas 90-100 mg/dl increased (p<0.05) ßEND concentrations in the ventral tegmental area (VTA) and 120-150 mg/dl increased both VTA (p<0.05) and nucleus accumbens (p<0.01) ßEND concentrations. None of the dosages significantly altered ßEND concentrations in the amygdala, preoptic area, or mediobasohypothalamus (MBH) after 30 min, although there were trends toward increases. BALs of 120-150 mg/dl (but not lower levels) also increased (p<0.05) MBH cytoplasmic POMC mRNA concentrations at the end of the 3 h infusion period (but not at 30 or 60 min) as well as at 3 h after termination of the infusions (i.e., 6 h after initiating treatment). These results demonstrate that atraumatic induction of physiologically meaningful BALs acutely activates the forebrain opiomelanocortinergic system, consistent with evidence the ß-endorphinergic mechanisms mediate some responses to alcohol ingestion. (NIH/NIAAA grant R01 AA10567 and Dept. Of Veterans Affairs)

277.10

DIFFERENTIAL ETHANOL (ETH) CONSUMPTION IN ROMAN HIGH-AVOIDANCE (RHA) AND LOW-AVOIDANCE (RLA) RATS. **O. Giorgi*, M.G. Corda, M. Orlandi, V. Valentini, G. Carboni, V. Frau and G. Di Chiara. Dept. of Toxicology, Univ. of Cagliari, vle. A. Diaz 182, 09126 Cagliari, *ITALY*.**

Selective breeding techniques have been used extensively to examine the role of genetic factors in the control of ETH intake. Moreover, preference for ETH has been observed also in lines of rats selectively bred for behaviors other than ETH consumption. This is the case of RHA and RLA rats, two lines selected and bred for rapid versus poor acquisition of two-way avoidance behavior in a shuttle box. RHA and RLA rats show many other behavioral differences related to emotional factors, RLA rats being emotionally more reactive. In addition, a number of differences in dopaminergic (DAergic) and GABAergic function in the CNS have been reported in these two lines. Given the role of DA and GABA in the regulation of ETH consumption, the present study was undertaken to characterize the differences in ETH intake and preference between RHA and RLA rats. ETH solutions were presented on alternate days in a free choice with water. The initial ETH concentration was 2% and 1% increments were applied every second day until a final concentration of 10% was reached by day 18. RHA rats consumed significantly larger amounts of ETH than did RLA rats. In addition, RHA rats, but not their RLA counterparts, displayed significant preference for ETH over water. To examine ETH intake and preference stability, animals were subsequently switched to daily presentations of 10% ETH for 18 consecutive days. The line-related differences in ETH intake and preference remained stable throughout the test period. These results indicate that RHA and RLA rats can provide a useful model to investigate the biochemical mechanisms involved in the behavioral responses to ETH. Studies aimed at characterizing the effects of ethanol on DAergic neurotransmission in the CNS of these two lines of rats are in progress in our laboratory.

277.11

ETHANOL PREFERENCE AND BEHAVIOR IN THE NAPLES HIGH- AND LOW-EXCITABILITY RAT LINES.

M.P.Pellicano and A.G.Sadile[1], (SPONSORED BY THE EUROPEAN BRAIN AND BEHAVIOR SOCIETY). Lab. Eating Behavior, Food Science & Technol. Inst., CNR, Avellino; [1]Lab. Neurophysiol. Behav. & Neural Networks, Dept. Human Physiol. "F.Bottazzi", SUN, Naples, I.

The aim of this project was to investigate the long-term effects of ethanol consumption on behavior in animal models. Adult male albino rats of the Naples High (NHE) and Low-Excitability (NLE) lines and of a random-bred control line (NRB) were housed in single cages with pelletted food and four bottles containing either tap water or 5, 10 and 20 % ethanol solution. A control group for each line consisted of rats with no access to ethanol. Food intake and fluid consumption were monitored three times a week during a 6-wk period at the beginning of the dark phase of the light-dark cycle. Body weight was measured every week. At the end of the experimental period, ethanol-exposed and control rats were exposed to a Lit-maze during three 10-min periods separated by a 24-h interval. Behavior was monitored and stored on videotape for off-line analysis of frequency of corner-crossings and rearings, and duration of rearing episodes. On day 1, total ethanol intake was higher in both NLE/NHE-rats compared to NRB controls. Ethanol preference for the different concentrations as percent of the total ethanol consumption, was lower for the 5% solution in both NLE/NHE rats, as compared to NRB-rats, whereas it was higher for 10 and 20 % in both strains. On day 2-42, total ethanol intake increased in all strains, but more in NLE/NHE-rats compared to NRB-rats. Ethanol preference was higher for 5 % solution in NHE and NRB-rats. In contrast, NLE-rats preferred the 20 % solution. Thus, the NLE/NHE rats appear as a useful model to study the neural substrates of ethanol preference, tolerance and dependence. **(Supported by CNR STRA094 and MURST95 grants).**

277.12

TASTE REACTIVITY CONCENTRATION-RESPONSE FUNCTIONS FOR ALCOHOL AND SUCROSE SOLUTIONS IN OUTBRED MICE. H.J. Kaczmarek, K.G. Hill, and S.W. Kiefer*. Dept. of Psychology, Kansas State Univ., Manhattan, KS 66506-5302.

Preliminary results from taste reactivity tests with outbred mice indicated that these animals did not respond differentially to a range of alcohol solutions (3%-12%, v/v) and that the response to alcohol resembled taste reactivity responses to single concentrations of sucrose, sodium chloride, quinine hydrochloride, and hydrochloric acid solutions. The lack of discriminative responding in mice to taste solutions was explored in two additional studies. Experiment 1 (n=16) involved taste reactivity testing to a range of alcohol concentrations (5%, 10%, 20%, 30%, and 40%) and Experiment 2 (n=26) involved five sucrose concentrations (0.01 M, 0.05M, 0.10 M, 0.50 M, and 1.0 M). Mice were presented with one solution per day for 60 s (rate = 0.2 ml/min); order of concentrations was determined randomly for each mouse. As a general characterization, predominant response profiles for mice, regardless of taste solution or concentration, consisted of tongue protrusions (ingestive), gapes, and forelimb flails (both aversive). Mice showed a significant increase in tongue protrusions for increasing concentrations of both alcohol and sucrose. Further, mice made significantly fewer gapes as the concentration of sucrose was increased. The present results demonstrate that concentration-dependent reactivity profiles, at least for tongue protrusions, are found in mice when a wide range of alcohol concentrations is used. Mice also show discriminative responding for sucrose solutions as reflected by increasing tongue protrusions and decreasing gape responses.

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277.13

NEUROCHEMICAL DIFFERENCES BETWEEN ETHANOL-NAIVE AND ETHANOL-EXPOSED WISTAR, ALCOHOL PREFERRING (P) AND ALCOHOL NON-PREFERRING (NP) RATS. A.D. Smith * and F. Weiss. Department of Neuropharmacology, The Scripps Research Institute, La Jolla, CA 92037.

Ethanol preference versus non-preference in genetically selected rat lines is believed to involve differences in dopamine (DA) and serotonin (5HT) function. Tissue studies have reported lower levels of DA and 5HT in the nucleus accumbens in P rats versus NP rats. This deficiency has been implicated in the heightened voluntary intake of ethanol in P rats. In the present study, quantitative in vivo microdialysis was used to investigate differences in the basal extracellular levels of DA and 5 HT in ethanol-naive and ethanol-exposed P, NP and Wistar rats. Rats were implanted with guide cannulae aimed at the nucleus accumbens. Animals were injected with saline or ethanol (1g/kg IP) for 5 days. Microdialysis was performed on the 6th day using the point of no net flux method. Alcohol-naive P rats showed higher basal extracellular 5 HT levels and no differences in DA levels compared to alcohol-naive NP and Wistar rats. 5HT levels in Wistar rats were also higher than NP rats. Repeated ethanol exposure resulted in increased basal DA extracellular concentration in Wistar and P rats with no changes in DA levels in NP rats. Basal 5 HT levels increased in Wistar and NP rats whereas a trend toward decreased 5HT levels was observed in P rats in accordance with findings of earlier tissue studies. The possibility of strain differences in the response of the DA system to ethanol challenge is currently under investigation in ethanol-naive and ethanol-exposed rats. (Supported by NIAAA AA 10531).

277.14

IN VIVO MICRODIALYSIS RECORDINGS OF STRIATAL DOPAMINE IN RATS SELECTIVELY BRED FOR DIFFERENTIAL CNS ETHANOL SENSITIVITY. A.F. Hoffman*, M.A. Segall, G.A. Gerhardt, T.A. French, and N. Weiner. Dept. of Pharmacology, University of Colorado Health Sciences Center, Denver, CO 80262.

Interactions between ethanol and central catecholamine systems have been reported to play a role in a variety of behavioral effects produced by alcohol. In the present study, we investigated the effects of ethanol on dopamine (DA) release in the striatum of both high alcohol sensitivity (HAS) and low alcohol sensitivity (LAS) rats. Animals were anesthetized with urethane (1-1.25 g/kg, i.p.) and stereotactically implanted with single 4 mm dialysis probes in the lateral striatum. Probes were perfused with an artificial CSF (aCSF) solution. Samples were collected every 20 minutes and analyzed by high pressure liquid chromatography coupled with electrochemical detection. Following an 80 minute period to allow for stabilization of metabolite levels, modified aCSF containing 100 mM KCl was applied for 20 minutes. After a 60 minute washout with normal aCSF, animals were injected with a 1.0 g/kg, i.p. dose of ethanol. Twenty five minutes later, the potassium stimulus was repeated. Preliminary results showed that basal DA levels were similar between HAS and LAS rats (27 ± 4 and 38 ± 10 nM, respectively). Basal levels of the DA metabolites 3,4-dihydroxyphenylacetic acid and homovanillic acid were slightly higher in HAS (6.7 ± 0.53 μM; 7.9 ± 1.1 μM) than in LAS rats (4.3 ± 0.52 μM; 5.7 ± 0.62 μM). Potassium-evoked release of DA in both lines was similar prior to ethanol administration. A 30% decrease in the potassium-evoked DA release was found 45 minutes following ethanol treatment in HAS rats, versus a 2% decrease in LAS rats. These results suggest that differences in both DA release and metabolism may exist between rats which are selectively bred for alcohol sensitivity. Experiments are in progress which will further elucidate both the nature and functional consequences of these differences. Supported by NS09199 and NIAAA 03527.

277.15

SHIFT-WORK PHOTOPERIODS INFLUENCE THE VOLUME OF ETHANOL SELF-ADMINISTERED "AFTER HOURS" IN RATS

D.V. Gauvin*, R.J. Briscoe, T.J. Baird, M. Vallett, S.A. Vanecek, & F.A. Holloway; Dept Psychiat & Behav Sci, O.U.H.S.C., Okla. City, OK 73190

Thirty-six male Sprague-Dawley rats were conditioned to self-administer 10%w/v ethanol (ETOH) in tap water using a modified Samson-sucrose fading procedure. Rats were given ad lib access to food and water and offered access to the ETOH solution for 1/2 hr a day in their home-cages (0600-0630h). Once consumption stabilized to ±10% variability over seven consecutive days, rats were placed in an isolated AAALAC-accredited colony room with a separate light-controlling timer. Lights were scheduled to cycle similar to the photoperiods experienced by humans working on a "southern-swing" shift for two consecutive months. Rats were given the 1/2 hour access to ETOH during a time-frame analogous to 1 hr after the human's work shift was over. On "days off" access was given at 0600-0830h. The amount of ETOH consumed, expressed in mls. or in g/kg, varied across the work shift cycle. Access to ETOH after the afternoon shift produced the highest voluntary ETOH consumption (approx. 2g/kg). Post day-shift access produced moderately high drinking (1.25-2 g/kg). Days off and post-midnight shifts produced the lowest rates of drinking (approx. 1.0 g/kg). For each work-shift schedule, rats drank more during the second month of exposure when compared to the first months drinking. These data suggest that the stress of shift work photoperiod changes may be a contributing factor to sustained increases in voluntary ETOH consumption. Supported by: NIDA trng. grant - 1T32DA07248 & NIAAA trng. grant-5T32AA07222 & res. grant-2RO1AA08333 to F.A.H.

277.16

DIFFERENTIAL ETHANOL AND AMPHETAMINE BEHAVIORAL SENSITIZATION IN DBA/2Abg, C57BL/6Abg, AND Abg:GHSC MICE. T. TRITTO*, S. CHENOWETH, and B.C. DUDEK. Psych. Dept., University at Albany, SUNY, Albany, NY, 12222

Repeated exposure to some drugs of abuse have been shown to produce behavioral sensitization which is thought to contribute to a drug's addiction liability. The present studies examined both EtOH (2.0 g/kg)- and AMPH (2.5 mg/kg)-induced behavioral sensitization in two inbred strains, DBA/2Abg (D2) and C57BL/6Abg (B6), and one heterogeneous stock, Abg:GHSC (GHS). The protocol was that used by Phillips et al. (1994) with the addition of a saline and a drug group which did not receive exposure to the activity test chamber until the final day of testing. This allowed for testing of a possible conditioning component. Cross-sensitization of AMPH to EtOH was also tested. Whereas both the D2 and B6 strains showed AMPH sensitization, only the D2 strain showed EtOH sensitization. Although there was a trend towards cross-sensitization in both inbred strains, this was not significant. The GHS stock did not show EtOH sensitization or cross-sensitization, and showed only conditioned AMPH sensitization. This lack of sensitization in this heterogeneous stock is consistent with previous findings in our lab and possible explanations for this lack fo sensitization are discussed. [Supported, in part, by NIH grant AA09038]

277.17

APPROACH AND AVOIDANCE CONDITIONING IN RATS BRED FOR ALCOHOL PREFERENCE. M.R. Blankenship*, P.R. Finn, J.E. Steinmetz. Dept. Of Psychology/Program in Neural Science, Indiana Univ., Bloomington, IN 47405

The development of an animal model for alcoholism has provided invaluable knowledge of the physiological and biochemical basis of the disease. However, these genetically selected characteristics give rise to a spectrum of, as yet, uncharacterized behaviors. Behavioral analysis of ETOH preferring (P) and ETOH nonpreferring (NP)(Li et al.,1994) rats appears to be a crucial step in identifying the factors that define the disease of alcoholism. It has been hypothesized that alcohol-seeking behavior may be driven by deficient reward systems or poor conditionability to negative consequences. In a paradigm that is designed to assess conditioning to both rewarding (approach) or aversive (avoidance) consequences in P and NP rats, several results suggest a distinct constellation of behaviors that may contribute to alcohol preference. When trained on the avoidance task first, P rats attain higher avoidance response rates than NP's ($p<.0004$). A three-way interaction of ETOH preference, task and session suggests that P rats learn faster on the approach task, while NP rats learn the avoidance task slowest ($p<.044$). When trained on the approach task first, NP rats reach higher response rates ($p<.021$). The fact that the order of task presentation is important in determining performance implies strong contextual carry-over effects. However, the difference between naive rats' performance may be based upon the contingencies of the tasks (P rats condition well to punitive contingencies and NP rats condition well to appetitive contingencies). Such differences may be reflected in the animal's predisposition to ETOH preference.

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277.18

ETHANOL, GENETICS AND THE C-FOS RESPONSE. B. Hitzemann, V. Patel, L.Cipp* and R. Hitzemann. Department of Psychiatry, SUNY at Stony Brook, NY 11794-8101 and Psychiatry and Research Services, VAMC, Northport, NY 11768.

The C57BL/6 (B6) and DBA/2 (D2) inbred mouse strains differ in their response to ethanol. For example, modest doses of ethanol increase locomotor activity in the D2 but not the B6 strain. The B6 and D2 strains also differ in a variety of paradigms which are thought to measure ethanol preference and/or reward. The mechanisms underlying these differences are not clear but may involve differential ethanol effects on the basal ganglia and related structures. In an attempt to support this hypothesis, we have compared the c-Fos response between strains after the administration of low to modest doses (0.25-1g/kg) of ethanol. For both strains and at all doses, a significant increase in c-Fos IR was detected in the dorsal striatum and the shell and core of the nucleus accumbens. In these regions there was a trend to a greater effect for the B6 strain. There was no detectable ethanol effect in the globus pallidus, the entopeduncular nucleus and the substantia nigra zona reticulata. Ethanol markedly increased (strain independently) c-Fos IR in the paraventricular thalamic nuclei. A marked difference between strains was noted in the central amygdaloid nucleus (B6 >> D2) and the bed nucleus of the stria terminalis (D2 > B6). Overall, although these data suggest that relatively low doses of ethanol have marked effects on synaptic activity, the reasons for the marked strain differences are less clear. Supported by AA 11034.

277.19

EFFECTS OF MORPHINE ON ETHANOL PREFERENCE IN HIGH ALCOHOL-SENSITIVE RATS. B. Hine.* Univ. of Puerto Rico School of Pharmacy, San Juan, PR 00936.

This experiment was conducted to determine whether morphine would increase 10% ethanol (ET) drinking in rats genetically selected for high alcohol sensitivity (HAS), that drink ET sparingly (<1.5 g/kg/day) in preference tests. Two groups of 7 singly-caged female HAS rats (U. of Colorado generation #27, 77-90 days old), equated on the basis of prior daily water intake, were administered three 1.5 mg/kg s.c. morphine sulfate (MS) or saline (SAL) injections during a standard ET preference test (Li- Alcohol.Clin.Exp.Res. 8:485,1984): once before 3 days of 10% ET as the sole fluid source and also at the end of the first and second weeks of the 3-week ET/water preference test.

This MS dose regimen produced a transient (24-hour) increase in ET consumption ($p<.05$:g/kg/day; $p=.052$:ET/water preference ratio) only after the second MS dose, relative to SAL. However, the major effect of MS was to decrease ET intake during the 3 days of 10% ET ($p=.036$) and to produce a 39-64% reduction in ET consumption/preference measures over the 3-week test period.

The relative decrease in ET preference might be due to a high sensitivity to the depressant effect of MS and/or biological characteristics requiring a higher basal ET preference in these rats for MS to increase drinking. Thus, the data are difficult to interpret, with respect to the opioid "deficiency" or "surefit" hypotheses of alcoholism. Supported by University of Puerto Rico (CIDIC) funds.

DRUGS OF ABUSE: COCAINE—GLUTAMATERGIC INFLUENCES

278.1

CONDITIONED EFFECTS OF COCAINE IN RATS: INFLUENCE OF MK-801 ON BEHAVIORAL AND GENOMIC RESPONSES. T.R. Franklin*, C. Walters, Y. Zhu, G. Aston-Jones and J.P. Druhan. Psychiatry Dept., Medical Colllege of Pennsylvania and Hahnemann University, Phila., PA 19102.

Studies in human cocaine abusers indicate that conditioned cocaine-like responses can be elicited by environmental stimuli previously associated with administration of the drug. In the present study, immunohistochemical localization of Fos protein was used as a genomic marker of cellular activation in rats to identify potential CNS sites involved in this conditioning process. Conditioned hyperactivity and Fos expression within the CNS were determined in rats exposed to a test chamber previously paired with 10.0 mg/kg cocaine. Rats in this 'Paired' group were found to exhibit higher levels of behavioral activity and increased numbers of Fos-positive cells within the lateral septum relative to 'Unpaired' rats that received cocaine in the colony room or 'Control' rats that never received cocaine. In contrast, there was no evidence of conditioned hyperactivity or conditioned Fos expression within the septum in rats that received injections of the NMDA receptor antagonist, MK-801 (0.25 mg/kg), prior to conditioning sessions. The correspondence between the expression of conditioned hyperactivity and genomic activation in the lateral septum, and the blockade of both effects by MK-801, suggests that the septum may play an important role in the expression of conditioned responses to cocaine-related stimuli. Quantification of Fos-positive cells in several other regions is currently underway to determine whether a similar correspondence can be found in other CNS sites. (Supported by NIDA grant #DA08381 to JPD).

278.2

NOVELTY AS AN APPETITIVE STIMULUS: PRETREATMENT WITH MK-801, AND PARALLEL EFFECTS WITH COCAINE. R. A. Bevins*, N. A. Brown, & M. T. Bardo. Psychology Dept., Univ. of Kentucky, Lexington, KY 40506.

The present work assessed whether repeated access to novel objects could serve as an appetitive (rewarding) stimulus in a place conditioning procedure. Using a three compartment box, rats were placed in a center gray area and allowed access to adjacent black or white compartments. Subsequent conditioning included eight 10-min confined exposures (1/day), four in each end compartment. Group Paired received access to an object in the nonprefered side; they were exposed to the other side without an object. The Unpaired Control received equal compartment and novel object exposure, except objects were presented in the home cage. When rats were re-tested for their side preference, Group Paired increased their time spent in the nonprefered side. The Unpaired Control did not show this change. Between group differences were again found in a separate experiment using a control that received no exposure to the objects. Cocaine (IV, 0.5 mg/kg), when used as the appetitive stimulus in the above procedures, produced a data pattern similar to that of the novel objects. Both Paired and Control novelty rats pretreated with the NMDA receptor antagonist MK-801 (0.1 mg/kg, IP) spent less time in the nonprefered side than untreated rats (i.e., place aversion). Thus, the apparent partial MK-801 blockade in Group Paired was the product of an aversion conditioned by MK-801 competing with an increased preference conditioned by the novel object. The present procedures could be used to study the suggested similarities between the appetitive properties of abused drugs and novelty.

NIH grants DA05623 to R.A.B. and DA07746 to M.T.B supported this work.

278.3

$GABA_B$ AGONISTS ATTENUATE THE REINFORCING EFFECTS OF COCAINE IN RATS. M. M. Andrews* and D.C.S.Roberts. Neuroscience Institute, Life Sciences Research Center, Carleton University, Ottawa, Canada, K1S 5B6

The effect of three $GABA_B$ agonists on cocaine self-administration in rats was examined. In the first study, rats were trained to self-administer cocaine (0.18, 0.38, 0.75, 1.5 mg/kg/inj) on a progressive ratio schedule. The response requirements for each injection during the session escalated according to the series: 1,2,4,6,9,12,15,20,25... The number of injections self-administered before responding ceased was defined as the breaking point. Pretreatment with baclofen (1.25, 2.5 or 5.0 mg/kg), SKF 97541 (0.05, 0.1 or 0.2 mg/kg) or CGP 44532 (0.125, 0.25 or 0.5 mg/kg) dose dependently reduced breaking points at doses that did not significantly affect food reinforced responding on an identical PR schedule. In a second series of experiments, rats were trained to self-administer cocaine on a discrete trials schedule. Animals were given the opportunity to respond for a single injection of cocaine (1.5 mg/kg/inj) during a 10 min trial. Discrete trials began at 30 min intervals continually for two weeks. Delivery of 45 mg food pellets was contingent on a response on a seceond lever which was continuously available. Injections of baclofen, SKF 97541 or CGP 44532 reduced cocaine intake for approximately 4 hours without affecting food intake. These data suggest that $GABA_B$ agonists might be useful in the treatment of cocaine addiction. Supported by NIDA Contract No. N01DA-3-7302.

278.4

U50,488, A KAPPA OPIOID AGONIST, AND COCAINE'S REWARD-RELEVANT EFFECTS. C.L. Hubbell*, T.M. Barringer, P.M. Gonzales, C.M. Moran and L.D. Reid. Lab. for Psychopharmacology, Rensselaer, Troy, NY 12180.

Rats were fixed with chronically indwelling, bipolar electrodes for the direct electrical intracranial stimulation (ICS) of the lateral hypothalamus. Subsequently, they were trained to press a bar in a Skinner box for ICS. During their initial days of pressing, the intensity of ICS was varied while observing rates of pressing. Two intensities were selected and used throughout the balance of testing: (a) a low intensity sustaining low rates of pressing and just above the threshold for intracranial reinforcement, and (b) a high intensity sustaining high, but not maximal, rates. Across days, a pattern of injections was introduced: placebos, cocaine (5.0 mg/kg), cocaine plus U50,488, cocaine, and placebos. This pattern was repeated until 3 doses of U50,488 (1.0, 3.0 & 10.0 mg/kg, 20 min before the session, i.p.) were tested. Cocaine, as expected, produced a significant increase in pressing. Any drug that might block cocaine's enhancement of pressing for ICS without reducing rates of pressing to below that without cocaine is apt to be a good candidate for a pharmacotherapy for cocaine abuse. It would block cocaine's reward-relevant effects without having deleterious side-effects. The low dose of U50,488 reliably enhanced pressing beyond that produced by cocaine alone. The medium intensity had no reliable effects on cocaine-enhanced rates of pressing. The high dose significantly reduced pressing to levels below that of placebos. None of the doses of U50,488, therefore, met the standard for a pharmacotherapy for cocaine-abuse. Supported by grant DA08937, National Insititue on Drug Abuse.

278.5

ANTISENSE OLIGODEOXYNUCLEOTIDE MODULATION OF GABA-TRANSAMINASE ALTERS COCAINE-INDUCED SEIZURES IN MICE. M.S. Abel* and N. Kohli. Dept. of Cell Biology & Anatomy, FUHS/The Chicago Medical School, North Chicago, IL 60064.

We have previously shown that antisense oligodeoxynucleotide (AS-ODN) blockade of GAD_{67} mRNA decreases the threshold for cocaine-induced seizures. We hypothesized that AS-ODN blockage of GABA-T mRNA would have the opposite effect, i.e. increase the threshold for cocaine-induced seizures. The lateral ventricles of male BALB mice (20-25g) were unilaterally infused with 1) AS-ODN (0.29, 0.87, 1.15, or 1.44 nmoles) in artificial CSF, 2) equivalent amounts of random ODN in artificial CSF, or 3) artificial CSF alone. After surgery, animals were allowed to recover for 15 hours before analysis of GABA levels and/or behavioral testing. Mice were injected i.p. with cocaine (50, 60, or 70 mg/kg), and seizure behavior was evaluated. The seizures consisted of severe tremors initially, followed by tonic-clonic extensions and contractions. At 0.29 and 0.87 nmoles of AS-ODN, all mice seized at all cocaine doses. Seizure latency and duration were not significantly different between groups. At 1.15 and 1.44 nmoles, AS-ODN had a protective effect (0-11% seized), as compared to controls (100% seized), at 70 mg/kg cocaine. There was a time-dependent effect of the AS-ODN, such that at 8 hours a qualitative difference in seizures was observed, and by 36 hours, the protective effect was gone. These data suggest that 15 hours after ICV injection, GABA levels are increased sufficiently to reduce either a) widespread tonic inhibition by GABA, or b) specific inhibitory GABAergic pathways.

Supported by FUHS/CMS

278.6

SLOW ONSET, LONG LASTING DOPAMINE REUPTAKE BLOCKERS AS POTENTIAL MEDICATIONS FOR THE TREATMENT OF COCAINE ABUSE. M. Froimowitz,* K.-M. Wu, and R.D. Spealman, Pharm-Eco Laboratories, Lexington, MA 02173 and Harvard Medical School, New England Regional Primate Research Center, Southborough, MA 01772.

The abuse potential of cocaine has been linked to its blockade of dopamine (DA) reuptake into presynaptic terminals in the central nervous system. An important factor in the abuse of cocaine is its rapid onset of action. In developing a medication to substitute for cocaine and reduce its illicit use, it is believed that a slow onset DA reuptake blocker would have more limited abuse potential. Using rational drug design and a pharmacophore model of DA reuptake blockers, a series of slow onset, long lasting compounds have been synthesized. A lead compound, CDTP #30,640, has been evaluated for its *in vivo* time course of action using locomotor activity in mice and schedule-controlled behavior in squirrel monkeys. The compound produced dose-related increases in behavior in both species but with a slow onset of action of about 30 min to initial effects. In addition, peak effects in monkeys occurred on the second and third days after administration with effects lasting as long as four to six days. Thus, CDTP 30,640 is a slow onset, long lasting DA reuptake blocker suitable for further evaluation as a potential medication for the treatment of cocaine abuse. This work was supported by DA48313 (to MF) and DA00499 and RR00168 (to RDS). The mouse locomotor assay was performed by the NIDA Intramural Research Program, Psychobiology Section.

278.7

SELECTIVE D1 (SCH23390) AND D2 (ETICLOPRIDE) DOPAMINE ANTAGONISTS ELEVATE INTRAVENOUS COCAINE SEIZURE THRESHOLDS IN RATS. C.E.Reigel* and A.T. Lovering. Dept. of Pharmacol., Texas Tech Univ. Health Sci. Center, Lubbock, TX 79430.

Although cocaine blocks the uptake of dopamine, dopaminergic involvement in cocaine-induced seizures has been largely ignored. D1 and/or D2 selective antagonists were administered intravenously at various doses in a volume of 0.2 ml/kg, 3 min prior to a 10 mg/kg-min infusion of cocaine. Cocaine infusions were continued until the onset of behavioral convulsions, thereby establishing cocaine seizure thresholds. SCH23390 produced a maximal 29.5 percent elevation of cocaine seizure threshold at 0.5 mg/kg. By 1.5 mg/kg, SCH23390 decreased cocaine seizure threshold 20.3 percent, possibly due to a loss of D1 specificity at this dose. Eticlopride produced a dose-dependent elevation of cocaine seizure threshold that reached a maximum of 84.2 percent at 0.5 mg/kg. The combination of D1 and D2 antagonists maximally elevated cocaine seizure threshold 84.6 percent at a dose of 0.5 mg/kg SCH23390 and 0.1 mg/kg eticlopride, comparable to the maximal elevation achieved by higher doses of eticlopride alone. Thus both D1 and D2 receptors appear to at least partially mediate the convulsant effects of cocaine, with a greater effect demonstrated at D2 receptors. The existence of a maximal ceiling for dopamine antagonist elevation of cocaine seizure threshold suggests that cocaine possesses an additional seizure mechanism. Effects of D1 and D2 antagonists were also determined against intravenous bupropion-, imipramine- and procaine-induced seizures. (Supported in part by NS 28118.)

278.8

MODIFICATION OF COCAINE SELF-ADMINISTRATION BY NITRIC OXIDE SYNTHESIS INHIBITION: A POTENTIAL TREATMENT FOR COCAINE ADDICTION? C.M. Pudiak* & M.A. Bozarth. *Addiction Research Unit, Department of Psychology, University at Buffalo, Buffalo, NY 14260.*

Studies have shown that animals reliably self-administer stimulants intravenously and that the neurotransmitter dopamine is critically involved in their reinforcing effects. Recently, nitric oxide-containing neurons have been identified in many dopamine-rich brain areas (e.g., striatum, ventral tegmental area), and neurochemical studies have shown that nitric oxide (NO) can modulate stimulant-evoked dopamine release. Results from this laboratory have shown that NO-synthesis inhibition blocks the development of cocaine sensitization and that such inhibition produces a shift in the time course of cocaine's effect on brain stimulation reward without altering cocaine's overall effect.

This study examined the role of NO in mediating cocaine reinforcement. Laboratory rats were tested for intravenous cocaine self-administration 3 hrs/day, 5 days/week with 2 days of no testing intervening between each 5-day block of testing. Once animals showed stable cocaine intake, a 5-day protocol was initiated. Animals self-administered cocaine on all days of testing, and on Day-2 and on Day-5 an intraperitoneal injection of Nω-nitro-L-arginine methyl ester (L-NAME; 3, 30, 300 mg/kg) or saline was administered 45 to 60 minutes into a 3-hr test session. Each injection was separated by a minimum of 72 hrs, and all doses were administered according to a Latin square design.

The results show that L-NAME significantly alters the reinforcement profile of cocaine by **decreasing** the amount of cocaine self-administered and by **increasing** the inter-response time between successive cocaine injections. These data suggest that L-NAME prolongs the rewarding effects of cocaine, probably through a pharmacokinetic action. This pharmacokinetic action appears to delay the onset of drug action, to diminish the peak CNS concentration, and to retard elimination from the CNS. Thus, the use of NO inhibitors may prove valuable in the treatment of cocaine addiction by modifying cocaine reinforcement through altered pharmacokinetics.

Supported by Pharmacia and Upjohn (Kalamazoo, MI).

278.9

ATTENUATION OF COCAINE-INDUCED INCREASE IN MESOACCUMBENS DOPAMINE AND BEHAVIORAL ACTIVATION BY SYSTEMIC TREATMENT WITH THE AMPA/KAINATE ANTAGONIST, LY-293558. C. W. Bradberry * and M. Selim. Yale Univ. Sch. of Med., Depts. of Psychiatry and Laboratory Medicine, and the West Haven Veterans Administration Hospital, West Haven, CT 06516.

This study investigated the impact of the systemically available antagonist of the AMPA/kainate excitatory amino acid receptor subtype, 6-(2-(1H-tetrazol-5-yl) ethyl) decahydroisoquinoline -3-carboxylic acid (LY-293558) on cocaine-evoked increase in extracellular dopamine (DA) in the nucleus accumbens (NAS) and locomotor activity, using *in vivo* microdialysis in awake animals. Systemic (IP) pretreatment with LY-293558 blocked the ability of cocaine-HCl (15 mg/kg, IP) to elevate extracellular DA in the NAS in a dose-dependent manner. At 0.1 mg/kg, LY-293558 resulted in a 20 % reduction of cocaine-induced elevation in DA levels ($F=1.24$, $p=0.3$, by two-way ANOVA, Vs. Controls). The 0.3 mg/kg dose reduced cocaine's response by 44% ($F=1.39$, $p=0.19$), whereas pretreatment with the 1.0 mg/kg dose completely blocked the effects of cocaine on extracellular DA (76% reduction, $F=3.06$, $p=0.002$). Concomitant observation of locomotor activity indicated a trend toward an attenuation of the cocaine-induced hyperlocomotion by both the 0.3 and 1 mg/kg doses of LY-293558 ($p<0.05$, by a one-tailed t-test). LY-293558 (0.3 and 1 mg/kg), by itself, had no effect on locomotion and did not alter basal activity. The highest dose of LY-293558 (1 mg/kg), when administered by itself, caused a reduction in basal DA levels in NAS microdialysates ($F=2.3$, $p=0.03$). These results are consistent with a growing body of literature which indicates that excitatory amino acid antagonists can inhibit both the behavioral and neurochemical actions of cocaine, and provide evidence for a potential role of AMPA/kainate antagonists in the pharmacotherapy of cocaine abuse.

Supported by DA 08073, DA 08227, VA Center Grant for the study of PTSD and the Yale VA Alcoholism Research Center.

278.10

THE EFFECTS OF THE DOPAMINE PARTIAL AGONIST TERGURIDE ON COCAINE SELF-ADMINISTRATION. L. Pulvirenti* [1], C. Balducci, M. Piercy and G.F. Koob Dept. of Neuropharmacol., Scripps Res. Inst., La Jolla, Ca 92037 and [1] "Mondino-Tor Vergata" Ctr for Exp Neurobiol, Un. of Rome "Tor Vergata", Rome, Italy.

Terguride (TG) is a partial dopamine agonist, acting at the D2 receptor site with high affinity and low intrinsic activity. TG acts as a functional antagonist in conditions of high dopamine tone and shows an agonistic profile in conditions of dopamine depletion. The present study was undertaken to assess the pharmacological effects of TG on cocaine reward magnitude and the abuse liability of TG. Intraperitoneal (IP) acute pretreatment with TG (0.025-0.4 mg/kg IP) induced a dose-dependent blockade of cocaine self-administration in rats. Using a within-session dose-effect curve of self-administration of cocaine at various doses (0.06-0.5 mg/injection), TG (0.2-0.4 mg/kg IP) was shown to produce a shift to the right of the inverted U-shaped dose-effect function curve of cocaine. This profile reflects an antagonistic action of TG on the reinforcing effects of cocaine, independent of the rate of responding. Acute pretreatment with TG (0.2-0.4 mg/kg IP) reduced the maximum FR of responding for cocaine thus indicating a reduction of the reward magnitude of cocaine. Finally, the reinforcing properties of TG were tested by substituting TG for cocaine. Animals trained to IV self-administer cocaine readily substituted apomorphine (a full dopamine agonist), but failed to substitute TG for cocaine. These results suggest that TG can block the reinforcing effects of cocaine but, that TG does not possess reinforcing properties by itself in rodents. (Supported by NIH DK26741)

278.11

THE EFFECTS OF FLUPENTHIXOL ON COCAINE-INDUCED LOCOMOTOR ACTIVITY IN MICE, G.J. Schaefer*, K.S. Regan, A. Shemer and J.B. Terrill, MPI Research, Mattawan, MI 49071, Pfizer, Inc., New York, NY 10017 and the National Institute on Drug Abuse, Rockville, MD 20857.

Cis:trans-flupenthixol $(HCl)_2$ (FLU) has been proposed as a treatment to reduce the use of cocaine. The present study was conducted to examine the interaction of FLU with cocaine. Male Swiss-Webster mice were divided into 12 groups of 10 animals each. On days 1-14, animals received an IP injection of saline or FLU (0.3 or 1.5 mg/kg). On days 1, 7 and 14 animals received an additional IP injection of either saline or cocaine (10, 50 or 70 mg/kg) two hours after the saline/FLU administrations. Evaluations of spontaneous locomotor activity (Omnitech Digiscan) on days 1, 7 and 14 revealed that cocaine alone produced a biphasic effect on horizontal activity. The maximum increase occurred at 10 mg/kg while the highest dose, 70 mg/kg, produced a response that was less than that of the saline. FLU alone decreased horizontal activity and also decreased activity when administered at both doses tested together with cocaine. In animals receiving cocaine alone, seizures and tremors were observed in animals on days 1 and 7, particularly in the animals receiving the high dose, and these tended to be blocked with 1.5 mg/kg FLU. Thus, both the hyperactivity as well as the gross neurological signs produced by cocaine were blocked by FLU.

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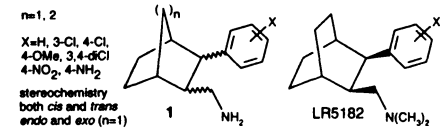
278.12

SYNTHESIS AND EVALUATION OF 2-PHENYL-(3-AMINOMETHYL)-ALKANES AS POTENTIAL COCAINE ABUSE TREATMENT AGENTS. H.M. Deutsch*, D.M. Collard, K. Burnham, S.G. Holtzman# and M.M. Schweri##. School Chemistry and Bio-chemistry, Georgia Tech, Atlanta GA 30332; #Emory University School of Medicine, Atlanta, GA 30322; ##Mercer University School Medicine, Macon GA 31207.



We have synthesized a series of 2-phenyl-(3-aminomethyl)-alkanes (**1**), modeled on the selective dopamine uptake inhibitor LR5182. The synthesized compounds were tested for their ability to inhibit [^{3}H]WIN 35,428 binding and [^{3}H]dopamine uptake and were also examined for antagonism against the behavioral effects of cocaine (COC). The compounds were initially evaluated by their DISCRIMINATION RATIO (DR) which is defined as the ratio of IC_{50} values for the inhibition of [^{3}H]dopamine uptake to [^{3}H]WIN 35,428 binding. Thus, compounds with high DR values would be relatively poor inhibitors of [^{3}H]dopamine uptake at a given [H]WIN 35,428 binding. IC_{50} values for binding for this series varied from 16 to 4200 nM. IC_{50} values for uptake varied from 34 to 8900 nM. Compounds with both high (6.5) and low (1.9) DR values were tested for their ability to substitute for cocaine in male Sprague-Dawley rats that were trained to discriminate between COC (10 mg/Kg) and saline. The synthesis, inhibition assays, structure-activity relationships and animal behavioral testing will be discussed in detail. Supported by NIDA (DA06305).

278.13

SYNTHESIS, PHARMACOLOGY AND MOLECULAR MODELING STUDIES ON A SERIES OF N-SUBSTITUTED 4',4"-DIFLUORO-3α-DIPHENYLMETHOXYTROPANE ANALOGS. A.H. Newman*, G.E. Agoston, J.H. Wu, R.H. Kline, S. Izenwasser, J.L. Katz, Psychobiology Section, NIDA-DIR, Baltimore, MD 21224.

A series of N-substituted-4',4"-difluoro-3α-diphenylmethoxytropane analogs were prepared from the N-nor analog via acylation followed by hydride reduction of the amide or by direct alkylation. All of the analogs displaced [^{3}H]WIN 35,428 from the dopamine transporter (K_i range = 8-2300 nM) and blocked dopamine reuptake (IC_{50} range=10-5400 nM) in rat caudate putamen. Further, none of the compounds demonstrated high affinity for serotonin or norepinephrine transporters. Several analogs (NH, NCH_3, N-butyl, N-allyl, N-butylphenyl) that demonstrated high affinity for the dopamine transporter (K_i range=8-30 nM), with a range of binding affinities at muscarinic m_1 receptors (K_i=6->500 nM) were evaluated as psychomotor stimulants and for their effects on cocaine-induced behavior. None of these analogs demonstrated efficacious locomotor stimulant actions in mice nor did they fully substitute for the discriminative stimulus effects in rats trained to discriminate 10 mg/kg of cocaine from saline. Interestingly, their effects on cocaine in the drug discrimination paradigm varied significantly and may be related to their binding affinities at muscarinic receptors. Specifically, those compounds with high affinity for both the dopamine transporter and muscarinic receptors potentiated the effects of cocaine. Whereas, when the muscarinic binding affinities were lower, the potentiation was lost. Molecular modeling studies of these compounds at both the dopamine transporter and muscarinic m_1 sites have been initiated as well as additional behavioral testing to further elucidate the roles of both the dopamine transporter and muscarinic receptors in the behavioral pharmacology of these compounds. These studies will provide an improved understanding of the mechanistic basis from which the development of a cocaine abuse therapeutic may result.

Supported by intramural funding of NIDA/NIH

279.1

Analysis of QTLs Affecting Response to Methamphetamine in the BXD Recombinant Inbred Mouse Strains J.K. Belknap*, J.E. Grisel, C.M. Wenger, & J.C. Crabbe VAMC and OHSU, Portland OR 97201

Genetic influences on phenotypes related to drug abuse tend to be quantitative in nature and therefore have been difficult to study using classic genetic analyses. Quantitative Trait Locus (QTL) mapping is a method used to examine genetic contributions to some of these traits by correlating allelic polymorphisms at a marker locus with variation in expression of a drug-related trait. A significant association indicates the probable existence of a gene near the marker that influences drug responsivity. Much of this research has been performed in the BXD panel of recombinant inbred (RI) strains which, along with the C57BL/6J (B6) and DBA/2J (D2) progenitors, have been genotyped for over 1600 markers. In the present study, B6, D2 and 24 of their RI strains were evaluated for effects of METH (0, 4, 8 and 16 mg/kg) on exophthalmos, body temperature, horizontal activity and the stereotypic climbing response. Strains differed in response to METH-- indicative of a significant genotypic contribution to variability in these traits. Some QTLs were correlated with several METH-induced responses suggesting common neural substrates underlying the responses to METH. For example, significant associations between an array of METH-related phenotypes and loci thought to modulate glutamatergic and cholinergic transmission were found (*Glud*, *Ache* and markers near genes encoding delta and gamma subunits of the acetylcholine receptor), consistent with pharmacological evidence supporting a role for these candidate genes. Finally, QTLs suggested by these analyses were compared with QTLs hypothesized to underlie cocaine responsivity (Tolliver et al, '94; Milner & Marley, '95) providing evidence for loci with pleiotropic influences on responsivity to multiple psychostimulants.

PHS #POI AA08621 & T32 AA07468, and NIDA # 271-90-7405.

279.2

PREDISPOSITION TO SELF-ADMINISTER AMPHETAMINE: THE ROLE OF INDIVIDUAL DIFFERENCES IN RESPONSE TO NOVELTY AND PRIOR EXPOSURE TO THE DRUG. Pierre, P. J.* and Vezina, P. Department of Psychiatry, University of Chicago, Chicago, IL 60637.

Prior exposure to stimulants leads to sensitized behavioral responding to subsequent administrations of these drugs and increases the likelihood that animals will self-administer them. A rat's response to a novel environment has also been suggested to predict this animal's predisposition to self-administer drug. The present experiment examined the relationship between response to novelty and drug preexposure and its contribution to an animal's subsequent lever pressing for drug. Rats showing a high (HR) or low (LR) locomotor response to novelty were given nine daily injections of amphetamine (1.5 mg/kg, i.p.) or saline. Starting one week later, rats were submitted to a ten day self-administration procedure in which presses on an active lever delivered 10 μg amphetamine/kg/infusion and presses on an inactive lever delivered no drug. On the first day of self-administration testing, lever press behavior did not reflect drug preexposure condition. However, with successive days, amphetamine preexposed rats maintained higher active lever pressing than saline preexposed rats, which showed decreasing pressing of both levers. Interestingly, only HR rats preexposed to amphetamine maintained increased active lever responding. LR rats exhibited decreasing levels of lever pressing over days regardless of drug preexposure condition. These data suggest that response to novelty may be a predictor more closely linked to an animal's propensity to become sensitized to the facilitatory effects of a drug rather than to an animal's liability to self-administer the drug. Supported by NIDA grant DA09397 to P.V.

279.3

INDIVIDUAL DIFFERENCES IN SUCROSE INTAKE ARE PREDICTIVE OF INTRAVENOUS AMPHETAMINE SELF-ADMINISTRATION LEVELS. **N.J. DeSousa[1]*, D.E.A. Bush[1], and F.J. Vaccarino[1,2].** Department of Psychology[1], University of Toronto, Toronto, ON, *M5S 1A1*, Canada; Clarke Institute of Psychiatry[2], Toronto, ON, *M5T 1R8*, Canada.

Rats exhibit significant individual differences in sucrose intake, and may be categorized as either low (LF) or high (HF) sugar feeders. Psychostimulant treatment has been shown to differentially affect nucleus accumbens (NAcc) dopamine (DA) release, sucrose intake, and exploratory locomotion in these groups. The present experiments examined the relationship between sucrose intake and psychostimulant self-administration.

In exp. 1, male Wistar rats were divided into LF and HF based on a median split of their sucrose intake in response to a saline injection. Rats were then surgically implanted with indwelling catheters into the jugular vein. Following recovery, they were placed in operant boxes containing two levers for 10 daily 30 minute sessions. Depression of the active lever resulted in an intravenous (i.v.) infusion of d-amphetamine (AMPH; 10 μg), while depression of the inactive lever had no programmed consequences. In exp. 2, an AMPH dose-response (1, 3, 10, 30, 100 μg/infusion) analysis was conducted using the same animals.

Results from exp. 1 showed that i.v. AMPH self-administration response rates were lower in LF than in HF across the 10 day acquisition period. Results from exp. 2 indicated that LF demonstrated a blunted AMPH dose-response with no horizontal shift in comparison to HF. These data support the hypothesis that the propensity to ingest sucrose is predictive of the activational effects of psychostimulant drugs. Supported by a Medical Research Council of Canada grant to F.J.V.

279.4

Novelty Seeking Predicts Individual Differences in Amphetamine Disrupted Bar Pressing. J.E. Klebaur*, M. Marion, R.A.Bevins, J. Carney, & M.T. Bardo. Depts of Psychology and Pharmacology Univ of Kentucky, Lexington, KY 40506-0044.

Past work has shown that individual differences can predict acquisition of amphetamine self-administration, locomotor response to stimulants, and development of amphetamine sensitization. The increase in activity observed when a rat is exposed to an inescapable novel environment is thought to reflect escape behavior due to stress. To assess approach to novelty in a free-choice test, we examined the ability of the novelty-induced place preference test to predict individual differences in amphetamine disrupted responding. Using this test to categorize a random population of rats as either high or low novelty seekers, it was found that high novelty seekers were more sensitive to the response suppressant effects of amphetamine than low novelty seekers. These results suggest that differences in response to novelty can predict sensitivity to the rate suppressant effects of amphetamine. (Supported by USPHS grant DA05312)

279.5

AGE-DEPENDENT EFFECTS OF AMPHETAMINE ON SEVERAL COMPLEX BEHAVIORS IN RHESUS MONKEYS. P. Morris, M.P. Gillam, S. Lensing, R.R. Allen, G.E. Schulze and M.G. Paule*. Division of Neurotoxicology, National Center for Toxicological Research, FDA, Jefferson, AR 72079-9502.

The effects of acute amphetamine hydrochloride (AMP) administration (0.01 to 3.0 mg/kg, intravenously) on several complex brain functions were studied in adolescent (3 years of age, N=11) and young adult (6 years of age, N=9) rhesus monkeys. Several food-reinforced operant behaviors, used to model motivation (M), learning (L), color and position discrimination (CPD) and short-term memory (STM) were monitored. The lowest dose of AMP that significantly disrupted these behaviors was consistently lower in young adults than it was in adolescents (M: 0.3 vs 1.0; L: 0.1 vs 1.0; CPD: 1.0 vs 3.0; STM: 0.3 vs 1.0; respectively, all in mg/kg). Thus the sensitivity to the behaviorally disruptive effects of AMP increases with age, such that young adults are on average about three times more sensitive to such effects than adolescents. Presuming that AMP causes its behavioral effects by interacting with catecholaminergic mechanisms, changes in the sensitivity to the behavioral effects of AMP may reflect the functional status of these systems in relation to age. Supported in part by Interagency Agreement #224-89-003 with NIDA.

279.6

DISSOCIABLE EFFECTS OF DORSAL VS. VENTRAL SUBICULUM LESIONS IN ANIMAL MODELS RELEVANT TO DRUG ABUSE AND SCHIZOPHRENIA. S. B. Caine*, R. B. Whitelaw, T. W. Robbins and B. J. Everitt. Department of Experimental Psychology, University of Cambridge, Cambridge, England, CB2 3EB.

Utilizing measures of locomotor activity, sensorimotor gating and iv drug self-administration, this study tested the hypothesis that the output pathways of the hippocampal formation originating in the dorsal and ventral subiculum differentially modulate the behavioral effects of systemically administered psychostimulant drugs. Previous studies showed that excitotoxic lesions of the hippocampus potentiated hyperactivity associated with increased nucleus accumbens extracellular dopamine after amphetamine challenge in rodents (Wilkinson et al., Behav. Brain Res. 55:143, 1993). Conversely however, selective lesions of the ventral subiculum of the hippocampal formation attenuated the psychostimulant and conditioned reinforcement enhancing properties of intra-accumbens amphetamine (Burns et al., ibid., p.119).

Bilateral excitotoxic lesions of the dorsal subiculum produced hyperactivity with enhanced responsiveness to amphetamine (shift to the left in the dose-effect function). In contrast, lesions of the ventral subiculum did not alter spontaneous activity but attenuated the psychostimulant effects of low dose amphetamine challenge. In the same animals as well as in drug naive animals, dorsal subiculum lesions increased acoustic startle reactivity without altering prepulse inhibition of startle (PPI). Conversely, ventral subiculum lesions did not alter startle reactivity but profoundly attenuated PPI. Importantly this deficit diminished in a session-dependent manner with repeated tests until baseline levels of PPI were normal; nevertheless these rats remained more sensitive to the PPI disrupting effects of a low dose amphetamine challenge. Finally, neither type of lesion unequivocally altered the self-administration of cocaine, though rates of drug intake tended to be lower in dorsal subiculum lesioned animals and higher in ventral subiculum lesioned animals across a range of cocaine doses, relative to sham-operated controls. The results suggest different roles for the dorsal and ventral subiculum in hyperactivity and sensorimotor gating, respectively, and perhaps subtle, opposing influences on cocaine self-administration. Supported by a Human Frontiers Science Program Long-term Fellowship to S.B.C.

279.7

REINFORCING EFFECTS OF d-AMPHETAMINE AND MORPHINE DEMONSTRATED USING AN INSTRUMENTAL-APPROACH PLACE CONDITIONING PROCEDURE. W. D. Eggman* and I. Lucki. Depts. of Psychiatry and Pharmacology, University of Pennsylvania, Philadelphia, PA 19104.

This study reports an instrumental place-preference (IPP) procedure designed to examine both the acute reinforcing effects of drugs as well as their ability to sustain a conditioned approach response. Using a two-chambered apparatus, animals were trained against their initial preference to locomote from a start box to a goal box to receive an injection of saline (SAL), d-amphetamine (AMPH; 0.32, 1.8 or 5.6 mg/kg) or morphine (MORPH; 5, 10 or 20 mg/kg). The time to enter the goal box (TEG) was recorded for each subject. Changes in TEG across training days provided a measure of the acute positive reinforcing effects of the training injections. A second preference test was then given to determine whether the side preferred initially was altered by IPP training.

IPP training with AMPH produced three distinct patterns depending on the training dose. The low dose of AMPH decreased TEGs, the medium dose of AMPH produced TEGs which did not differ from SAL, and the highest dose of AMPH tended to produce an increase in TEGs. In contrast, all doses of MORPH produced TEGs of shorter latencies than SAL; although the highest dose was not significantly different. During post-training preference tests, all AMPH and MORPH animals demonstrated a robust preference for the goal box; the side preference of SAL animals did not differ significantly from the pretest. These results suggest that an instrumental training procedure can demonstrate acute appetitive (low doses of AMPH and all doses of MORPH) and aversive (high dose of AMPH) effects of drugs of abuse. In addition, since all animals demonstrated a significant preference for the drug-paired environment regardless of acute effects of their training dose, these results are consistent with a differentiation between the reinforcing effects of drug doses and their ability to induce drug "craving". Supported by NIDA DA 05186.

279.8

ROLE OF BEHAVIORAL AND PHARMACOLOGICAL VARIABLES IN THE RETENTION OF TOLERANCE TO AMPHETAMINE HYPOPHAGIA. K.M. Hughes* and D.L. Wolgin. Dept. of Psychology, Florida Atlantic Univ., Boca Raton, FL 33431.

Tolerance to amphetamine hypophagia is lost when drug injections are suspended while feeding tests are continued (Wolgin & Hughes, in press). In this experiment, we determined whether the loss of tolerance is a function of drug withdrawal *per se*. Male Sprague-Dawley rats received amphetamine (2 mg/kg, ip) before daily milk tests, to induce tolerance. The rats were then assigned to one of three groups. During the next 21 trials (the retention phase), the After Group received amphetamine after the milk tests, so that feeding was no longer disrupted by drug-induced stereotyped movements. The Saline Group was withdrawn from the drug entirely, and received only saline injections, while the Before Group continued to receive amphetamine injections prior to milk. Dose-response tests revealed that the Before Group retained tolerance, whereas the Saline Group lost tolerance. The After Group lost tolerance at doses of 2 and 4 mg/kg, but retained tolerance at 1 mg/kg. These results demonstrate that the loss of tolerance to the higher doses of amphetamine was not due to drug withdrawal *per se*, but may have been due to the unlearning of behavioral strategies previously acquired under the drug.

Supported by USPHS grant DA 04592 from NIDA

279.9

THE EFFECTS OF NUCLEUS ACCUMBENS CCK_B RECEPTOR ACTIVATION ON AMPHETAMINE SELF-ADMINISTRATION. D.E.A. Bush[1]*, N.J. DeSousa[1] and F.J. Vaccarino[1,2]. Department of Psychology[1], University of Toronto, Toronto, ON, *M5S 1A1*, Canada; Clarke Institute of Psychiatry[2], Toronto, ON, *M5T 1R8*, Canada.

The mesolimbic dopamine (DA) pathway terminating in the nucleus accumbens (NAcc) is a critical substrate involved in the mediation of reward. Cholecystokinin (CCK) is co-localized with DA in a subset of mesolimbic neurons and its actions at CCK_B receptors inhibit NAcc-DA neurotransmission. The present experiment examined the effects of NAcc microinjections of pentagastrin, a selective CCK_B receptor agonist, on i.v. *d*-amphetamine (AMPH) self-administration. Six male Wistar rats were surgically implanted with indwelling catheters into the jugular vein, and with bilateral guide cannulae aimed at the rostral portion of the NAcc. Rats were trained to press a lever for 100μL infusions of AMPH (50 μg/kg/i.v. infusion) during daily 3 hour sessions. Animals received intra-NAcc microinjections (0.5μL) of pentagastrin (0, 0.05, 0.5, and 5.0 nM) in a counterbalanced order following 3 day periods of stable responding. Results showed that intra-NAcc pentagastrin dose-dependently (0.05 and 0.5 nM) increased response rates for i.v. AMPH. The compensatory increase in AMPH self-administration in the presence of intra-NAcc pentagastrin is consistent with the hypothesis that NAcc CCK_B receptor activation attenuates dopamine-mediated reward. Supported by a grant from the Medical Research Council of Canada to F.J.V.

279.10

ROLE OF HIPPOCAMPUS ON AMPHETAMINE INDUCED CONDITIONED LOCOMOTOR RESPONSE. S.Mandillo, A.Felici, S.Cabib*, A.Oliverio and A.Mele. Dip. Genetica e Biologia Molecolare, Università di Roma "La Sapienza" and Ist. di Psicobiologia e Psicofarmacologia, C.N.R., Rome, Italy.

It has been shown that hippocampus plays a focal role in different kind of associative learning. The purpose of this study was to assess whether lesions of the main hippocampal efferent pathway would have in some way affected development of context dependent sensitization to amphetamine. Electrolytic lesion of the fimbria fornix was performed in CD1 mice. Sham and lesioned mice were then divided into three groups: the first group received saline injections before the exposure to 45 min activity session and saline 2 hrs after being returned to the home cage (CONTROL), the second group received saline injection before activity and amphetamine (2.5mg/kg) in the home cage (UNPAIRED), the last group received amphetamine (2.5mg/kg) before activity and saline in the home cage (PAIRED). After five days of treatment the animals was given a saline injection before the exposure to the test cage. Electrolytic lesions of the fimbria fornix enhanced spontaneous activity in mice, response to amphetamine was also enhanced even if not a significant way. Conditioned response on day six in the PAIRED group however was not modified by the lesion. Experiments with more selective lesions of hippocampal-accumbens pathways are on going in order to assess the role of hippocampus in amphetamine induced conditioning and sensitization (A.F. is supported by a "Mariano Scippacercola Foundation" fellowship).

279.11

c-fos ANTISENSE OLIGONUCLEOTIDE INFUSION INTO MOUSE STRIATUM BLOCKS METHAMPHETAMINE-INDUCED LOCOMOTOR ACTIVITY IN A REVERSIBLE MANNEER. T. Umekage[a]*, M. Namima[b], K. Fukushima[a], S. Sugita[a] and Y. Watanabe[a]. [a]Dept. of Pharmacology, [b]Center for Laboratory Animal Science, The National Defense Medical College, Tokorozawa, 359, Japan

Dramatic behavioral changes occur after exposure to a psychomotor stimulant such as methamphetamine (MAP). It has been reported that amphetamine induces immediate early gene c-fos in striatum neurons. c-fos encodes transcription factors that may lead to activation of late effector genes and permanent changes in brain function. To investigate the involvement of immediate early gene in MAP-administrated mice, we have examined locomotor activity and the expression of c-Fos protein after c-fos-antisense oligodeoxynucleotide (ODN) infusion into mice striatum. MAP(2mg/kg)-induced locomotor activity was significantly suppressed by stereotactic injection of c-fos antisense ODN (2mM,1μl) into male ddy mice striatum. The suppression of activities was observed transiently and reproductively. The effect was associated with an elimination of expression of c-Fos immunoreactivity within the infused region of the striatum. These results suggest that c-Fos may be the key molecule regulating the functional output in the MAP-induced mouse motor mechanism.

279.12

A DOSE-EFFECT COMPARISON OF TWO MEASURES OF PSYCHOMOTOR ACTIVATION PRODUCED BY INTRAVENOUS AMPHETAMINE OR COCAINE. Hans S. Crombag*, Kaitlin E. Browman, Karen Lapidos, Aldo Badiani and Terry E. Robinson.
Department of Psychology and Neuroscience Program, The University of Michigan, Ann Arbor, MI 48109.

There is almost no data available on the effects of dose of intravenously (IV) administered psychostimulants on psychomotor activation. We sought here to remedy this situation in anticipation of dose-effect studies on psychomotor sensitization produced by IV drug administration. Thus, we characterized the effects of dose of IV cocaine (COC) or amphetamine (AMPH) on two indices of psychomotor activation: rotational behavior in rats with unilateral 6-OHDA lesion and locomotor activity in intact rats It was found that for doses ranging from 0.125 to 16.0 mg/kg (IV) there was a progressive increase in rotational behavior. A progressive increase in locomotor crossovers was only observed for doses between 0.25 and 2.0 mg/kg. There was a decrease in locomotor crossovers for doses greater than 4.0 mg/kg due to the emergence of stereotyped behaviors. Finally, when a challenge dose was given to test for the development of sensitization only rotational behavior showed significant sensitization. Thus, with IV administration an increase in drug effect is characterized by a linear increase in rotational behavior over a wide range of doses, whereas this is not true for locomotor activity. Therefore, the rotational behavior model may be particularly useful when studying phenomena associated with changes in drug effect, such as sensitization.
(Supported by Grant # DA 04294)

279.13

THE EFFECTS OF INTRA-PREFRONTAL CORTEX D-AMPHETAMINE ON SUBCUTANEOUS D-AMPHETAMINE-INDUCED HYPERACTIVITY. O. Ben-Shahar* and A. Ettenberg. Behavioral Pharmacology Lab, Dept of Psychology, University of California, Santa Barbara, CA 93106

The mesolimbic dopaminergic pathway (with it's cell bodies in the ventral tegmental area (VTA) and terminals in the nucleus accumbens (NAC)) has been implicated in the neural mediation of the hyperlocomotor response to amphetamine. This pathway is also involved in the sensitization to amphetamine-induced hyperactivity, observed after repeated administration of amphetamine. Dopamine cells within the VTA also project to the medial prefrontal cortex (mPFC) and recent anatomical, physiological and behavioral research has identified a functional link between these two projection areas of the VTA (i.e. the NAC and the mPFC). The present study was therefore devised to examine the effects of intra-mPFC amphetamine on amphetamine-induced hyperactivity. On each of 3 consecutive days, rats received bilateral intra-mPFC injections, of either 5 µg d-amphetamine /0.5 µl saline or 0.5 µl saline, and a SC injection of either 1 mg d-amphetamine/ml/kg or 1ml/kg saline. Immediately after the drug treatment each rat was placed into the locomotor chamber for 60 mins. Intra-mPFC d-amphetamine did not block the acute locomotor response to SC d-amphetamine, however, it did attenuate the sensitized hyperlocomotor effects of SC amphetamine that developed over trials/days.

This work was supported by NIDA grants DA05041 and DA08042 awarded to AE

279.14

EFFECT OF METHAMPHETAMINE ON VARIOUS ASPECTS OF MOTOR BEHAVIOR AT COLD AND AMBIENT TEMPERATURES R. A. Mohaghegh*, T. A. Ansah and D. C. Shockley. Meharry Medical College, Department of Pharmacology, Nashville, TN 37208.

We have previously shown that methamphetamine (MAMPH) induced a spectrum of thermic and motor activities at different ambient temperatures. However, the data from motor effect of MAMPH were only evaluated subjectively. In this experiment, we studied the effect of i.p. MAMPH (0.01 to 10 mg/kg at 22 ± 1°C, and 1.25 to 10 mg/kg at 7 ± 1°C) on motor behavior of rats in a quantitative way. We also compared the effect of a toxic dose of MAMPH (5.0 mg/kg), amphetamine (10 mg/kg), and cocaine (60 mg/kg) at 21 ± 1 and 7 ± 1°C.
The motor behavior of male Sprague-Dawley rats were recorded and analyzed via Digiscan Animal Activity Monitor every five minutes for four hours; one hour after saline and three hours after either MAMPH, amphetamine or cocaine, or equivolume saline. MAMPH at low doses increased the horizontal, vertical, and ambulatory activities of the rat, while, MAMPH, amphetamine, and cocaine increased the stereotypic activities only at high doses. Further analysis of the data are in progress. This research was supported in part by T32-MH 19843-02 and DA06686.

279.15

LOCOMOTOR EFFECTS OF AMPHETAMINE THROUGHOUT THE LIGHT DARK CYCLE Christopher Lewis, O. Gaytan, A.Swann*, and N. Dafny Dept. of Neurobiology and Anatomy, and Dept. of Psychiatry, UT Medical School at Houston, P.O. Box 20708, Houston, TX 77225

Low doses of amphetamine (Amp) cause increases in the level of locomotor activity such as forward ambulation and rearing. The amount of locomotor activity varies significantly between the light and dark phases of the rat. and so it became of interest to study whether the locomotor effect elicited by amphetamine (i.e., forward ambulation and rearing) differs if the time of drug administration is varied. 96 male Sprague-Dawley rats (about 200 g each) were housed in computerized activity monitoring system test cages after 7 days of acclimation to L/D cycle (07:00/19:00). Continuous recording was begun (10 min bins) for 3 days as follows: Days 1 &2 - baseline activity data; and Day 3 - rats were randomized for s.c. treatment of saline, 0.6, or 1.2 mg/kg Amp at one of the following times (08:00, 14:00, 20:00, or 02:00). Data collected immediately after injection (day 3) were compared to baseline activity data (days 1-2) at the corresponding time to obtain the drug effect. Data were subjected to a two-factor (dose and treatment time) ANOVA. The motor indices of total distance and vertical activity are presented. Compared to vehicle, both Amp doses markedly elevated the counts of the locomotor indices studied across all treatment times ($p < 0.01$). There was no dose-by-time interaction in the two-factor ANOVA for either total distance or vertical activity, indicating an absence of circadian rhythmicity in the dose-response relationship. However, the magnitude of the Amp effect on vertical activity was significantly greater when injected at 20:00 ($p < 0.01$) as compared to all other injection times. In conclusion, the magnitude of the Amp effect on rearing is dependent on the time of injection, but the locomotor response to different Amp doses was similar for both indices at all 4 injection times.

279.16

COMPARISON OF CS$^+$-INDUCED CHANGES IN DOPAMINE IN THE NUCLEUS ACCUMBENS OF RATS FOLLOWING EITHER SELF- OR YOKED- ADMINISTRATION OF *d*-AMPHETAMINE. P. Di Ciano, C.D. Blaha and A.G. Phillips*. Department Psychology, University of British Columbia, Vancouver, B.C., Canada V6T 1Z4.

We have previously demonstrated (Di Ciano et al., Soc Neuro Abst 21:2103, 1995) that repeated pairings of either cocaine or *d*-amphetamine with a compound environmental stimulus (odour, light) produced conditioned increases in both locomotor activity and extracellular dopamine (DA) concentrations in the nucleus accumbens (NAc), when rats were exposed to the compound CS$^+$ in the absence of the drug. In the present study, *in vivo* chronoamperometry was used to monitor extracellular concentrations of DA in the NAc, when rats were exposed to a CS$^+$ (flashing light) that had been paired repeatedly with either self-administered or yoked *d*-amphetamine injections (0.25 mg/kg/injection). On the seventh, and final consecutive day of *d*-amphetamine treatment, *d*-amphetamine produced significant increases in the DA signal in both the self-administering rats and those that received yoked infusions, of ~9 nA and ~7 nA, respectively, by the end of the 3 hour session. When presented with the CS$^+$ as a prime and at 30 min intervals, in the absence of drug on the eighth day, rats that had previously self-administered the drug showed significant increases in DA concentrations that continued to increase steadily over the 3 hour session, reaching a maximum of ~6 nA by the end of the session. These conditional changes in the DA signal were significantly lower than those observed during self-administration of the drug. By comparison, the yoked group showed a pattern of conditional increases, similar to the self-administering rats, which plateaued at ~7 nA; a change that was not significantly different from those seen during drug sessions. The present study provides support for the hypothesis that DA in the NAc is involved in the conditioned incentive effects of drugs of abuse. In addition, the present experiment also suggests that these conditioned incentive effects are different following either self- or yoked- administration of *d*-amphetamine. *Supported by MRC, Canada.*

279.17

IBOGAINE INTERFERES WITH THE ESTABLISHMENT OF AMPHETAMINE PLACE PREFERENCE LEARNING. [1]I. Moroz, [1]L.A. Parker* and [2]S. Siegel [1]Department of Psychology, Wilfrid Laurier University, Waterloo, Ontario and [2]Department of Psychology, McMaster University, Hamilton, Ontario.

The present series of experiments assessed the ability of a single injection of ibogaine (40 mg/kg), a proposed anti-addictive agent, to modify the establishment of amphetamine-induced place preference learning, when administered 24 hours prior to amphetamine conditioning. Ibogaine pretreatment blocked the establishment of an amphetamine place preference after one or two conditioning trials, but was not effective after four trials (Experiments 1 and 2). The attenuation of ibogaine's ability to interfere with place preference conditioning after multiple trials may be the result of the development of tolerance to this effect of ibogaine (Experiment 3). Supported by the Natural Sciences and Engineering Research Council of Canada.

279.18

AMPHETAMINE INFUSIONS INTO THE VENTROLATERAL STRIATUM PRODUCE CONDITIONED PLACE PREFERENCE. D.A. Baker*, S.E. Specio, L.T.L. Tran-Nguyen, T.L. Archer, J.L. Neisewander. Department of Psychology, Arizona State University, Box 871104, Tempe, AZ 85287-1104.

The effects of amphetamine infusions into the ventrolateral striatum (VLS) on conditioned place preference (CPP) and motor behaviors were investigated in rats. Five 2-day conditioning trials were conducted over consecutive days in a CPP apparatus with 2 distinct compartments. On one day of the trial, rats received bilateral infusions of amphetamine (0, 5, 10, or 20 µg/0.5 µl/side) into the VLS and were then confined to a compartment for 30 min. On alternate days, rats received sham infusions and were then confined to the other compartment for 30 min. The order of these treatments and the compartment paired with amphetamine were counterbalanced. Motor behaviors, including locomotion, oral stereotypies, sniffing, rearing, and grooming, were assessed during conditioning. CPP was measured 24 hr following the last day of conditioning. Intra-VLS infusion of amphetamine did not alter locomotion, sniffing, rearing, or grooming relative to saline controls. However, intra-VLS infusion of the 10 µg dose of amphetamine produced an increase in oral stereotypies relative to saline controls. Intra-VLS infusion of the 10 µg dose also produced CPP, whereas the other doses of amphetamine produced a trend towards CPP. These results suggest that intra-VLS administration of amphetamine produces CPP at a dose that also produces oral stereotypies. (Supported by DA07730 and HHMI)

279.19

LACK OF MU-OPIOID AGONIST MODULATION OF THE DISCRIMINATIVE STIMULUS EFFECTS OF COCAINE AND d-AMPHETAMINE IN RATS D. R. Woolfolk* and S. G. Holtzman, Department of Pharmacology, Emory University School of Medicine, Atlanta, Georgia 30322

Mu-opioid receptor agonists increase the activity of dopamine neurons and could, therefore, modulate the behavioral effects of drugs mediated by the dopamine system. Morphine has been shown to potentiate the discriminative stimulus effects of cocaine in squirrel monkeys (Spealman & Bergman, 1992). The ability of morphine (mor), methadone (meth), buprenorphine (bup) and nalbuphine (nalb), to modulate the discriminative stimulus effects of d-amphetamine (amph) and cocaine (coc) in rats was tested. Two separate groups of Sprague-Dawley rats were trained to discriminate 1.0 mg/kg amph or 10 mg/kg coc from saline in an avoidance/escape procedure. Rats were pretreated (sc) with saline, mor or meth (0.3-3.0 mg/kg), bup (0.03-0.3 mg/kg), or nalb (3.0-10 mg/kg), then dose-response curves for amph (0.1-1.0 mg/kg, ip) or coc (1.0-10 mg/kg, ip) were generated by cumulative-dosing. In both groups of rats, there was substantial intersubject variability. For example, mor and meth enhanced the stimulus effects of coc in some coc-trained rats, but not in others. The 0.03 mg/kg dose of bup enhanced coc's cue in some rats, while the higher doses of bup blocked these effects in some rats, as did nalb. Neither the potency of coc, nor that of amph, as discriminative stimuli was altered significantly by any of the opioids when results were averaged across animals. These results suggest that any enhancement of dopaminergic neurotransmission that occurs from mu-opioid receptor stimulation by the agonists tested in this study is not sufficient to consistently change the stimulus effects of amph and coc in rats. (Supported in part by NIH Grant DA00541 and by K05 DA00008.)

279.20

PMMA AS A DISCRIMINATIVE STIMULUS. R. Young,* M. Dukat, L. Malmusi, and R.A. Glennon. Department of Medicinal Chemistry, MCV/VCU, Richmond, VA 23298.

PMMA (N-methyl-1-(4-methoxyphenyl)-2-aminopropane), a structural hybrid of paramethoxyamphetamine and methamphetamine, has been previously shown to lack amphetamine-like or hallucinogen-like discriminative stimulus effects in animals. PMMA does, however, produce MDMA ("Ectasy" or N-methyl- 1-(3,4-methlenedioxyphenyl)-2-aminopropane)-appropriate responding in animals trained to discriminate MDMA from vehicle. In order to further characterize this seemingly unique agent, we trained a group of six Sprague-Dawley rats to discriminate 1.25 mg/kg of PMMA (ED50 = 0.44 mg/kg) from saline. The PMMA stimulus failed to generalize to the CNS stimulant S(+)amphetamine or to the hallucinogen DOM(1-2,5-dimethoxy-4-methyl-phenyl)-2-aminopropane). Stimulus generalization occurred to (±)MDMA (ED50 = 1.32 mg/kg) and S(+)MDMA (ED50 = 0.48 mg/kg). Partial generalization occurred with R(-)MDMA. The PMMA stimulus also generalized to the α-ethyl homolog of PMMA (EH/PMMA, ED50 = 1.32 mg/kg). Other agents examined included R(-)PMMA, S(+)PMMA, conformationally restricted analogs of PMMA, and the N-desmethyl analogs of PMMA, i.e., (±)PMA, R(-)PMA, and S(+)PMA. Taken together, the results of these studies suggest that PMMA is an MDMA-like agent that lacks the amphetamine-like stimulant character of MDMA. These findings support our previous suggestion that PMMA be considered the structural parent of the MDMA-like family of designer drugs. (Supported in part by NIDA grant DA-01642).

SYMPOSIA MONDAY PM

280

SYMPOSIUM. MODULATION OF NEURONAL EXCITABILITY AND BEHAVIOR. J.L. Feldman, UCLA & R.M. Harris-Warrick, Cornell Univ. (Chairpersons); R.L. Calabrese, Emory Univ.; & D.A. McCormick, Yale Univ.

Modulation of neuronal excitability has been extensively studied at the cellular level, but the consequences of these changes in neuronal firing patterns for behavior are less well understood. The aim of this symposium is to show how modulation of cellular properties of identified neurons alters function at the network and higher levels of organization and leads to changes in behavior. Harris-Warrick will introduce the topic and discuss possible cellular targets of neuromodulation in behavioral networks. Calabrese will discuss experimental and modeling approaches to study a two-neuron mutually inhibitory oscillator network driving heartbeat in the leech. He will describe the role of several identified K^+ currents in the generation of the heartbeat rhythm and its modulation by the peptide FMRFamide. Harris-Warrick will describe the effect of dopamine on several currents in identified neurons of the pyloric motor pattern generator in the lobster stomatogastric ganglion, and will show how these currents affect phasing of neuronal activity in the rhythmic motor pattern. Feldman will describe the modulation of the respiratory motor pattern in the rat, showing how a set of neuromodulators affects respiratory motoneurons and their pre- and postsynaptic interactions to provide a cascade control of coarse and fine tuning of breathing. McCormick will describe the ionic mechanisms for modulation of excitability of thalamic and cortical neurons and their neuronal circuits during different waking and sleep states, when both network interactions (such as the propensity to generate seizures) and responsiveness to sensory input are profoundly changed. We hope that this symposium will encourage discussion of the behavioral consequences of neuromodulation.

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SYMPOSIUM. CELL CYCLE REGULATION AND CNS DEVELOPMENT: IS ONE DIVISION LIKE ANY OTHER? M. Elizabeth Ross, Univ. of Minn.(Chairperson); Patrick O'Farrell, UCSF; Yuh-Nung Jan, UCSF & HHMI; Susan McConnell, Stanford Univ.; Nathaniel Heintz, Rockefeller Univ. & HHMI.

Early observers of the ventricular neuroepithelium considered the region to be comprised of "indifferent" cells that acquire neuronal identity only after they cease to divide. However, neural proliferation must be exquisitely controlled in a stage and area specific manner as the CNS develops to provide appropriate cell numbers for regional morphogenesis, implying a need for intermediate phases of progenitor fate determination. Substantial evidence has accumulated to suggest that neural identity is progressively established even as CNS progenitors proliferate. This symposium will examine recent advances in cell cycle regulation in the context of normal development and discuss special challenges to proliferation posed by CNS histogenesis. Mechanisms integrating neurodevelopment with decisions to progress through, or exit from the cell cycle will be discussed and emerging models for the interrelationship of cell cycle regulation, neurodifferentiation and programmed cell death will be presented. **O'Farrell** will provide an overview of molecular interactions governing cell division and discuss the relationship between developmental programming and the cell cycle in *Drosophila*. **Jan** will discuss neuronal determination in *Drosophila* and propose that an organizer of cell division provides position cues for mitotic spindle orientation and asymmetric gene expression in neural precursors. **McConnell** will discuss the role of asymmetric divisions in the control of neuronal fate in the cerebral cortex and will present evidence for the progressive restriction of developmental potential over time in the ventricular zone. **Heintz** will consider the role of cell cycle progression in CNS apoptosis. Evidence will be presented supporting involvement of cell cycle proteins in programmed cell death under some, but not all circumstances.

NEURONAL DEATH IV

284.1

ROLE OF PRION PROTEIN IN RESISTANCE TO OXIDATIVE STRESS AND REGULATION OF SUPEROXIDE DISMUTASE, D.R. Brown* and H.A. Kretzschmar. Department of Neuropathology, University of Göttingen, Robert-Koch-Strasse 40, Göttingen, D-37075, Germany.

The function of the cell surface prion protein is unknown. Mice lacking PrP^C expression develop and behave normally. In the present study cell culture of cerebellar cells was used to examine differences between PrP deficient mice and wild type mice. Cell culture studies have revealed that cerebellar cells lacking PrP^C are more sensitive to oxidative stress and undergo cell death more readily than normal cells. Assays of protein extracts from mouse brain indicate that Cu/Zn superoxide dismutase has reduced activity in PrP^C deficient mice. Oxidative stress applied to cultured cerebellar cells normally increases superoxide dismutase activity but cells from PrP knockout mice show no upregulation of Cu/Zn superoxide dismutase in cultures of normal cerebellar cells. Our findings suggest that PrP^C may regulate the activity of superoxide dismutase. PrP^C expression may be important for cellular resistance to oxidative stress. Our results suggest that PrP^C functions to aid cells resist oxidative stress that would otherwise activate the cell suicide pathway. Funded by the Bundesministerium für Bildung, Forschung und Technology (01KI 9461/8), the Wilhelm Sander-Stiftung (89.036.2) and the Fritz Thyssen-Stiftung (1992/2/64) of Germany.

284.2

PROTECTIVE ROLE OF HEME OXYGENASE-1 IN OXIDATIVE STRESS INDUCED NEURONAL INJURY. W.D.LE*, W.J.XIE, R.KONG, X.ZHAO, S.H.APPEL. DEPT. OF NEUROLOGY, BAYLOR COLLEGE OF MEDICINE, HOUSTON, TX 77030

Heme oxygenase-1 (HO-1) is an inducible stress protein in response to variety of oxidative challenge and stimuli. In primary hippocampal and hybrid neuronal cultures, HO-1 expression was significantly elevated in response to an increase in the production of reactive oxygen species in a time-dependent manner after exposure to synthetic human ß-amyloid$_{1-40}$ and hydrogen peroxide. The induction of HO-1 could be seen in both astrocytes and neurons in mixed hippocampal cultures. The levels of HO-1 in primary and cell line cultures however were dropped when the cultured cells started degeneration. To understand the role of HO-1 in oxidative stress-induced neuronal injury, we used HO-1 antisense oligonucleotide to inhibit expression of HO-1 in hybrid neuronal cell cultures. After 24 hr treatment with HO-1 antisense, the expression of HO-1 was dramatically decreased as documented by immunostaining and immunoblot. Although low concentration of HO-1 antisense did not affect the cell viability, high concentration of the antisense caused cell death. Down regulation of HO-1 expression with HO-1 antisense increased the neuronal cells 3-5 times more vulnerable to ß-amyloid and hydrogen peroxide induced cytotoxicity. These findings support the role of HO-1 as an important cellular antioxidant defense protein in protecting neurons from oxidative stress-induced injury.

284.3

PKC-DEPENDENT MODULATION OF NEURONAL APOPTOSIS AND OXIDATIVE STRESS BY BDNF. B.J. Gwag*[1] and D.W. Choi[2], [1]Dept. of Pharmacology, Ajou Univ. School of Medicine, Suwon 441-749, Korea; [2]Center for the Study of Nervous System Injury and Dept. of Neurology, Washington Univ. School of Medicine, St. Louis, MO 63110.

Near-pure neuronal cortical neuronal cell cultures undergo cycloheximide-sensitive neuronal apoptosis following serum deprivation. Inclusion of 100 ng/ml brain-derived neurotrophic factor (BDNF) or insulin-like growth factor I (IGF-I) attenuated serum deprivation-induced neuronal apoptosis. Concurrent treatment with 0.3 - 10 μM Go 6976, a selective inhibitor of PKC α and β1, reversed this protective effect of BDNF, but not that of IGF-I. Wortmannin, an inhibitor of phosphatidylinsositol-3-kinase (PI-3-kinase), partially reversed both the protective effects of BDNF and IGF-I.

Free radical stress was induced in mixed cultures of cortical neurons and glia by exposure to 30 μM Fe^{2+} or 1 mM buthionine-sulfoximine (BSO), leading to submaximal neuronal necrosis over 24 hrs. Inclusion of BDNF or IGF-1 potentiated such free radical-mediated neuronal necrosis. This cell death potentiation by BDNF or IGF-I was prevented by addition of cycloheximide, suggesting a requirement for new protein synthesis. As with the protective effects of the growth factors on serum deprivation-induced apoptosis, the harmful effects of BDNF but not IGF-1 were blocked by inclusion of Go 6976, and both BDNF and IGF-I injury potentiation was partially sensitive to wortmannin. Present data provide evidence that both the attenuation of neuronal apoptosis and the potentiation of neuronal necrosis induced by BDNF are sensitive to inhibition of PKC α and β1 as well as possibly, PI-3-kinase. Supported by NIH NINDS grant NS 30337 (DWC).

284.4

FREE OXYGEN RADICALS MEDIATE EXCITOTOXIC AND APOPTOTIC NEURONAL DEGENERATION J.H.M. Prehn* Dept. of Pharmacol. & Toxicol., FB 16, Philippe-Univ., Ketzerbach 63, 35032 Marburg, Germany

Overactivation of glutamate receptors (excitotoxicity) and activation of a conserved cell death program (PCD) have been implicated in the degeneration of neurons in stroke, Parkinson's and Alzheimer's disease. The present study was undertaken to evaluate the involvement of reactive oxygen species in both types of cell death.

Cultured rat hippocampal neurons were derived from neonatal (P1) Fischer rats and cultured for 10 - 14 days. Excitotoxic injury was induced by a 10 min exposure to the selective glutamate agonist NMDA (30-300 μM), and apopototic cell death by exposure to staurosporine (0.01-0.5 μM). Production of free oxygen radicals (superoxide anions) was monitored using the specific fluorescent probe dihydroethidin (HEt) in combination with digital microfluorimetry. Exposure to 300 μM NMDA induced an immediate increase in the rate of conversion of HEt to its oxidized form ethidine. This was followed by extensive neuronal degeneration assessed 18 hours later. Exposure to 30 μM NMDA neither induced a significant increase in free radical production, nor any significant cell death. In contrast to the acute response to NMDA, exposure to staurosporine (0.5 μM) induced a delayed production of free oxygen radicals with peak production 120 min after staurosporine exposure (increase of 81.5±7.0 %; $p<0.001$) and persistent elevation for 6 hours. Administration of the antioxidants U74500A (1-10 μM), dihydrolipoic acid (1-10 μM) or vitamin E (10-100 μM) significantly reduced both staurosporine- and NMDA-induced neuronal death.

These results demonstrate the importance of increased free oxygen radical production in the initiation of both NMDA receptor-mediated excitotoxic injury and PCD. Antioxidant therapy may therefore be useful in the treatment of neurodegenerative disorders

284.5

NEURONS AND MICROGLIA COLLABORATE VIA AN EXTRACELLULAR PROTEOLYTIC CASCADE TO PROMOTE NEURONAL DEGENERATION. S. E. Tsirka*, A. D. Rogove, T. H. Bugge, J. L. Degen and S. Strickland
Department of Pharmacology, Program in Genetics, and MSTP Program, University Medical Center at Stony Brook, Stony Brook, NY 11794-8651, Division of Developmental Biology, Children's Hospital Research Foundation, Cincinnati, OH 45229

Mice lacking the serine protease tissue plasminogen activator (tPA) are resistant to excitotoxin-mediated hippocampal neuronal degeneration. We have used genetic, cellular, and biochemical analyses to study the role of tPA in neuronal cell death. Mice deficient for the zymogen plasminogen, a known substrate for tPA, are also resistant to excitotoxins, implicating an extracellular proteolytic cascade in degeneration. The two components of this cascade, tPA and plasminogen, are both synthesized in the mouse hippocampus. tPA mRNA is found in neurons and microglia, with microglia contributing the majority of enzymatic activity upon injury. In contrast, plasminogen mRNA is found exclusively in neurons, suggesting a collaboration of neighboring cell types in effecting degeneration. Consistent with the model that extracellular proteolysis is required at the time of excitotoxic injury, infusion of plasminogen activator inhibitor-1 into the hippocampus of wild-type mice confers resistance to excitotoxin-induced neuronal degeneration.

This work was supported by fellowships from the International Human Frontier Science Program Organization (S.E.T.), the Danish Medical Research Council (T.H.B.), the MSTP program (A.D.R.), by an Established Investigator Award from the American Heart Association (J.L.D.), and by grants from the NIH (S.S., J.L.D.).

284.6

DIFFERENTIAL SCREENING FOR NMDA-INDUCED GENES IN PRIMARY NEURONAL CULTURES. J.R. Naranjo* and B. Mellström, Cajal Institute, C.S.I.C., Madrid, Spain

Neuronal death produced by over-stimulation of excitatory amino acid receptors is a well established phenomenon. A rapid induction of immediate early genes occurs after receptor stimulation and announce a second wave of changes in gene expression that might direct the final events leading to cell death or survival after excitatory amino acid receptor stimulation. In order to identify those genes we prepared cDNA libraries from control neuronal primary cultures and from cultures four hours after a pulse of NMDA. Here we present data indicating that the gas1 (growth arrest specific gene 1) gene, whose product is known to be a marker of the G_0 phase of the cell cycle, is induced in cultured cortico-hippocampal neurons committed to die after a brief exposure to NMDA. Induction of gas1 also occurred in rat brain areas that show delayed neuronal death after intraperitoneal injection of kainic acid. Blockage of Gas1 function with antisense gas1 oligodeoxynucleotide or by expression of antisense gas1 mRNA in stably transfected NB69 neuroblastoma cells, prevented NMDA-induced excitotoxic neuronal death and reduced staurosporine-induced apoptosis, respectively. In addition, overexpression of Gas1 in NIH3T3 cells produced a marked reduction in the number of viable cells by an apoptotic mechanism. Interestingly, Gas1 function was attenuated by coexpression of the human Bcl-2 protein.

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284.7

GLUTAMATE INDUCED RELEASE OF NEUROTOXIN IN RAT HIPPOCAMPAL NEURONS: K.W.Yoon*, P.T.Shah & M.L.Klein, Dept. of Neurosurgery & Surgical Research Institute, St. Louis University Health Science Center, St. Louis, MO ,63110-0250.

The central neurotoxicity of the excitatory amino acid glutamate may be responsible for neuronal damage associated with certain acute insults including hypoxia-ischemia, epilepsy & trauma.Exposure of dissociated rat hippocampal culture (1-2 week in vitro) to glutamate (500μM ,1min) produced toxicity which could be transferred to a naive non-treated culture After 1 min exposure , glutamate was washed away & the naive culture treated with ECF collected at 5 min from treated culture.This toxicity is only transiently expressed with the maximum effect seen when ECF is transferred within 5 minutes following glutamate insult while no significant toxicity can be demonstrated at 30 minutes. Glutamate concentration in the transferred ECF was measured & found to be < 30nM/ ml at 5 min. NMDA & non-NMDA antagonists MK-801(10μM) & CNQX(30μM) blocked the neuronal cell death when added to the donor culture(90.81±9.76 % reduction in cell death,n=6). However ,MK-801 & CNQX did not block neurotoxicity in the recipient culture indicating involvement of some other neurotoxin.

We have previously shown that traumatized neuronal culture can release a neurotoxin involving cyclooxygenase dependent mechanism.However, Indomethacin(10 & 100μM)had no significant effect on toxicity when added to both donor & recipient culture.Dantrolene(30μM) significantly blocked toxicity when added to the recipient culture(88.23±20.21% reduction in cell death,n=5). Therefore glutamate may propagate neurotoxicity by inducing release of a soluble & transferable toxin. This toxin may be different from the previously described trauma induced toxicity.

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284.8

LASTING SER-73 PHOSPHORYLATION OF c-JUN, SUPPRESSION OF ATF-2 AND ACTIVATION OF c-JUN N-TERMINAL KINASES (SAPK) IN ADULT RAT BRAIN FOLLOWING SEIZURES, ISCHEMIA AND AXOTOMY

T. Herdegen* (1), T. Kallunki (3), A. Martin (2), T. Buschmann (2), M. Karin (3); Univ. Kiel (1) and Univ. Heidelberg (2), Germany; (3) Univ. San Diego, USA.

c-Jun expression is related to cell death in embryonic and to regeneration/survival in adult neurons following neuronal damage. We have visualized the activated form of c-Jun by a specific antibody against the phosphorylated Ser-73 of c-Jun (p-c-Jun), expression of ATF-2 and the activity of their regulating kinases JNKs (SAPKs) by in-gel- and kinase assays. In the untreated rat brain, JNK has a basal activity, but p-c-Jun is not detectable in areas with constitutive c-Jun expression. Following axotomy of retina and substantia nigra, ischemia and seizures p-c-Jun is exclusively present in neurons with c-Jun expression up to 30 days, whereas ATF-2 completely disappears. JNK-2 (less JNK-1) is activated for days in homogenates of axotomized, ischemic or postiktal areas. Induced c-Jun is phosphorylated and ATF-2 is suppressed, too, in dying DRG neurons following UV-irradiation, and both, p-c-Jun and cell death is prevented by the pretreatment of the calcineurin-inhibitor FK-506. These data suggest a novel interplay between JNK, c-Jun phosphorylation and ATF-2 suppression due to neurodegeneration in the adult brain.

284.9

APOPTOTIC DNA FRAGMENTATION IS ASSOCIATED WITH MODULATION OF BCL-2, BCL-X, BAX, AND C-FOS EXPRESSION FOLLOWING FOCAL CEREBRAL ISCHEMIA IN RATS. F. Gillardon, C. Lenz, S. Krajewskit, J. C. Reedt, W. Kuschinsky, and M. Zimmermann*, Physiologisches Institut, Universität Heidelberg, 69120 Heidelberg, Germany, and ‡La Jolla Cancer Research Foundation, La Jolla, CA92037, USA.

Permanent occlusion of the middle cerebral artery (MCA) in rats was used to assess the effects of focal cerebral ischemia on the expression of members of the *bcl-2* gene family which have been implicated in the regulation of programmed cell death. Six hours of intraluminal MCA occlusion lead to ischemic cell damage within the ipsilateral caudate putamen, basolateral cortex, and parts of the thalamus as detected by nitro blue tetrazolium staining of rat brain sections. In the infarcted cortex and thalamus fragmentation of genomic DNA was frequently observed using *in situ* end-labeling of DNA breaks by terminal transferase, whereas only scattered labeled nuclei were visible in the infarcted caudate putamen. Immunohistochemical analysis revealed activation of c-Fos in neurons of the infarcted cortex and thalamus and in the non-infarcted cingulate cortex. A decrease in neuronal immunoreactivity for Bcl-2, and Bcl-X and an increase in immunostaining for Bax was observed within the ischemic cortex and thalamus. Intensity of immunostaining did not change in neurons located at the border (penumbra) of the infarcted brain regions. Within the infarct core (caudate putamen) however, protein levels of all Bcl-2 family members declined and c-Fos remained absent. RT-PCR analysis revealed a pronounced decrease in *bcl-2* mRNA levels in the ischemic hemisphere, whereas the amount of *bax* mRNA was significantly elevated despite an ischemia-induced suppression in total RNA synthesis. Thus, a shift in the ratio of cell death repressors Bcl-2 and Bcl-X_{LONG} to cell death promotor Bax following focal cerebral ischemia may lead to a derepression of the cell death programme. A concomitant sustained activation of c-Fos may contribute to neuronal apoptosis within the ischemic cortex and thalamus.

284.10

BAX IS REQUIRED FOR MOTONEURON DEATH FOLLOWING AXOTOMY AND DURING DEVELOPMENT J.L. Elliott*[1], C.M. Knudson[2], S.J. Korsmeyer[2] and W.D. Snider[1]. CSNSI, Depts. of Neurology[1], and Pathology[2]. Washington University School of Medicine, St. Louis, Mo. 63110.

Members of the bcl-2\ced-9 family, acting as either inhibitors (bcl-2, bcl-xl) or promoters (bcl-xs, bax, bad, bak), have been implicated as important regulators of cell progression along an apoptotic death pathway. Recent investigations have established the importance of bcl-2 and bcl-x in promoting neuronal survival, whereas the important pro-apoptotic bcl-2 family members in vivo are unknown. We have generated mice with targeted disruptions of bax alleles to determine whether bax is critical for programmed neuronal death in vivo.

We have used the paradigm of neonatal facial axotomy to test the role of bax in regulating motoneuron death after target deprivation. The right facial nerve was transacted in post-natal (PN) 1 wild type bax+/+, heterozygous bax+/-, and homozygous bax -/- mice. 7 days later, the animals were sacrificed and motoneuron counts were performed. Both bax +/+ and bax+/- mice demonstrated a similar degree of facial motoneuron survival (14% and 12% respectively) on the axotomized side. In contrast, 86% of facial motoneurons survived axotomy in bax -/- mice. Remarkably, facial motoneurons in bax -/- mice were alive even at 4 weeks (the longest time period assessed) after a PN1 axotomy, although they had become markedly atrophic. Total facial motoneuron counts were performed in bax +/+, bax +/-, and bax -/- mice. Bax -/- mice exhibited a 51% increase in the number of facial motoneurons as compared to wild type mice. Bax +/- mice had a small but significant increase (13%) in facial motoneurons vs.wild type.

The results of this study demonstrate that a single gene product, bax, is necessary for motoneuron death in the setting of trophic factor deprivation. Although motoneurons lacking bax survive neonatal axotomy, even for extended time periods, they become markedly atrophic. Mice deficient in bax also demonstrate an increase in motoneuron number in a gene dose dependent fashion, suggesting bax regulates naturally occurring cell death.

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284.11

BAX IS REQUIRED FOR DEATH OF SYMPATHETIC NEURONS BY TROPHIC FACTOR DEPRIVATION AND DURING DEVELOPMENT. T.L. Deckwerth*‡, C.M. Knudson§, S.J. Korsmeyer§, and E.M. Johnson, Jr.‡ Washington University School of Medicine, Departments of Molecular Biology and Pharmacology‡, Medicine and Pathology§, St. Louis, MO 63110, USA.

Members of the BCL2 family of proteins either promote or repress programmed cell death. Given the existence of multiple death-promoting members, a question exists as to whether a single member can be of paramount importance in controlling apoptosis. We tested whether BAX, a death-promotor, is required for neuronal death by trophic factor deprivation and during development. Wild type neonatal sympathetic neurons depend on nerve growth factor (NGF) for survival and die by apoptosis when deprived of NGF. In contrast, *Bax*-deficient sympathetic neurons showed long-term survival in the absence of NGF. These salvaged neurons displayed remarkable soma atrophy and a reduced elaboration of neurites; yet, they proved capable of responding to re-addition of trophic factor with soma hypertrophy and enhanced neurite outgrowth. Developmental cell death of sympathetic neurons was also diminished in the absence of BAX. These results demonstrate that NGF deprivation-induced death of sympathetic neurons is *Bax*-dependent and that BAX regulates naturally occurring developmental neuronal death. Supported by NIH grants NS24769, AG12947, and CA49712.

DEGENERATIVE DISEASE: OTHER—MOLECULAR BIOLOGY

285.1

ABNORMALLY PHOSPHORYLATED TAU (τ) AND PRION PROTEIN AMYLOIDOSIS. B. Ghetti*[1], P. Piccardo[1], M. R. Farlow[1], S.R. Dlouhy[1], F. Tagliavini[2], G. Giaccone[2], and O. Bugiani[2]. [1]Indiana University School of Medicine, Indianapolis, IN 46202; [2] Istituto Carlo Besta, Milano, Italy.

Prion protein (PrP) amyloid as well as neurofibrillary tangles, neuropil threads, and abnormal neurites are consistently present in Gerstmann-Sträussler-Scheinker (GSS) disease with mutations in the *PRNP* gene at codon 198 (F198S) and 217 (Q217R) as well as in PrP cerebral amyloid angiopathy with mutation at codon 145 (Y145Stop). In these disorders, PrP parenchymal or vascular amyloid deposition is severe in the cerebral and cerebellar cortex. We have analyzed the neurofibrillary changes of GSS disease with mutation at codon 198 (Indiana kindred) using monoclonal antibody AT8 which recognizes abnormally phosphorylated epitope Ser-202/Thr-205 on PHF τ protein. Serial coronal brain slices from five patients of the Indiana kindred were labeled with antibodies AT8 and 3F4. The latter recognizes epitopes corresponding to residues 109-112 of human PrP. AT8 immunoreactivity is strong in the neocortex, caudate nucleus, putamen, claustrum, and amygdala, it is weak in the globus pallidus and thalamus, it is virtually absent in the cerebellum. 3F4 immunopositivity is strong in the neocortex, caudate nucleus, putamen, claustrum, and cerebellum. In the caudate nucleus and putamen, PrP deposition is not associated with severe amyloidosis. Thus, the topography of neurofibrillary changes and PrP deposits overlap in the cerebrum, but not in the cerebellum. Our data indicate that in some variants of hereditary PrP amyloidosis, abnormal phosphorylation of τ occurs in the cerebrum and it may be linked to the presence PrP degradation products. (Supported by PHS R01 NS 29822).

285.2

CHEMICAL CHAPERONES STABILIZE THE NATIVE CONFORMATION OF THE PRION PROTEIN. J.Tatzelt*, S.B. Prusiner, W.J.Welch. University of California, San Francisco, CA 94143-0518.

Prion diseases include kuru, Creutzfeldt-Jakob disease (CJD), Gerstmann-Sträussler-Scheinker syndrome (GSS) and fatal familial insomnia (FFI) of humans, as well as scrapie and bovine spongiform encephalopathy (BSE) of animals. A conformational change in the cellular prion protein (PrP^C) is thought to be a decisive factor in these diseases. Formation of the pathogenic scrapie isoform (PrP^{Sc}) likely involves a transition from the α-helical form PrP^C into one predominantly consisting of β-sheet. Here we show that incubation of scrapie-infected mouse neuroblastoma (ScN2a) cells with various reagents known to stabilize proteins in their native conformation results in a substantial reduction in the rate and extent of PrP^{Sc} formation. Such reagents include the cellular osmolytes glycerol and trimethylamine N-oxide (TMAO), and the organic solvent dimethylsulfoxide (DMSO). These so-called "chemical chaperones" inhibited PrP^{Sc} formation as a function of both dose and time of exposure. Chemical chaperones did not appear to affect the existing population of PrP^{Sc} in ScN2a cells; instead, they interfered with the conversion of newly synthesized PrP^C to PrP^{Sc}. We suspect that the chemical chaperones act to stabilize the α-helical form(s) of either full length PrP^C or degradation intermediates of PrP^C. Stabilization of the α-helical form would reduce or prevent the acquisition of a β-sheet conformation and the subsequent formation of detergent-insoluble PrP aggregates.

285.3

PRIONPROTEIN FRAGMENT INDUCES $[Ca^{2+}]_i$ INCREASE IN MICROGLIA. J. W. Herms*, A. Madlung, D. R. Brown and H. A. Kretzschmar. Department of Neuropathology, University of Göttingen, Robert-Koch-Strasse 40, Göttingen, D-37075, Germany.

Microglia in a reactive state are known to be associated with neurodegenerative changes in prion diseases. Using the synthetic PrP fragment PrP 106-126 , known to be toxic for neurons as a model for investigating neurodegeneration in these diseases, we have previously shown that exposure to PrP 106-126 alters microglial activity and viability. It is thought that PrP 106-126 is toxic to neuronal cultures by inducing increased release of oxygen radicals from microglia (Brown et al. Nature 380:345-347).

To further eludicate how the peptide activates microglia, we investigated the effects of PrP 106-126 on intracellular free Ca^{2+} concentration ($[Ca^{2+}]i$) in cultured, LPS-treated microglia by using Fura-2 imaging. Exposure to 100 µM PrP 106-126 induced a marked increase in $[Ca^{2+}]i$ within 10 ± 6 minutes in the microglia of normal (C57black/SV129) as well as PrP-deficient mice. From basal levels of 52 ± 25 nM in both populations, the increase was significantly lower in PrP-deficient mice with 150 ± 40 nM (n=6) than in control mice (376 ± 190 nM (n=11)). Predominantly these cells showed a transient calcium increase, which decreased to near basal values within 20 minutes after maximal response was observed.

Based on the assumption that microglia activation may be the initial process leading to loss of neurons in prion diseases, our findings demonstrate the earliest reaction of microglia to a PrP-fragment known so far. This finding may help to elucidate the sequence of events leading to neuronal damage in prion diseases. *(Supported by BMBF grant 01KI 9461/8 to HAK)*

285.4

SIMILAR POSTTRANSLATIONAL MODIFICATIONS OF THE PRION PROTEIN IN FAMILIAL, SPORADIC AND IATROGENIC CREUTZFELDT-JAKOB DISEASE (CJD). *P. Parchi, S. Capellari, S.G. Chen, B. Ghetti[1], J. Mikol[2], C. Vital[3], E.J.Cochran[4], J.Q Trojanowski[5], D.W.Dickson[6], R.B. Petersen, P. Gambetti*. Div. Neuropath., CWRU, Cleveland, OH, 44106, Indianapolis, IN[1], Paris[2] and Bordeaux[3], France, Chicago IL[4], Philadelphia, PA[5], Bronx, NY[6].*

Prion diseases are caused by an infectious agent that shows strain variations and is thought to consist solely of the protease-resistant form of the prion protein (PrP^{res}). Recent data suggested that self-propagation of PrP^{res} with distinct conformations could be the molecular basis of prion strains. Previously, we examined PrP^{res} in sporadic CJD and in two familial prion diseases, fatal familial insomnia (FFI) and CJD^{178}. In order to determine the full spectrum of PrP^{res} characteristics in CJD we now analyzed PrP^{res} in other subtypes of familial CJD and in iatrogenic CJD. We examined 5 subjects carrying the E200K mutation, 3 with insertion mutations (96,120,and 144 bp), 1 with the V210I mutation and 1 with iatrogenic CJD (growth hormone), and we compared the data with those obtained from 25 sporadic CJD, 11 FFI and 7 CJD^{178}. Western blot and epitope mapping showed that in all cases PrP^{res} comprised 3 major glycoforms of a N-terminal truncated fragment. Based on the electrophoretic mobility only two sizes of the fragment were observed. PrP^{res} type 1 (size of unglycosylated form~20-21 kDa) was detected in most sporadic CJD, in 2 insertional mutations and in the E200K, D178N-129V, V210I mutations, whereas PrP^{res} type 2 (size of unglycosylated form~18-19 kDa) was found in atypical sporadic CJD, in the 120 bp insertion, in the subject with iatrogenic CJD and in FFI. Within each of the two groups, the ratio of the 3 PrP^{res} glycoforms did not significantly differ between sporadic, iatrogenic and familial CJD except for the E200K and D178N mutations which showed an underrepresentation of the unglycosylated form. These data provide indirect evidence for the existence of two major prion strains in humans causing CJD. Supported by AG08012, AG08155, AG08992 and the Britton Fund.

285.5

E200K MUTATION: EFFECT ON PRION PROTEIN

S.Capellari, P.Parchi, C.B.Urig, S.L.Richardson, C.Russo, P.Gambetti and R.B.Petersen Institute of Pathology,* Case Western Reserve University, Cleveland, OH, 44106

Prion diseases are neurodegenerative illnesses that can be sporadic, infective or inherited. On the basis of their clinical and neuropathological manifestations they are divided in three main groups: Creutzfeld-Jakob disease (CJD), Gerstmann-Sträussler Scheinker and Fatal Familial Insomnia. They are all characterized by the presence, in the CNS, of a protease resistant isoform that derives from a normal protein referred to as the cellular prion protein (PrP^c). Inherited prion diseases are autosomal dominant disorders caused by mutations in the prion protein gene.

A replacement of Lys for Glu at residue 200 (E200K) in PrP^c is the most common cause of inherited CJD. To understand the effect of this mutation on PrP^c synthesis and processing, we transfected human neuroblastoma cells with the normal or the E200K mutated human PrP coding sequence. Both mutant PrP (PrP^M) and PrP^c are detectable in the cell lysate as well as on the cell surface and migrate in gels as three major isoforms: unglycosylated, monoglycosylated, and fully (di) glycosylated. The E200K mutation has a profound effect on the unglycosylated isoform which shows: 1. an aberrant electrophoretic mobility, 2. decreased solubility in detergents, 3. lower quantity at the cell surface, and 4. decreased susceptibility to PIPLC cleavage. Glycosylation partially corrects these changes. The E200K results in the slight increase of a 12kDa aminoterminal truncated fragment. The mutation also affects glycan processing: the fully glycosylated PrP^M migrates faster and appears to be more heterogeneous than PrP^c due to differences in the complex glycans. Changes similar to those of the mutated cells are observed in brains from affected subjects with the E200K mutation. The accumulation of the 12 kDa PrP peptide in these brains is especially striking. These findings confirm the validity of our cell model.

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285.6

IDENTIFICATION OF A CYCLIN-DEPENDENT KINASE-5 BINDING PROTEIN USING THE YEAST TWO-HYBRID SYSTEM. S. Guidato*, D. M. McLoughlin, J.-P. Brion* and C. C. J. Miller. Departments of Neuroscience and Neurology, The Institute of Psychiatry, De Crespigny Park, Denmark Hill, London SE5 8AF, UK. * Laboratory of Pathology and Electron Microscopy, Université Libre de Bruxelles, 808 route de Lennik, Bldg C-10, 1070 - Brussels, BELGIUM.

Cdk5 is a neuronal cdc2-like kinase that display neuronal specific activity. In common with other members of the cdk family, cdk-5 requires association with another protein (activator) for activity. Known activators of cdk5 are p35 and a homologue p39. A further protein, p67 has also been shown to modulate cdk-5 activity. In order to understand further the mechanisms regulating cdk-5 activity, we have attempted to identify proteins that interact with cdk-5. We utilised the yeast two-hybrid system with cdk-5 as a "bait" to screen a human brain library for putative regulators of cdk-5. One such cdk-5 interacting protein was identified. Immunoprecipitation experiments confirmed that cdk-5 interacts with this protein in co-transfected COS cells. Studies on the *in vivo* expression of this cdk-5 binding protein and its effect on cdk-5 activity are currently underway. Cdk-5 is a kinase known to phosphorylate both neurofilaments and tau; abberant phosphorylation of these proteins is seen in some neurodegenerative diseases such as Alzheimer's disease, motor neurone disease and Lewy body pathologies. Understanding the mechanisms that regulate cdk-5 activity therefore has relevance to these neurodegenerative diseases.

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285.7

THE MACHADO-JOSEPH DISEASE GENE PRODUCT MJD1 IS A CYTOPLASMIC PROTEIN EXPRESSED WIDELY IN THE CENTRAL NERVOUS SYSTEM. H.Paulson, S.Das, P.Crino, M.Perez, S.Patel, K.Fischbeck and R.Pittman*. Depts. of Neurology and Pharmacology, Univ. of Penn., Philadelphia PA 19104

Machado-Joseph disease is one of at least five neurodegenerative triplet repeat diseases caused by an expanded CAG/polyglutamine tract within the protein coding region of the disease gene. Using RT-PCR, we isolated multiple splice variants of the *MJD1* gene from patient lymphoblastoid cells and normal human brain. A nearly full length splice variant was used to create a fusion protein, against which antiserum was generated. On Western blot, the antiserum specifically recognized normal and expanded MJD1 protein from patient lymphoblastoid cells and from transfected cells. In nonneuronal tissues, several other species were detected which may correspond to splice variants. In contrast, in human brain the antiserum detected a single major species, a doublet corresponding to full length MJD1, that was present in all regions examined by Western blot (cortex, caudate/putamen, pons and cerebellum). Immunohistochemistry demonstrated cytoplasmic staining in select populations of neurons thoughout the brain, including anterior horn cells in the spinal cord, Purkinje cell and granule cell layers in the cerebellum, and infrequent neurons within the caudate and putamen. Immunofluorescence of a motor neuron-hybrid cell line transfected with MJD1 cDNA confirmed a cytoplasmic localization. Further studies with this antiserum may help elucidate the function of MJD1. Funded in part by postdoctoral grants from the Howard Hughes Medical Institute and the American Academy of Neurology.

285.8

LOSS OF NEURONS AND SYNAPSES IN THE BASAL GANGLIA OF MICE WITH TARGETED DISRUPTION OF THE HUNTINGTON'S DISEASE GENE. J.R. O'Kusky[1]*, J. Nasir[2] and M.R. Hayden[2]. Departments of [1]Pathology and Laboratory Medicine and [2]Medical Genetics, University of British Columbia, Vancouver, B.C., Canada V5Z 1M9.

We have created a targeted disruption in exon 5 of Hdh (Hdh^{ex5}), the murine homologue of the HD gene. Morphometric analyses were performed in light and electron microscopy to determine the numerical density and total number of neurons in the caudate-putamen, globus pallidus, subthalamic nucleus and substantia nigra of 7 Hdh^{ex5} heterozygotes and 7 normal littermates at 4 months of age. In Hdh^{ex5} heterozygotes there was a significant decrease in the total number of neurons in the globus pallidus (29%, $p< 0.001$) and subthalamic nucleus (51%, $p< 0.001$). Total glial cell density was significantly increased in both regions (18-20%, $p<0.02$), although there was no hypertrophy of astrocyte processes by electron microscopy to indicate active gliosis. In the subthalamic nucleus there was a significant decrease in the density of symmetric synapses (45%, $p< 0.01$) with no significant difference in the density of asymmetric synapses, suggesting a relatively selective loss of pallido-subthalamic afferents. Sporadic degenerating axon profiles were observed by electron microscopy in the globus pallidus and subthalamic nucleus in approximately two-thirds of the Hdh^{ex5} heterozygotes, but in none of the control mice. These results indicate that the Hdh gene plays a role in the normal development of the murine basal ganglia. Furthermore, the data suggest that disinhibition of the subthalamic nucleus through loss of pallido-subthalamic afferents contributes to the motor deficits previously documented in these mice.

285.9

DECREASED THYROTROPIN RELEASING HORMONE (TRH) RECEPTOR mRNA IN SPINAL MOTONEURONS OF WOBBLER MOUSE EARLY IN MOTONEURON DISEASE IS FOLLOWED BY A LATER INCREASE, L.L.Vacca-Galloway* and S.H. Zhang. Dept. of Anatomy, Univ. of Hong Kong, Hong Kong.

In situ hybridization, applied to spinal cord frozen sections (after anesthesia and perfusion) from old (age 3-4 mo) and young (age 3 wk) Wobbler and control littermate mice, shows lower numbers of TRH receptor (r) mRNA ventral horn neurons (VHNs) in Wobbler specimens. Lower amounts of TRHr mRNA are detected by photometry in young Wobbler specimens. Prior autoradiography shows a lower TRHr level in Wobbler VH compared with controls. In old Wobblers amounts of TRHr mRNA are greater in normal-looking VHNs compared with controls, whereas detection of TRHr by autoradiography shows lower levels. The increase of TRHr mRNA may result from VHN shrinkage known to occur in old Wobblers; decrease in young Wobblers may be due to VHN swelling. Alternatively translation is faulty. Vacuolated VHNs also produce TRHr mRNA. Early decrease of TRHr message in VHNs correlates with early sprouting of nearby TRH processes; later (secondary?) increase with later sprouting. Funded by Croucher Foundation (360/031/0814) and Research Grants Council (338/031/0010).

285.10

THE TRANSGENIC SOD-1 MOUSE MODEL OF ALS: SELECTIVE OXIDATIVE STRESS IN MOTOR NEURONS Smith M.A., Dal Canto M., Richey P.L. and Perry G.* Institute of Pathology, Case Western Reserve University, 2085 Adelbert Road, Cleveland, Ohio 44106 USA

Genetic evidence linking a small number of genetically-based amyotrophic lateral sclerosis (ALS) cases to an alteration in the antioxidant enzyme Cu/Zn superoxide dismutase (SOD-1) suggests at the minimum that oxidative damages can lead to ALS. Such a notion is supported by the development of a mouse transgenic for mutant SOD-1 that rapidly develops many of the clinical and pathological correlates of ALS.

Over the past two years our laboratory has become increasingly focussed on the role of oxidative protein modifications in age-related neurodegenerative diseases. Here, we present evidence for oxidative modifications in motor neurons in ALS and transgenic mice overexpressing mutant SOD-1. Using our recently developed *in situ* hydrazine technique we localized free carbonyl groups, as a measure of oxidative damage, in motor neurons and axons in the transgenic and control mice. Further, there was a striking increase in the level of heme oxygenase-1 (HO-1) in the transgenic mice that was limited to motor neurons showing vacuolation and neurofibroblast accumulation. Since HO-1 catalyses the rate limiting step converting heme, a prodxidant, to bilirubin, an antioxidant, HO-1 induction indicates oxidative stress for the degenerating neurons resulting from mutant SOD-1 expression.

These data suggests a link between oxidative stress and the ALS phenotype of cytoskeletal disruption and motor neuron death. Understanding the types and extent of oxidative damage in ALS will provide new insight as to how one could prevent neuron damage by providing a rational therapeutic strategy that is likely to include antioxidants and free radical or carbonyl scavengers.

Supported by grants from the NIH, AHAF, and AFAR.

285.11

GAIN-OF-FUNCTION NEUROTOXIC EFFECTS OF MUTANT SOD-1 PROTEINS IN A PRIMARY CULTURE MODEL OF AMYOTROPHIC LATERAL SCLEROSIS. H.D. Durham[1]*, J. Roy[1], L. Dong[2], and D.A. Figlewicz[2,3], [1]Montreal Neurological Institute, McGill University, Montreal, Canada H3A 2B4; [2]Dept. of Neurology and [3]Dept. of Neurobiology & Anatomy, Univ. of Rochester Medical Center, Rochester NY 14642.

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disorder characterized by loss of cortical, brainstem, and spinal motor neurons. Mutations in the CuZn superoxide dismutase (SOD-1) gene underlie some familial cases of ALS (FALS). To examine the mechanism of neuronal death in SOD-1 FALS, 4 different SOD-1 cDNAs bearing mutations found in FALS patients (*mSOD*), or wild type human SOD-1 cDNA (*SOD^{wt}*), were subcloned into an expression vector. cDNA constructs were microinjected into nuclei of motor neurons in primary cultures of murine spinal cord, achieving expression of human SOD-1 protein in >80% of injected cells. Many features of motor neuron disease seen in human ALS and in mSOD transgenic mice were reproduced within 2 weeks, including hyperphosphorylation and bundling of neurofilaments, appearance of small vacuoles and inclusions, and cell death with evidence of apoptosis. Approximately 80% of spinal motor neurons injected with mSOD constructs died by postinjection day 12, compared to less than 20% injected with *SOD^{wt}* or vector. mSOD was not lethal to dorsal root ganglion neurons over the same time period, indicating differential susceptibility of neurons in culture to toxicity of mSOD. A novel observation was the abnormal aggregation of mSOD protein in up to 50% of mSOD-expressing motor neurons. The aggregates, never observed in motor neurons expressing *SOD^{wt}* enzyme, were not located in lysosomes or peroxysomes, suggesting they were cytosolic. It is concluded that mutations in different regions of the SOD-1 gene predispose the protein to intracellular aggregation which contributes to the toxicity of mSOD in motor neurons. [supported by MDA-Canada, MDA-USA, and NIHRO1 NS34101-01]

CALCIUM CHANNELS: PHYSIOLOGY, PHARMACOLOGY, AND MODULATION III

286.1

RAPID MODULATION OF N- AND L-CALCIUM CHANNELS IN CEREBELLAR GRANULE NEURONS BY INSULIN-LIKE GROWTH FACTOR-1 IS DEPENDENT ON TK ACTIVATION OF PI 3-KINASE. L.A.C. Blair* and J. Marshall, Pharmacology, Yale University, New Haven, CT, and Molecular Pharmacology and Biotechnology, Brown University, Providence, RI.

IGF-1 rapidly increased the activity of a subset of voltage-activated Ca channels in cultured cerebellar granule cells. Following superfusion of low, 20 ng/ml, concentrations of IGF-1, Ca channel currents carried by 20 mM barium increased ~2-fold in 10sec; no cells responded to addition of vehicle saline alone. The increase in activity was specifically mediated by IGF-1 receptors. Insulin produced only modest responses at 20,000 ng/ml, a concentration that can activate IGF-1 receptors. Additionally, the tyrosine kinase (TK) inhibitor Lavendustin A (1μM) fully blocked the IGF-1-induced increase, while its inactive analog Lavendustin B had no effect. IGF-1-increases in Ca channel activity were also dependent on activation of phosphatidylinositol 3-OH kinase (PI 3-kinase). Responses were inhibited by pretreatment with the membrane permeable PI 3-kinase inhibitors wortmannin (100nM) and LY294002 (10-50μM); vehicle additions were without effect. Similarly, PI 3-kinase activity assayed in vitro increased >5-fold after 10 sec exposure to 20 ng/ml IGF-1, but failed to increase if cells had been pretreated with Lavendustin A, wortmannin or LY294002; Lavendustin B was without affect. Finally, IGF-1 apparently modulated only a subset of Ca channels. The entire IGF-1-dependent increase in Ca channel activity could be accounted for by ω-CgTx GVIA- and DHP-sensitive channels. Furthermore, each Ca channel subtype appeared to be modulated within a specific voltage range, suggesting both altered voltage-dependence and, as opposed to insertion of new channels, modification of existing active channels.

286.2

LOW- AND HIGH-VOLTAGE-ACTIVATED Ca^{2+} CURRENTS IN GUINEA PIG PIRIFORM CORTEX NEURONS. L. Magistretti* and M. de Curtis, Dept. of Exper. Neurophysiology, Istituto Nazionale Neurologico "C. Besta", Milan, Italy.

Olfactory cortex is known to be organized in four layers, each with different cell composition. In layer II only pyramidal and semilunar neurons are found at high density. In layer III both pyramidal and multipolar neurons are present at lower density, whereas layer IV (or endopiriform nucleus) contains essentially only multipolar neurons. These different neuron types have received little attention as to their intrinsic membrane properties. Calcium (Ca^{2+}) conductances have been proposed to play a crucial role in shaping delayed EPSPs and in promoting epileptiform potentials in the piriform cortex. Here we characterize the voltage-activated Ca^{2+} currents of piriform-cortex layer II neurons. The study will be extended to layer III and IV neurons.

The anterior piriform cortex of 15 to 30-day-old guinea pigs was cut into 500 μm-thick coronal slices and layer II was dissected out under microscopic control. Neurons were acutely isolated with enzymatic treatement (1 mg/ml pronase, 15 min at 34°C) followed by gentle mechanical dissociation. Voltage-clamp recordings were performed with the whole-cell mode of the patch-clamp technique. Voltage-activated Ca^{2+} currents, isolated by opportune intra- and extracellular blockers and ionic substitutes, were recorded in 55 cells. High-voltage-activated (HVA) Ca^{2+} currents were present in all cells, and could be evoked with test pulses (tp) positive to -40/-30 mV from a holding potential (hp) of -50 mV. With hp of -100 mV, a sizeable low-voltage-activated (LVA) current could be evoked in 30% of the cells with tp positive to -70 mV. LVA current i) could be isolated either by subtraction or pharmacologically; ii) decayed monoexponentially with τ of ca. 25 ms at -30 mV; iii) was fully steady-state inactivated at -60 mV and fully reprimed at -105 mV. LVA current density was 7.3 ± 3.4 pA/pF at -30 mV. LVA current detection did not depend on the amount of available cell membrane, since membrane capacitance was 10.3 ± 3.5 in the group of cells displaying LVA current and 11.5± 2.6 in the other group. These data suggest that two neuronal sub-populations exist in layer II piriform cortex as to the expression of LVA currents.

286.3

PROTEIN KINASE C REGULATES CALCIUM CHANNELS LOCALIZED TO A VESICULAR POOL IN *APLYSIA* BAG CELL NEURONS.

B. H. White*[1], T. A. Nick[2], T. J. Carew[2], and L. K. Kaczmarek[1]. Dept.s of Pharmacology[1] and Psychology[2], Yale University, New Haven, CT 06520

Cultured bag cell neurons from adult *Aplysia* express two types of calcium channels, one of which (BCCa-I) resides in vesicles distributed primarily to somata and growth cones [White and Kaczmarek (1996) *Soc. Neurosci. Abstr.*: 508.5]. We have analysed the expression of this channel in the bag cell neurons of juvenile *Aplysia* (5-10 g) using channel-specific antibodies and find that vesicular expression of BCCa-I occurs in juvenile bag cell neurons but is chiefly restricted to cell somata. Further, the percentage of cultured bag cell neurons displaying a vesicular distribution of BCCa-I ranged from 4% to over 80% in juvenile animals, raising the possibility that juvenile calcium current magnitudes may also vary.

Bag cell neurons have two calcium conductances, one of which is observed only after stimulation of protein kinase C (PKC). Simultaneous sampling of calcium currents in bag cell neurons from different juvenile animals by whole-cell patch clamp techniques showed that the percentage of cells containing BCCa-I vesicles was significantly correlated with the magnitude of the PKC-sensitive calcium current ($p=0.02$), but not the PKC-insensitive current. These results strongly suggest that BCCa-I channels carry the PKC-sensitive calcium current in bag cell neurons. They are consistent with a model in which the PKC-recruited channels are translocated from the vesicular pool to the plasma membrane.

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286.4

THE p38 MAP KINASE PATHWAY MEDIATES INHIBITION OF NEURONAL CALCIUM CURRENT BY BRADYKININ.

M.A. Wilk-Blaszczak* and F. Belardetti. Dept. Pharmacology, UT Southwestern, Dallas, TX 75235.

In NG108-15 cells, bradykinin (BK) produces slow inhibition of the voltage-dependent Ca^{2+} current ($I_{Ca,V}$) and activation of a voltage-independent K^+ current. We have previously determined that these effects are mediated by two G proteins, G_{13} and $G_{q/11}$, respectively (*Neuron*, 12, 109; 13, 1215, 1994). We have now investigated whether the effect of G_{13} on $I_{Ca,V}$ is mediated by a member of the MAPK (mitogen-activated protein kinase) family. Using whole-cell patch-clamp recording (10 mM EGTA in pipette), we find that blockade of the classical ERK (extracellular signal-regulated kinase) pathway with PD98059 (20 μM in pipette, n=6) did not depress the inhibition of $I_{Ca,V}$ produced by BK (or by Leu-Enk, which is mediated by G_{oA}). In contrast, inclusion in the pipette of SB203580 (20 μM; n=11), an inhibitor of p38 MAPK, but not of SKF106978 (an inactive analog; 20 μM, n=11), blocked inhibition of $I_{Ca,V}$ by BK (but not by Leu-Enk). Application of SB203580 did not block activation of the K^+ current by BK (n=6). The p38 MAPK pathway may be important in mediating slow inhibition of $I_{Ca,V}$ by neurotransmitters. Supp. in part by NIH GM47721

286.5

CALCIUM-DYNAMICS IN VISUAL INTERNEURONS AFTER PHARMACOLOGICAL STIMULATION IN VITRO

T.G.Oertner*, T.M.Brotz and A.Borst Friedrich-Miescher-Laboratory of the Max-Planck-Society, Spemannstr.37, D-72076 Tübingen, Germany.

Intracellular calcium is thought to play a significant role in dendritic processing of synaptic signals. Using the large motion sensitive tangential cells in the lobula plate of the blowfly (*Calliphora erythrocephala*) as our model system, we are interested in the functional role previously described calcium transients play for dendritic information processing in these cells (Borst, A. & Egelhaaf, M. *PNAS* 89, 4139, 1992, Egelhaaf, M. & Borst, A. *J. Neurophysiol.* 73, 2540, 1995). A pharmacon blocking specifically dendritic calcium channels would be an important tool in this analysis. A recently developed *in vitro* preparation of the fly brain allows for the study of calcium dynamics in visual interneurons of the blowfly under defined conditions (Brotz, T. M., Egelhaaf, M. & Borst, A. *J. Neurosci. Meth.* 57, 37-46, 1995). After injecting the cells with the calcium-sensitive dye fura-2™ we stimulate the cells iontophoretically with the cholinergic agonist carbachol. The spatio-temporal changes in relative fluorescence (up to -30 %) representing the intracellular Ca^{2+}-concentration are optically monitored simultaneously with intracellular recordings of the membrane potential. The onset of the calcium transient in the dendritic region next to the stimulus electrode, the soma, and the axon terminal is correlated with the electrical signal while a slower wave of increasing calcium concentration spreads along the dendrite with the diffusion of carbachol in the tissue. From that we conclude that the cell is equipped with different types of voltage-gated calcium channels: Calcium channels with a low activation threshold which are concentrated at the soma and the axon terminal, and high threshold Ca^{2+}-channels in the dendrite. The calcium influx can be blocked by Co^{2+} ions (2mM) or by removing extracellular calcium. More specific blocking agents are currently under evaluation to characterize the pharmacology of Ca^{2+}-channels in blowfly tangential cells.

This work was supported by the Max-Planck-Society.

286.6

CONDUCTANCE AND PHARMACOLOGICAL PROPERTIES OF 2 CALCIUM CHANNELS IN PHYSIOLOGICAL LEVELS OF EXTERNAL CALCIUM.

P. J. Church* and E.F. Stanley SMS, NINDS, NIH, Bethesda, MD 20892

Due to the relatively low conductance of Ca^{2+} through HVA calcium channels little is known about the transport rates of single calcium channels in the physiologically relevant 1-4mM Ca^{2+}_{ext} range. To examine single channel currents in this range, we recorded from dissociated chick ciliary ganglion neurons using the on-cell patch clamp technique with low noise, quartz micropipettes.

An L-type calcium channel current was identified based on voltage dependence of activation and inactivation, sensitivity to the dihydropyridines S(-)-BayK8644 and Nifedipine, insensitivity to ω-conotoxin GVIA and a unitary conductance of 26pS in 110mM Ba^{2+}. This channel had a unitary conductance of 8.8pS in 110mM Ca^{2+} and 2.4pS in 2mM Ca^{2+}. A second current fluctuation was present in Ca^{2+}, but not Ba^{2+}. This current had a voltage dependence similar to the L-type, but was insensitive to S(-)-BayK8644, Nifedipine, and ω-conotoxin GVIA and had a unitary conductance of 12pS in 110mM Ca^{2+} and 2.4pS in 2mM Ca^{2+}.

Fitting the concentration dependence of both conductances with a Langmuir isotherm gave dissociation constant (K_D) estimates of approximately 5mM. Thus, Ca^{2+} transport through these channels is particularly sensitive to changes in external Ca^{2+} concentrations in the physiological range and approaches saturation at about 10mM. Supported by the NINDS, NIH, Intramural Research Program.

286.7

COMPARISON OF THE ATP MEDIATED INHIBITION OF N- AND P/Q-TYPE CALCIUM CURRENTS IN BOVINE ADRENAL CHROMAFFIN CELLS.

K.P.M. Currie* and A.P. Fox. Dept Pharm. & Physiol. Sci., Univ of Chicago, Chicago IL 60637.

N- and P/Q-type calcium channel currents (I_{Ca}) were recorded from adrenal chromaffin cells in the presence of nisoldipine to block any L-type I_{Ca}. The remaining I_{Ca}, isolated using selective toxins, was 48 ± 2% N-type and 45 ± 2% P/Q-type I_{Ca} (n = 21). Extracellular ATP, acting through P_{2Y} purinoceptors, inhibited I_{Ca} in a pertussis toxin sensitive manner typical of many neurotransmitters. However, the N- and P/Q-type currents were differentially targeted. Peak N-type I_{Ca} was inhibited by 67 ± 2% and P/Q-type current by 38 ± 2% (n = 24; $p < 10^{-6}$). Inhibition of I_{Ca} by neurotransmitters is comprised of a voltage insensitive component and a voltage sensitive component which can be relieved by depolarizing pre-pulses to very positive potentials. The P/Q-type current, in addition to being inhibited to a lesser extent, exhibited a smaller proportion of inhibition that was voltage sensitive. The voltage sensitive pathway suppressed the peak N-type current by 46 ± 2% compared to the P/Q-type current which was inhibited by 19 ± 2% (n = 17; $p < 10^{-6}$). In contrast the voltage insensitive pathway inhibited both current components to a similar degree (22 ± 2% and 18 ± 1% (n = 17) for the N- and P/Q currents respectively). The voltage sensitive inhibition was investigated further and it was found that the pre-pulse voltage required to give half maximal relief of the inhibition was shifted about -15 mV (to +42 mV) for the P/Q-type current relative to the N-type current (n = 4). The duration of the pre-pulse and the time between pre- and test pulse were also varied to determine rates of unblock and reblock of the channels respectively. Preliminary data suggested only small differences in these parameters for the two current components. N- and P/Q-type I_{Ca} are both involved in transmitter release and are targets for inhibitory neuromodulation. Here we show that although the mechanism of inhibition is qualitatively similar for the two current types there are subtle differences, especially in the voltage sensitive inhibitory pathway.

This work was supported by NIH

286.8

EFFECTS OF VOLTAGE AND NOREPINEPHRINE ON SINGLE N-CHANNEL GATING IN FROG SYMPATHETIC NEURONS.

Hye Kyung Lee & Keith S. Elmslie.* Department of Physiology, Tulane University Medical School, New Orleans, LA 70112.

N-type calcium current is steeply voltage dependent with minimal to maximal activation occurring over a range of ~40 mV. Norepinephrine (NE) shifts activation to higher voltages and increases the voltage range required to reach full activation. We recorded from single N-channels to understand the voltage dependence of channel gating and how gating is altered by NE.

Single N-channel events were measured after filtering at 1 kHz using 50% threshold detection. P_o increased from 0.1 at +20 mV to 0.8 at +50 mV. This increase in P_o was due in part to changes in mean open time (τ_o), which increased 3 fold from +10 mV to +40 mV (n = 4). This was unexpected since τ_o has been shown to be voltage-independent for many channels.

Addition of 100 μM NE to the pipet solution induced a voltage-dependent inhibition of single N-channels. Ensemble currents showed a slow component to activation and current amplitude was facilitated following strong depolarization (+70 mV). Facilitation was due to an increase of P_o, which resulted in part from a decrease in the latency to first opening. However, a change in τ_o also appeared to contribute to the increase in P_o. τ_o measured at +40 mV was 1.9 ms in control patches (n=5), but decreased to 1.3 ms in NE patches (n=3). Strong depolarization returned τ_o to control values (1.8 ms, n=3). We are investigating the decrease in τ_o to determine if it is consistent with predictions of the 'willing-reluctant' model of N-channel gating.

Supported by LEQSF, NIH & PhRMA.

286.9

BIOPHYSICAL AND PHARMACOLOGICAL PROPERTIES OF Q-TYPE Ca^{2+} CHANNEL CURRENTS IN RAT NEUROHYPOPHYSIAL TERMINALS. G. Wang & J. Lemos, Worcester Foundation for Biomedical Research, Shrewsbury, MA 01545

Voltage-dependent Ca^{2+} channels play an essential role in depolarization-secretion coupling at nerve terminals. We have previously reported the co-existence of three (*L, N & Q*) types of Ca^{2+} channel currents in the isolated terminals of the rat neurohypophysis and that all three are important for vasopressin release (Wang et al., 1994, Neurosci. Abs. 20: 629). The present study focuses on characterizing the *Q*-type Ca^{2+} channel current using the whole-terminal, perforated patch-clamp method. The whole-terminal *Q*-type Ba^{2+} current is high-voltage-threshold (-10 mV) activated and transient. Its inactivation time constant is 22.7 ms, *vs.* 30.7 ms for the *N*- and 172.6 ms for the *L*-type currents. The *N*- and *Q*-type Ba^{2+} currents have very similar steady-state inactivation curves, with initiation of inactivation at -80 mV and complete inactivation at -60 mV, both of which are much more negative than that for the *L*-type current. Pharmacologically, the *L*-type current is much more sensitive to Cd^{2+} and to dihydropyridines than is the *N* or *Q*. In terms of the sensitivity of the currents to ω-conotoxins GVIA/MVIIC and to Aga IVA, the nerve terminals can be divided into two groups. One group exhibited three approximately equivalent Ba^{2+} currents: one highly sensitive to nicardipine, one to GVIA, and the other to MVIIC/Aga IVA. However, the other group demonstrated, in addition to a similar proportion of nicardipine-sensitive Ba^{2+} current, a much larger proportion of GVIA-sensitive but a relatively smaller proportion of MVIIC-/Aga IVA-sensitive Ba^{2+} current. Binding rate constants for MVIIC to the *Q*-type Ca^{2+} channel is $1.7 \times 10^5\ M^{-1}\ s^{-1}$ *vs.* $2.5 \times 10^5\ M^{-1}\ s^{-1}$ to the *N*-type Ca^{2+} channel. These results suggest that (1) *N*- and *Q*-type Ca^{2+} channel currents in the neurohypophysial terminals have similar gating kinetics but different pharmacological sensitivity; (2) there is heterogeneity in the *N*- and *Q*-type Ca^{2+} channel densities among the neurohypophysial terminals. This heterogeneity of channel types might be due to differential localization in vasopressin- *vs.* oxytocin-releasing terminals. (Supported by NIH grant NS29470)

286.10

β-adrenergic receptor-mediated inhibition of a long-lasting form of L-type calcium channel facilitation in rat hippocampal neurons. R.K. Clouses* and N.V. Marrion. Vollum Institute, OHSU, Portland, OR 97201.

We have characterized a novel, long-lasting form of prepulse facilitation exhibited by L-type Ca channels and its modulation by β-adrenergic receptor activation in acutely dissociated hippocampal CA1 neurons. Single channels, sensitive to nimodipine (1 μM), were recorded from cell-attached patches using low concentrations of Ba or Ca (5-10 mM) as the charge carrier, in the absence of dihydropyridine agonists. Following a depolarizing prepulse to +40 mV (50-200 ms), a large increase in sustained channel activity at membrane potentials between -60 and -20 mV was observed. We have called this behavior "delayed facilitation" because it typically began several hundred milliseconds after the prepulse and decayed over several seconds. Prepulses caused a mean seven-fold increase in open probability measured over a six second time segment by increasing both the channel opening frequency and the proportion of longer duration openings (τ = 3.0 ms). Delayed facilitation was inhibited by the β-adrenergic receptor agonist, isoproterenol (1 μM). Isoproterenol reduced the channel opening frequency and the proportion of longer openings to control levels. Control sustained activity was not affected nor was standard activation of L-type Ca channels, measured during 150 ms depolarizing steps to 0 mV. Isoproterenol-induced inhibition of delayed facilitation was reversed by the β-adrenergic receptor antagonist, propanolol (10 μM), and mimicked by application of 8-CPT cAMP, a membrane permeable analog of cAMP. Thus, an increase in cAMP mediated by activation of β-adrenergic receptors selectively inhibits delayed facilitation of L-type Ca channels in the hippocampus.
(Supported by NIH grant 29806)

286.11

INHIBITION OF NEURONAL APOPTOSIS BY A CHARGED 1,4-DIHYDROPYRIDINE L-TYPE CALCIUM CHANNEL BLOCKER. R.P. Mason*, P. Leeds†, M.F. Walter, C. Hought, K.-G. Zhangt, P.E. Mason, and D.-M. Chuangt. Neurosciences Research Center, Medical College of Pennsylvania & Hahnemann University, Allegheny Campus, Pittsburgh, PA 15212 and †National Institute of Mental Health, NIH, Bethesda, MD 20892.

Amlodipine (AML) is a charged L-type Ca^{+2} channel blocker widely used in the treatment of cardiovascular disorders. Increases in intracellular Ca^{+2} levels that have been observed in association with apoptosis may be attenuated by Ca^{+2} channel blockers. In this study, the neuroprotective effect of AML was examined using an age-induced apoptosis model (J. Neurochem. 66:928;1996) consisting of a primary culture of rat cerebellar granule cells (CGC). AML significantly delayed age-induced apoptosis of CGC beyond the normal average of 17 days *in vitro* (DIV). The neuroprotective effect of AML was biphasic with a maximum at 1-10 nM; increasing doses resulted in a loss of neuroprotection. The observed neuroprotection was most effective when AML was repeatedly administered after 6 DIV. Other L-type Ca^{+2} channel blockers were also neuroprotective with a rank order of potency of AML≥nimodipine>nifedipine>> inactive nifedipine metabolite. Within the effective concentration range, neither AML nor nimodipine significantly affected the increase in intracellular Ca^{+2} levels triggered by NMDA receptor activation which is involved in age-induced apoptosis. However, AML (1-1000 nM) attenuated in a dose-dependent manner the intracellular calcium increases elicited by KCl depolarization. Moreover, AML exhibited dose-dependent antioxidant activity at nanomolar concentrations in reconstituted liposomes. The results of these studies suggest that L-type Ca^{+2} channel blockade and antioxidation mechanisms contribute to the potent neuroprotective effects of AML. These findings have therapeutic implications for the use of AML in neurodegenerative disorders.

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286.12

AMYOTROPHIC LATERAL SCLEROSIS (ALS) IgGs INCREASE K^+-EVOKED [^{3}H]NORADRENALINE RELEASE FROM RAT CEREBRAL CORTEX SYNAPTOSOMES C. Grassi*°, M. Martire°, C. Cannizzaro°, M. Lo Monaco°, E. Carbone^ and G.B. Azzena° Institutes of *Human Physiology, °Pharmacology and *Neurology, Catholic University "S. Cuore", Rome, ^Dept. of Neuroscience, University of Turin, Italy.

ALS is a neurodegenerative disease characterized by spinal cord and cortical motoneuron death. Experimental evidence suggests that neuronal vulnerability may be related to increased levels of intracellular Ca^{2+} and that autoantibody interaction with voltage-operated Ca^{2+}-channels (VOOCs) may be involved in the pathogenesis of sporadic ALS. Aim of the present study was to verify whether ALS IgGs can modify noradrenaline (NA) release from rat brain synaptosomes by affecting calcium influx through VOOCs. Cortical synaptosomes were incubated at 37 °C with 0.05 μM [^{3}H]NA for 15 min and then perfused with medium containing IgGs (0.1-0.2 mg/ml), purified from sera of either control subjects or ALS patients. Synaptosomes were depolarized with 9 mM KCl for 90 s, fractions were collected every minute and the areas under the time course release curves were measured. ALS IgG treatment (0.2 mg/ml) enhanced NA release from 8.9±0.4 to 12.3±0.7 (means of the areas ± S.E.M., n=6), with a mean increase of 38.2%. This effect was dose-dependent, the maximum increase induced by 0.1 mg/ml IgGs being 11.4%. Pre-incubation of synaptosomes with ALS IgGs produced greater increases in NA release (up to 170%), along with changes in basal release and NA uptake. The blockade of N-type Ca^{2+}-channels (ω-conotoxin GVIA, 3.0 μM) reduced NA release by only 18-25% of control values. These data suggest that ALS IgGs can enhance NA release from rat synaptosomes, probably by increasing Ca^{2+} influx through different VOOCs subtypes.
Supported by CNR and TeleThon grants.

ISCHEMIA: NEUROPROTECTION

287.1

HIGH DOSE BACLOFEN PROTECTS BRAIN AFTER TEMPORARY MCA OCCLUSION Catherine Jackson-Friedman, Patrick D. Lyden* *Veteran's Administration Medical Center, San Diego, CA 92161*

We have previously shown that GABA-A agonists are neuroprotective (Stroke 23:1463-1470, 1992). Although neuroprotection has not been shown with low doses of the GABA-B agonist, Baclofen, we decided to study a higher dose since Baclofen inhibits excitatory transmission.

Methods: Using the method of Zea Longa we occluded the middle cerebral arteries (MCA) of male, Sprague Dawley rats anesthetized with Halothane. The animals were kept normothermic during ischemia. To generate a quantal bioassay, a range of occlusion durations were used. Five minutes after occlusion onset we injected either placebo, 10mg/kg, or 20mg/kg Baclofen intraperitoneally. Behavior was rated normal/abnormal at 48 and 72 hours post ischemia. After intravenously infusing 200μl of 2% Evans Blue brains were examined post mortem to verify MCA occlusion.

Results: The ED_{50} is the duration of MCA occlusion that renders 50% of the subjects abnormal. The ED_{50} for vehicle was (n=19) 24.7±1.96 min, for Baclofen 10mg/kg (n=16) 31.9±2.79 min, and for Baclofen 20mg/kg (n=18) 81.4±4.62 min ($p<.05$ for each dose vs. control). Subjects with intracerebral hematoma or subarachnoid blood were excluded: 1/20 control, 12/28 at 10mg/kg Baclofen, and 9/27 at 20mg/kg Baclofen (Chi Square, $p<.01$).

Conclusion: Higher doses of Baclofen can provide significant neuroprotection after MCA occlusion in a rodent suture model. However, there was an increase in the number of hemorrhages in Baclofen treated groups which may nullify this protective effect. (Supported by the Medical Research Service, Department of Veteran's Affairs).

287.2

NEUROPROTECTION BY LUBELUZOLE THROUGH THE SIGNAL TRANSDUCTION PATHWAYS OF ANOXIA AND NITRIC OXIDE. J. A. Benjamins*, M. TenBroeke, J. Kue, and K. Maiese. Dept. of Neurology, Center for Molecular Medicine, Wayne State Univ. Sch. of Med., Detroit, MI, 48201

Neuronal survival following ischemic injury is determined through the induction of several biological pathways. Some neuroprotective agents, such as metabotropic glutamate agonists, exert their effects through the signal transduction pathways of nitric oxide (NO) (Maiese, et al. *J Neurochem* (in press)). Lubeluzole, a benzothiazole compound, has recently been demonstrated to be efficacious in both clinical and experimental models of cerebral ischemia. In primary hippocampal neurons, we examined whether Lubeluzole modulated the signal transduction mechanisms of NO, a "down-stream" mediator of anoxic neurodegeneration. Neuronal injury was assessed by using a 0.4% trypan blue dye exclusion method 24 hours following exposure to anoxia (95%N_2, 5%CO_2) or treatment with sodium nitroprusside (300μM) or SIN-1 (300μM). Approximately 70% of the neurons die over a 24 hour period following exposure to anoxia or NO. Co-administration of increasing doses of Lubeluzole (0.3nM to 1000nM) during anoxia or NO exposure increased neuronal survival from approximately 30% (anoxia or NO only) to a maximum of approximately 70% (Lubeluzole and anoxia /NO). Statistically significant protection was demonstrated with the Lubeluzole concentrations of 500nM to 1000nM (n=6, $p<0.001$). Protection was stereospecific since the R-Isomer did not alter neuronal survival. Post-treatment with Lubeluzole (750nM) at 2, 4, 6, 12, and 24 hours following anoxia or NO application significantly increased neuronal survival for a period of 4 to 6 hours following neuronal injury (n=6, $p<0.001$). We conclude that neuroprotection by Lubeluzole involves the direct modulation of the NO pathway during a "protective window" period. Further investigation of the signal transduction mechanisms below the level of NO generation may elucidate the specific cellular events responsible for ischemic neurodegeneration.

Supported by the Alzheimer's Association, AHA (National), Janssen Research Foundation, NIH NINDS, and the United Cerebral Palsy Foundation.

287.3

THE REDOX AGENT PYRROLOQUINOLINE QUINONE (PQQ) IS NEUROPROTECTIVE IN A SLICE MODEL OF HYPOXIA/ISCHEMIA

F.E. Jensen *, and F. Williams. Div. Neurosci., Children's Hosp.[1], Harvard Med. Sch., Boston, MA 02115

PQQ is a putative essential nutrient that has been shown *in vitro* to diminish NMDA-evoked current and neurotoxicity by direct oxidation of the NMDA receptor redox site (*J. Neurosci.*1992;12:2362-2369). PQQ is a putative essential nutrient with redox cycling ability. We have previously shown that administration of PQQ following hypoxia/ischemia in a rodent stroke model significantly reduces infarct size (*Neurosci.* 1994;62:399-406). While *in vitro* neuronal culture results indicate a direct effect of PQQ at the NMDA redox site, the mechanism of action *in vivo* or in the presence of intact synapses is still unclear. The present study was undertaken to establish whether PQQ interacted with the redox site in a hippocampal slice model of hypoxia/ischemia, where transmission at endogenous synapses remains intact. Slices (400μm thick) were prepared from adult rats and incubated in ACSF bubbled with 95%O_2/5%CO_2. Hypoxia/ischemia was simulated by an 8 min exposure of the slices to glucose-free ACSF bubbled by 95%N_2/5%CO_2. Extracellular recordings of area CA1 population spikes were obtained in response to Schaffer collateral stimulation every 30 sec before, during, and after O_2/glucose deprivation. Control slices rapidly became isoelectric and failed to recover (n=7). In contrast, slices exposed to PQQ (200μM, n=7) throughout the experiment had significantly better recovery to near baseline responses ($p<0.01$). The additon of SOD (20μg/ml) and catalase (10μg/ml) did not attenuate or alter the neuroprotective effect of PQQ (n=5). These results are the first to show a protective effect of the redox agent PQQ in a slice model of hypoxia/ischemia, and suggests the possibility that PQQ may act on synaptically mediated excitotoxic events. Supported by AHA, NS31718, NS32570 (FEJ).

287.4

PROTECTION AGAINST CA1 HYPOXIC NEURONAL INJURY WITH INHIBITORS OF ADP-RIBOSYLATION. R.A. Wallis* and K.L. Panizzon. Dept. of Neuro., UCLA Sch. of Med. and Sepulveda VAMC, Sepulveda, CA 91343. The production of nitric oxide induces ADP-ribosylation of multiple proteins. We have previously found that inhibitors of both mono- and poly-ADP-ribosylation are protective against trauma and NMDA exposure, but not against injury from AMPA or kainate. Since NMDA receptor activation plays a prominent role in hypoxic injury, we assessed the neuroprotective potential of ADP-ribosylation inhibitors against CA1 hypoxic injury using the hippocampal slice. Slices exposed to hypoxia showed evidence of severe neuronal injury with mean orthodromic and antidromic PS recovery to 3 ± 3% and 3 ± 2% of initial amplitude. Inhibitors of both mono-, and poly-ADP-ribosylation were strongly protective against hypoxic injury. With the mono-ADP-ribosylation inhibitor, novobiocin 500 μM, recovery of CA1 orthodromic and antidromic PS improved to 95 ± 3% and 96 ± 2%. Similarly, *meta*-iodobenzylguanidine 20 μM, showed increased orthodromic and antidromic PS recoveries of 93 ± 2% and 93 ± 3%. The inhibitor of poly-ADP-ribosylation, 3-methoxybenzamide 500 μM, also improved orthodromic and antidromic PS recovery to 92 ± 2% and 94 ± 2%. Likewise, 3'-aminobenzamide 1 mM, demonstrated hypoxic protection with orthodromic and antidromic PS recovering to a mean 77 ± 13% and 79 ± 14%. Although nicotinamide 10 mM, acts as an inhibitor of poly-ADP-ribosylation and provides protection from traumatic injury, nicotinamide actually exacerbated hypoxic injury, suggesting that other nicotinamide effects are deleterious during hypoxia. In conclusion, the findings of this study suggest that the processes of both mono- and poly-ADP-ribosylation are important in the evolution of CA1 hypoxic injury. Supported by the Dept. of Veterans Affairs and the American Health Assistance Foundation.

287.5

NGF INDUCTION BY CLENBUTEROL AND PROTECTIVE EFFECTS IN A RAT MODEL OF MCA-OCCLUSION C. Culmsee, I. Semkova, H. Vedder*, M.K.H. Schäfer, E. Weihe, & J. Krieglstein Inst. Pharmacol. & Toxicology, Philipps Univ. Marburg, D-35032 Marburg, Germany

Delivery of neurotrophins to the CNS is one of the largest obstacles in developing effective therapies of neurodegenerative disorders, because these proteins are unable to cross the blood-brain barrier. In our laboratory the induction of NGF-synthesis by clenbuterol has been shown to protect rat hippocampal neurons from excitotoxic damage *in vitro*. Based on these findings we attempted to find out whether the lipophilic β_2-adrenergic agonist clenbuterol could also protect brain tissue from ischemic damage in a model of permanent middle cerebral artery (MCA) occlusion in Long Evans rats. After clenbuterol administration the changes of NGF-mRNA in the ischemic rat cortex were investigated by reverse transcription-polymerase chain reaction (RT-PCR) and *in situ*-hybridisation. The concentration of NGF-protein in rat brain tissue was determined with two-site NGF-immuno assay. Pretreatment with clenbuterol (0.1-0.5mg/kg in rats) administered intraperitoneally 3 h before permanent MCA-occlusion produced statistically significant ($p<0.001$) reductions of infarct volume. Analysis of the RT-PCR-product showed an increase in NGF-mRNA 6 h after clenbuterol treatment *in vivo* (0.1-10 mg/kg). Additionally, a marked increase in NGF-mRNA in the dentate gyrus of the lesioned hemisphere was found with *in situ*-hybridization of rat brain slices. The results concerning NGF-mRNA induction were supported by evaluation of protein levels in the rat brain tissue: a three-fold increase in NGF-protein concentration was measured 10 h after clenbuterol administration. In conclusion, clenbuterol induces NGF-mRNA and NGF-protein synthesis in rat brain tissue and furthermore, provides neuroprotective effects against brain damage caused by ischemia. The results suggest that induction of growth factors in brain tissue by systemically administered lipophilic drugs could become a useful therapeutic strategy for neurodegenerative disorders and stroke.

287.6

POST-ISCHEMIC INFUSION OF SUPEROXIDE DISMUTASE OR SOD:TET451 REDUCES CEREBRAL INFARCTION FOLLOWING TEMPORARY FOCAL ISCHEMIA/REPERFUSION IN RATS. J.W. Francis*, J.M. Ren, L. Warren, R.H. Brown, Jr., and S.P. Finklestein. Cecil B. Day Neuromuscular Research and CNS Growth Factor Research Laboratories, Mass. Gen. Hospital, Charlestown, MA 02129.

Oxygen free radicals play a major role in neuronal cell injury following cerebral ischemia/reperfusion. The free radical scavenging enzyme, Cu/Zn superoxide dismutase (SOD-1) ameliorates various types of brain injury resulting from temporary CNS ischemia. We have compared the cerebroprotective properties of human SOD-1 (hSOD-1) with a novel recombinant SOD-1 hybrid protein, SOD:Tet451, composed of hSOD-1 linked to the neuronal binding fragment of tetanus toxin (TTxC). Following 2 hours of temporary middle cerebral artery occlusion, rats infused with equivalent activities of either hSOD-1 or SOD:Tet451 for the initial 3 hours of reperfusion showed reductions in cerebral infarct volume of 43% and 57%, respectively, compared to saline-treated controls ($p<0.01$). Serum hSOD-1 concentrations in rats receiving SOD:Tet451 were 7-fold higher than those in rats receiving the native enzyme. Animals treated with SOD:Tet451 also demonstrated an extended persistence of hSOD-1 in the bloodstream during drug washout as compared to animals given free enzyme. Immunohistochemical examination of brain sections from an SOD:Tet451-treated ischemic rat showed positive immunoreactivity in the ipsilateral cerebral cortex using either anti-TTxC or anti-human SOD-1 antibodies. Our results document that both hSOD-1 and SOD:Tet451 significantly reduce brain infarct volume in a model of transient focal ischemia/reperfusion in rats. Additionally, our findings suggest that the cerebroprotective effects of SOD-1 may be enhanced by neuronal targeting as seen with the hybrid protein, SOD:Tet451. This work was supported in part by the Amyotrophic Lateral Sclerosis Association, the American Paralysis Association, and NIH/NINDS grant P01 NS#10828.

287.7

LONG-LASTING INCREASED HYPOXIC TOLERANCE BY PRECEDING *IN VIVO* CHEMICAL HYPOXIA - 'CHEMICAL PRECONDITIONING'. M. W. Riepe[1] *, K. Kasischke[1], A. Raupach[1], F. Esclaire[2], J. Hugon[2] and A. C. Ludolph[1]. Department of Neurology, Humboldt University (Charité), 10098 Berlin, Germany and Department of Histology, University of Limoges, Limoges, France[2].

Sublethal ischemia preceding sustained ischemia is cytoprotective in heart and brain - 'ischemic preconditioning'. We show that clinically inapparent systemic pretreatment with a chemical inhibitor of mitochondrial complex II, 3-nitropropionic acid (3-np), increases hypoxic tolerance in rat hippocampal slices.

In slices prepared from untreated control animals ("c-slices") neuronal density after 15 min of hypoxia is 52 ± 15 % (mean ± SD) of c-slices without hypoxia. In slices prepared from animals treated by a single i.p. injection of 20 mg/kg body weight 3-np ("p-slices") posthypoxic neuronal density improved to 97 ± 23 % ($p < 0.01$). The amplitude of the CA1 population spike (psap; stimulation of Schaffer collaterals with 0.1 Hz) in c-slices and p-slices at the end of 15 min hypoxia is about 10 %. Upon 45 min of recovery psap is 31 ± 9 % in c-slices and 90 ± 7 % ($p < 0.01$) in p-slices (1 hour time interval between *in vivo* treatment and preparation of slices). With four days interval, posthypoxic recovery was 66 ± 30 % ($p < 0.05$) in p-slices. Increased hypoxic tolerance is partly abolished by additional treatment with glibenclamide prior to hypoxia and is completely abolished by additional treatment with NMDA antagonists.

We conclude that mild selective chemical hypoxia increases hypoxic tolerance in experimental animals - 'chemical preconditioning'. Increased hypoxic tolerance is diminished by glibenclamide, an antagonist at K_{ATP}-channels, and APV, an antagonist at NMDA-receptors.

Supported by a grant from the Deutsche Forschungsgemeinschaft to MWR and ACL.

287.8

LESIONS OF DEEP PREPIRIFORM CORTEX RESULT IN NEUROPROTECTION DURING ISCHEMIA VIA ATTENUATION OF RISES IN EXTRACELLULAR GLUTAMATE CONCENTRATION.

R.P. Simon, K. Kawaguchi and M. Huerbin*. Department of Neurology, University of Pittsburgh, Pittsburgh, PA 15261.

A small region of deep prepiriform cortex (area tempestas, AT), acting in part via excitatory amino acid (EAA) neurotransmission, modulates epileptic activity throughout the limbic system. We have shown that pharmacologic blockade of EAA receptors at AT attenuates cell death during limbic seizures independent of reduction in seizure duration (Neurosci 61:817-822, 1994). We therefore investigated a possible role of EAA neurotransmission at AT in modulating neuronal injury in a different form of excitotoxic injury, that occurring during global ischemia induced by 10 minutes of 4 vessel occlusion. EAA neurotransmission at deep prepiriform cortex was modulated either by bilateral infusion of NBQX at AT (0.5 µl containing 5 nmol) or by lesioning AT by stereotactic injection of kainate bilaterally at 72 hours before ischemia (anticonvulsant pretreatment precluded seizures). NBQX injected 1 mm medial to the target served as an anatomic control. Morphologic endpoints were assessed in the CA1 and CA3 hippocampal subsectors. Neuronal injury was quantified with HSP72 immunocytochemical staining at 24 hours following ischemia and neuronal cell death by cell counting of viable cresyl violet stained neurons at 72 hours after ischemia. Both NBQX infusion and AT lesions produced attenuation of neuronal injury and death in target neurons. NBQX injection 1 mm medial to AT was not neuroprotective. In a separate set of experiments lesioned animals and controls had microdialysis probes placed in the hippocampus during the ischemic insult. The controls, but not the lesioned animals, demonstrated the well known glutamate peak; neuroprotection was confirmed in lesioned rats at 7 days. Thus, excitatory neurotransmission at AT effects ischemic cell death in hippocampal neurons and does so via modulation of ischemia-induced glutamate concentrations. (Supported by NS-35965; RPS)

287.9

Protection Against Focal Ischemic Infarction Elicited By Stimulation of the Cerebellar Fastigial Nucleus Results From Excitation of Intrinsic Neurons. S. Glickstein*, E.V. Golanov, K. Kobylarz and D.J. Reis. Dept. of Neurol. & Neurosci., Cornell Univ. Med. Coll. New York, NY 10021.

Electrical stimulation of the cerebellar fastigial nucleus (FN) of rat for 1h reduces, by over 50%, the focal ischemic infarction produced by occluding the middle cerebral artery (MCAO) (Reis et al., 1991), even 2 wks later. Electrical stimulation of FN excites intrinsic neurons **and** axons, each having different actions: chemical stimulation of intrinsic neurons lowers arterial pressure (AP) and rCBF (Chida et al., 1990), while electrical stimulation of FN, after excitotoxic destruction of intrinsic neurons, elevates AP and rCBF, the fastigial pressor response (FPR) (Miura & Reis, 1969). We assessed whether the neuroprotection elicited by FN stimulation is mediated by intrinsic neurons or fibers of passage. In anesthetized spontaneously hypertensive rats ibotenic acid (IBO) (23 nmol in 300 nl, each side) was microinjected into FN (n=14) or into cerebellar dentate nuclei (DN, n=5), stimulation of which is not neuroprotective. Five days later animals were reanesthetized, instrumented, an electrode inserted into FN, and the MCA occluded. After determining the threshold for the FPR (elevation of AP by 15 mmHg), the FN was stimulated for 1h (1 sec on/1 sec off, 0.5 msec, 50 Hz, 5x threshold) before MCAO. Twenty four h later rats were sacrificed and lesions measured. In sham controls the FN was not stimulated prior to MCAO. IBO microinjected into FN destroyed >90% of neurons in the nucleus while injections in DN destroyed the majority of DN but spared FN neurons. In 5 uninjected controls sham stimulation of the FN resulted in a FPR whose threshold was 30±6 μA) and a lesion of 137±37 mm^3. The FPR threshold after DN lesions was 35±2 mA (NS) and infarction volume was reduced by by 45% to 75±27 mm^3 ($p<0.05$), salvage comparable to that produced by FN stimulation alone (Golanov et al., 1996). In rats with FN lesions the FPR threshold was unchanged (35±2 mA) (indicating preservation of local axons) but FN stimulation no longer reduced lesion size (120±33 mm^3, n=14, $p>0.05$). We conclude: the central neurogenic neuroprotection elicited from FN is mediated by activation of intrinsic neurons while the FPR results from excitation of axons projecting into or through the nucleus.

287.10

Electrical Stimulation of the Cerebellar Fastigial Nucleus Reduces the Delayed Neuronal Death Produced by Global Cerebral Ischemia. F. Liu*, E.V. Golanov and D.J. Reis. Dept. Neurol. & Neurosci., Cornell Univ. Med. Coll. New York, NY 10021.

Electrical stimulation of the cerebellar fastigial nucleus (FN) reduces, by 50%, the volume of a focal cerebral infarction elicited by occlusion of the middle cerebral artery (Reis et al., 1991). We investigated whether FN stimulation would reduce the delayed death of hippocampal neurons of the CA1 subfield elicited by transient global ischemia. Sprague-Dawley rats were anesthetized, instrumented to record cerebral blood flow (rCBF) and EEG, electrodes placed in FN and the vertebral arteries coagulated. Animals were ventilated with 100% O_2 (PaO_2=463±113mmHg). Blood gases and body temperature were monitored and maintained. Groups of 5 rats were subjected to reversible occlusion of common carotid arteries for 10, 20, 30 or 40 min. Neuronal death directly related to duration of occlusion. 40 min of occlusion destroyed ~90% of neurons, a time used thereafter. Occlusion immediately reduced rCBF to 4±7% and flattened the EEG in 8±3 sec. After clamps were released, rCBF and EEG were restored and animals returned to the home cages. Five days later, they were sacrificed, brains removed, sectioned, stained, and the density of pyramidal neurons in the CA1 measured. In sham (non-stimulated) animals, 40 min of ischemia reduced the number of neurons from 210±5 to 33±11/mm ($p<0.05$; n= 6). Electrical stimulation of the FN (1 sec on/1 sec off, 50 Hz, pulses 0.5 msec, 70-100 μA) for 1h immediately before occlusion increased the number of surviving neurons (to 134±9 neurons/mm ($p<0.05$) representing a salvage of ~57%. FN stimulation 24h before ischemia comparably protected hippocampal neurons (141±6/mm, $p<0.05$). FN stimulation also reduced the intensity of the local cellular inflammatory reaction in the CA1 reducing numbers of infiltrating cells and reactive microglia. We conclude: (a) electrical stimulation of FN can reduce the delayed neuronal degeneration of hippocampal neurons of the CA1 subfield elicited by transient forebrain ischemia; (b) the protective effect persists for at least 24h; (c) conditional central neurogenic neuroprotection elicited from FN is effective against global as well as focal ischemia.

287.11

Neuroprotective Electrical Stimulation of the Cerebellar Fastigial Nucleus Suppresses Expression of Peri-Infarction Depolarizing Waves. E.V. Golanov* and D.J. Reis. Div. of Neurobiol., Dept. of Neurol. & Neurosci., Cornell Univ. Med. Coll., New York, NY 10021.

Electrical stimulation of the cerebellar fastigial nucleus (FN) in rat for 1h reduces, up to 50%, the volume of the focal cerebral infarction produced by middle cerebral artery occlusion (MCAO) performed even two weeks later. MCAO also immediately generates recurrent peri-infarction depolarizing waves (PIDS) which appear to exacerbate ischemic damage (Hossmann, 1994). We investigated whether FN stimulation could modify the expression of PIDS. Sprague-Dawley rats were anesthetized, paralyzed, and ventilated controlling blood gases and body temperature. Cerebral blood flow (rCBF), EEG and DC potentials were recorded over that region of the cortex salvaged by FN stimulation for 3h after permanent MCAO occlusion. Within 2 to 3 min after MCAO PIDS appeared over the ischemic penumbra consisting of typical negative DC potentials which peaked at -25.2±2.8 mV 23.1±7.2 sec after their onset and recovered by 192.0±126 sec (mean ± SEM; n=5). PIDS appeared randomly at a frequency of 0.038/min for a total of ~6 events over the 3h of observation and were accompanied by EEG flattening and reduction in rCBF. One h of FN stimulation synchronized the EEG but did not alter DC potentials. However, MCAO performed 1h later resulted in a ~50% reduction in the numbers of PIDS (5.8±2.9 to 2.6±1.3; $p<0.05$) and in lengthening of the latency to the first wave (from 3.2±1.4 to 8.3±2.1 min; $p<0.05$). Comparable stimulation of the rostral ventrolateral medulla (RVL) (n=5), which does not salvage cortex (Golanov et al., 1994) did not affect PIDS. Stimulation of FN for 1h abolished all PIDS in 5 rats in which MCAO was performed 72h later. Site-specific stimulation of FN almost immediately initiates a marked suppression of the expression of PIDS. Such suppression may, along with reduced expression of proinflammatory molecules in cerebral microvessels (Galea et al., *Soc. Neurosci. Abstr.*, 1996), add to the conditioned central neurogenic neuroprotection elicited from FN.

287.12

CONDITIONAL STIMULATION OF THE CEREBELLAR FASTIGIAL NUCLEUS (FN) REDUCES INFLAMMATORY REACTIVITY OF BRAIN MICROVESSELS. E. Galea*, D.L. Feinstein, E.V. Golanov, D.L. Reis Dept. Neurol. and Neurosci., Cornell Univ. Med. Coll., New York, NY 10021.

Electrical stimulation of the FN results in up to a week-long protection against brain infarctions. FN also inhibits the expression of inducible nitric oxide synthase (iNOS) in microvessels (MV) in the salvaged zone (Galea et al., J Cereb Blood Flow Metab 15: Suppl 1, S91, 1995). We investigated whether FN stimulation alone could suppress cytokine-stimulated expression of endothelial iNOS measured by quantitative RT-PCR *in vitro*. Spontaneously hypertensive rats were stimulated in the FN (1s on/1s off, 0.5 ms/pulse, 50 Hz, 70 μAmp) or sham-stimulated for 1h. Twenty four h later MV were isolated by gentle homogenization, centrifugation in 18% dextran, and filtration through 5-μm nylon screens. Alkaline phosphatase activity, a brain endothelium marker, was comparably enriched in control (3.3-fold) and FN-conditioned MV (4.4-fold) respect to whole brains (n=4). MV were incubated in DMEM with IL-1β (0.1-100 ng/ml), TNF-α (1-100 ng/ml) or IFN-γ (100 U/ml) at 37°C for 2-24h. After 24h of incubation, mitochondrial dehydrogenases in control and FN-conditioned MV were respectively reduced to 52%±14 (n=3) and 47%±17 (n=3) of values in fresh MV, indicating comparable decrease of metabolic viability over time in both fractions. Maximal iNOS mRNA expression was observed after 5-h exposure to IL-1β (10 ng/ml). However, while expression was 9.6±0.9 times over basal in control MV (n=4), it was reduced to 2.25±0.77 over basal in FN-conditioned MV (n=4). These results suggest that FN stimulation may render brain microvasculature refractory to inflammatory activation. Such conditioned suppression of iNOS may contribute to central neurogenic neuroprotection elicited from FN. *Supported by NHLBI (HL18974).*

VISUAL CORTEX: EXTRASTRIATE—DORSAL STREAM I

288.1

FIXATIONAL EYE MOVEMENTS AFFECT THE VISUAL RESPONSES OF NEURONS IN THE SUPERIOR TEMPORAL SULCUS (STS) W. Bair*, L.P. O'Keefe, J.A. Movshon. HHMI and Center for Neural Science, New York Univ., New York, NY 10003.

We measured eye movements (EMs) made by an awake, fixating, macaque monkey, and analyzed their effects on the neuronal discharge of 40 directionally selective neurons in the STS. The monkey maintained fixation within 1.5 deg of a small fixation spot; he fixated before (0.2 s) and during (2 s) the presentation of a visual stimulus to the neuron's receptive field, which lay 5–15 deg from the center of gaze. On many trials, the monkey made one or more small EMs, beginning 0.1–0.3 s after initial fixation. The average frequency of these small movements was near 0.5/trial, and peaked 0.5–1.0 s after stimulus onset.

We examined neuronal responses around the time of EMs that occurred while the visual stimulus was present in the receptive field. Mean EM duration was 25 ms; mean amplitude was 0.7 deg. EMs often occurred as pairs of oppositely directed micro-saccades separated by 250 ms. The distribution of EM directions was roughly uniform, but underrepresented movements toward the target, perhaps because the animal was trained not to make saccades to the stimulus. Between 40 and 80 ms after an EM, neuronal discharge was often altered, in a manner that depended on the direction of eye and stimulus movement. When the eye movement induced retinal slip in the neuron's preferred direction, response was often increased, especially when the ongoing visual stimulus elicited relatively little response. When the slip was in the neuron's non-preferred direction, response was strongly reduced, especially if the ongoing visual stimulus elicited a strong response.

EMs during fixation influence neuronal discharge in a direction-specific manner. Nominally identically stimuli may thus differ on the retina, and this difference will influence spike train statistics, including estimates of correlation between neurons.

Supported by the Howard Hughes Medical Institute.

288.2

FIRST- AND SECOND-ORDER MOTION PROCESSING IN SUPERIOR TEMPORAL SULCUS NEURONS OF THE ALERT MACAQUE. L. P. O'Keefe* and J. A. Movshon. Center for Neural Science and Howard Hughes Medical Institute, New York University, New York 10003.

Extrastriate visual cortical areas in the superior temporal sulcus (STS) provide behaviorally relevant signals about visual motion, but it is unclear whether these neurons provide relevant signals about both first- and second-order motion. Our earlier recordings in anesthetized macaques suggested that directional signals for second-order motion stimuli were weak or absent in most MT neurons. We have now examined responses of STS neurons to first- and second-order motion in an alert macaque.

We measured responses of single neurons in the STS of an alert macaque monkey to several first- and second order motion stimuli. For each neuron, we measured directional tuning and contrast response with two types of first-order gratings and two types of "drift-balanced" second-order gratings created by spatiotemporal modulation of the contrast or flicker rate of a random texture. Nearly all neurons gave vigorous, direction-selective responses to first-order motion. Responses to second-order motion varied widely, with most neurons exhibiting weaker responses and less selectivity for the direction of second-order gratings. We measured contrast reponses from each neuron in the preferred and null direction for each class of motion, and generated neurometric functions representing their sensitivity to these targets. Thresholds for first-order motion were usually between 0.005 and 0.1, while thresholds for second-order motion spanned a range of contrasts roughly 10 times higher. In nearly half the neurons, thresholds for second-order motion were too high to be reliably established.

While some neurons in the STS carry reliable information about the motion of second-order targets, overall levels of responsiveness and selectivity are much lower for these stimuli than for first-order targets. We are examining the behavioral significance of these motion signals in parallel psychophysical experiments.

Supported by HHMI.

288.3

ORGANIZATION OF DISPARITY SELECTIVITY IN MACAQUE AREA MT. G. C. DeAngelis, I. M. Groh*, and W. T. Newsome. Dept. of Neurobiology, Stanford University, Stanford CA 94305.

Primate visual area MT contains a high percentage of directionally selective neurons that are organized into columns according to preferred direction of motion (Albright et al. 1984). In addition, most neurons in macaque MT are selective for binocular disparity (Maunsell and Van Essen 1983). We have examined whether neurons in MT are also organized systematically according to disparity preference.

Experiments were carried out with two adult rhesus monkeys that performed a visual fixation task. We recorded multi-unit activity and local field potentials (LFP) at equally-spaced intervals (100 μm apart) along electrode penetrations though area MT. Whenever possible, we also recorded the activity of isolated single units. At each recording site, we measured direction tuning (at the estimated preferred disparity) and disparity tuning (at the measured preferred direction) by presenting coherently moving random-dot patterns. Stimuli were rendered in depth using the red-green anaglyph technique.

Along oblique penetrations through area MT, we found regions of strong disparity selectivity (usually 500-1000 μm long) interspersed among poorly-tuned regions of roughly similar dimensions. Within the strongly-tuned regions, nearby recording sites usually had similar preferred disparities, and we often observed a smooth progression of preferred disparities from near to far, or vice-versa. At sites where single units were recorded, the disparity tuning of these units closely matched that of the multi-unit activity and the LFP. Within regions of MT where multi-unit and LFP activity were poorly tuned for disparity, single units also tended to be poorly tuned. We have not yet observed any systematic relationship between patterns of disparity and direction tuning.

Our results suggest that neurons with strong and weak disparity selectivity are compartmentalized in area MT. Regions of strong selectivity appear to contain an orderly map of disparity preferences.

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288.4

REPRESENTATION OF THREE-DIMENSIONAL SURFACES IN PRIMATE VISUAL AREA MT. G. J. Carman* and T. D. Albright. Sloan Center and Vision Center, Salk Institute, San Diego, CA 92186.

Premise: The rapid and accurate perception of the three-dimensional (3D) depth, orientation, and shape of the surfaces of general objects may be supported by explicit representations of these geometric attributes within the primate visual pathway. **Methods:** We recorded from neurons in the superior temporal sulcus of three alert monkeys as they viewed 3D surfaces presenting controlled motion and/or stereo cues to the depth, orientation, and shape of their surfaces. Neurons were initially characterized by their tuning for direction, disparity, and speed using surfaces positioned within the classical receptive field. Neurons were then characterized by their response to surfaces extending outside the classical receptive field. **Results:** Neurons responded vigorously to the combination of motion and stereo cues such as occurs during natural viewing of 3D objects. We observed three types of neuronal selectivity, defined by the position of surfaces relative to the zero-displacement and zero-disparity horopter: (1) near cells, responsive to surfaces nearer than the horopter; (2) horopter cells, responsive to surfaces about the horopter; and (3) far cells, responsive to surfaces farther than the horopter. These selectivities were clustered, suggesting a systematic organization into columns of preferred stereo disparity as well as of preferred direction of motion. Furthermore, response to surfaces within classical receptive fields could be modulated by surfaces extending into the surrounds, in a manner consistent with normalization of response by viewing distance, which may play a role in perceptual constancy. **Conclusion:** Our findings support the hypothesis that neurons in area MT integrate motion and stereo cues to provide a representation of 3D depth of surfaces in the visual field. (Supported by NIH EY0617, NIH EY07605, and Salk-Sloan Center for Theoretical Neuroscience, San Diego.)

288.5

MT RESPONSES DO NOT MATCH PERCEPTUAL REPORTS DURING VIEWING OF COLOR-SEGMENTED MOTION DISPLAYS

Lisa J. Croner* and Thomas D. Albright, Salk Institute, La Jolla, CA 92037.

The use of color as a segmentation cue strongly enhances human subjects' ability to discriminate direction of targets moving in dynamic noise (Croner & Albright, 1994, *I.O.V.S. (Suppl.)*, 35:1643). We previously found that direction discrimination capacity of individual MT neurons (which are thought to be important for perceptual choice based on motion signals) is not influenced by color segmentation cues when monkeys do not make directional judgements (Croner & Albright, 1995, *Soc. Neurosci. Abstr.*, 21:281). In this study, we investigated whether MT responses correlate with the segmentation-enhanced percept when monkeys perform a direction discrimination task.

We recorded activity of single neurons in area MT of fixating rhesus monkeys while the animals discriminated direction in visual stimuli presented in the neurons' receptive fields. Stimuli were dynamic displays of bright dots, a variable fraction of which moved in the same direction at the same speed (motion signal), while the remaining dots were displaced randomly (noise). Two stimulus configurations were used. In the *homochromatic* condition, all dots were the same color. In our novel *heterochromatic* condition, signal dots were a different color from noise dots. ROC analysis generated neurometric functions expressing each neuron's capacity to discriminate motion in its preferred versus anti-preferred direction. These were compared with simultaneously obtained psychometric functions showing the monkeys' perceptual performance.

As for human subjects, segmentation by color enhanced the monkeys' ability to discriminate signal direction. For the majority of neurons studied, however, homochromatic and heterochromatic discrimination thresholds were statistically indistinguishable. Thus, the capacity of individual MT neurons to discriminate direction is not influenced by color segmentation cues, even when concurrent perceptual reports demonstrate that color segmentation improves directional discriminability.

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288.6

MEASUREMENTS OF INFORMATION RATES IN MONKEY MT NEURONS IN RESPONSE TO TIME-VARYING STIMULI. G. Buračas*, A. Zádor, M. DeWeese, and T. Albright, VCL, MNL, and Sloan Center, Salk Institute, P.O.Box 85800, San Diego, CA 92186.

The traditional notion of a tuning curve relates the time-average firing rate of a neuron to the constant characteristics of the stimulus in its receptive field; this firing rate is typically measured in windows of several hundreds of milliseconds. How does the appropriate time window change when the stimulus changes with time? Inspired by work on a motion-sensitive neuron in the fly (Bialek et al, Science, 1991 252:1854-7), we presented visual stimuli with rich temporal structure to alert fixating macaque monkeys while recording extracellularly from neurons in area MT. The stimulus consisted of a Gabor patch whose direction of motion performed a random walk. For one stimulus ensemble, the spike train exhibited modulation on a 10 millisecond time scale (cf. Bair and Koch, 1996); under these conditions, the spike train encoded nearly all of the 30 bits/second available in the stimulus. This information could have been extracted only if spikes were detected with a precision of order 10 millisecond. By contrast, a traditional analysis based on counting the number of spikes in much longer epochs would have underestimated the information rate by an order of magnitude. These results suggest that the notion of a tuning curve can be extended to the analysis of time-varying stimuli, provided that the window size is appropriately small. These experiments may have implications for understanding cortical processing of natural time-varying stimuli. *Supported by EY07605 and Salk-Sloan center for Theoretical Neurobiology.*

288.7

SELECTIVE EFFECTS OF LESIONS OF CORTICAL AREAS MT AND MST IN THE MACAQUE MONKEY. K. K. Rudolph* and T. Pasternak. Depts. of Neurobiology & Anatomy, Brain & Cognitive Science, and Center for Visual Science, University of Rochester, Rochester, NY 14642.

Areas MT and MST, components of the dorsal pathway, are thought to represent intermediate stages in the processing of visual motion. Lesions of these areas disrupt performance on some motion discrimination tasks but leave others intact. To explore in depth the nature and the selectivity of these lesion effects, we trained monkeys with unilateral ibotenic acid lesions of areas MT/MST to discriminate differences in the direction and/or orientation of a variety of random-dot and grating stimuli. On each trial, two comparison stimuli appeared successively in the same retinal location and the monkeys were rewarded for judging them as "same" or "different". Performance was assessed by either reducing the contrast of gratings, broadening the direction range of dot stimuli, or by masking gratings and dot stimuli with dynamic noise. Thresholds were measured in intact and lesioned hemifields at about the same time. MT/MST lesions produced a large, permanent deficit in the discrimination of direction of moving gratings masked by noise but did not affect contrast thresholds. Similarly, performance on tasks requiring integration of local motion signals was affected only in the presence of random directional noise. This susceptibility to noise was motion specific, since the discrimination of grating orientation in the presence of spatial noise was unaffected. Such effects were not observed in monkeys with lesions of area V4, a major component of the ventral pathway implicated in processing stimulus form. Our results suggest that the removal of areas MT and MST, rich in directionally selective neurons, substantially reduces the brain's ability to extract motion signal in the presence of noise. These results also strongly support the notions of specialization of MT/MST for motion processing and segregation of motion processing to the dorsal pathway.

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288.8

LESIONS OF AREAS MT/MST IN THE MACAQUE MONKEY LIMIT SHORT-TERM MEMORY FOR MOTION. T. Pasternak* and K. K. Rudolph. Depts. of Neurobiology & Anatomy, Brain & Cognitive Science, Center for Visual Science, University of Rochester, Rochester, NY 14642.

Despite strong evidence of functional specialization of areas MT/MST for motion processing, lesions of these areas appear to spare a number of measures of motion perception, including contrast sensitivity and range thresholds for discriminating opposite directions of motion (see Rudolph and Pasternak, NSC 1996). To assess how robust these apparently preserved directional signals are in the absence of MT/MST neurons,, we used tasks requiring the retention of motion information over time. Monkeys with unilateral ibotenic acid lesions of areas MT/MST were trained to fixate while performing a short-term memory task with stimuli placed into the lesioned and intact portions of the visual field. On each trial, a sample stimulus moving in one of eight directions was followed by a test stimulus moving in the same or opposite direction, and the animal made a same-different judgment. The retention of direction information was assessed by increasing the direction range of dot stimuli, or by decreasing grating contrast, and measuring thresholds over a range of temporal delays interposed between sample and test stimuli. Performance declined with increasing delays in both hemifields. However, the rate of this decline was a factor of 2-3 more rapid in the lesioned hemifield. As a result, the deficit, which was minimal at shorter delays (up to 1.5 sec), increased dramatically at longer delays. Thus, in the absence of MT/MST neurons, retention of information about motion direction is severely limited.

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288.9

THE HUMAN KINETIC OCCIPITAL (KO) AREA INVESTIGATED WITH fMRI. S. Van Oostende[1], S. Sunaert[1], P. Van Hecke[2], G. Marchal[1] and G.A. Orban[3]*. [1]Afdeling Radiologie, UZ-Gasthuisberg, [2]Biomedische NMR-Eenheid, Campus Gasthuisberg, [3]Lab. Neuro- en Psychofysiologie, KULeuven, Campus Gasthuisberg, B-3000 Leuven, Belgium.

Previous PET studies (Orban *et al.* PNAS, 92:993-997, 1995; Dupont *et al.* Soc.Neurosci.Abstr. 21:663,1995) have revealed an area in lateral occipital cortex specialized in processing of kinetic contours. In the present fMRI study we investigated this area in single subjects. In all experiments subjects viewed randomly textured pattern (RTP) stimuli (3 deg central) while fixating a point. Subtraction of uniform motion (all pixels move together) from kinetic gratings (strips of pixels move in opposite direction) revealed an activation of KO in 14 subjects bilaterally (R > L). This region is 20 mm posterior from area MT defined by uniform motion minus static (all pixels stationary). Comparing different dynamic stimuli to static (5 subjects), revealed that KO was more activated by kinetic gratings than by uniform motion, transparent motion or luminance gratings, dynamic noise being the least effective. This profile was different from that of MT. In a final experiment (6 subjects) we showed that KO is located laterally from V3, visualized by the comparison of horizontal and vertical meridians. These results confirm that KO is specialized in the processing of kinetic boundaries and is distinct from known visual areas.

288.10

CONTRAST MODULATION IN A PASSIVE VIEWING TASK OF MOTION REVERSAL : A PET STUDY. L. Cornette[1,2], P. Dupont[1,2], I. Joniau[3], A. Rosier[1,2], W. Spileers[3], L. Mortelmans[2] and G.A. Orban[1]. [1]Lab. Neuro- en Psychofys., Medical School, KULeuven, GHB. [2]PET Centre, Dep. Nuclear Med., UZ, GHB. [3]Dep. Ophtalmol. UZ, GHB, B-3000 Leuven, Belgium.

In this $H_2{}^{15}O$-PET study, we specifically investigated human brain activations during reversal phase of contrast modulated motion. Ten male subjects were engaged in a passive viewing task. The stimulus was a 5 degree square random dot pattern (13.08 cd/m^2) on a luminated background (4.3 cd/m^2), with a density of 29 dots/degree^2. Dots were moving at 4 degree/sec on a single horizontal axis, while subjects were fixating a central fixation point. The following contrast-modulated (0 to 8.7%) conditions were presented : reversal of motion (a), continuous motion to the right (b), and a static random dot pattern (c). As control, reversal and static conditions were repeated without contrast modulation (d) (e). Human brain regions ($p_{uncorr} < 0.001$) activated by simple reversal (d minus e) are : dorsal cuneus (V3), BA 2/40 (2v), superior parietal lobe and lateral frontal eye field. The absence of prominent activation in what is considered the homologue of monkey MT/V5 might be due to the use of motion in only one direction axis. Contrast modulated continuous motion (b minus c) yields activation only in dorsal cuneus. Accentuating motion reversal phase by means of contrast modulation (a minus c) reveals new foci ($p_{corr} < 0.05$) : anterior bank of the parieto-occipital sulcus (BA 19, homologue of monkey area PO), lateral sulcus (BA 47 and BA 44) and claustrum. As PO receives magnocellular input and as it is only activated by adding contrast modulation on a reversal stimulus, we suggest PO is specifically activated by changes in motion direction. Activation of BA 47 (and claustrum) might reflect sustaining voluntary fixation (*Brain*, 1994, 117:1073). BA 44 activation could be related to supramodally perception of fast temporal variations in a stimulus. (Supported by NFWO 3.0227.95)

288.11

SELECTIVE DEFICITS OF FIRST OR SECOND ORDER MOTION IN STROKE PATIENTS PROVIDE. FURTHER EVIDENCE FOR SEPARATE MECHANISMS. L M. Vaina[1]* and A. Cowey[2] Boston University, Dept. of Biomedical Eng. & Neurology, Boston, MA, USA[1] and Dept. of Experimental Psychology, Oxford University, England[2]

Psychophysical studies suggest that there may be at least two motion systems: a 1st-order system responds to spatio-temporal variations in luminance, and a 2nd-order system responds to spatio-temporal variations in attributes such as contrast. The 2nd-order motion is visible to human observers but invisible to 1st-order motion mechanisms. Single cell recording studies in the macaque also dissociate 1st-order and 2nd-order motion, and suggest that while 1st-order motion is first registered in V1, the detection of 2nd-order motion is accomplished in MT. We have previously reported on a stroke patient with a unilateral lesion permanently impaired on 2nd-order motion but not on 1st-order motion (Vaina et. al, 1993; Vaina& Cowey, 1996).

Here we report a double dissociation of deficits between 1st-order and 2nd-order motion in two patients (F.D. and R.A.) with focal unilateral brain lesions confirmed by MRI. F.D.'s lesion involved the dorsolateral caudal aspect of the temporal lobe while RA's involved mostly the rostro-medial occipital cortex. We used several 1st- and 2nd-order stimuli to measure FD and RA's ability to discriminate direction in local and global motion. The results revealed a double dissociation of deficits: F.D. was impaired on all 2nd-order motion tasks, but normal on the 1st-order tasks. R.A. displayed the opposite deficit. In both patients this dissociation was stable over two years of repeated testing and all the deficits were present only in the visual field contralateral to the lesion. Our results provide further evidence for the view that 1st- and 2nd-order motion are mediated by separate neuronal circuitry, but do not support the view that MT is crucial for the detection of 2nd-order motion. The locus of the lesions suggests that probably 2nd-order, but not 1st-order motion is mediated by a region in the visual cortex adjacent and dorsal to area MT (F.D.'s lesion), and cortical areas such as V1 and V3 are probably not significantly involved (R.A.'s lesion) in this motion. *Supported by NIH grant EY-2RO-1-07861-06*

288.12

A MODEL USING MOTION-OPPONENT OPERATORS TO COMPUTE TRANSLATIONAL AND ROTATIONAL HEADING PARAMETERS FOR A MOVING OBSERVER C. S. Royden*, Computer Science Department, Wellesley College, Wellesley, MA 02132.

As people move about the world, the images of objects move on the retina. This image motion contains much information about the observer's trajectory. Psychophysical studies have shown that people can judge their motion accurately for both straight line and curved-path motion. This implies that they must be able to compute both translation and rotation components of heading from visual information. Longuet-Higgins and Prazdny (*Proc. R. Soc. Lon. B*, 1980) showed how one can use local differences in the image motion to compute the translation and rotation components of observer motion. One can approximate the calculation of the observer translation components using motion-opponent operators with properties similar to cells in the Middle Temporal area (MT) of primates. I present a model that uses motion-opponent operators to compute the translation parameters for observer motion. The outputs of these operators feed forward in a radial pattern to large-field cells, similar to those in the primate Medial Superior Temporal visual area (MST). Observer translation is computed as the center of expansion of the maximally responding cell in this second layer. Observer rotation is computed by comparing the output of the motion-opponent cells with that of pure direction-selective cells to find the component of flow perpendicular to the radial translation pattern. The result of this comparison feeds into large field cells that prefer patterns associated with particular observer rotations. These cells also have receptive fields similar to some found in MST. Computer simulations show that this model computes heading parameters well for a variety of conditions.

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LEARNING AND MEMORY: SYSTEMS AND FUNCTIONS IV

289.1

LEARNING-RELATED ACTIVATION OF VISUAL CORTEX BY AN AUDITORY STIMULUS: EXPLANATION THROUGH ANALYSIS OF NEURAL INTERACTIONS

AR McIntosh[1]*, RE Cabeza[1], NJ Lobaugh[2] S Houle[3], [1]Rotman Research Inst of Baycrest Centre, [2] Psychol Research, Hosp for Sick Children, [3]PET Centre, Clarke Inst; Univ of Toronto, ON, Canada, M6A 2E1

The complete perception of our world is formed by the interactions between sensory modalities. To help characterize these areas, PET was used to measure regional cerebral blood flow in 11 subjects as they learned an association between visual and auditory stimuli. Moreover, using path analysis, the interactions mediating this association were quantified. When the auditory stimulus (FM tone) had a high probability of preceding a visual stimulus (circular geometric figure), activation of dorsal extrastriate cortex was observed when the tone was presented by itself. When expressed in terms of a functional network, learning-related changes in feedback effects from tertiary visual and dorsolateral prefrontal cortices seemed to mediate this change in extrastriate activity. Specifically, suppressive effects from prefrontal cortex reduced, and enhancement from tertiary visual cortex increased, during tone presentation as the association between stimulus events was learned. The approach of mapping activity across the entire brain and expressing data in terms of neural interactions thus provides key insights into some of the processes involved in cross-modal interactions. (Supported by NSERC grant to ARM)

289.2

REPRESENTATIONS OF STIMULUS AND RESPONSE DURING DIFFERENTIAL SENSORY LEARNING IN HUMANS: SPATIAL PATTERN ANALYSIS USING PARTIAL LEAST SQUARES. NJ Lobaugh*[1], N Rajah[2], JM Jennings[2], S Houle[3], AR McIntosh[2]; [1]Psychol Research, Hosp for Sick Children, [2]Rotman Research Inst Baycrest Ctr, [3]PET Ctr. Clarke Inst; Univ of Toronto, ON, Canada M6A 2E1

Integration of information between sensory modalities is a key property of nervous system operations and is fundamental to learning and memory. Using PET measures of regional cerebral blood flow (rCBF), we have shown that a tone predicting visual stimuli leads to activation of dorsal extrastriate cortex. To further explore this activation, the present study used a differential associative learning paradigm. Healthy young subjects (n=11) pressed a button to one of two visual stimuli. Two tones were also presented; one (T1) predicted a visual stimulus (target or distractor) and the other its absence (T2). PET rCBF measures were taken during training: the distractor was presented alone on the first and last of 8 scans, and the intervening 6 scans alternated between T1 alone or T2 alone. Differential reaction times (RTs) to the two tones emerged during training. A new image analysis method, Partial Least Squares (PLS, McIntosh, et al, Neuroimage, in press) was used to extract sets of distributed activity patterns related to 1) experimental design, and 2) learning-related changes in RT (brain-behaviour). The first PLS pattern related to design included right parahippocampal and dorsolateral prefrontal cortices, and reflected task acquisition. Dorsal extrastriate regions were part of a second pattern that distinguished tones from visual stimuli; activity of this area may be related generally to conditioned expectancy. A third pattern, related to the predictive significance of T1 and T2, included bilateral posterior thalamus and retrosplenial cortex. In the brain-behaviour PLS, dorsal extrastriate and retrosplenial cortices, and posterior thalamus defined a spatial pattern that differentiated T1 from T2. These three regions may code the signal value of the tones, in preparation for the appropriate response. (Supported by NSERC grant to ARM).

289.3

NEURAL MACHINERY INVOLVED IN WORKING MEMORY: A WHOLE BRAIN FMRI STUDY. VS Mattay*, AKS Santha, J Callicott, A Bertolino, K Tallent, T Goldberg, R Coppola, JA Frank and DR Weinberger. CBDB/NIH, Bethesda, MD

Converging evidence suggests that the prefrontal cortex plays a predominant role in working memory with support from multiple regions of association cortex. We explored the potential of fMRI to outline this distributed network within individuals and to assess the level and variability of recruitment of the different regions involved. We studied eight normal volunteers (4 males and 4 females, mean age =28yrs) with whole brain isotropic multislice gradient echo EPI while they performed two working memory tasks with increasing working memory load (2 back and 2 back with catch) and a sensorimotor control task intermingled with the resting state. Student's t test at 97.5 % confidence level with the probability threshold adjusted by Bonferroni correction for about 15000 cortical voxels in the brain, was used to identify activated voxels. Analysis of the working memory - control task 'activation' maps shows that while there was considerable intersubject variation in the total and regional number of activated voxels, all subjects showed prominent activation in prefrontal cortex [(Brodman areas 9, 10 and 45) ~ 33 % of 'activated' voxels] and parietal cortex [(Brodman areas 5,7, 39 and 40) ~ 45 % of 'activated' voxels]. Additional regions of activation with individual variations included the insular, anterior and posterior cingulate, extrastriate, premotor and supplementary motor regions. The extent of activation increased globally and in the above cortical regions with increasing memory load in all subjects, with individual variations as to which region showed more activation with respect to the other regions. The results display the potential of fMRI to map the neural machinery involved in working memory and also to map the relative individual differences in the level of recruitment of the different cortical areas required in performing working memory tasks. The variation in each individual's activation pattern with increasing working memory load may reflect alteration in the cognitive strategy that is unique to each individual as they adapt to the increased load.

289.4

PREFRONTAL MEDIATION OF EPISODIC MEMORY PERFORMANCE. A.D. Wagner*, J.D.E. Gabrieli, J.E. Desmond, S. Joaquim, and G.H. Glover. Depts. of Psychology and Radiology, Stanford University, Stanford, CA 94305.

Functional neuroimaging studies have revealed activation in right anterior prefrontal cortex (at or near BA 10) during performance of episodic memory tasks. This activation may reflect processes associated with retrieval search and effort, rather than processes associated with retrieval success (e.g., Schacter et al., 1996). Here, prefrontal activation during various levels of episodic memory performance was examined at the individual subject level using fMRI. A levels-of-processing study manipulation served to vary probability of successful yes-no recognition at test. There were three memory conditions: (1) High Performance, consisting of memory judgments for well-learned words; (2) Low Performance, consisting of memory judgments for less well-learned words; (3) New, consisting of memory judgments for unstudied words. In three scans, activation during each of these memory conditions was compared to Baseline (reading unstudied words). In a fourth scan, activation during the High and Low Performance conditions was directly compared. Each scan consisted of 6 memory/baseline cycles during which functional images were collected continuously from 8 coronal slices through prefrontal cortex (y=+15 to +64 mm) using a T2*-weighted gradient echo spiral sequence (1.5T, 6 mm thick slices, 1 mm inter-slice spacing, TR=720, TE=40, flip angle=65). Results revealed that, relative to Baseline, all three memory conditions led to (a) right anterior prefrontal activation, (b) right posterior prefrontal activations (near BA 45, 47, 46, and 9), and (c) in some subjects, left anterior and posterior prefrontal activations. The right anterior activations tended to be stronger in the High vs. Baseline comparison than in the Low vs. Baseline and New vs. Baseline comparisons. However, some subjects demonstrated comparable or greater activation in these latter conditions. Further, the direct comparison of High vs. Low yielded minimal activation differences in all but one subject. These data suggest that right anterior prefrontal activation reflects processes associated with retrieval search and effort, with retrieval context affecting when these processes are engaged. Supported by the NSF and NIH (AG12995).

289.5

EPISODIC RETRIEVAL OF CONTENT AND TEMPORAL CONTEXT ACTIVATE SIMILAR REGIONS OF THE PREFRONTAL CORTEX: A FUNCTIONAL MRI STUDY. LT Eyler Zorrilla,* M D'Esposito, GK Aguirre, E Zarahn. Depts. of Psychology and Neurology, U of Penn, Phila, PA 19104

Lesion studies in humans and animals support a double dissociation between content memory and memory for temporal context, emphasizing the importance of medial temporal lobe (MTL) in the former and lateral prefrontal cortex (PFC) in the latter. In a previous study (unpublished observations) we have confirmed the role of the PFC in judgments of recency using fMRI with healthy volunteers. However, neuroimaging studies of episodic content memory by many researchers have failed to find MTL involvement in retrieval, and instead consistently have shown PFC activation. We therefore directly compared the activation associated with retrieval of temporal context to that seen in retrieval of content in order to determine whether unique regions were activated during performance of either task. Nine subjects learned two lists of words prior to scanning. On recognition trials, subjects indicated whether the word had appeared on either list; on list discrimination trials, they indicated on which list the word appeared. These tasks were performed during fMRI scanning with a 1.5 T magnet. Data were analyzed using a general linear model for correlated observations. No areas of unique activation were revealed when recognition and list discrimination were compared directly. These results suggested that similar regions are activated by retrieval of content and temporal context. This hypothesis was tested directly in an additional subject who performed a sensorimotor control task in addition to the memory trials. Results confirmed that both types of retrieval led to significant increases in activity in the same regions of prefrontal cortex relative to the control. Our findings suggest that similar cognitive processes may be involved in memory for "what" and "when" and that the PFC contributes to episodic retrieval of both content and temporal context. Supported by McDonnell-Pew and NIH grant NS01762.

289.6

FUNCTIONAL MAPPING OF IMPLICIT AND EXPLICIT MEMORY USING REPETITIVE TRANSCRANIAL MAGNETIC STIMULATION (rTMS). **T.A. Blaxton*, E.M. Wassermann, E.A. Hoffman, H.S. Oletsky, M. Hallett, W.H. Theodore. National Institute of Neurological Disorders and Stroke, NIH, Bethesda, MD 20892.**

We used rTMS to map memory function in 11 temporal lobe epilepsy patients with unilateral disease (8 left, 3 right). All were left language dominant as determined by amytal exam. rTMS was delivered during encoding to left and right frontal (speech arrest site and contralateral reflection), mid/superior temporal (1cm above top of ear), and posterior temporal (2-3cm posterior to mid/superior site) regions as subjects named color photographs of objects and read words aloud during 3 s presentions. rTMS was delivered randomly on half of the study trials at 10 Hz and ≤100% of motor threshold intensity. 3050 ms pulse trains began 50 ms before stimulus presentation. Memory for items named correctly during encoding was tested using nonstimulated implicit category member production and explicit semantic cued recall with category label cues. Items were counterbalanced across memory tasks, stimulation conditions, and stimulated brain regions across subjects. Memory was better for pictures than for words on both tests. Comparing memory performance on stimulated versus nonstimulated study trials, rTMS delivered to both left and right frontal regions disrupted explicit recall of pictures. Implicit picture priming (relative to nonstudied baseline) was disrupted by left and right frontal as well as mid/superior temporal stimulation. Posterior temporal rTMS did not affect performance on either memory task, although left posterior temporal stimulation disrupted naming during encoding. These disruption mapping results converge substantially (though not completely) with prior findings from activation mapping studies. **(This work supported by the NIH/NINDS.)**

289.7

AGE-RELATED CHANGES IN REGIONAL CEREBRAL BLOOD FLOW (rCBF) DURING MEMORY ENCODING AND RETRIEVAL. R. Cabeza*, E. Tulving, A. R. McIntosh, C. L. Grady, J. M. Jennings, S. Kapur, L. Nyberg, S. Houle, F. I. M. Craik. Rotman Research Institute of Baycrest Centre, University of Toronto

Changes in rCBF in healthy young and older adults were measured by positron emission tomography (PET) while they were either reading, encoding, recognizing, or recalling word pairs. Compared to reading, encoding in young subjects was associated with higher rCBF (activation) in the left prefrontal, medial temporal, and anterior cingulate regions. Activation in these regions was weaker in old adults. Areas more active during recognition than during reading in young subjects were the right prefrontal, anterior cingulate, and inferior parietal cortices. Older subjects activated these regions to a lesser degree, but activated the precuneus region, which was not active in young subjects. Compared to reading, recall in young subjects was associated with activity in right prefrontal, anterior cingulate, parietal, lenticular, thalamic, and cerebellar regions. Older subjects showed less activity in these regions, but activated a left prefrontal region that was not active in young subjects. In sum, old subjects showed weaker activations than young subjects in several regions, including frontal, medial temporal, anterior cingulate, and parietal regions, but activated some regions that were not active in young subjects. The first result is consistent with evidence of neural decay in the aging brain. The second result suggests a reorganization of brain function, like the one observed after brain damage. Since old subjects performed as well as young subjects, despite their weaker activations, this functional reorganization may reflect a compensatory mechanism.

This study was supported by a fellowship of the International Human Frontier Science Program to R. Cabeza.

289.8

THE NEURAL BASIS OF VISUAL SKILL: AN FMRI STUDY OF MIRROR READING. R. A. Poldrack*[1], J. E. Desmond[1,2], G. H. Glover[2], and J. D. E. Gabrieli[1]. Departments of Psychology[1] and Radiology[2], Stanford University, Stanford CA, 94305

The reading of mirror-reversed text has been extensively examined as an example of perceptual-motor learning, but its neural basis has remained poorly understood. fMRI was used to examine the brain systems involved in mirror reading performance. Mirror-reversed and normal letter strings were presented in alternating blocks of 10 items. Half of the strings in each block were words and half were pronounceable pseudowords. Subjects performed a lexical decision for each item, indicating whether it was a word or not.

Functional data were acquired from ten 6mm coronal slices at 1.5 T using a gradient echo spiral sequence (TR = 900, TE = 40, flip angle = 70°). A method for online registration was used to place the slices from Y = -25 mm to Y = -95 mm in Talairach space. Data were analyzed using the cross-correlation method described by Friston et al. [Human Brain Mapping, 1994]. Two scans were performed for each subject, each with a unique word list.

Comparison of mirror reading to normal text reading revealed several areas of significant ($p < .01$) activation consistently across subjects. There was extensive activation bilaterally in the middle and superior occipital gyri (corresponding to Brodmann's areas [BA] 18 and 19). There was also consistent right parietal activation in the superior parietal lobule (corresponding to BA 7) and less extensive activation in this area on the left. There were no extensive activations for normal text reading compared to mirror reading. These data suggest that multiple visual and visuospatial systems are involved in mirror reading, and that these systems are a superset of those involved in the processing of normal text.

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289.9

FUNCTIONAL MRI STUDIES OF MENTAL ROTATION AND OBJECT IDENTIFICATION PROCESSES. B. Rypma*, M.A DeBell, J.D.E. Gabrieli, V. Prabakharan, M.F. Zabinski, J.E. Desmond, G.H.Glover. Depts. of Psychology and Radiology, Stanford Univ., Stanford, CA 94305.

Recent brain imaging studies of mental rotation have shown that parietal lobe is activated during mental rotation. We sought to determine the role of parietal mental rotation mechanisms in object identification by contrasting tasks which produce angle-dependent mental rotation functions (determination of the left-right, or parity, status of objects) with tasks that do not produce mental rotation functions (object identification). In the present study, brain regions responsible for mental rotation and object identification were mapped using functional Magnetic Resonance Imaging. Each subject was scanned three times in tasks that required subjects to either determine if two misoriented Snodgrass-Vanderwart pictures had the same or different identities or to determine if the two objects would face the same or different directions. Each of these tasks was compared against a sensorimotor control task in which subjects were required to press a button as soon as an object pair appeared on the screen. Brain images were collected in 10 oblique coronal planes perpendicular to the AC-PC line. FMRI was obtained with T2* -weighted gradient echo spiral pulse sequence (1.5T, TR=900, TE=40, flip angle=70 degrees, interslice spacing=1.5 mm, slice thickness=5.0 mm). Activations associated with determining the identity status of the two objects was strongest in temporal lobe and cerebellar regions. Activation associated with parity determination was also found in temporal and cerebellar regions but was considerably greater in parietal regions. These results suggest (1) that processes related to mental rotation and identity determination may be independent and (2) that brain regions associated with mental rotation may be activated mainly when tasks require parity determination but minimally activated when subjects are required to determine the identity of objects.

This research was funded by grants from NIH and NIA.

289.10

BLOOD FLOW INCREASES IN LEFT DORSAL PREMOTOR CORTEX DURING SENSORIMOTOR INTEGRATION LEARNING. M. Iacoboni, R.P. Woods, J.C. Mazziotta*. UCLA Brain Mapping Division, Neuropsychiatric Institute, UCLA School of Medicine, Los Angeles, CA 90095.

We have previously observed that learning in visuo-motor integration produces blood flow increases in left prefrontal, dorsal premotor, and motor areas in the human brain. We have also observed that visuo-motor learning is not associated with blood flow decreases (Iacoboni et al., J Neurophysiol, in press).

Here we investigate blood flow changes associated with practice in a sensorimotor task in which auditory stimuli were used. We measured reaction times and regional cerebral blood flow (rCBF) changes with positron emission tomography (PET) and $H_2{}^{15}O$ in seven normal subjects performing a response choice task to lateralized tones of 1000 Hz. Twelve rCBF measurements were performed in each subject. Reaction times shortened with practice ($p<0.04$). Serial rCBF increases fitting the reaction time slope ($p<0.05$, corrected for multiple spatial comparisons) were observed in the dorsal premotor cortex of the left hemisphere. No significant rCBF decreases were observed.

Our data suggest a modality-independent role of the left dorsal premotor cortex in human sensorimotor integration learning. Our data also support the notion that, at variance with other types of learning frequently associated with blood flow decreases (Raichle et al, Cereb Cortex 1994; 4: 8-26), human sensorimotor integration learning seems to be associated with blood flow increases only.

Supported by International Human Frontier Science Program.

289.11

FUNCTIONAL NEUROANATOMY OF MOTOR SEQUENCE LEARNING IN HUMANS: A PET STUDY. M. Honda*, M-P. Deiber, V. Ibañez, P. Zhuang, C. Toro, M. Hallett. Human Motor Control Section, NINDS, NIH, Bethesda, MD 20892

The serial reaction time paradigm (SRT) involves implicit and explicit motor sequence learning. To examine the neural basis of each learning process, we studied 12 healthy right-handed volunteers using positron emission tomography (PET). The modified SRT was employed in which each session consisted of 10 repetitions of a test sequence of 10 visually presented numbers. Subjects were requested to push a separate button in response to each number. Sessions including the same test sequence were repeated 7 times in total. The effect of explicit and implicit learning was assessed separately by the correct generation of the test sequence after each session and the RT change without any successful generation of the sequence, respectively. Regional cerebral blood flow during each session was measured by PET scanning using $H_2{}^{15}O$ and analyzed in correlation with each behavioral indicator.

The correlation map with correct generation of the sequence revealed significant involvement of bilateral inferior parietal lobes, precuneus, premotor cortices, dorsal prefrontal cortices, and contralateral SMA and cingulate cortex, in the explicit learning. In contrast, the correlation map with RT during pure implicit learning phase showed significant involvement of the contralateral primary sensory-motor cortex adjacent to the hand representation. These results suggest that the different cortical areas are involved in the two different kinds of motor sequence learning.

289.12

A PET STUDY OF MOTOR MEMORY CONSOLIDATION. R Shadmehr*, HH Holcomb, K Akhavan-Toyserkani. Depts. Biomedical Eng. & Radiology, Johns Hopkins Univ., Baltimore, MD 21205, & Maryland Psychiatric Res Cent, Univ. Maryland, Baltimore, MD 21228.

Elsewhere in this volume we report psychophysical evidence that learning a motor skill (control of arm movements in a haptic field) sets in motion neural processes that continue to evolve after practice has ended. The consolidation of the skill could be disrupted within a window of a few hours after initial learning, but not after that period. Here we report on the changes in the neural representation of the skill during this consolidation period. PET was used to measure rCBF of 8 subjects during 5 conditions: moving the robot in a null field, in a random field, early learning of a curl field, late learning of the curl field, and 6 hours later in the same field. Based on previous results, during the 6 hours a consolidation process may have taken place. In comparing the rCBF while subjects were moving in the field in the late stage of learning vs. when same movements were performed 6 hours later, we found a significant reduction bilaterally in the dorsolateral prefrontal cortex ($z = 4.82$ and $z = 4.54$, corrected $p < 0.006$ and $p < 0.02$ for right and left sides respectively, SPM95). In comparison, bilateral reductions were not observed in a separate study of 8 subjects where a similar number of movements in the field were performed without the passage of 6 hours (Brashers-Krug et al, in prep). One possibility is that the prefrontal lobe may play a critical role in the initial binding of visual to motor information, but that with time, this role may be lessened as more direct connections between the visual and motor regions come to represent the learned skill. Funded by Whitaker Foundation, NIH, and ONR.

PARKINSON'S DISEASE

290.1

CONTROVERSIAL ISSUE: BENEFICIAL EFFECTS OF NITRIC OXIDE RADICALS (NO) IN THE CENTRAL NERVOUS SYTEM. C.C. Chiueh, P. Rauhala, & I. Sziraki*. Unit on Neurotoxicology & Neuroprotection, Laboratory of Clinical Science, NIMH, NIH, 10/3D-41, Bethesda, MD 20892

It has been proposed that free radicals may be involved in degenerative brain disorders such as idiopathetic Parkinson's disease and MPTP- or 6-OH-dopamine-induced parkinsonism. Similar to MPP+, oxidant stress-induced nigral injury has been consistently demonstrated *in vivo* with hydroxyl radicals (OH) generated by iron complexes [i.e., ferrous citrate & sodium nitroferricyanide (sodium nitroprusside)].

Intracerebral infusion of NO or NO donor compounds did not induce lipid peroxidation and neuronal death *in vivo*. In fact, NO released from non-iron containing NO donors protected nigral neurons against oxidative nigral injury elicited by OH generated by ferrous citrate because exogenous NO is a potent inhibitor of brain lipid peroxidation catalyzed by submicromolar iron (Rauhala et al., *Free Radical Biol. Med.* in press). Moreover, NO also suppressed sodium nitroprusside-induced brain lipid peroxidation through inhibition of OH generation.

In conclusion, NO may be a potent endogenous antioxidant to protect iron-containing nigral neurons against progressive free radical damage. Debate of the controversial issue of whether NO radica ls friend or foe to injured brain neurons (Choi, 1993) could alter current concept for developing neuroprotective strategies and agents. (supported by NIMH IRP: Z01-MH-02648-04 LCS)

290.2

INHIBITION OF NEURONAL NITRIC OXIDE SYNTHASE PREVENTS MPTP INDUCED PARKINSONISM IN BABOONS. P. Hantraye*, E. Brouillet, R. Ferrante, S. Palfi, R. Dolan, R.T. Matthews, M.F. Beal. URA CEA-CNRS 2210, SHFJ, DRIPP, DSV, Orsay FRANCE, GRECC, VA medical Center, Boston University School of Medicine, Bedford MA 02118 and Neurochemistry Lab, Neurology Service, Mass Gen Hosp and Harvard Med Scholl, Boston MA02114.

MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) produces clinical, biochemical and neuropathologic changes reminiscent of those which occur in Parkinson's disease (PD). 7-nitroindazole (7-NI) is a relatively selective inhibitor of the neuronal isoform of nitric oxide synthase (NOS) which blocks MPTP neurotoxicity in mice. We now show that 7-NI protects against profound striatal dopamine depletions and loss of substantia nigra tyrosine-hydroxylase positive neurons in MPTP treated baboons. The use of an automated video-based movement analysis system showed that 7NI completely blocked the severe hypokinesia and bradykinesia induced by MPTP. Furthermore, the use of the Object retrieval detour task, a task dependent on the functional integrity of the frontostriatal pathway and highly sensitive to striatal dopamine depeletion, demonstrated that while MPTP animals were unable to perform, MPTP/7NI or 7NI treated animals showed responses similar to control animals. These results strongly implicate a role of nitric oxide in MPTP neurotoxicity, and suggest that NOS inhibitors selective of the neuronal isoform might be useful in treating PD. Funded by CEA, CNRS, NS-16367, NS-10828, NIH AG-12922-01.

290.3

NEURAL EFFECTS AND REGULATION OF TRANSCRIPTION OF GDNF, A TGFß-SUPERFAMILY MEMBER. C. Suter-Crazzolara*, L. Farkas T. Lenhard and K. Unsicker. Dept. of Anat. & Cell Biol., Univ. Heidelberg, INF 307, D-69120 Heidelberg, Germany.

GDNF is a potent neurotrophic factor for several neuron populations in the CNS and PNS. We shall present data concerning mechanisms that may be involved in the protective actions of GDNF. To test the hypothesis that GDNF may act in part through the regulation of $Ca2^+$-binding proteins we have begun to study effects of GNDF on calretinin (CalR) expression in embryonic striatal and toxically impaired hippocampal neurons. We show that GDNF induces, de novo, CalR immunoreactivity in striatal neurons and elevates CalR mRNA in glutamate-exposed hippocampal neurons. Similar, but weaker effects can be elicited by NT-3, NT-4, FGF-2 and CNTF. Another important aspect of the physiological actions and putatative therapeutic applicability of GDNF concerns its regulation of expression in glial cells, a principal source of GDNF. Towards this end, we have studied regulation of GDNF mRNA in C6 glioma cells by a large battery of cytokines, hormones, and neuropeptides using a competitive PCR approach. Our data suggest that FGF-2, in contrast to many other molecules, can up-regulate GDNF mRNA levels. Finally, to analyze the regulation of GDNF at molecular levels in more detail, we have begun to study the regulatory promoter elements of the human GDNF gene. **Supported by DFG (Un34/16-2)**

290.4

CHANGES IN SPONTANEOUS MOTOR BEHAVIORS ASSOCIATED WITH ANATOMICAL DECREASES IN IMMUNOHISTOCHEMICAL AND NEUROCHEMICAL INDICES OF DOPAMINE IN 6-HYDROXYDOPAMINE TREATED RATS. C.G. van Horne*, M. Hebert, S. Eken, G.A. Gerhardt, and B.J. Hoffer. Dept. of Pharmacology, U. of Colorado H.S.C., Denver, CO. 80262, and Dept. of Neurosurgery, Brigham and Women's Hospital, Boston, MA. 02146

Previous studies of the unilateral 6-hydroxydopamine (6-OHDA) lesion model of Parkinson's disease (PD) have focused primarily on drug-induced rotational behaviors related only to changes in the nigrostriatal portion of the ascending midbrain dopaminergic system. Therefore, we investigated spontaneous motor behaviors as well as drug induced rotations and measured immunohistochemical and neurochemical alterations in the ventral tegmental area (VTA) and ventral striatum/nucleus accumbens (VS/NA) in addition to the nigrostriatal pathway. Our objectives were to produce both full and selective lesions and to compare the behavioral changes to the anatomical distribution and neurochemical extent of the lesions. Male Fischer 344 rats were injected with 6-OHDA into either the medial forebrain bundle (full lesions) or into the cell body region of the substantia nigra (SN, selective lesions). Apomorphine-induced (0.05mg/kg, s.c.) rotational behavior was recorded for 90 minutes. Spontaneous activity was recorded in 10 minute bins for 60 minutes in automated activity chambers (Omnitech Inc.) and reported as activated activity (AA, first 10 minutes) and habituated activity (HA, a 10 minute average of the last 3, 10 minute bins). The density of tyrosine hydroxylase (TH) immunohistochemical staining was evaluated by image analysis for the SN, VTA, VS/NA, medial dorsal striatum (DS), and lateral DS. Whole tissue levels of dopamine (DA) in the SN, VTA, NA, and striatum were determined by HPLC. The fully lesioned group had significantly greater rotations compared to the selective group and also demonstrated significantly reduced HA scores for total distance, cm/movement, movement number, and stereotypy. Rearing activity was significantly lower during the AA and there was no significant difference in velocity for either time point. Although the selective group showed significantly greater TH densities in the VS/NA and VTA compared to the fully lesioned group, significantly higher levels of DA, however, were only found in the VTA and not the VS/NA. Thus, several differences in the behavioral profiles of the fully and selectively lesioned rats are associated with anatomical variations in the density of TH staining and DA levels. (Supported by NS09199)

290.5

TIME-COURSE OF STRIATAL, PALLIDAL AND THALAMIC α1, α2 AND β2/3 $GABA_A$ RECEPTOR SUBUNIT CHANGES INDUCED BY UNILATERAL 6-OHDA LESION OF THE NIGROSTRIATAL PATHWAY. H.J. Caruncho*, I. Liste, G. Rozas, E. Lopez, M.J. Guerra and J.L. Labandeira. Dept. of Morphological Sciences. Univ. of Santiago School of Medicine. 15705-Santiago de Compostela. Galicia. Spain.

A semiquantitative immunocytochemical analysis of several $GABA_A$ receptor subunits in the brain of unilaterally 6-OHDA lesioned rats showed an increase in the lesioned striatum for the α2 and β2/3 subunits 3 and 7 days after the lesion when compared with the unlesioned side, whereas after 4 and 12 weeks of lesioning these changes were not significant. The same was observed for the α1 subunit that was decreased in the ipsilateral globus pallidus. In addition, the α1 subunit was decreased in several relay thalamic nuclei in the lesioned side, whereas the α2 subunit was increased in the intralaminar thalamic nuclei of the lesioned side. Again, these changes were apparent 3 and 7 days after the lesion but dissapeared in samples from rats sacrifized 4 and 12 weeks after the lesion. These results suggest the existence of an early adaptation to the lesion in terms of changes in $GABA_A$ receptor expression. The compensation of these changes after 4 or 12 weeks is due not only to a partial reversion of the changes in the lesioned side but also to changes in the unlesioned side in comparison with the expression found in control (unlesioned) rats. Supported by grants from XUGA and the spanish DGICYT.

290.6

ANTIPARKINSONIAN ACTIVITY OF THE NMDA2B ANTAGONIST RO 25-6981 IN RATS

P.-A. Löschmann, U. Wüllner*, J.B. Schulz, G. Fischer[1], J.A. Kemp[1], T. Klockgether, University of Tübingen, Dept. of Neurology, 72076 Tübingen, Germany; [1]Hoffmann La Roche Ltd, Preclinical Research, 4070 Basel, Switzerland

Experimental and clinical evidence indicate that degeneration of nigral dopamine (DA) neurons leads to secondary changes of neuronal activity in the basal ganglia involving increased glutamatergic excitation in the striatum and the basal ganglia output nuclei. Competitive antagonists of the NMDA subtype of glutamate receptors potentiate the antiparkinsonian activity of direct DA agonists or L-DOPA in MPTP treated primates and 6-OHDA lesioned rats. However, unselective inhibition of NMDA receptors throughout the central nervous system limits the therapeutic benefit of such compounds and leads to side-effects such as ataxia and psychosis. A more specific approach would involve the selective inhibition of glutamatergic pathways overactive in Parkinson's disease. The message for the 2B subunit of the NMDA receptor is differentially expressed in the brain with high densities within the basal ganglia. Ro 25-6981 has been characterized as a non-competitive antagonist with selectivity for the NMDA receptors containing the NR2B subunit (see Trube et al., this meeting). This compound has no affinity for the known sites of non-competitive antagonists such as PCP or MK-801. In 6-OHDA substantia nigra lesioned rats, Ro 25-6981 alone induced contralateral rotations while the competitive non-selective NMDA antagonist CPP had no effect. Contralateral rotations induced by direct DA agonists such as apomorphine or lisuride or L-DOPA (plus benserazide) were dose-dependently potentiated by co-treatment with Ro 25-6981. Compared to CPP, this effect was observed over a wider range of doses. NMDA2B antagonists could be useful adjuvants for treatment of Parkinson's disease with a reduced propensity to induce side effects known for unselective NMDA antagonists.

290.7

NEURONAL CELL DEATH BY APOPTOSIS *IN VIVO* IN THE 1-METHYL-4-PHENYL-1,2,3,6-TETRAHYDROPYRIDINE (MPTP) MOUSE MODEL OF PARKINSON'S DISEASE (PD).

A.M. Janson*. Dept. of Neuroscience, Karolinska Institutet, Doktorsringen 17, S-171 77 Stockholm, Sweden.

Apoptosis may represent an important mechanism of cell death in some experimental models of neurodegeneration as well as in human neurodegenerative disorders. Although the MPTP mouse model of PD has been studied extensively over the last decade, the mechanism by which the neuron ultimately dies remains unknown.

A single dose of MPTP (40 mg/kg, sc.) was given to 10 weeks' old male C57 BL/6 mice. The nuclear alterations were studied in midbrain sections and analyzed with stereological unbiased quantitative techniques at various time points after the lesion. Two different in situ labeling techniques analyzing DNA fragments were compared. The first one uses DNA polymerase I, which detects the 3'-OH-ends of single-stranded DNA breaks. The second technique employs terminal deoxynucleotidyl transferase labeling the 3'-OH-ends of both single- and double-stranded DNA breaks.

The results for the first time indicate a role of apoptotic-like cell death mechanisms in the MPTP mouse model *in vivo*, possibly also related to the pathogenic mechanism in PD.

Supported by the Swedish Medical Research Council (grant 10816).

290.8

PET IMAGING REVEALS REDUCED [C-11](+)McN5652 BINDING TO 5-HT TRANSPORTERS IN PARKINSON'S DISEASE.

Z. Szabo*, T. Preziosi, R. Hoehn-Saric, U.Scheffel, S. Palmon, W.B. Mathews, H.T. Ravert, R.F. Dannals. Departments of Radiology, Neurology and Psychiatry, The Johns Hopkins University. Baltimore, MD 21205.

Post-mortem studies indicate multisystemic (DA, 5-HT, NE, acetylcholine) damage in Parkinson's disease (PD). The purpose of this study was to assess deficits in 5-HT transporters (5-HTT) in the brain of living PD patients by measuring the binding of the 5-HTT specific [C-11](+)McN5652[1] using positron emission tomography (PET). Six healthy subjects and four patients with mild PD were injected with 18±3 mCi high specific activity[C-11](+)McN5652. Sequential PET scans were acquired over 95 minutes and regional time-activity curves were processed using metabolite corrected arterial plasma activity as the input function. The impulse response function at 75 minutes post injection (*irf*) was calculated by matrix regularization to represent radioligand retention. Compartmental parameters of uptake (K_1), release (k_2) and distribution volume ($DV = K_1/k_2$) were obtained as well.

In PD both K_1 and *irf* were reduced while k_2 was increased. Reduction of radioligand uptake (K_1) was more prominent in cortical (27-45%) than subcortical regions (15-27%). By contrast, increases in radioligand release (k_2) were more pronounced in subcortical (180-980%) than cortical regions (44-107%). In subcortical regions (pons, hypothalamus, caudate nucleus, putamen, and thalamus) reduction in *irf* exceeded reduction in K_1. By ANOVA, the variance of *irf* was partially attributable to K_1, but the main effect of disease remained significant after removal of the covariance effect of K_1 ($F=21.7$, $p<0.001$). Thus, both delivery and specific binding of [C-11](+)McN5652 are reduced in PD. In brain areas abundant in 5-HTT reduction of specific binding exceeds reduction of delivery, providing evidence for decreased 5-HTT in PD.

[1]McN5652 = 1,2,3,5,6,10b-hexahydro-6-[4-(methylthio)phenyl]pyrrolo [2,1-a]isoquinoline.

(Supported by the Johns Hopkins PET Center)

290.9

TRANSGENIC MICE WITH INCREASED NUMBER OF DOPAMINE TRANSPORTERS (DAT) SHOW GREATER SENSITIVITY TO MPTP. V. Kostic[1], D. Donovan[2], R. Yokoyama[1], S. Przedborski[1]*, and G.R. Uhl[2]. [1]Dept. Neurol., Columbia Univ., New York, NY 10032; and [2]NIDA-IRP and Dept. Neurol. & Neurosci., JHUSM, Baltimore, MD 21224.

MPTP damages dopamine (DA) neurons of the substantia nigra pars compacta (SNpc) as seen in Parkinson's disease. A major step in the MPTP toxic pathway is the selective accumulation of its active metabolite MPP+ in DA neurons by DAT. Blockade of DAT prevents MPTP effects, but whether more DATs can enhance MPTP DA toxicity in vivo is unknown. Thus, we assessed MPTP DA toxicity in transgenic mice expressing the rat DAT gene in catecholamine neurons. These mice exhibit increased SNpc [3H]mazindol binding compared to their non-transgenic littermates, thus indicating that these transgenic mice have greater SNpc DAT density. Adult mice were injected with MPTP (15 mg/kg, 4q2 hr) and 1 week after the last injections the number of tyrosine hydroxylase (TH)-positive neurons through the entire SNpc was counted. This MPTP regimen caused ~34% reduction in SNpc TH-positive neuron number in the non-transgenic animals. However, this MPTP regimen caused ~50% reduction in SNpc TH-positive neuron number in the transgenic mice compared to saline-injected controls. The difference in the percent of MPTP-induced SNpc cell loss between the transgenic and non-transgenic mice is highly significant ($p<0.01$). This study demonstrates that increased density in DAT enhances the susceptibility of SNpc DA neurons to MPTP toxicity. This finding may have major implications for Parkinson's disease.

This work is supported by the NINDS, MDA, PDF, and Lowenstein Foundation.

290.10

STIMULATION THRESHOLD ANALYSIS REDUCES RISK TO INTERNAL CAPSULE IN PALLIDOTOMY PROCEDURES. R.C. Frysinger*, J. M. Bronstein, E. Behnke, T. Fields and A. De Salles. *Depts. of Neurobiology, Neurology, Surgery and the Brain Research Institute, UCLA Med. School, Los Angeles, CA 90095.* Accurate placement of radio frequency lesions in the globus pallidus internus (GPi) is required for maximal remission of motor symptoms of Parkinson's disease (PD) and avoidance of damage to the neighboring internal capsule (IC) and optic radiations (OR).

We performed intraoperative stimulation at up to 5 sites along each of 35 electrode penetrations in 31 non-demented patients aged 34-77 years with medically refractory PD undergoing ventroposterior pallidotomy (trains of constant voltage biphasic 1 msec pulses at 2 Hz and 50 Hz). Voltage was increased at each frequency to a limit of 4 volts or to a perceptible motor response. Phasic motor activation at 2Hz was assumed mediated through IC. One case showed transient post-operative facial weakness after a lesion at a site with a 2 Hz threshold of 1V. The subsequent 28 cases had 2 Hz thresholds above 1.5 volts (mean=2.73V, s.d=±0.65), and a good clinical response free of symptoms of IC damage. Positive PD signs at 50 Hz were assumed mediated through GPi. Thresholds below 3V were consistently associated with positive outcomes (2.33, ±0.95V). Proximity to OR was indicated by visual phenomena associated with either frequency at less than 4 V amplitude. Threshold analysis in conjunction with accurate and flexible MR based stereotactic targeting can help to simplify the pallidotomy protocol and minimize the number of electrode penetrations required. *Supported by NIH NS01596 and an AAN Research Fellowship to JMB*

290.11

Anatomical Configuration of Fetal Mesencephalic Grafts in
MPTP Monkeys R.A.E. Bakay * Emory University School of Medicine, Atlanta GA 30322

The organotypic patterns of tyrosine hydroxylase (TH) reactive cells in successful mesencephalic grafts is well established. The location of these cells in the outer edges of the graft has not been explained.The center of the graft contain the other cells in the mesencephalon. Immunocytochemical studies document the cells to be primary GABA immunoreactive. Cellular pattern and connections resemble Substantia Nigra pars reticulata.

NIH

290.12

CORRELATION ANALYSIS OF MULTINEURON RECORDINGS IN HUMAN THALAMUS AND GLOBUS PALLIDUS. J.M. Hurtado, C.M. Gray*, L.B. Tamas, K.A. Sigvardt, S. Starsinic, H. Richard and E.A. Franz. Center for Neuroscience, Univ. of Calif., Davis, CA 95616 and Pacific Neurosciences Institute, Lafayette, CA 94549.

The neurosurgical treatment of the symptoms of Parkinson's disease by thalamotomy and pallidotomy incorporates electrophysiological recording of neuronal activity to verify the site of lesion placement. We have developed and employed a method for recording from two independently movable microelectrodes or tetrodes during this procedure. This technique increases the accuracy of the mapping protocol while also decreasing its duration. Moreover, it has provided the first opportunity to investigate the incidence and properties of both local and long-range interactions among the neurons in these structures. The data collected to date demonstrates a wide range of neuronal firing characteristics in the Putamen, GP_e, and GP_i of the Basal Ganglia, and D_m, V_m, and V_c of the Thalamus. Cells in these various regions can be distinguished on the basis of the incidence and properties of rhythmic firing, the incidence and magnitude of synchronized firing, as well as spontaneous firing rates, propensity to fire in bursts, action potential duration, and responses to kinesthetic or tactile stimulation. Cells that rhythmically fire at tremor frequencies of 4-6 Hz, as well as higher frequencies of 10-20 Hz, synchronize their activity with little or no average phase lag. Supported by NIH (CMG, KAS) and NSF (CMG).

290.13

EFFECTS OF VISUAL INFORMATION AND PRACTICE ON NORMAL AND PARKINSONIAN SUBJECTS. W. A. Hening*[1,2], M. Rolleri[2], and J. Gordon[3,4]. Neurology Depts., UMDNJ-RW Johnson Med Sch[1], New Brunswick, NJ and VAMC[2], Lyons, NJ; Ctr for Neurobiology & Behavior[3], Columbia Univ, NYC; and NY Medical College[4], Valhalla, NY

Background: Some investigators have reported reduced accuracy when Parkinson's disease patients (PDPs) make movements without visual task information. In previous work, we found that PDPs achieved normal accuracy when allowed practice. In those studies, however, subjects were given some practice with visual input in each session before testing We now asked whether practiced PDPs would be innaccurate if required to make movements when a session began without visual input. Design/Methods: 8 PDPs (Hoehn & Yahr stages I[1], II[5], and III[2]) tested at end of medication doses and 9 controls, all experienced, responded to 4 targets presented on a computer monitor requiring hidden movements of 8 to 32 cm over a digitizing tablet. Blocks had either simple (S: 5 of each successively) or choice (C: 20 randomized targets) trials. The first 4 blocks (2 S, 2 C) presented minimal task information (MTI): no knowledge of results and no screen feedback of hand position or display of target after the "go" signal. Subjects were then given 40 practice trials with visual information, followed by further S and C blocks with MTI. Results: PDPs were less accurate than controls, but the difference was significant only before the visual information. Both subject groups undershot the large target, while PDPs used more in-course corrections. Conclusions: When MTI is presented before practice with visual information, even experienced PDPs are less accurate. PDPs use more trajectory updating to improve accuracy, adaptively using slower movements. For all subjects, accuracy is compromised before practice.

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290.14

IMPAIRED TACTILE DISCRIMINATION OF GRATING ROUGHNESS AND ORIENTATION IN PARKINSON'S DISEASE. K. Sathian*, A. Zangaladze, J.L. Vitek & M.R. DeLong
Dept. of Neurology, Emory Univ. Sch. Med., Atlanta, GA 30322.

Somatosensory abnormalities, both tactile and kinesthetic, have been reported in Parkinson's disease (PD) by Schneider et al. (Neurology, 37:951-6, 1988). We explored this issue further in a psychophysical investigation of tactile perception at the index fingerpad in patients with PD, who were candidates for or had undergone pallidotomy. We used gratings of alternating ridges and grooves, which have proven useful in characterizing tactile perception psychophysically and neurophysiologically (Sathian, TINS, 12:513-519, 1989).

Three tasks were used to assess discrimination thresholds, corresponding to 75% correct performance in two-alternative, forced-choice paradigms. One task measured spatial acuity as the groove width required to discriminate the orientation of gratings impressed into the immobilized fingerpad. In the other two tasks, subjects actively scanned gratings that differed in either groove or ridge width, and their ability to discriminate grating roughness was assessed. Patients with PD were impaired on all three tasks, relative to age-matched normal controls. Our results imply a general impairment of tactile perception in PD and provide impetus to investigation of its neural basis.

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291.1

LOCALIZATION OF A FINE-GRAINED CATEGORY OF SHAPE: AN EXTRASTRIATE LETTER AREA REVEALED BY fMRI. M. J. Farah[1], T. A. Polk[1], M. Stallcup[1], G. K. Aguirre[2], D. Alsop[3], M. D'Esposito[2], J. Detre[2]*, and E. Zarahn[4]. Depts. of Psychology[1], Neurology[2], Radiology[3], and Neuroscience[4], Univ. of Penn., Philadelphia, PA 19104.

The principle of localization of function has been extended within the visual system to include cortical areas dedicated to perceptual categories such as color, motion, and even face perception. Are finer category distinctions also localized, i.e. represented in segregated regions, or are they intermixed in a distributed manner within a system of coarse-grained localization? We used fMRI to study the degree to which shape representation is localized or distributed at the level of the categories letter, digit, and geometric shape (e.g. triangle, circle, star) in left occipitotemporal cortex.

Five normal right-handed subjects passively viewed two sets each of strings of letters, digits, geometric shapes and fixation points, each displayed for 150msec, one per second, in eight 40s blocks per run (1.5T, TR 2, 5" surface coil over left occipitotemporal cortex). Multiple runs per subject were collected (with the block order counterbalanced across runs) and data were analyzed using a variant of the general linear model for correlated observations proposed by Worsley & Friston (1995). Statistically significant segregation was observed in individual subjects. Letter-specific and shape-specific areas were observed in most subjects within the left occipitotemporal region under study ($p<0.05$, corrected). No digit-specific areas were observed in any subjects in this region. Some areas were significantly activated by all three stimulus types compared with fixation ($p<0.05$, corrected).

These results demonstrate that in some individuals there is segregation of cortical shape representation according to the category of shape, with letters being represented in areas that do not represent simple geometric shapes or even digits and geometric shapes being represented in areas that do not represent letters or digits. The fact that letters and digits cannot be distinguished by their visual features suggests that experience plays a role in establishing the segregation of letter processing.

Supported by the Krasnow Institute, McDonnell-Pew, and NINDS.

291.2

ABSTRACT, NOT VISUAL, ORTHOGRAPHIC KNOWLEDGE ENCODED IN EXTRASTRIATE CORTEX: AN fMRI STUDY. T. A. Polk[1], M. Stallcup[1], G. K. Aguirre[2], D. Alsop[3], M. D'Esposito[2], J. Detre[2], E. Zarahn[4], and M. J. Farah[1]*. Depts of Psychology[1], Neurology[2], Radiology[3], and Neuroscience[4]. Univ. of Penn., Philadelphia, PA 19104.

In a previous PET study, Petersen et al. (*Science* 249:1041, 1990) found areas of left medial extrastriate visual cortex that were significantly more activated by passive viewing of words and pseudowords than consonant strings. Are these areas responding to the *visual* form of orthographically legal stimuli or to their *abstract* form—the specific sequence of abstract letters they contain, independent of their visual form (case, font, size, etc.)? These alternatives can be distinguished by the use of aLtErNaTiNg case stimuli, which correspond to naturally occurring abstract, but not visual, word forms.

During an fMRI session, subjects passively viewed two sets each of concrete words, pronounceable pseudowords, consonant strings, and fixation points, each displayed for 150msec, one per second, in eight 40s blocks per run (1.5 T, TR 2, 5" surface coil over left temporal cortex). Multiple runs per subject were collected (with the block order counterbalanced across runs) and data were analyzed using a variant of the general linear model for correlated observations proposed by Worsley & Friston (*Neuroimage* 2:173, 1995). Left medial extrastriate visual areas were found to be significantly more activated by alternating case words and pseudowords than consonant strings in individual subjects ($p<0.05$, corrected).

These results demonstrate that the so-called "word form" area in extrastriate cortex encodes abstract word forms based on orthographic regularities, rather than just the visual form of word-like stimuli.

Supported by the Krasnow Institute for Advanced Study, the McDonnell-Pew Program in Cognitive Neuroscience, and the National Institute of Neurological Disease and Stroke.

291.3

FLUENCY ASSOCIATED WITH SIZE OF BROCA'S AREA IN SCHIZOPHRENIA. L.L. Phillips[a], J.M. Kuldau[a,b], E.R. Gautier[a], P. Zuffante[c], R.M.Bauer[c], M.J. Byerly[b], A.A. Mancuso[d] & C.M. Leonard[a]. Depts. of Neuroscience [a], Psychiatry[b], Health & Clinical Psychology[c] & Radiology[d], University of Florida, Gainesville, FL 32610.

Previous studies have suggested an involvement of the frontal lobes in the deficits of verbal fluency and other cognitive abilities associated with schizophrenia. This study used magnetic resonance imaging to investigate the relationship of pars triangularis, a frontal lobe structure involved in the generation of speech, with measures of verbal fluency (FAS), phonemic awareness, IQ and the positive and negative symptoms (PANSS) of 26 male schizophrenic patients (mean age = 40.3). Other brain measures included the midsagittal area, right and left hemispheric widths, brain length, corpus callosum area and planum temporale surface. Analyses revealed no significant differences in any of the anatomical variables from those of 17 age and sex matched controls. As expected, the schizophrenic subjects had significant deficits on tests of phonemic awareness, fluency, and IQ. Only pars triangularis demonstrated a significant relationship with any of the cognitive variables. This relationship was indicated in two ways: (1) the surface area of the left pars triangularis decreased with declining verbal fluency in the schizophrenic patients ($r = 0.53$, $p < 0.02$); and (2) the right pars triangularis was positively correlated with positive symptoms. The absence of the expected fluency/Broca's area correlation in the controls may reflect their ability to distribute cognitive load over wide functional networks. Such flexibility may be impaired in the schizophrenic patients due to developmental neural errors in association cortex.

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291.4

SPATIO-TEMPORAL BRAIN DYNAMICS OF AUDITORY LANGUAGE COMPREHENSION PROCESSES: COMPARISON BETWEEN POSITRON EMISSION TOMOGRAPHY AND ELECTRICAL EVENT-RELATED POTENTIAL MAPPING. B. Doyon, G. Thierry, J.F. Démonet*. INSERM U 455, Service de Neurologie, CHU PURPAN, 31059 TOULOUSE Cedex, France.

Démonet et al. (1), in a previous Positron Emission Tomography (PET) study described different patterns of brain activation associated with two monitoring tasks, a lexical semantic task using real words and a phonological task using consonant-vowel pseudo-words. The present study replicated the same cognitive experiment and compared PET findings with auditory event-related potentials (ERP) recorded over 32 channels, in twelve normal right-handed volunteers. The phoneme task consisted to discriminate between the successive occurence of phonemes /d/ and /b/ in tetra-syllabic pseudo-words versus others configurations. The semantic task consisted to discriminate, in a list of adjective-noun pairs, the animals of small size preceded by an adjective bearing a 'positive' feature versus others configurations. Although the general shape of ERPs were similar in both tasks, the waves differed in time course. ERPs elicited by words occurring earlier than those associated with pseudo-words. This advance probably reflects fast and massively parallel mode of processing, for the lexical semantic task and serial, delayed processing for phonological analysis, both involving interconnected neural assemblies. The spatial distribution of such an advance observed throughout the scalp matched the pattern of regional cerebral blood flow (rCBF) changes observed between tasks in the PET study, and dipole analysis (BESA) confirmed this matching. Overall, across-task rCBF differences might reflect distinct modes of activation of a large-scale neural ensemble, differentially operated over time, rather than spatially segregated neural entities specialized for phonology or semantics.

1. Démonet JF et al. Brain 1992, 115: 1753-1768.

291.5

FUNCTIONAL MAGNETIC RESONANCE IMAGING OF PHONOLOGICAL PROCESSING. J.A. Frost, J.R. Binder, T.A. Hammeke, P.S. Bellgowan, J.A. Springer, R.F. Newby, R.W. Cox, S. Jaradeh*. Medical College of Wisconsin, Milwaukee, WI 53226.

Functional magnetic resonance imaging was used to identify brain areas involved in phonological manipulation of speech sounds. Whole-brain imaging was performed on a 1.5T GE scanner using 3-axis gradient and insertable transmit/receive RF coils with a gradient echo echo-planar sequence (40 ms TE, 4 s TR, 24 cm FOV, 64 x 64 matrix, and 7 mm slice thickness). Ten healthy right-handed human subjects (5 female, 5 male) ages 16-30 were scanned during three auditory tasks. During the phoneme **deletion** task, subjects deleted /s/ and /t/ from non-words and blended the resulting non-word. In the phonetic **discrimination** control task, subjects detected occurrences of /s/ and /t/ in syllable sequences. During the phonological **working memory** control task, subjects detected repeat occurrences of non-words. Statistical parametric maps, thresheld at $p < .0001$, were generated showing t values for the deletion-discrimination and deletion-working memory comparisons.

Compared with the discrimination control task, the deletion task activated cortex on both banks of the intraparietal sulcus bilaterally, involving the medial supramarginal and angular gyri and the lateral superior parietal lobule. Other bilateral activation foci, more extensive on the right, were identified in the inferior frontal gyrus and the precentral sulcus, involving the pars opercularis and pars triangularis. The deletion task requires phonetic perception, working memory, and phoneme segmentation, deletion and blending. While the discrimination task controls for low-level phonetic perception, it does not place great demands on working memory. Therefore, some of the regions active in this comparison may be due to the working memory component of the deletion task.

When the deletion task was compared with the working memory control task, the main activation focus was in the left precentral sulcus extending into the pars opercularis and pars triangularis, suggesting that this region is critical for phonological processes involving segmentation, deletion, and blending.

(Supported by NIH NS33576)

291.6

INTERACTIONS BETWEEN SEMANTIC PROCESSING AND RESPONSE MODE IN BRAIN ACTIVATION: DIFFERENCES IN FUNCTIONAL CONNECTIVITY. J.M. Jennings*, A.R. McIntosh, S. Kapur, E. Tulving, & S. Houle. Rotman Research Institute of Baycrest Centre; Clarke Institute of Psychiatry; University of Toronto; Toronto, ON, Canada, M6A 2E1.

We investigated the proposition that brain regions associated with semantic processing would be the same regardless of how subjects made a response. Subjects underwent 6 PET scans, in which they made semantic or lexical word judgments, responding "yes" or "no" in three different modes: mouse-clicking, spoken response, or silent thought. Analyses showed a significant increase in rCBF associated with semantic processing in the left inferior frontal cortex, the anterior cingulate, and the right cerebellum for all three response conditions. However, there was also a significant interaction: semantic processing was associated with greater rCBF to the left superior temporal gyrus in the mouse-click condition, the left medial frontal gyrus in the spoken response condition, and the right dorsolateral prefrontal cortex in the silent thought condition. To understand why the effects of semantic processing and response mode combined in this non-additive, interactive manner, we examined the functional connectivity between the common left inferior frontal region and the rest of the activated brain by calculating reference pixel correlations. This analysis revealed a different set of correlations for each response mode. For example, rCBF in the left medial frontal gyrus was strongly associated with rCBF in the left inferior frontal cortex for the spoken response condition but not the other response modes. We suggest that there is a core network associated with semantic processing, which is activated regardless of response mode, however, additional and distinct areas of the brain are recruited into this network depending on how subjects have to organize their responses. In short, cognitive and behavioral processes combine non-additively, a result which is best understood through the examination of neural interactions.

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291.7

IMPAIRED RETRIEVAL OF CONCEPTUAL AND LEXICAL KNOWLEDGE FOR ACTIONS FOLLOWING LEFT PREMOTOR/PREFRONTAL DAMAGE.

JA Fiez*, D Tranel, H Damasio, AR Damasio, Div. Cog. Neurosci., Dept. Neurology, Univ. of Iowa College of Medicine, Iowa City, IA 52242.

In a previous study, we reported that a subject with damage to the left premotor region had impaired retrieval of words for actions (verbs), but normal retrieval of words for concrete entities (nouns). To pursue this result, we developed a standardized Action Recognition and Naming Battery in normal subjects which can be used to measure the ability of brain-damaged subjects to retrieve both conceptual and lexical knowledge for actions. This standardized test battery was administered to 68 brain-damaged subjects selected from our Patient Registry. All had single, stable lesions in various sectors of the telencephalon.

In 10 subjects with left frontal lesions, we replicated our initial result regarding the association of action word retrieval failure with damage to the left frontal region. Neuroanatomical analysis in these subjects revealed that the lesions clustered in the inferior motor and premotor regions, with the maximal lesion overlap occurring in the inferior frontal gyrus and the inferior sector of the precentral gyrus (5 subjects). The overlap tapered both into more anterior prefrontal regions, and posteriorly into the supramarginal gyrus. In all 10 subjects, the defect in word retrieval was paralleled by defects in the retrieval of conceptual knowledge for actions. In other words, defects in the retrieval of conceptual and lexical knowledge tend to co-occur regularly with regard to actions, which contrasts with frequent dissociations we have obtained between these two capacities with regard to some concrete entities (e.g., animals). The findings suggest that as far as actions are concerned, the separation of neural systems dedicated to conceptual and lexical knowledge retrieval may be minimal.

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291.8

NEURAL CORRELATES OF LEXICAL ACCESS AS SHOWN BY fMRI.

M. Kraut, S. Kremen, B. Soher, R.N. Bryan, B. Gordon, and J. Hart, Jr.* Depts. of Neurology, Radiology, Biomedical Engineering, Cognitive Science and the Zanvyl Krieger Mind/Brain Institute, The Johns Hopkins University, Baltimore, MD 21287.

We have used functional magnetic resonance imaging (fMRI) to help determine what cortical regions are activated during visual processing of letter strings to determine their lexicality.

We studied 9 normal young adults, 6 men and 3 women, using a behavioral paradigm with a baseline condition of visual detection and an activated condition of word/nonword lexical decision. fMRI data were gathered using 10 mm thick coronally oriented sections ranging from the occipital pole to the temporal pole. Scanning parameters were TR/TE/α=60/60/40°. Regions of activation in each section were detected by cross correlation analysis (Bandettini et al., J. Magn. Res. Med. 30:161, 1993), and were superimposed upon collocated T1 weighted anatomic images.

Eight of nine subjects studied exhibited significant signal changes. 6/8 showed changes in the inferior occipitotemporal gyri bilaterally, 8/8 in inferior parietal lobe (5 bilateral, 3 left only) and 7/8 in the superior temporal gyrus (2 bilateral, 5 left only). There was activation in the superior parietal lobule and middle and inferior temporal gyri on a less consistent basis.

These data strongly suggest that lexical processing engages a network of discrete cortical regions. The patterns of signal change indicate bilateral activation of the ventral occipitotemporal region--presumably in an early stage of visual processing and not necessarily specific to lexicality. Signal changes in the left inferior parietal and superior temporal regions likely reflect processes that are engaged in lexical judgments.

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291.9

KNOWLEDGE ABOUT NATURAL KINDS AND ARTIFACTS: A PET STUDY OF LEXICAL ACCESS.

S.F. Cappa*, D. Perani, T. Schnur, F. Grassi, F. Fazio
Universities of Milano and Brescia, INB CNR, S. Raffaele Scientific Institute, Milan, Italy

The double dissociation between disorders of recognition of natural kinds and artifacts has been suggested to reflect a selective disruption of access to structural knowledge in the case of living stimuli, and of access to functional information for man-made tools. In a previous PET study, we found that the recognition of animal pictures was associated with bilateral inferior temporo-occipital cortex activation, while a significant activation of left dorsolateral frontal cortex was present with artifacts (Perani et al., Neuroreport 1995). In this PET experiment, we contrasted directly the access to structural and functional-associative knowledge related to natural kinds and artifacts from visual words. Twelve right handed male volunteers underwent 12 consecutive scans of the following six conditions, repeated twice: 1. Baseline; 2. Detection of the letter "e" in legal pseudowords; 3. Living, structural: decide whether the animal corresponding to the word presented on the screen had a long or short tail with respect to the body; 4. Living, associative: decide if the animal was typically found in Italy; 5. Artifact, structural: decide whether the object was longer-than-wider or vice-versa; 6. Artifact, associative: decide whether the object was typically used as a kitchen tool. Scans were obtained using a PET tomograph GE-Advance (General Electric Medical System, Milwaukee,WI). Image manipulations and statistical analysis were performed using statistical parametric mapping (SPM-95,MRC Cyclotron Unit, London,UK).While letter detection in pseudowords was associated with an extensive bilateral activation of occipital and parietal areas, access to semantic knowledge engaged extensively the left dorsolateral and medial frontal cortex, and the posterior cingulus. Furthermore, access to structural knowledge was associated with significant bilateral activation in the inferior parietal and inferior temporal lobe. Whereas access to associative knowledge activated right fusiform gyri and posterior cingulas bilaterally. Significant differences were also present according to semantic category: right lateral occipital for natural kinds, left medial occipital for artifacts. The present findings are in agreement with the clinical evidence for both category and modality effects in semantic memory disorders.*Sources of funding:* C.N.R., Italy; HFSPO.

291.10

CORTICAL ORGANIZATION FOR LANGUAGE IN NATIVE DEAF AND HEARING SIGNERS. Part I: AMERICAN SIGN LANGUAGE.

D. Corina[1], D. Bavelier[2], P. Jezzard[3], I. Rauschecker[2], A. Braun[4], R. Turner[5], H. Neville*[6], UWashington, Seattle WA[1]; Georgetown U., Washington DC[2]; NIMH[3] & NIDCD[4], Bethesda MD; Inst. Neurol., London UK[5]; UOregon, Eugene OR[6].

The study of the cortical organization for ASL in two groups of native signers, deaf (D) and hearing (HOD), provides an opportunity to determine the contributions of language modality and sensory experience in cortical organization for language. Changes in blood oxygenation/flow level (MR imaging at 4T: 8 sagittal slices (5 mm), gradient echo MBEST, 64x64, TR-4000ms, TE-28ms, FOV-16cm) were recorded while 12 genetically, congenitally deaf subjects and 10 hearing subjects born to deaf parents viewed filmed ASL sentences alternating with nonsigns formally similar to ASL signs.

Processing ASL in D Ss led to a bilateral pattern of activation. Left prefrontal regions (Broca and DLPC) and left posterior temporal areas classically associated with language were also robustly active in the right hemisphere. Surprisingly, activation along the superior temporal sulcus (STS) was more robust in the right than in the left hemisphere. In addition, whereas the left angular gyrus (AG) usually recruited during reading was not active, the right AG was active. Finally, no activation was observed in the supramarginal gyrus, an area linked to spoken language comprehension.

This pattern of activation contrast with standard views on the neuro-biology of language. To determine whether this reorganization reflects the visuo-spatial processing demands of ASL or the unique sensory experience of deaf subjects, HOD Ss were run in the same paradigm. As in D Ss, HOD Ss displayed a bilateral pattern of activation. However, comparison of these groups show subtle differences in superior temporal and parietal activation which may be attributed to differences in sensory/language experience.

291.11

CORTICAL ORGANIZATION FOR LANGUAGE IN NATIVE DEAF AND HEARING SIGNERS. Part II: WRITTEN ENGLISH.

D. Bavelier*[1], D. Corina[2], P. Jezzard[3], V. Clark[3], A. Braun[4], R. Turner[5], H. Neville[6], Georgetown U., Washington DC[1]; UWashington, Seattle WA[2]; NIMH[3] & NIDCD[4], Bethesda MD; Inst. Neurol., London UK[5]; UOregon, Eugene OR[6].

In our companion abstract (see, Corina et al.), we described the cortical organization for ASL in native signers. In the present study, the role of the language modality is further assessed by examining the same subjects as they processed another visually presented language, written English. Ss were scanned while reading English sentences that alternated with consonant strings. Eight monolingual native English speakers (HG) were also included.

When processing English both HG and HOD Ss displayed a left lateralized pattern of activation including the classical language areas in the prefrontal cortex (Broca and DLPC), the left STS and left angular gyrus. The same HOD Ss when tested on ASL (see Part I) displayed a clear bilateral pattern of activation. Thus, the increased right hemisphere activation during the processing of ASL cannot be attributed simply to the sensory modality through which language is perceived.

In contrast to the results for Hg Ss and HOD Ss, deaf subjects when reading English showed a bilateral pattern of activation, with a tendency for a larger activation in the right posterior STS and right angular gyrus. Future research will need to determine the contributions of various factors to this pattern of results, including increased reliance on the visual features of English, delayed learning of English or the unique experience of acquiring solely a visual language as a natural language. (Part I and Part II supported by NINDS DC00128 and the McDonnel Pew Foundation)

292.1

NMDA RECEPTOR ANCHORING TO ACTIN CYTOSKELETON MEDIATED BY α-ACTININ IN DENDRITIC SPINES. M. Wyszynski*, A.H. Beggs, A. Rao, A.M. Craig, E. Nigh, and M. Sheng, Howard Hughes Medical Institute and Dept. of Neurobiology, Mass General Hospital and Harvard Medical School, Boston, MA 02114; Dept of Medicine, Childrens Hospital, Boston, MA. 02115; Dept of Cell and Structural Biology, University of Illinois, Urbana, IL 61801.

The NMDA receptor subclass of glutamate-gated cation channels is implicated in mechanisms of activity-dependent plasticity and excitotoxicity. NMDA receptors are clustered at postsynaptic sites in neurons, and their activity shows mechanosensitivity and dependence on integrity of the actin cytoskeleton. In this study, α-actinin2 is identified as a brain postsynaptic density protein which colocalizes with NMDA receptors in dendritic spines, and which specifically binds via its central rod domain to the C-terminal cytoplasmic tail of the NMDA receptor subunit NR1. α-actinins belong to the spectrin/dystrophin superfamily of actin binding proteins, and actin is a major component of dendritic spines, thus this interaction could mediate NMDA receptor anchoring to the postsynaptic actin cytoskeleton. The NR2 subunits of NMDA receptors are known to bind the postsynaptic density protein PSD-95. The interaction between α-actinin and NR1 signifies therefore that different subunits of the heteromeric NMDA receptor interact with distinct sets of intracellular proteins to mediate NMDA receptor connections to cytoskeleton and intracellular signaling molecules. (Supported by National Research Service Award CA66268-02 (M.W.), the Charles H. Hood Foundation (A.H.B.), the Lucille P. Markey Charitable Trust and NIH grant NS33184 (A.M.C). M.S. is an assistant investigator of the Howard Hughes Medical Institute.)

292.2

SUB- AND MAIN CONDUCTANCE STATES OF A MUTANT NMDA-CHANNEL HAVE DIFFERENT MONOVALENT CATION SELECTIVITIES. R. Schneggenburger* and P. Ascher Neurobiologie, Ecole Normale Supérieure, 75005 Paris, France.

There are numerous examples of ion channels with multiple conductance states, and it has generally been assumed that the different states have a similar ionic selectivity. We have obtained data indicating that this assumption may not hold in all cases. Recordings of NMDA-induced whole-cell currents were made in HEK cells expressing a mutant NMDA-channel, NR1(N598Q)-NR2A. Under biionic conditions (150 mM Cs^+ inside, 150 mM Na^+ outside or vice versa), pronounced current noise was observed at the apparent reversal potential. This phenomenon was absent in symmetrical Na^+ solutions, indicating that NMDA might activate two conductances with different ionic selectivities.

In outside-out patches (2 μM $[Ca]_o$) from oocytes expressing the mutant NMDA-channels, NMDA induced openings to a sub- and a main conductance level. In symmetrical 150 mM Cs^+-solutions the slope conductances were ≈ 50 pS and ≈ 100 pS and the two reversal potentials were close (within ≤ 2 mV) to 0 mV. However, in biionic conditions, the reversal potential of the sub level shifted to ≈ +15 mV (Cs^+ inside, Na^+ outside) or to ≈ -15 mV (Na^+ inside, Cs^+ outside), whereas the reversal potential of the main conductance level remained close to 0 mV. Thus, while the permeability ratio P_{Na}/P_{Cs} is ≈1 for the main conductance level, it is ≈ 1.8 for the pore of the subconductance level. *Supported by the CNRS (URA 1857), by the EC (BIO2-CT93-0243) and by an EC fellowship (HCM programme) to R.S.*

292.3

A MUTATED RESIDUE IN THE M3-M4 LINKER OF THE NMDA RECEPTOR FILLS THE GLYCINE BINDING POCKET. M.W. Wood*, H.M.A. Vandongen, K.S. Jones, and A.M.J. VanDongen. Department of Pharmacology, Duke University Medical Center, Durham, NC 27710.

One property distinguishing the NMDA receptor from other ionotropic glutamate receptors is its requirement for the co-agonist glycine. A recent topology model of ionotropic glutamate receptors includes a hairpin pore structure similar to that found in voltage-gated K^+ channels. The model places the region linking the third and fourth putative transmembrane domains (TM3 and TM4) in the extracellular space. This conclusion is incompatible with studies describing functional phosphorylation sites in the M3-M4 linker of non-NMDA glutamate receptors. We engineered a canonical consensus site for protein kinase A (PKA) into the M3-M4 linker of the NR1 subunit of the NMDA receptor at a position analogous to a functional PKA site described for GluR6. The mutation resulted in a decrease in the apparent affinity for the co-agonist glycine by two orders of magnitude. Ablation of the serine residue within the artificial PKA site further decreased the glycine affinity, eliminating the possibility that the phenotype arose from phosphorylation of this site. Point mutations of the residues comprising the PKA site implicate a single position as the dominant residue affecting glycine affinity (Ala714 to Leu). Reversion of this residue within the artificial PKA site restores most of the affinity. Additional mutations at position 714 also vary glycine affinity. The side chain volume of residues at this position correlates with the resulting affinity. An engineered N-linked glycosylation site at position 711 maps this region to the extracellular space. Mutation of position 714 to a cysteine increases the receptor's sensitivity to a thiol selective reagent, supporting extracellular localization. This series of experiments supports the three transmembrane topology model for the NMDA receptor and implicates alanine 714 as a residue within the binding pocket for the co-agonist glycine.
This work was supported by Grant NS31557 from N.I.N.D.S

292.4

UPREGULATION OF AMPA RECEPTOR SUBUNIT GLUR2 FLOP mRNA DURING CEREBELLAR GRANULE CELL MATURATION IN VITRO COINCIDES WITH THE APPEARANCE OF AMPA NEUROTOXICITY.
P. Longone*, F. Impagnatiello, E. Costa and A. Guidotti. The Psychiatric Institute, Department of Psychiatry, University of Illinois at Chicago, Chicago, IL 60612.

The neurotoxic response of cerebellar granule neurons to the application of AMPA (50μM) in the presence of cyclothiazide (10μM) increases from 2 days *in vitro* (DIV), when it is almost absent, to 6 DIV, when it becomes approximately 100%. We examined whether the increased susceptibility to the neurotoxic effect of AMPA is related to changes in the expression of AMPA GluR2 flip/flop splice variant mRNAs. The mRNAs were measured by competitive RT-polymerase chain reaction (RT-PCR). Primer sequences were chosen to amplify the flip/flop splice region of this subunit. The primer set was demonstrated to amplify the correct size mRNA fragment. Total RNA was reverse transcribed and co-amplified with the internal standard which utilizes the primers for the canonic sequence, except for an internal restriction site (BglII) created by site-directed mutagenesis. To determine the amount of the GluR2 flip and flop mRNA forms, the resulting amplification product was cut with a restriction enzyme specific for each of the two splice forms (AvaI R2 flip, StuI R2 flop). The mRNA content of GluR2 at 2 DIV (flip+flop: 13±1.7 attomol/μg RNA; n=3) increased by ~ 2-fold at 6 DIV. The flip/flop ratio shifted from 2.5 at 2 DIV to 0.3 at 6 DIV. Thus, whereas the flip variant of GluR2 mRNA content does not change significantly, the flop variant increases dramatically (about 5-fold) from 2 to 6 DIV. Our results agree with the view that the upregulation of AMPA receptor subunit GluR2 mRNA expression and the shift in its splice variant flip/flop ratio may participate in the increased neurotoxic susceptibility of cerebellar granule cells to AMPA. To examine whether in addition to changes in AMPA subunit GluR2 flip/flop mRNAs, the other AMPA receptor subunit mRNAs undergo similar modifications, we have constructed the appropriate primers and internal standard for quantitative RT-PCR analysis of GluR1, 3, and 4 flip/flop mRNA subunit expression. The regulation of their expression during culture maturation is now under investigation.
NS 2813 0-06.

292.5

LOCALIZATION OF NMDAR-ASSOCIATED PROTEINS IN CULTURED HIPPOCAMPAL NEURONS [1]A. Rao*, [2]E. Kim, [2]M. Wyszynski, [2]M. Sheng and [1]A.M. Craig. [1]Dept. Cell and Structural Biology, University of Illinois, Urbana, IL 61801 and [2]HHMI and Dept. Neurobiol., MGH and Harvard Med. School, Boston, MA 02114.

Several proteins that interact with the NMDA receptor have recently been identified using the yeast two-hybrid system (Kornau et al., 1995, Science, 269:1737-1740; Niethammer et al., 1996, J. Neurosci. 16:2157-2163). Amongst these, the related proteins PSD-95 and chapsyn-110 bind the NR2 subunit of the NMDA receptor, while the actin-binding protein α-actinin binds the NR1 subunit. We describe here the subcellular localization and developmental profile of these three proteins in cultured embryonic rat hippocampal neurons as determined by immunocytochemistry. In mature cultures, all three proteins were concentrated at excitatory glutamatergic synapses, colocalized with clusters of GluR1 or NR1. None of these proteins were present at inhibitory synapses containing GABA receptor clusters. PSD-95 and chapsyn-110 clusters were observed at essentially all GluR1- or NR1-containing synapses. In contrast, α-actinin colocalized with GluR1 and NR1 at excitatory spiny synapses on pyramidal cells but not at excitatory shaft synapses on GABA cells. α-Actinin, therefore, is not necessary for clustering of GluR1 or NR1 on dendritic shafts, but may have a role in the formation of spiny excitatory synapses. Consistent with this idea, α-actinin was concentrated at spiny synapses as soon as they could be detected during development of the neurons in culture. PSD-95 and chapsyn-110 were present at both spiny and shaft GluR1 and NR1 clusters from early times in development, consistent with a role for these two proteins in formation of excitatory synapses. Supported by NIH and the Markey Charitable Trust.

292.6

RESCUE OF NMDAR1 KNOCK OUT MICE USING TRANSGENIC MOUSE TECHNOLOGY. T. Iwasato, D. F. Chen, T. Sasaoka*, S. Tonegawa, *Center for Learning and Memory, MIT, Cambridge MA02139*

Gene disruption of the key subunit of the NMDA receptor (NMDAR1) results in neonatal lethality, which has limited the use of the knock out mouse technology in investigation of the role of the NMDA receptor in the process of neural development and synaptic plasticity. To rescue the lethality of NMDAR1 knock out mice, we have generated several lines of transgenic mice using constructs carrying NMDAR1 cDNA under the control of a constitutive promoter or a putative NR2D promoter. They were crossed with NMDAR1 heterozygous mice (NR1+/-) to generate transgenic-homozygous mutant mice (NR1-/-,tg). We have focused on two lines of transgenic mice. One of them (tgA) carries NMDAR1 cDNA under the control of a constitutive promoter and expresses transgene in every tissue including brain. About half of the NR1-/-,tgA mice could survive to the adult stage. The rescued NR1-/-,tgA mice are slightly smaller and breed less efficiently than other littermates. The general anatomical structure of brain appeared normal. Whisker related patterns were shown to be restored in the level of brainstem, thalamus, and cortex. The other line of transgenic mice (tgB) carries NMDAR1 cDNA under control of a putative NMDAR2D promoter. However, we found it expressed the transgene in the cortex in addition to the subcortical region, probably because of the positional effect of the integration site of the transgene. About 20% of NR1-/-,tgB mice could survive much longer than knock out mice (NR1-/-), though almost all died at or before weaning time. These rescued NR1-/-,tgB mice were smaller in size than other littermates. Like NR-/-,tgA mice, the general anatomical structure of brain appeared normal. In contrast, these mice did not have the whisker related patterns in the levels of principal nucleus of brainstem, thalamus or cortex. These two mice (NR1-/-,tgA and NR1-/-,tgB) should be useful to study the mechanisms of the activity-dependent refinement process in the developing central nervous system.
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292.7

GENERATION OF TRANSGENIC MICE EXPRESSING AN EMBRYONIC SUBUNIT OF THE NMDA RECEPTOR IN THE POSTNATAL FOREBRAIN S. Okabe*, C. Collin, N. Meiri+, J. Bengzon, M. B. Kennedy**, M. Segal#, and R. D. G. McKay LMB and LAS+, NINDS/NIH, Bethesda, MD 20892, **Div. of Biology, California Inst. of Tech., Pasadena, CA 91125, #Weizmann Inst., 76100, Israel.

To test the effects of expressing an embryonic NR2 subunit in mature forebrain neurons, we created mice carrying NR2D gene under the control of the promotor of the α subunit of the Ca^{++}/calmodulin-dependent protein kinase (CaMKIIα). Transgene-derived transcripts were abundant in adult cortex, hippocampus, and striatum. Western blotting of transgenic hippocampus showed that transgene-derived NR2D protein amount increased significantly from postnatal 2 to 8 weeks, and remained stable thereafter. Histological examination revealed no gross anatomical abnormality, which is consistent with the postnatal up-regulation of the transgene. Adult (8-17-week-old) transgenic mice expressed large amount of NR2D with a concomitant reduction of NR2B and autoactive CaMKII. Juvenile (3-week-old) transgenic mice had moderate expression of NR2D protein and the NR2B and autoactive CaMKII amount was normal. The age-dependent shift of NMDA receptor composition and modulation of post-synaptic kinase activity were correlated with the change of synaptic plasticity in hippocampus, which will be presented in the accompanying paper (C. Collin et al.).

Supported by NINDS/NIH

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ALTERED HIPPOCAMPAL PLASTICITY BUT UNIMPAIRED SPATIAL LEARNING IN TRANSGENIC MICE OVEREXPRESSING NR2D RECEPTORS. C. Collin[1], S. Okabe[1], N. Meiri[2], M. B. Kennedy[3], M. Segal[4], and R. D. G. McKay[1]. [1]LMB, [2]LAS, NINDS/NIH, Bethesda MD, 20892; [3] CALTECH, Pasadena, CA 91125; [4]The Weizmann Institute, Rehovot 76100, Israel.

Behavioral and electrophysiological plasticity were assessed in transgenic mice overexpressing the NR2D subunit of the NMDA receptor (see Okabe et. al.). Two transgenic (Tg) lines expressed significantly different patterns of exploratory activity in an open field compared to control mice; they moved less and crossed the center of the field less than controls. These differences were not caused by a motor impairment, as all groups behaved the same on a rotary rod. Also, there were no differences between the controls and the transgenic mice in acquisition and retention of spatial memory in a water maze. Slices of hippocampus of 3 week (juvenile) and 2-3 months (adult) control and Tg mice were recorded in an interface chamber at 30°C, using extracellular electrodes. Field EPSP's from stratum radiatum were not different between control and Tg slices. Tetanic stimulation (100Hz for 1 sec) produced a smaller long term potentiation (LTP) in Tg (19±6% above baseline) than in control (55±7% above baseline) adult slices. By contrast, juvenile slices of Tg's and controls expressed similarly sustained LTP. Depotentiation was induced in both adult and juvenile slices by stimulation at a rate of 1 Hz for 10 minutes, 30 minutes after the LTP evoking tetanus . Only the control slices of both age groups expressed marked depotentiation. Furthermore, in non-tetanized, juvenile slices long term depression (LTD) can be evoked by 1-5 Hz stimulation. LTD was seen only in the control and not in the Tg slices. The Tg slices were able to express non-NMDA mediated LTP, evoked by a 200Hz stimulation in presence of an NMDA antagonist, 2-APV. These studies indicate that NR2D overexpressing mice are deficient in NMDA-mediated LTP and LTD, but express normal cognitive behavior.

Supported by NINDS/NIH

292.9

TRANSMEMBRANE TOPOLOGY OF THE NMDA RECEPTOR SUBUNIT NR1: THE M3-M4 LOOP REGION IS EXTRACELLUAR AND PARTICIPATES IN GLYCINE BINDING. J. Kuhse, H. Hirai, J. Kirsch, B. Laube, U. Ernsberger* and H. Betz. Max-Planck-Institute für Hirnforschung, Deutschordenstr. 46, 60528 Frankfurt/Main, Federal Republic of Germany.

The N-methyl-D-aspartate (NMDA) subtype of ionotropic glutamate receptors is a hetero-oligomeric membrane protein composed of homologous subunits. Here, we investigated the transmembrane topology of the ligand-bindig NR1 subunit of recombinant NR1/NR2B receptors and the contribution of its M3-M4 loop to ligand binding. The cellular localization of epitope-tags engineered into the NR1 protein was analyzed by confocal laser scanning microscopy after heterologous expression in a human cell line. Epitopes in the N-terminus and the putative M3-M4 loop were found to be exposed extracellularly, whereas a C-terminal epitope was localized intracellularly. Substitution of aromatic residues at positions 735 or 736 of the M3-M4 loop produced a 15 to 30-fold reduction in apparent glycine affinity without affecting the binding of glutamate and the competitive glycine antagonist 7-chlorokynurenic acid; mutation of both residues caused a >100-fold decrease in glycine affinity. These results substantiate an extracellular location of the M3-M4 loop of the NR1 subunit and provide the basis for modelling the glycine binding pocket of the NMDA receptor.

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292.10

THE ROLE OF N-TERMINAL RESIDUES OF NR2B IN THE MODULATION OF NMDA RECEPTORS Gallagher, M.J., Huang, H., and Lynch, D.R*., Department of Pharmacology and Pediatrics, University of Pennsylvania, Children's Seashore House, Philadelphia, PA 19104-4318

There are several compounds which modulate the NMDA receptor in a subunit specific manner. The drugs ifenprodil and haloperidol, and the endogenous polyamine spermidine all exert preferences for receptors containing NR2B over NR2A. We have determined using chimeric NR2A/NR2B subunits and site-specific mutants that two residues of NR2B (E201 and R337) are directly involved in the modulation of NMDA receptors. Glycine-independent spermidine stimulation and haloperidol high affinity inhibition of NMDA receptors are both affected by mutation of E201. Replacing E201 with the negatively charged aspartate (E201D) caused only moderate reduction in polyamine stimulation, while mutation to the positively charged arginine (E201R) eliminated polyamine stimulation. None of the mutations of E201 caused any change in affinity for ifenprodil, which provides additional evidence that ifenprodil and polyamines interact at distinct sites on the NR2B subunit. Mutation of another residue Arginine 337, previously shown to be crucial for ifenprodil inhibition, has no effect on either haloperidol inhibition or polyamine stimulation, demonstrating the ifenprodil specificity of this residue. We have also mutated the corresponding residues of NR2A (N200 and Q336) in an effort to confer NR2B specific properties to the NR2A subunit. The receptor NR1A/Q336(NR2A) exhibited 3-5 fold greater apparent ifenprodil affinity than native NR2A containing receptors. The mutant N200E(NR2A) showed significant polyamine stimulation, while haloperidol inhibition was not improved. (This work was supported by the Grants F32-DA05675, DA07130, and NS01789 from the National Institutes of Health)

292.11

IMPAIRED MOTOR PERFORMANCE AND CEREBELLAR SYNAPTIC PLASTICITY IN MICE LACKING THE mGluR4 SUBTYPE OF METABOTROPIC GLUTAMATE RECEPTOR R. Pekhletski*[1], R. Gerlai[2], L.S. Overstreet[3], X.-P. Huang[1], N. Agopyan[2], N.T. Slater[3], W. Abramow-Newerly[2], J.C. Roder[2], and D.R. Hampson[1]. [1]Faculty of Pharmacy, Univ. of Toronto, Toronto, Canada. [2]Samuel Lunenfeld Res. Inst. & Depart. of Med. Genetics, Univ. of Toronto, Toronto, Canada. [3]Depart. of Physiol. & the Northwestern Univ. Inst. of Neurosci., Northwestern Univ. Med. School, Chicago, USA.

The glutamate analog, L-2-amino-4-phosphobutyric acid (L-AP4) is a selective ligand at a subset of metabotropic glutamate receptors (mGluRs). Although previous studies have suggested that receptors for L-AP4 may be involved in the regulation of glutamate release, the function of these receptors in the brain remains largely unknown. To provide a better understanding of the biological role of L-AP4 receptors, we have generated and studied knockout mice (KO) lacking the mGluR4 subtype of mGluR.

The mGluR4 mutant mice displayed normal spontaneous motor activity in the open field test and no impairment on the bar cross test suggesting that disruption of the mGluR4 gene does not cause gross motor abnormalities, impairments of novelty-induced exploratory behaviours, or alterations of fine motor coordination. In contrast, the mutant mice were significantly impaired compared to their wild type littermates on the rotating rod motor learning test, suggesting that the mGluR4 KO mice may have a decreased ability to learn this motor task.

Patch-clamp recordings from Purkinje cells in cerebellar slices demonstrated that, unlike wild type littermates, the mutant mice were insensitive to the suppressant effects of L-AP4 on synaptic transmission. This was accompanied by significant impairments in measures of presynaptic short-term synaptic plasticity including a reduction in paired-pulse facilitation and post-tetanic potentiation. These findings indicate that an important function of mGluR4 is to provide a presynaptic mechanism for maintaining synaptic efficacy during repetitive activation by limiting exhaustion of synaptic vesicle stores. This work was supported by the MRC, Canada and the NIH, USA.

292.12

A TETRAMERIC SUBUNIT STOICHIOMETRY FOR A GLUTAMATE RECEPTOR/CHANNEL COMPLEX

I. Mano and Y. I. Teichberg*
Department of Neurobiology, the Weizmann Institute of Science, Rehovot 76100, Israel.

Until recently, the glutamate receptors (GluRs), as other ligand-gated channels, were thought to form pentameric structures assembled from subunits, each having four transmembrane segments. However, as evidence has been presented that GluRs harbor only three transmembrane segments in addition to a potassium-channel-like P-loop segment, the generally accepted view that the GluR channel complexes are made of five subunits was here questioned. We study the properties of the wild-type (WT) GluR1 subunit of the α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptors coexpressed in *Xenopus* oocytes with the L646A GluR1 mutant, which present identical levels of expression and apparent affinity to KA, exhibits the same current-voltage relation, and its ability to form heterocomplexes with another AMPA receptor subunit - GluR2 - is indistinguishable from that of the WT GluR1. However, this mutant remarkably differs from the WT in its ~100 fold reduced sensitivity to co-application of the desensitizing agonist Quisqualate (QA). Comparing the contribution of WT-like receptors to the mixed response we show that desensitization of a kainate (KA) -activated channel complex requires the interaction of QA with only one subunit. Using the analysis developed by MacKinnon to determine channel stoichiometry, we provide now evidence suggesting that homomeric GluR1 channel complexes have a tetrameric structure. Thus, the GluR pore structure and subunit stoichiometry present the hallmark of voltage-gated channels.

Supported by grants from the Minerva Foundation (Munich, Germany), The Harry Stern Family Foundation, The Golden Charitable Trust and the Leo and Julia Forchheimer Center for Molecular Genetics.

293.1

PSI EXPRESSION IN PLATELETS AND NEURAL CELL LINES. R. Vidal and B. Frangione*.Dept. Pathology, New York University Medical Center,New York, NY10016

Alzheimer's β protein (Aβ) circulates as a complex preferentially with apoJ, a carrier for Aβ in both plasma and CSF. Platelets are the main source of soluble β protein precursor (βPP) or Nexin II. βPP as well as apoJ are stored in platelet's α-granules and are released into extracellular fluid following activation. Carboxyl end immunoreactivity of the presenilin 1 (PS1) protein, (to which most cases of early-onset FAD are linked), has been reported in senile plaques of familial and sporadic AD. We studied PS1 expression in platelets, megakaryocytes and neural cell lines by RT-PCR. PCR products were characterized by restriction enzyme analysis and DNA sequencing and the purity of platelet's RNA was estimate after RT-PCR for the beta chain gene of HLA-DR (marker of mononuclear cells). PS1 transcripts were found to be present in all samples analyzed, with full-length PS1 as the predominant isoform. Transcripts with and without the VRSQ sequence (exon 3) were observed in almost equimolecular amounts. Alternative splicing of exon 8 (a possible hotspot for PS1 mutations) resulting in a shorter isoform more prominent in leukocytes, was observed to constitute a very low percentage of PS1 transcripts. Similar results were obtained using RNA isolated from human neuroblastoma cell lines (SHSY5Y and NT-2) and cells of the thrombocytic lineage. We also found a novel splice variant that results in the loss of exon 10 (codons 319-376). Loss of this exon does not alter the reading frame of the PS1 molecule. The significant of this new variant is difficult to predict without knowledge of the PS1 function. Expression of PS1 was assessed by immunoblotting analysis using a polyclonal anti-N terminus PS1 antibody, recognizing mainly a 44/50 KDa band in neuroblastoma and megakaryocytic cell lines. The relationship of presenilin mutations to the amyloidogenesis of AD is unclear. As with βPP, the expression of PS1 is not limited to regions of the brain affected by the neuropathological changes observed in AD. The analysis of the expression of presenilins should be considered as a step towards the understanding of their physiological function and their role in AD.

Supported by NIH grant AG05891

293.2

EXPRESSION OF PRESENILIN 1 (PS-1) AND 2 (PS-2) IN MOUSE BRAIN, AND IN NORMAL AND ALZHEIMER'S DISEASE HUMAN BRAINS. D.P. Huynh*, V.V. Ho, and S.M. Pulst. CSMC Burns and Allen Research Institute, UCLA, 8700 Beverly Boulevard, Los Angeles, CA 90048.

We investigated the expression of two familial Alzheimer's disease (AD) proteins (presenilins) in mouse brains, and in normal and AD human brains. Affinity-purified antibodies (ABs) were generated in rabbits and chicken against three peptides of presenilin 1 and to two peptides of presenilin 2. In Western blot analyses, all PS-1 ABs detected a single 53 kDa protein and PS-2 ABs detected a 43 kDa protein in mouse, human brains, as well as in neuronal and glial human cell lines. In mouse CNS, expression of PS-1 and PS-2 was seen in areas known to be affected in AD such as CA1-CA4 areas, entorhinal cortex, dentate gyrus, subiculum, amygdaloid nuclei, and pyramidal neurons of the neocortex. However, neurons normally not affected in AD such as thalamic nuclei, Purkinje cells, DRG, and spinal cord also expressed PS-1 and PS-2. In normal human brains, pyramidal neurons in the neocortex and hippocampus were stained, but no staining was seen in glial cells. In AD brains, identical staining patterns were observed, but reactive astrocytes were strongly stained. In conclusion, we have produced several highly specific presenilin antibodies that will be useful molecular tools in future investigations of presenilin function. The strong staining of reactive astrocytes in FAD brains suggest a role for these cells in the pathogenesis of FAD. Expression of presenilins in areas not affected by AD suggests that other proteins may interact with presenilins to cause cell type specific neuronal death. *Supported by the Carmen and Louis Warschaw Endowment Fund for Neurology and the Ruth and Laurence Harvey Endowment Fund for Neuroscience.*

293.3

S182 / PRESENILIN-1 PROTEIN AND mRNA IN AD

D Schemchel, EG Stopa, BS Rubin, V Kuo-Leblanc, PC Song, JC King, K Boteva, N Mitsuda, JR Gilbert and MP Vitek*

Bryan Alzheimer's Disease Research Center, Duke Univ. Med. Ctr., Durham, NC; Dept. Anatomy and Cell Biol., Tufts Univ. Sch. of Med., Boston, MA; Dept. Pathology, Brown Univ. Sch. of Med., Providence, RI; GlaxoWellcome Res. Inst., Res. Trian. Park, NC 27709

Some early onset familial Alzheimer's disease is associated with mutations of the S182 or Presenilin-1 gene located on chromosome 14 and of the homologous STM2 or Presenilin-2 gene located on chromosome 1. As little is known about the function or location of either gene's products, we probed their distribution in human brains. In the present study, 8 micron sections taken from 10 different brain areas (2-8 hrs PMI) of 7 AD and 5 age-matched controls were immunohistochemically stained with specific antibodies to Presenilin protein. *In situ* hybridization with a cRNA probe specific for PS-1 was performed on these same sections.

In all cases, neuronal staining was prominent where PS-protein immunoreactivity displayed a perinuclear distribution. Some immunoreactivity was also associated with senile plaques. The *in-situ* hybridization signal was present in most neurons throughout the 10 brain regions examined. In AD patients, this signal was clearly reduced as a function of neuronal loss. A consistent finding in both AD and control cases was the increased staining for PS-1 mRNA in the CA2 region of the hippocampus when compared to CA1 and CA3.

Our data indicate that PS-1 protein and mRNA are widely expressed in neurons throughout the brain and that gene expression may be reduced in sporadic Alzheimer's disease. Supported by AG 10682, AG 05128, AG 12851.

293.4

Presenilin Fragments in Cerebro-Spinal Fluid and Brain Tissue

T.Wisniewski, W.Dowjat, A.Golabek, D.Miller *, B.Frangione Dept. of Neurol. and Pathol., NYU Medical Center, NY 10016

The majority of early-onset familial Alzheimer disease (FAD) has been linked to a gene located on chromosome 14, presenilin-1 (PS-1). Numerous mutations in this gene have been associated with early-onset FAD. To investigate the involvement of PS-1 in AD we raised polyclonal antibodies to the amino terminus of PS-1 (residues 48-70) and the carboxyl terminus (residues 448-467). Previously we have shown that this carboxyl terminal antibody immunoreacts with AD neuritic plaques of both sporadic and FAD patients suggesting that PS-1 may have a general role in the pathogenesis of AD and not just in the rare FAD cases. Further immunohistochemical studies with these antibodies in sporadic AD and Down Syndrome (DS) patient's brains have found carboxyl terminal immunoreactivity in both the amyloid deposits of neuritic plaques and cerebral vessels. In biochemical extractions of neuritic plaque amyloid from sporadic AD and DS patient brains we found by Western blotting, that proteins which specifically immunoreact with our anti-PS-1 antibodies, co-purify with amyloid β (Aβ). In AD amyloid that is partially purified by homogenization in SDS and sucrose gradients we found a 17 kDa fragment band which is recognized by the anti-carboxyl PS-1 antibody, as well as a PS immunoreactive 44 kDa band that most likely represents the intact protein. On the other hand, in both normal and AD cerebrospinal fluid (CSF), we detect a 27 kDa protein that is labeled by the amino terminal anti-PS-1 antibody. These results suggest that PS is normally degraded to at least two fragments (or these are products of different transcripts): a 27 kDa amino terminal protein that is released in the CSF and a 17 kDa carboxyl terminal fragment found in brain tissue. Supported by NIH grants AG05891 and AG00542

293.5

PROTEOLYTIC PROCESSING AND UBIQUITIN-DEPENDENT DEGRADATION OF ALZHEIMER-ASSOCIATED PRESENILIN 1 AND 2. T.-W. Kim*, R. D. Moir, W. P. Pettingell, O. G. Hallmark, D. M. Kovacs, W. Wasco, R. E. Tanzi. Genetics and Aging Unit, Massachusetts General Hospital, Harvard Medical School, MA 02129.

The presenilin genes (PS1 and PS2) appear to be responsible for the majority of early onset familial Alzheimer's disease (FAD). The biological roles of the presenilins and how their pathogenic mutations confer FAD are unknown. In this study, we set out to examine the processing and degradation pathways of PS1 and PS2. For regulated expression of PS2, we have established inducible cell lines expressing either wild-type or mutant forms of PS2 under the tight control of the tetracycline-responsive transactivator. Western blot analysis revealed that FLAG epitope-tagged PS2 was detected as a 53~55 kDa as well as high molecular weight forms. We have found that 55 kDa PS2 is proteolytically processed into two stable cellular polypeptides including a 35 kDa N-terminal fragment and a 20 kDa C-terminal fragment which was localized to the detergent-insoluble cellular fraction (e.g. cytoskeleton). PS2 was found to be polyubiquitinated *in vivo*; an inhibitor of the 26S proteosome complex led to the accumulation of polyubiquitinated forms of PS2. Interestingly, in cells expressing PS2 with the Volga/German FAD mutation (N141I), the 20 kDa fragment accumulated at higher concentrations than in cells expressing wild-type PS2. Similar to that found for PS2, 45~50 kDa full-length PS1 was also processed to generate 27 kDa N-terminal- and a detergent-insoluble 19 kDa C-terminal fragments. The proteosome blocker also caused the accumulation of the polyubiquitinated PS1, indicating that both PS1 and PS2 are degraded via the ubiquitin-proteosome pathway. Our studies raise the possibility that abnormal processing and/or inefficient degradation of PS1 and PS2 are involved in pathogenic mechanisms of presenilin mutations.

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293.6

SUBCELLULAR LOCALIZATION OF PROTEOLYTIC FRAGMENTS DERIVED FROM NORMAL AND AD-ASSOCIATED PRESENILINS.

D. M. Kovacs*, S. D. Schmidt, T.-W. Kim, W. Wasco and R. E. Tanzi. Genetics and Aging Unit, Massachusetts General Hospital, Boston, MA 02129 USA.

The large majority of familial Alzheimer's disease (FAD) is caused by mutations in one of two recently identified genes, presenilin 1 (PS-1) and presenilin 2 (PS-2). We have recently reported (*Nature Med. 2:224, 1996*), that PS-1 and PS-2 mRNAs are expressed in similar neuronal populations of rat and human brains, with virtually identical regional distributions. Both PS proteins were mainly detected in the nuclear envelope, ER, and Golgi membranes of transiently transfected mammalian cell lines. These data indicate that the pathogenic effect of mutations in the presenilins begins in the intracellular membranes of neuronal populations. However, FAD-associated mutations did not alter significantly the subcellular localizations of those two proteins. We have since shown both PS-1 and PS-2 to undergo proteolytic cleavage into two fragments. The C-terminal products of both PS-1 and PS-2 have been detected in the detergent-resistant cellular fraction, indicating a potential association with the cytoskeleton (Kim et al., submitted). To further investigate this possibility, we have performed immunofluorescent studies of stabily and transiently transfected cell lines to compare the subcellular localization of the presenilin N- and C-terminal fragments produced by wild type versus mutant PS constructs. In addition, we have assessed the effects of the cytoskeletal disrupting agents, nocadazole and cytochalasin D, on the subcellular distributions of PS-1 and PS-2, and found a significant redistribution of both proteins in the presence of these agents.

Supported by the French Foundation, NIA, and NINDS.

293.7

METABOLISM OF THE ALZHEIMER'S DISEASE PRESENILIN-1 PROTEIN IN TRANSFECTED AND PRIMARY CELL CULTURES.

Cai'ne Wong, Henrike Hartmann, Matthias Staufenbiel+ and Bruce A. Yankner*. Dept. Neurology, Harvard Medical School and the Children's Hospital, Boston, MA. +Sandoz Pharma Ltd., Basel, Switzerland.

Mutation of the presenilin-1 (PS-1) gene has been implicated as the most common cause of early onset familial Alzheimer's Disease (FAD). The PS-1 sequence predicts a 54 kD protein with at least 7 transmembrane domains. Immunoprecipitation and Western blotting of PS-1 cDNA-transfected COS cells revealed predominant 40-43 kD and 30-33 kD species. The same species were detected in cells transfected with mutant PS-1 cDNAs. Similar species were also detected in non-transfected COS, neuroblastoma, FAD and normal fibroblast, and human cortical cultures. In addition, we have identified a 12-16 kD protein that is immunoreactive with the PS-1 antibody, is increased with PS-1 transfection, and is absent in antibody preabsorbed controls. This 12-16 kD protein is the major PS-1 immunoreactive species in primary human fibroblast and cortical cultures, but is a relatively minor species in transfected COS cells. The 12-16 kD fragment is likely to be either a proteolytic product or an alternatively spliced variant of PS-1. The abundance of this species in primary cell cultures suggests that it may have functional biological significance. *Support by NINDS, Alzheimer's Association and Sandoz Pharma Ltd.*

293.8

ENDOPROTEOLYTIC PROCESSING AND PROTEIN TOPOLOGY OF PRESENILIN 1.

G Thinakaran,*[1,4] DR Borchelt,[1,4] A Doan,[2] HH Slunt,[4] L Spitzer,[4] T Ratovitsky,[4] MK Lee,[1,4] C Nordstedt,[5] M Seeger,[6] SE Gandy,[6] JA Hardy,[7] AI Levey,[8] DL Price,[1,4] and SS Sisodia[1,2,4] [1]Departments of Pathology, [2]Neuroscience and [3]Neurology, and [4]The Neuropathology Laboratory, The Johns Hopkins University School of Medicine, Baltimore, MD 21205; [5]Karolinska Institute, Stockholm, Sweden; [6]Cornell University Medical College, New York, NY; [7]University of South Florida, Tampa, FL; [8]Emory University School of Medicine, Atlanta, GA.

The majority of early-onset cases of FAD are linked to mutations in two related genes, *Presenilin 1 (PS1)* and *Presenilin 2 (PS2)*. We have used three highly specific antibodies, generated against non-overlapping epitopes of PS1 to examine the expression and metabolism of PS1 *in vivo*. In stably transfected cell lines, PS1 is synthesized as a 43 kDa polypeptide. However, full-length PS1 undergoes proteolytic processing *in vivo*, and the preponderant PS1-related species that accumulate in the cell lines, as well as brains of rodents, primates, and humans are ~27-28 kDa N-terminal and ~16-17 kDa C-terminal derivatives. The site of cleavage is being determined by biochemical and molecular approaches, but our epitope mapping studies indicate that cleavage occurs in a region in which ~58% of PS1 missense mutaions occur. Furthermore, an FAD-linked PS1 variant encoded by mRNA that lacks exon 9 is not subject to endoproteolytic cleavage.

We have also examined the protein topology of PS1 *in vivo* using a novel chimera-based strategy and have demonstrated that the N-terminus of PS1 is oriented towards the cytoplasm. Using this approach, we have also demonstrated that hydrophobic residues 280-300 within the proposed "loop" domain of PS1 can be used as a transmembrane helix. We are currently assessing the membrane orientation of the hydrophilic "loop" domain of PS1 using N-glycosylation antibody tagging methods in combination with antibody decoration approaches.

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293.9

IDENTIFICATION AND CHARACTERIZATION OF A PRESENILIN 1 INTERACTING PROTEIN

A. Doan[1]*, G. Thinakaran[2,4], A. Lanahan[1], D. L. Price[1,2,3,4], S. S. Sisodia[1,2,4] and P. E. Worley[1,3]. Depts. of [1]Neuroscience, [2]Pathology and [3]Neurology and [4]Neuropathology Laboratory, The Johns Hopkins University School of Medicine, Baltimore, MD 21205.

The majority of individuals with Familial Alzheimer's disease (FAD) have mutations in two genes: *presenilin 1* (PS1, chromosome 14) and *presenilin 2* (PS2, chromosome 1). In an attempt to identify proteins that interact with PS1, N-terminal and loop fragments from PS1 were screened in the yeast two-hybrid system using a rat forebrain, random primed cDNA library. We report that the hydrophilic "loop" region of PS1 interacts with a fragment of the rat homologue of human filamin (ABP-280) in the two-hybrid system. The candidate interacting filamin-related polypeptide was coimmunoprecipitated with full-length PS1 from HEK293 cells using polyclonal antibodies directed to the loop region of PS1. We are currently characterizing this interaction further via coimmunoprecipitation and colocalization in brain and cell culture. We are also in the process of identifying additional PS1 interacting proteins. Characterization of the cellular biochemistry of the interactions of these candidate proteins with PS1 will provide important insights into the biology of Alzheimer's disease.

Supported by NIH, Alzheimer's Association, Develbiss Fund, Adler Foundation, and Medical Scientist Training Grant

293.10

ENDOPROTEOLYSIS AND SATURABLE ACCUMULATION OF HUMAN PRESENILIN 1 DERIVATIVES IN TRANSGENIC MICE.

D. Borchelt[1]*, M. Lee[1], G. Thinakaran[1], H.H.Slunt[2], T. Ratovitski[2], G. Kim[2], A. Levey[3], S. Gandy[4], N.A. Jenkins[5], N.G. Copeland[5], D.L. Price[1,6,7], and S.S. Sisodia[1,7]. Dept of Pathology[1], Neuropathology Lab[2], Neurology[6], and Neuroscience[7], Johns Hopkins Sch of Med, Baltimore, MD 21205; Emory Univ.[3], Atlanta, GA; Cornell Univ. Medial College[4], New York, NY; Mammalian Genetics Lab[5], National Cancer Inst., Frederick, MD.

The majority of cases of early-onset familial Alzheimer's disease are linked to mutations in two related seven-nine transmembrane proteins termed the presenilins 1(PS1]) and 2 (PS2], respectively. To begin to examine the metabolism of the presenilins *in vivo*, and to develop model systems to investigate pathogenic mechanisms, we have produced transgenic mice expressing human (Hu) wt and mutant PS1, using cDNA transgenes driven by the murine prion protein promoter. Our initial analysis of ten lines of mice expressing wt Hu PS1, revealed that 42 kDa wt Hu PS1 proteins are normally cleaved to generate stable 27 kDa (N-terminal) and 18 kDa (C-terminal) fragments. Among these lines of mice, the levels of Hu PS1 mRNA and full-length 42 kDa polypeptide were proportional; increased levels of transgene-derived mRNA led to increased the levels of the 42 kDa precursor. However, the levels of accumulated 27 and 18 kDa derivatives plateaued (~3-fold over nontransgenic littermates), indicating that a saturable process governs the production or accumulation of these derivatives. We are examining the influence of wt and mutant PS1 on the processing of the amyloid precursor protein (APP) as well as the consequences of expressing these proteins in the murine nervous system. Supported by the NIH (AG05146 and NS20471), the Develbiss Fund, and the Alzheimer's Association.

293.11

FUNCTIONS OF PRESENILINS: GENERATION AND CHARACTERIZATION OF PRESENILIN 1-NULL MICE.

P.C. Wong[1,4], H. Zheng[2], H. Chen[1,4], M.E. Trumbauer[3], A.J. Roskams[1,4*], H.Y. Chen[2], L.H. Van der Ploeg[2], D.L. Price[1,2,3,4] and S.S. Sisodia[1,2,4]. Depts. of [1]Pathology, [2]Neuroscience and [3]Neurology, and [4]Neuropathology Laboratory, Johns Hopkins University School of Medicine, Baltimore, MD 21205 and [5]Dept. of Genetics and Molecular Biology, Merck Research Laboratories, Rahway, New Jersey 07065.

Mutations in the presenilin 1 (PS1) and presenilin 2 (PS2) genes segregate with the majority of early-onset familial Alzheimer's disease (FAD) pedigrees. PS1 and PS2 are integral transmembrane proteins homologous to *sel-12*, a *C. elegans* protein involved in intracellular signaling pathway mediated by members of the Notch/lin-12 family of receptors. Little is known about the physiological functions of PS or the effects of PS mutations on the cellular abnormalities characteristic of AD. In order to begin to examine the *in vivo* role of PS1 during development and aging, we ablated the expression of the PS1 gene by homologous recombination in embryonic stem cells and have generated mice heterozygous for the targeted allele. Mice homozygous for PS1 targeted allele are being generated to analyze the impact of the PS1 null state on the development, structure and functions of the nervous system. Efforts are underway to generate mice with functionally inactivated PS2 genes. Supported by grants from NIH (AG05146 and NS20471), Develbiss Fund and Alzheimer's Association.

293.12

EXPRESSION OF FAD-LINKED MUTANT PRESENILIN 1 IN TRANSGENIC MICE.

M.K. Lee[1], D. Borchelt[1], G. Thinakaran[1], H. Slunt[1], G. Kim[1], T. Ratoviski[1], L. Martin[1], S. Gandy[2], A. Levy[3], N. Jenkins[4], N. Copeland[4], D.L. Price[1,5,6*], and S.S. Sisodia[1,6]. Dept. Of Pathology[1], Neurology[5], and Neuroscience[6], Johns Hopkins School of Medicine, Baltimore, MD 21205; Cornell Univ. Medical Coll.[2], New York, NY; Emory Univ.[3], Atlanta, GA; Mammalian Genetics Lab.[4], NCI, Frederick, MD.

Mutations in genes encoding related proteins, termed presenilins 1 (PS1) and 2 (PS2), are linked to the majority of cases with early-onset familial Alzheimer's disease (FAD). To examine the pathogenic mechanism(s) of FAD, we have produced transgenic mice expressing either wild type (wt) or mutant human PS1, using cDNA transgenes driven by murine prion protein promoter. Initial immunohistochemical and light microscopic analysis of mice expressing human PS1 and PS1 with the A246E missense mutation reveals somatodendritic and axonal localization of human wt or mutant PS1 proteins. Remarkably, our initial biochemical analyses reveal that, relative to the wt human PS1, endoproteolytic processing and/or accumulation of the mutant PS1 is qualitatively different. We have also generated additional transgenic mice expressing the FAD-linked M146L PS1 variant and a PS1 variant that lacks exon 9. The expression, metabolism and subcellular distributions of these PS1 variants in transgenic mice will be discussed. Supported by the NIH, the Develbiss Fund, and the Alzheimer's Association.

293.13

APOPTOSIS AND THE ALZHEIMER'S DISEASE GENE PRESENILIN-1 Henrike Haftmann*, Jorge Busciglio, Cai'ne W. Wong, Matthias Staufenbiel+, and Bruce A. Yankner. Dept. Neurology, Harvard Medical School and the Children's Hospital, Boston, MA, + Sandoz Pharma Ltd., Basel, Switzerland

Most early-onset familial Alzheimer's disease (AD) is caused by mutation of the presenilin 1 (PS-1) or presenilin 2 (PS-2) genes. A recent report suggested that transfection of a cDNA encoding a C-terminal fragment of the mouse homolog of PS-2 protected against apoptosis (Vito et al., 1996). We therefore investigated if PS-1 overexpression is able to rescue cells from apoptotic cell death. COS cells were transiently transfected with PS-1 cDNA; controls were transfected with either the vector alone or with the vector encoding ß galactosidase (ßGal). Western blot analysis with an antibody to PS-1 revealed two endogenous bands - a major species migrating at 30-33 kD and another species at 40-43 kD. Transfection of PS-1 cDNA increased the levels of these species, which were absent in antibody preabsorption controls. Comparison of the major transfection-induced 43 kD band with the 125 kD ßgal band showed similar transfection efficiencies. To test the effect of PS-1 overexpression on apoptosis, cells were treated with either tert-butyl-hydrogen peroxide (tbh) or staurosporine. The cells were then fixed and immunostained with the PS-1 or ßgal antibodies and apoptotic cells were identified by characteristic morphological criteria. Under these conditions, PS-1 did not protect against apoptosis induced by either drug. We also investigated fibroblast cell lines from the FAD1 family and from age-matched controls for susceptibility to apoptosis induced by tbh or staurosporine. Both groups showed similar sensitivity to cell death induced by these agents. In conclusion, our data do not support the hypothesis that PS-1 exerts a protective effect against apoptotic cell death.

293.14

PS2 PARTICIPATES IN CELLULAR APOPTOSIS. B. Wolozin*, P. Vito, K. Ganjei, K. Iwasaki, E. Lacana and L. D'Adamio. Section on Geriatric Psychiatry, NIMH and T-cell Molecular Biology Unit, NIAID, Bethesda, MD 20892.

Recent discoveries have shown that mutations in the presenilin genes on chromosomes 1 & 14 cause familial Alzheimer's disease (AD), however, the function of these genes is not known. The importance of PS2 for AD lead us to directly examine the role of PS2 levels in neuronal apoptosis. Undifferentiated PC12 cells (udPC12) or neuronally differentiated PC12 cells (nPC12) were transfected with pcDNA3 vectors containing mouse PS2 in sense or antisense orientation as well as ALG3 or ϕ-64 inserts, which are dominant negative mutants for PS2. Two days after the transfection, the udPC12 cells were treated with agents known for their cytotoxicity: glutamate, hydrogen peroxide, the free radical MPP^+, A23187 or ß-amyloid$_{1-42}$, while the nPC12 cells were deprived of NGF. Following the treatment the cells were either analyzed using the MTT assay or fixed and stained for apoptotic nuclei using the TUNEL method. Transfection with antisense PS2, ALG3 and ϕ-64 protected udPC12 cells against glutamate toxicity, but did not affect sensitivity to the other insults. Antisense PS2, ALG3 and ϕ-64 also partially protected the nPC12 cells against NGF-withdrawal, increasing the reactivity in an MTT assay 3-fold and reducing the number of TUNEL positive cells below baseline. Conversely, transfection with sense presenilin increased the number of apoptotic neurons. Thus, expression of PS2 is necessary for some forms of apoptosis, and reduction of PS2 levels by transfection with vectors containing either PS2 antisense, ALG3 or ϕ-64, provides protection against glutamate toxicity of withdrawal of trophic support.

NEUROPSYCHIATRIC DISORDERS: POSTMORTEM I

294.1

PROTEIN KINASE C IN THE POSTMORTEM BRAIN OF TEENAGE SUICIDE VICTIMS, G. N. Pandey*, Y. Dwivedi, S. C. Pandey, R. Conley, R. Roberts, and C. Tamminga Psychiatric Institute, Department of Psychiatry, University of Illinois at Chicago, Chicago, IL 60612

Several lines of evidence indicate that $5HT_{2A}$ receptors are upregulated in the postmortem brain of suicide victims. $5HT_{2A}$ receptors have been shown to be linked to the phosphatidyl inositol signaling system, of which protein kinase C (PKC) is an important component. The role of PKC in suicide is presently unclear. To investigate the role of PKC, we studied [^{3}H]phorbol dibutyrate (PDBU) binding to PKC in the frontal cortex (Brodmann's areas 8 and 9) of teenage suicide victims and normal controls. Postmortem brain samples were obtained from the Brain Collection Program of the Maryland Psychiatric Research Center. K_D and B_{max} of [^{3}H]PDBU binding to PKC were determined in membranal and cytosolic fractions of the brain by the radioligand binding technique. We observed that B_{max} of [^{3}H]PDBU binding to PKC was significantly decreased in the membranal fraction of the frontal cortex obtained from suicide victims (n = 16) compared to normal control subjects (n = 19); however, B_{max} in the cytosolic fraction and K_D in both membranal and cytosolic fractions remained unaltered. These results suggest an abnormality of PKC in the brain of suicide victims and that PKC may have an important role in the pathophysiology of teenage suicide. Further studies are under way to determine whether the decrease in PKC is isozyme specific.

294.2

MORPHOMETRY OF LOCUS COERULEUS NEURONS IN ALCOHOLISM AND SUICIDE. M.D. Underwood*, D.K. Pauler, V.V. Arkhipov, J.J. Mann, D.A. Brent and V. Arango. Department of Neuroscience, Columbia University, New York, NY 10032.

We have reported fewer locus coeruleus (LC) neurons in alcoholics, suicides and alcoholics who died by suicide compared to controls (*Alcohol. Clin. Exp. Res.* 1996, In Press). We then sought to determine whether LC neuron morphometry is distinct in alcoholism and suicide.

Fixed brainstems were sectioned and Nissl-stained. Controls (n=11; ages: 17-69y) were free of drugs, by toxicological screen, and known psychiatric or neurologic disorders. Diagnosis of alcohol-dependence (n=7; ages: 32-70y) was determined by psychological autopsy or investigative report. Suicide as cause of death, with alcoholism (n=7; ages: 20-58) or without (n=6; ages: 23-84), was determined by the coroner. LC neurons were examined at three coronal levels: the decussation of the trochlear nerves, 2.5mm rostral and 5mm caudal. Neuron measurements (area, perimeter, form factor) were made with an image analysis system (Imaging Research, Inc.). Potential covariates age and postmortem interval were not significant ($p>0.05$). Neuron area in the left LC was larger than the right LC ($p<0.05$) in controls; this effect was more pronounced in females than in males. Neuron area was greater in rostral portions than caudal of the LC ($p<0.05$). In alcoholics, the area of LC neurons at the rostral level was smaller than controls (897±33 *vs.* 816±64 μm^2, $p<0.05$). LC neuron area at other levels and in suicides did not differ. Neuron shape depended on rostral-caudal level, but did not differ between groups. Reduced noradrenergic neurotransmission in alcoholics, but not suicides, may be due to neuronal degeneration or selective loss of larger neurons in rostral portions of the LC. Suicides and alcoholics appear to have distinct noradrenergic morphometric abnormalities. (Supported by the American Suicide Foundation, and PHS grants AA09004, MH47097 and MH46745)

294.3

CORTICAL α_1-ADRENERGIC BINDING IN ALCOHOLISM AND SUICIDE. M.J. Bakalian,* M.D. Underwood, J.J. Mann, N. Paykina, S.A. Kassir, M. Douglass, S. Gapon, D.A. Brent, S.P. Ellis, S. Li and V. Arango. Department of Neuroscience, Columbia University, New York, NY 10032.

We previously reported reduced β_1-adrenergic binding (Allen *et al.*, Soc. Neurosci. Abstr., 1993) as well as reduced α_2-adrenergic binding (Mann *et al.*, Soc. Neurosci. Abstr., 1996) in the prefrontal cortex (PFC) of alcoholics who died by suicide and other causes, compared to controls. Using quantitative autoradiography, we sought to compare binding to α_1-adrenergic sites in the same three groups of subjects (n=14 matched triplets). Assays were performed as previously described (Arango *et al.*, Brain Res., 1993) using PFC coronal sections from the right hemicerebrum and 4nM ^{3}H-prazosin. The coroner or medical examiner ruled whether the cause of death was suicide. Alcohol dependence was determined by psychological or physical autopsy. Quantitative analysis of α_1-adrenergic binding was performed by identifying isodensity bands that correspond in gyri to layers I II, III, IV-V and VI. In sulci, there are three bands over layers I-II, III and IV-VI. Data were tested using variance component analysis with age, sex and PMI as covariates. Binding was lower (8% to 16%) in alcoholics than in controls in dorsolateral (area 9), ventrolateral (areas 46 and 47) and orbital PFC (area 12 sulcus). Similarly, binding in suicide alcoholics was lower (9% to 14%) than in controls in gyral area 9 and sulcal area 12. Binding decreased with age, but not with PMI, in the dorsolateral and lateral PFC. No sex differences were detected. In conclusion, the suicide and nonsuicide alcoholic groups did not differ from each other but had lower binding than controls, suggesting that the effect of alcohol overrides any increase in binding present in nonalcoholic suicides (Brain Res., 1993).

Supported by PHS grants AA09004, MH40210, MH47097 and MH46745.

294.4

ALTERATIONS IN α_2-ADRENERGIC BINDING DISTINGUISH ALCOHOLIC SUICIDE VICTIMS FROM ALCOHOLICS. J.J. Mann,* M.D. Underwood, D.A. Brent, S.A.Kassir, V.L. Johnson, M. Douglass, S.P. Ellis, S. Li, and V. Arango. Department of Neuroscience, Columbia University , New York, NY 10032.

Suicide rates are markedly elevated for alcoholics. In addition to changes in the serotonergic system, other neurotransmitter systems seem to be involved in suicidal behavior and alcoholism. We previously reported that β_1-adrenergic binding is reduced in the brain of alcoholics and then sought to determine whether α_2-adrenergic binding is also altered. We performed quantitative autoradiography in coronal sections of the right prefrontal cortex (PFC), just anterior to the genu of the corpus callosum. We compared alcohol-dependent suicide victims to alcohol-dependent nonsuicides as well as normal controls. Toxicological screens were negative for all drugs, except alcohol. We matched cases on age, sex and postmortem interval (PMI, n=14 triplets). Sections (20μm) were pre-incubated, pre-washed and incubated in 4nM ^{3}H-*p*-amino clonidine (^{3}H-PAC) to label α_2-receptors as described previously (Arango *et al.*, Brain Res., 1993). The distribution of α_2-adrenergic receptors was the same in both groups and formed five isodensity bands corresponding to layer I, layer II, upper layer III, layers III-IV and layers V-VI. Binding was densest in layer I and upper layer III and least dense in layers V-VI. Data were examined using a variance component analysis with age, sex and PMI as covariates. The nonsuicide alcoholic group did not differ from the control group in the level of α_2-adrenergic binding in any of the areas studied. The alcoholic suicide group had approximately 25% less binding than the controls in lateral and ventral PFC ($p<0.01$). Binding decreased with age, but not with PMI. No sex differences were detected. Lower α_2-adrenergic binding in ventrolateral PFC may reflect a component of altered noradrenergic function that contributes to suicidal behavior, so prevalent in alcoholics. (AA09004, MH47097 and MH46745)

294.5

PATTERNS OF 5-HT$_{1A}$ BINDING IN ALCOHOLISM AND SUICIDE. V. Arango, M.D. Underwood, S.A. Kassir, D.A. Brent, A. Khaibullina, V. Arkhipov, S.P. Ellis, S. Li, and J.J. Mann. Dept. of Neuroscience, Columbia University, NY 10032.

Serotonin alterations have been reported in suicidal behavior. Serotonin is also involved in the regulation of alcohol preference and intake. Using quantitative autoradiography, we compared 5-HT$_{1A}$ binding in the prefrontal cortex (PFC) of alcohol-dependent individuals (suicide and nonsuicide) and normal controls with negative toxicological screens for other drugs. Alcohol-dependence was determined by physical and/or psychological autopsy. Subjects were matched for postmortem delay (PMI), age and sex (N=16 triplets). Coronal sections (20μm) were incubated with 2nM ^{3}H-8-OH-DPAT as described previously (Arango *et al.*, Brain Res., 1995). 5-HT$_{1A}$ receptor distribution across cortical layers was similar in all groups, forming five isodensity bands. Binding was approximately 30% higher in ventral and medial cortex than in dorsal and lateral cortex. The alcoholic group had 20% less 5-HT$_{1A}$ binding than the controls in lateral PFC (area 46, p=0.036), but not in other areas. When a variance component analysis was done, with an additional 56 subjects of matched nonalcoholic suicides (n=28) and controls (n=28), a total of 104 subjects, the alcoholic nonsuicides had reduced binding in all areas of ventrolateral PFC (45 and 46). The alcoholic suicides had reduced binding in area 45. Binding increased with age in lateral and orbital prefrontal cortex and decreased with PMI in the same areas. Females had more binding than males in lateral, orbital and medial PFC. We conclude that: 1. 5-HT$_{1A}$ binding is decreased in some brain regions of alcoholics; and 2. Binding in alcoholics changes in the opposite direction to that in nonalcoholic suicides. This study provides further evidence linking serotonin to alcoholism, suggesting a serotonergic deficiency in alcoholic suicide victims. (AA09004, MH40210, MH47097 and MH46745)

294.6

SUICIDE AND THE HUMAN 5-HT$_{1D\beta}$ RECEPTOR AND GENE. Y. Huang*, R. Grailhe, M. Zhou, R. Hen, V. Khait, V. Arango and J.J. Mann. Dept. of Neuroscience, New York State Psychiatric Institute, New York, N.Y. 10032.

Knockout of 5-HT$_{1B}$ gene in mice results in reduced latency aggression and increased aggression. We decided to study the 5-HT$_{1D\beta}$ gene and receptor binding in the brain of suicide victims to determine whether this gene is involved in suicide. Here we report the detection of two 5-HT$_{1D\beta}$ receptor gene polymorphisms of the coding sequence and the allelic association of 5-HT$_{1D\beta}$ receptor genes with receptor-binding indices in both suicide and control groups. Single-strand conformational polymorphism (SSCP) analysis and DNA sequencing technique were used to screen the entire coding region of the 5-HT$_{1D\beta}$ gene for possible variations in genomic DNA isolated from postmortem human brain tissue (N=58). Amplification of the genomic DNA was accomplished with three primer pairs. To optimize the sensitivity of the SSCP analysis, PCR fragments were further digested with different restriction endonuclease enzymes (Eae I, Sty I, Sac II and Bmy). The binding indices (B_{max}, maximal binding sites; K_d, binding affinity) of the 5-HT$_{1D\beta}$ receptor were determined in Brodmann area 9 of postmortem brain tissue using 5-hydroxyl[^{3}H]tryptamine as the ligand and sumatriptan as the displacer. B_{max} and K_d did not differ in suicides and controls (44.0 vs 41.3 fmol/mg pr. and 1.3 vs 1.1 nM respectively). Two common polymorphic patterns were identified in the 5-HT$_{1D\beta}$ gene with allelic frequencies of 0.76 and 0.24 in the control subjects (N=29), and they did not differ from frequencies of 0.69 and 0.31 in the suicide subjects (N=29). The SSCP variants were caused by a silent C to T substitution at nucleotide 129 and a silent G to C substitution at nucleotide 861 of the coding region. No significant correlation between B_{max} and genotype was observed. The data suggest that there are two silent mutation points in the coding sequence of 5-HT$_{1D\beta}$ receptor gene and that 5-HT$_{1D\beta}$ receptor-binding characteristics and gene polymorphism are not correlated. This study did not find evidence of an abnormality in the gene or gene product for the 5-HT$_{1D\beta}$ receptor in suicide victims. (Supported by MH40210 and MH46745)

294.7

DECREASED CYTOSOLIC [^{3}H]cAMP BINDING IN BIPOLAR DISORDER (BD) POSTMORTEM BRAIN. S. Rahman, P.P. Li*, L.T. Young, S.J. Kish, O. Kofman and J.J. Warsh. Clarke Institute of Psychiatry,University of Toronto, Toronto, ON, Canada

Although hyperfunction of the G protein-coupled adenylyl cyclase pathway has been implicated in BD (Avissar and Schreiber, Biol. Psychiatry, 31:435, 1992), direct evidence to support altered cAMP signaling in this disorder is still elusive. cAMP-dependent protein kinase (PKA), a tetrameric protein composed of 2 regulatory (R) and 2 catalytic subunits, is the primary target of intracellular cAMP signaling. PKA R-subunit levels are modulated by changes in cytosolic cAMP levels consequent to activation of receptors coupled to this second messenger pathway. We assessed here whether [^{3}H]cAMP binding, a measure of PKA R- subunit levels, is altered in postmortem BD brain regions in which higher Gα_s protein levels have been previously identified. [^{3}H]cAMP binding was measured by the procedure of Takeda *et al.* (J. Biochem. 105:327, 1989). In temporal cortex membrane fractions, [^{3}H]cAMP binding was saturable and specific with K_d values of 0.89 ±0.1 nM (site 1) and 3.6 ± 0.47 nM (site 2), and B_{max} of 154 ± 28 fmol/mg protein (site 1) and 283 ± 37 fmol/mg protein (site 2), respectively. Cytosolic [^{3}H]cAMP binding was best described by single site binding with K_d and B_{max} of 0.73 ± 0.04 nM and 232 ± 7 fmol/mg protein, respectively. Compared with age and postmortem-delay matched controls (n=5-10), specific [^{3}H]cAMP (5 nM) binding was significantly reduced (F= 6.545, *df* [1,80], p = 0.012) in cytosolic fractions across all examined regions of BD brain (frontal [-22%], temporal [-23%], occipital [-22%] and parietal cortex [-15%], and cerebellum [-36%] and thalamus [-13%]), but there were no differences in [^{3}H]cAMP binding in the membrane fractions from these same regions. Lithium levels measured in BD temporal cortex did not correlate significantly with [^{3}H]cAMP binding. The reduced cytosolic [^{3}H]cAMP binding may reflect modulatory changes secondary to altered cAMP signaling in BD brain. The possibility that antemortem lithium and/or other mood stabilizer treatment may contribute to the above changes cannot be ruled out, however. (Supported by the grants from the MRC (JJW&LTY). SR is a Clarke Institute Research Foundation fellowship recipient and LTY an Ontario Career Scientist awardee)

294.8

METHODOLOGICAL PROBLEMS WITH THE DOPAMINE D$_4$ SUBTRACTION METHOD IN POSTMORTEM BRAIN STUDIES. M. Li, H. Fang, D. Helmeste and S.W. Tang.* Depts. of Psychiatry and Pharmacology, University of California, Irvine, Irvine CA 92717.

The ability to quantify dopamine D$_4$ receptors in various psychiatric and neurological disorders is hampered by the lack of a specific dopamine D$_4$ ligand. A compromise using the differential labeling properties of ^{3}H-raclopride (D$_2$ and D$_3$) and ^{3}H-nemonapride (D$_2$, D$_3$ and D$_4$) was proposed by Seeman et al (1993) and applied to postmortem schizophrenic brain tissues. Several groups have since replicated this method, producing variable results. Our data show that the conditions of assay, specifically the selection of agents used to define specific binding, appear to be the most critical. Sulpiride, as originally described by Seeman et al. (1993), appeared to be the most specific, yet produced no D$_4$ differential consistently in seven normal control brains. Haloperidol and butaclamol baseline both included a substantial σ component and produced an apparent "D$_4$ differential", part of which is σ. Data indicated that additional binding sites are also defined by these compounds, but not by sulpiride. A full Scatchard analysis of tissues is also essential as a 6 nM concentration of ^{3}H-raclopride and a 1 nM concentration of ^{3}H-nemonapride yield a positive differential in all 7 brains, with 6 nM ^{3}H-raclopride being close to (75% of Bmax) but not at the saturation point. Our results caution the application of the subtraction method to measure dopamine D$_4$ receptors (postmortem brain tissues obtained from NNRSB, VAMC, LA).

(Non-commercial/non-grant funding sources)

294.9

EFFECT OF NEUROLEPTICS ON THE SUBREGIONAL DISTRIBUTION OF GLUTAMATE DECARBOXYLASE$_{65}$-IMMUNOREACTIVE TERMINALS IN THE HIPPOCAMPUS OF CONTROL AND SCHIZOPHRENIC SUBJECTS (SZs). M.S. Todtenkopf and F.M. Benes.* Laboratory for Structural Neuroscience, McLean Hospital, Belmont, MA; Department of Psychiatry and Program in Neuroscience, Harvard Medical School, Boston, MA.

Recent findings of increased GABA$_A$ receptor binding on NPs in sector CA$_3$ and a reduced density of nonpyramidal neurons (NPs) in sector CA$_2$ of SZs has prompted interest in the question of whether there may be a loss of GABAergic activity in the regio inferior of patients with this disorder. In order to test this hypothesis, an extensive and reliable method for localizing the 65 kD isoform of glutamate decarboxylase (GAD) has been developed and applied to a cohort of controls (CONs) (N = 11) and SZs (N = 11) matched for age (59 and 57 yrs, respectively), postmortem interval (18 and 20 hrs, respectively) and gender. The data revealed no difference in the density of GAD$_{65}$-immunoreactive (IR) terminals on pyramidal cells (PNs), NPs or in neuropil (NPL) of sectors CA$_{1-4}$. Similarly, no differences were observed in the strata moleculare, radiatum or oriens of any of CA subfields. Regression analysis, however, revealed a highly significant ***positive correlation*** between neuroleptic dose (CPZ) and the density of GAD$_{65}$-IR terminals on PNs (r = 0.81; p = 0.002) and NPs (r = 0.78; p = 0.005) of sector CA$_4$ and in the stratum oriens of sectors CA$_3$ (r = 0.70; p = 0.016) and CA$_2$ (r = 0.77; p = 0.009). Overall, these data are consistent with a model in which subtle losses of inhibitory activity, particularly in sectors CA$_3$ and CA$_2$ of SZs may be compensated for, at least in part, by CPZ-induced increases of GABAergic terminals in the stratum oriens of these same sectors. Supported by MH42261, MH00423 and MH31154.

294.10

RELATIVE DISTRIBUTION OF PYRAMIDAL (PN) AND NONPYRAMIDAL (NP) NEURONS IN THE HIPPOCAMPUS OF SCHIZOPHRENIC (SZ) AND MANIC DEPRESSIVE (MD) BRAIN. F.M. Benes, E. Kwok, S.L. Vincent.* Laboratory for Structural Neuroscience, McLean Hospital, Belmont, MA; Department of Psychiatry and Program in Neuroscience, Harvard Medical School, Boston, MA.

Recent postmortem studies have suggested that there may be a decrease of GABAergic neurons and/or activity in layer II of the anterior cingulate and prefrontal cortices of SZs. The recent finding of a preferential upregulation of the GABA$_A$ receptor on NPs in CA$_3$ has suggested that this subregion may also be a potential site for defective GABAergic modulation in SZ. To explore this possibility, a comparison of the relative distribution of PNs and NPs in sectors CA$_{1-4}$ from normal control (N = 11) and SZs (N = 10) cases matched for age, postmortem interval and gender has been undertaken. All neurons in a single cross-section of the hippocampus (approximately 3000 neurons/case) at the level of the lateral geniculate nucleus were determined to be either a PN or NP. For PNs, there were no differences in the average number, density or size in any of the sectors examined. In contrast, there was a 40% reduction in the number (p = 0.05) and a 50% decrease in the density (p = 0.01) of NPs in sector CA$_2$, but not CA$_1$, CA$_3$ or CA$_4$. MD patients (N = 5) also showed a selective reduction in the total number and density of NPs in sector CA$_2$ only; however, both neuroleptic-treated and neuroleptic-free SZs and MDs showed a reduction of NPs in CA$_2$, indicating that this finding is probably not related to an antipsychotic drug effect. Taken together, these data are consistent with the idea that a possible reduction of disinhibitory GABAergic activity in CA$_3$ of SZs may be related to a selective loss of NPs in sector CA$_2$. Overall, however, these data implicate the regio inferior as a central site for GABAergic dysfunction in ***both*** SZ and MD brain. Supported by MH00423, MH42261 and MH31862.

294.11

NEURONAL SIZE, SHAPE, AND ORIENTATION IN THE NORMAL LEFT AND RIGHT HIPPOCAMPUS ARE ALTERED IN SCHIZOPHRENIA. D.W. Zaidel *, M.M. Esiri ‡, and P.J. Harrison § *Dept. of Psychology, UCLA, Los Angeles, USA; ‡Clinical Dept. of Neuropathology, Radcliffe Infirmary, Oxford, UK; §University Dept. of Psychiatry, Warneford Hospital, Oxford, UK.

The morphological cytoarchitecture of the hippocampus, on either side, is poorly delineated in normal (NRM) or in schizophrenic (SCZ) individuals. We studied the two hippocampi in 14 chronic SCZ and 17 NRM using 10 microns nissl-stained sections. All brains were neuropathologically normal; groups were matched for age, post-mortem delay, and fixation time, and were coded for data collection. Neurons in several regions within hippocampal subfields CA1 - 4 and subiculum were captured on a computer with a video camera attached to a microscope (objective x10). Total magnification was x250. The digitized images were analyzed semi-automatically for size, shape, and orientation on a Macintosh computer. Minimal left-right differences in NMR were observed. In SCZ, neuronal size was consistently smaller in subfields of the left hippocampus than left NRM. Cell shape was significantly different in SCZ in subfields of both hippocampi, being less triangular (more ovoid) than in NRM. There were no group differences in variability of neuronal orientation. The findings provide further indications of abnormal hippocampal cytoarchitecture in SCZ and suggest that some changes in the disease may affect the two hippocampi asymmetrically.

(Supported by the Wellcome Trust, Stanley Foundation, and NIH NS20187.)

294.12

COGNITIVE DEFICITS WITHOUT A CHOLINERGIC CAUSE: CHOLINE ACETYLTRANSFERASE ACTIVITY IN THE PARIETAL CORTEX OF 95 ELDERLY SCHIZOPHRENICS. P. Powchik*, K.L. Davis, and V. Haroutunian, Dept. Psychiatry, Mount Sinai School of Medicine and Bronx VA Medical Center, New York.

Cognitive deficits are among the prominent symptoms of schizophrenia in chronically ill elderly patients. The neurobiological substrates of these cognitive deficits are unknown. Although a variety of neuropathological and neurochemical abnormalities have been reported in postmortem specimens from elderly schizophrenics, none have been found to account adequately for these cognitive deficits. Because Alzheimer's disease and the cortical cholinergic deficits associated with it is the most common cause of cognitive dysfunction in the elderly, it is possible that a disproportionate number of elderly schizophrenics suffer from either Alzheimer's disease and/or cortical cholinergic deficits associated with it. We compared the activity of choline acetyltransferase, a marker of cholinergic function, and neuropathological indices of Alzheimer's disease in the parietal cortex of 20 normal elderly controls, 95 elderly schizophrenics, and 135 Alzheimer's disease cases. Neither choline acetyltransferase, nor neuropathological indices of Alzheimer's disease accounted for the antemortem or postmortem assessed cognitive deficits in these patients. This study of a large, antemortem assessed and diagnosed cohort of elderly schizophrenics demonstrates that neurological lesions other than those associated with Alzheimer's disease or cortical cholinergic dysfunction must be responsible for the severe cognitive deficits suffered by this population.

This work supported by a VA Merit Review awarded to V. Haroutunian

294.13

SENILE DEGENERATION IN THE BRAINS OF COGNITIVELY IMPAIRED, ELDERLY SCHIZOPHRENICS. A.J. Dwork, E.S. Susser, C. Waniek, J. Keilp, D. Liu*, M.A. Kaufman, I. Prohovnik New York State Psychiatric Institute and Columbia University, New York, NY.

Schizophrenia is frequently associated with cognitive impairment that increases with age and contributes significantly to poor social functioning. In order to determine whether senile degenerative changes contribute to this impairment, we evaluated neuritic (Alz 50-immunoreactive) senile plaques (nSP) and neurofibrillary tangles (NFT) in neocortex and hippocampus from 66 chronically institutionalized schizophrenics, aged 45-105, and 26 similarly aged, chronically institutionalized individuals with mood disorder. These were compared to autopsy specimens from 14 patients without psychiatric disease. Schizophrenics judged by review of medical records to have definite cognitive impairment (DCI) were older and had significantly more nSP and NFT than did those without DCI. Restriction of the analysis to schizophrenics aged <85 eliminated the age difference, but the differences in nSP and NFT persisted. Levels of nSP and NFT in the schizophrenics without DCI were significantly and substantially lower than in nonpsychiatric patients without cognitive impairment. This difference was not found when the nonpsychiatric patients were compared to the mood disorder patients without DCI, who were from the same institutions as the schizophrenics and had received similar somatic treatments. It thus appears that cognition in chronically institutionalized schizophrenics shows an increased sensitivity to senile degeneration, and that this cannot be ascribed to institutionalization or to the side effects of therapy. *AG10638, MH50727*

294.14

LOSS OF SUBICULAR MAP-2 IMMUNOREACTIVITY IN PSYCHIATRIC DISORDERS G. Rosoklija, A.P. Hays, N. Latov, S.A. Sadiq, M. Kaufman, C. Waniek, J. Keilp, I. Prohovnik, A.J. Dwork* New York State Psychiatric Institute and Columbia University.

Loss of subicular MAP-2 immunoreactivity in schizophrenia has been reported in two small studies. We now report a larger study that confirms this finding but finds it not to be specific for schizophrenia.The current study examines MAP-2 immunoreactivity in the brains of 96 chronically institutionalized, mostly aged psychiatric patients, 51 with schizophrenia and 45 with dementia, mood disorder, and other psychiatric conditions. These were compared to 14 nonpsychiatric patients from a general hospital. MAP-2 immunoreactivity was evaluated subjectively by 2 observers blind to the source of the specimens and by 2 computerized densitometry protocols conducted by a similarly blinded operator. In all nonpsychiatric cases, subicular MAP-2 immunoreactivity was similar to that in CA4. Among the psychiatric cases, there was much greater variability, with subicular MAP-2 levels that were both higher and lower than in CA4. Consistent with our earlier finding in schizophrenia, low levels of subicular and CA1 MAP-2 were present in approximately one-third of psychiatric patients, regardless of diagnosis (schizophrenia, dementia, mood disorder, and "other"). Among dementia cases, results were similar for those with and without neuropathologically verified Alzheimer's disease. We conclude that there is an association between MAP-2 abnormalities and disturbed mentation that may be constant across various illnesses.

Supported by AG10638, MH50727, and the Stanley Foundation.

TRANSPORTERS III

295.1

α_1 ACID GLYCOPROTEIN DOES NOT MODIFY [^{3}H]-CITALOPRAM BINDING OR [^{3}H]-5-HT UPTAKE BY THE HUMAN SEROTONIN TRANSPORTER *IN VITRO*. M. J. Owens*, W. N. Morgan and C. B. Nemeroff. Lab. of Neuropsychopharmacology, Dept. Psychiatry & Behavioral Sciences, Emory University School of Medicine, Atlanta GA 30322.

α_1 acid glycoprotein is the major serum protein responsible for nonspecific protein binding of basic drugs. Although a normal constituent of serum, α_1 acid glycoprotein is an acute phase protein produced in large amounts by the liver, and perhaps other organs including the brain, under stressful conditions. Our group (Arch Gen Psychiat 47:337-342, 1990) and others have observed a significant increase in serum α_1 acid glycoprotein concentrations in depressed patients. Additionally, others have reported that this protein can directly interact with, and modify the function of, the serotonin transporter (SERT). Because of the role of the SERT in the mechanism of action of antidepressants and, perhaps, the pathophysiology of depression, we have scrutinized the effects of human α_1 acid glycoprotein on [^{3}H]-citalopram binding and [^{3}H]-5-HT uptake in cells stably expressing the human SERT. α_1 acid glycoprotein (10^{-9} - $10^{-4.5}$ mol/L) did not alter either the K_d or B_{max} of [^{3}H]-citalopram binding. These concentrations of α_1 acid glycoprotein did dose-dependently decrease free [^{3}H]-citalopram concentrations as predicted from clinical pharmacokinetic studies. Identical concentrations of α_1 acid glycoprotein also failed to alter either the K_m or V_{max} of [^{3}H]-5-HT uptake. Although direct modulation of the SERT by α_1 acid glycoprotein is very intriguing, our studies suggest that any allosteric modulation is not a direct action of α_1 acid glycoprotein on the SERT molecule. Supported by DA-08705, MH-51761, and MH-42088.

295.2

Mammalian Brain PROT is Substantially Enriched in Highly Purified Synaptic Vesicles. R. T. Fremeau, Jr.*, D. Kleyen, and B. Domin, Dept. of Pharmacol., Duke University Medical Center, Box 3813, Durham, NC 27710.

The mammalian, brain-specific, high affinity L-proline transporter (PROT) is expressed by a subpopulation of glutamatergic neurons in rat brain (Velaz-Faircloth et al., J. Biol. Chem. 270:15755-15761,1995). Ultrastructural immunogold-silver labeling studies revealed that the PROT protein is localized to presynaptic excitatory nerve terminals in the dorsal striatum and stratum oriens of the CA1 region of the hippocampal formation (Fremeau et al., Soc. Neurosci. Abstr., Vol. 21, part 3, p. 2062, 1995). Only a subset of the morphologically-identified excitatory nerve terminals were immunolabeled. Surprisingly, in the labeled terminals the majority of immunogold-silver particles were found over synaptic vesicles. To further explore the unexpected vesicular localization of PROT, we isolated highly purified synaptic vesicles from nerve terminals and monitored the distribution of PROT during the purification in comparison to several synaptic vesicle and plasma membrane markers. We report that PROT is substantially enriched in the highly purified synaptic vesicle fraction compared to the markers for the plasma membrane. These results confirm that the immunoreactive PROT protein is targeted to synaptic vesicles in specific excitatory nerve terminals. PROT is the only protein characterized at the molecular level that is localized to synaptic vesicles in a specific subset of excitatory nerve terminals. To develop a model system for examining the subcellular targeting of the PROT protein we generated stably transfected MDCK cell lines. Immunofluorescent staining revealed that PROT is localized to a population of intracellular vesicles in the cytoplasm of MDCK cells. Little, or no plasma membrane staining was observed. These results indicate that the PROT protein contains within its primary amino acid sequence information necessary for targeting to intracellular vesicles. Supported by NIH NS32501.

295.3

MOLECULAR CLONING AND CHARACTERIZATION OF THE *rosA* MUTANT IN DROSOPHILA, C. Geng, M. G. Burg*, G. Koliantz, Y. Guan, and W. L. Pak. Dept. of Biological Sciences, Purdue University, West Lafayette, IN 47907

Among the collection of mutants in the Pak laboratory, those that are known to be defective in photoreceptor cell function include a class of mutants known as the receptor oscillation (*ros*) mutants, which are characterized by rapid oscillations in the electroretinogram (ERG) in response to a light stimulus. We report the molecular cloning of the *rosA* (receptor oscillation A) gene using a positional cloning strategy. The *rosA* locus was mapped to the 24F6-25A1 region of chromosome 2L. We obtained P1 clones that were reported to cover this region from the *Drosophila* Genome Project. We performed Northern blot analysis using individual fragments from these genomic clones as probe and identified two DNA fragments that detected a significant reduction in a 3.7 kb transcript in several *rosA* mutants. These fragments were used as probe to isolate clones from a *Drosophila* adult head cDNA library. The largest cDNA clone obtained (3.6 kb) was used to rescue the *rosA* mutant phenotype via P-element mediated germline transformation, using a heat shock promoter to drive the expression of cDNA. All transformant lines generated (N=14) demonstrated a heat-induced rescue of the *rosA* mutant phenotype. The largest open reading frame in the cDNA encodes a protein of 777 amino acids which shares homology to a class of membrane transporters. These results indicate that the *rosA* gene product is a membrane transporter that is required for normal photoreceptor cell function. Supported by NIH grants MH49727 and EY00033.

295.4

CLONING AND EXPRESSION OF A NEURONAL-SPECIFIC K-Cl COTRANSPORTER IN RAT BRAIN. J. A. Payne, T. J. Stevenson, and L. F. Donaldson*. Depts. of Human Physiology and Biological Chemistry, Univ. of Calif. Sch. of Med., Davis, CA 95616.

Using a combination of database searching, PCR, and library screening, the full-length cDNA of a K-Cl cotransporter isoform (KCC2) was identified in rat brain that is specifically localized in neurons. A cDNA of 5566 bases was obtained from overlapping clones that encoded a protein of 1116 amino acids with a core molecular mass of 124-kDa. Over its full-length, the amino acid sequence of KCC2 is 67% identical to the widely distributed K-Cl cotransporter isoform (KCC1) but only 26% identical to other members of the cation-chloride cotransporter gene family. Structural analysis indicates 12 putative transmembrane (TM) segments with 4 putative glycosylation sites on an external intermembrane loop between TM5 and TM6. HEK-293 cells stably transfected with the full-length cDNA expressed a ~50-fold greater furosemide-sensitive ^{86}Rb influx than control cells. This ^{86}Rb influx absolutely required external Cl^- but not external Na^+. Rat tissue Northern blot analysis revealed a very highly expressed ~5.6-kb transcript in brain only. Reverse-transcriptase PCR revealed that KCC1 was present in rat primary astrocytes and rat C6 glioma cells but that KCC2 was absent from these cells, suggesting that KCC2 is not of glial cell origin. *In situ* hybridization studies demonstrated that the KCC2 transcript was expressed at very high levels in neurons throughout the central nervous system, including pyramidal neurons of the hippocampus, granular cells and Purkinje neurons of the cerebellum, and many groups of neurons throughout the brainstem. It is proposed that KCC2 is a major Cl^- efflux pathway in neurons and helps maintain low cell $[Cl^-]$ and an inwardly-directed Cl^- electrochemical gradient for the proper function of ligand-gated Cl^- channels (i.e. $GABA_A$ receptor) in postsynaptic inhibition. (Supported by start-up funds)

295.5

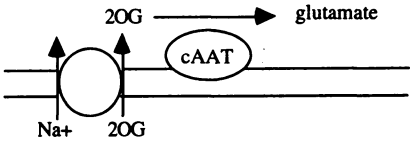
MODULATION OF CREATINE TRANSPORTER ACTIVITY BY PROTEIN KINASES. Tea N. Kekelidze* and Mario D. Saltarelli. Department of Neurology, Emory University School of Medicine, Atlanta, GA 30322

The creatine kinase/phosphocreatine (CK/PC) system plays an important role in cellular ATP homeostasis. An adequate supply of creatine is necessary for the maintenance of PC levels and is achieved through the action of a high-affinity, sodium- and chloride-dependent creatine transporter (CREAT), which has been recently cloned and identified as a member of norepinephrine/GABA transporter gene family. The deduced amino acid sequence of CREAT demonstrates the presence of putative sites for phosphorylation by protein tyrosine kinase (PTK) and protein kinase C (PKC). Creatine transport activity may provide a site for the regulation of creatine homeostasis. When rat muscle (L6) and glial (C6) cell lines were grown in creatine-free media, sodium-dependent high-affinity [^{14}C]creatine uptake (V_{max}) increased markedly in a time-dependent manner. To examine the role of protein kinase-mediated regulation of CREAT activity, we assessed the effects of the PTK inhibitor herbimycin and the PKC activator phorbol 12-myristate 13-acetate (PMA) on sodium-dependent [^{14}C]creatine uptake in L6 myoblasts. When L6 cells were incubated with 10 nM PMA for 40 min, specific [^{14}C]creatine uptake decreased by 30±3% (X±S.E.M; N=5; $p<0.002$) when compared to parallel wells incubated in either the inactive 4α-phorbol or vehicle. The effect of PMA on [^{14}C]creatine uptake was both time-and concentration dependent. Additionally, when cells were incubated for 24 hr in the presence of 50 nM herbimycin during exposure to creatine-free medium, specific [^{14}C]creatine uptake was reduced by 22±3% (X±S.E.M; N=5, $p<0.002$) when compared to vehicle controls. However, when L6 cells were incubated with herbimycin (1-24 hr) in 5 mM creatine medium, specific [^{14}C]creatine uptake was unaltered, demonstrating that herbimycin is selectively inhibiting the mechanism which leads to CREAT up-regulation in creatine-free medium. These data suggest that CREAT activity may be modulated by two distinct protein kinases. Supported by NINDS NS01651 (MDS) and a Markey CNS Fellowship (TNK).

295.6

CYTOSOLIC ASPARTATE AMINOTRANSFERASE BINDS TO NEURONAL PLASMA MEMBRANES. John C. Lehmann*, Denis Kapkov and Angela Harty, Dept. Neurosurgery MS 407, Medical College of Pennsylvania and Hahnemann University, Philadelphia PA 19102-1192.

Two genes encode aspartate aminotransferase(AAT), one which is incorporated into mitochondria (mAAT) and one which is found in the cytosol (cAAT). High cAAT-like immunoreactivity has been used in the past to map putative glutamatergic neurons. cAAT converts 2-oxoglutarate (2OG) into glutamate. A high affinity sodium dependent carrier exists in the plasma membrane for 2OG.



Data obtained from Western blots suggests that cAAT binds to the neuronal plasma membrane in brain with a heterogeneous regional distribution, correlating with the known density of glutamatergic innervation of the brain. The hypothesis is entertained that cAAT is bound to the internal surface of the glutamatergic neuronal membrane to rapidly convert 2OG, released from astrocytes, to neurotransmitter glutamate. Extracellular glutamate exerts a negative feedback inhibition of 2OG uptake. EY10140

295.7

Transport-related current by D-amphetamine in HEK-293-cells stably expressing the human dopamine transporter.

H.H.Sitte[1], S.Huck[2*], C.Pifl[1].
[1]Inst.of Biochemical Pharmacology and [2] Inst. of Neuropharmacology, University of Vienna, A-1090 Vienna, Austria.

The dopamine transporter is a member of the family of Na^+/Cl^--dependent neurotransmitter-transport molecules. Interest in this plasmalemmal protein stems from its role as a terminating factor of dopaminergic neurotransmission and from its involvement in the action of the psychostimulants amphetamine and cocaine. To study the mechanism of translocation of substrates we measured transport-coupled charge transfer by the patch-clamp technique in the whole-cell configuration.

Human embryonic kidney 293-cells were transfected with the cDNA of the human dopamine transporter in the exprcssion vector pRc/CMV and stably expressing cell clones were selected by neomycin resistance. From the different cell lines obtained we used a clone for electrophysiological studies which expressed a maximal dopamine transport rate of 1.6 ± 0.3 fmol/min/cell (at 37° C). At a holding potential of -70 mV, dopamine and amphetamine induced inward currents in a concentration-dependent manner when added by rapid superfusion system. The EC_{50} of the effect of amphetamine was 0.4 μM. At 10 μM, D-amphetamine induced an inward current of 8-12 pA/100 pF (n=5). At 30 μM, the maximal inward current induced by D-amphetamine was higher than that induced by dopamine. Effects of dopamine and D-amphetamine were blocked by 30 μM cocaine, which per se did not evoke an inward current.

These results show that D-amphetamine behaves electrophysiologically like a substrate for the dopamine transporter and suggest that D-amphetamine is translocated into the cell at least at the same rate as the natural substrate dopamine.

295.8

NEURONAL AND ASTROCYTIC EXPRESSION OF EAAC1, A HIGH-AFFINITY GLUTAMATE TRANSPORTER, IN THE CEREBRAL CORTEX. E. Conti*, A. Minelli, M. Melone, S. DeBiasi[‡], and J.D. Rothstein°. Inst. Hum. Physiol., Univ of Ancona, Ancona, Italy; [‡]Dept. Physiol. and Biochem., Univ. of Milano, Milano, Italy; and °Dept of Neurology, Johns Hopkins Univ., Baltimore, MD, 21287, USA.

The cellular localization, distribution, morphology, and transmitter content of neocortical cells expressing EAAC1, a high-affinity glutamate transporter, have been studied by immunocytochemistry with antipeptide antibodies (Rothstein et al., 1994) raised in rabbits against the C-terminal amino acid sequence (DKSDDTISFTQTSQF) of the transporter (Kanai et al., 1992, 1995; Bjoras et al., 1996). Adult rats were perfused through the ascending aorta with 4% paraformaldehyde for light microscopy, or with 4% paraformaldehyde and 0.5% glutaraldehyde for electron microscopy.

Light microscopic analysis showed that: i) EAAC1-positive neurons are present throughout the cortical mantle, although they are more numerous in layers V-VI and II-III; ii) EAAC1-positive neurons are mostly pyramidal, but some are non-pyramidal; and iii) some EAAC1-positive glial cells are present in the white matter underlying the neocortex. Co-localization studies performed using the paired surface method (Kosaka et al., 1985) showed that: i) most EAAC1-positive pyramidal neurons are Glu-positive; ii) most EAAC1-positive non-pyramidal neurons are GAD-positive; and iii) most EAAC1-positive glial cells of the white matter are GFAP-positive. At the electron microscopic level, EAAC1 immunoreactivity in the cerebral cortex is present in neuronal perikarya and dendritic processes, but not in axon terminals, and in some distal astrocytic processes.

The present results suggest that the function of EAAC1 may not be limited to the termination of Glu-mediated synaptic transmission. Indeed, the expression of EAAC1 in Gluergic and GABAergic neurons and in astrocytes, but not in Gluergic axon terminals, seems to indicate that this transporter plays a major role in the overall regulation of Glu metabolism. (Funded by MURST and NIH)

295.9

Na^+, K^+, AND H^+ FLUX COUPLING IN A GLUTAMATE TRANSPORTER
I.I. Wadiche*, N. Zerangue, and M.P. Kavanaugh Vollum Institute, Oregon Health Sciences University, Portland, OR 97201

Although glutamate uptake kinetics are slow relative to the fastest events in excitatory synaptic transmission (Wadiche et al. Neuron 14:1019 1995), glutamate transport controls steady-state concentration of transmitter, which can in turn influence receptor activity over long time scales (Trussel & Fischbach Neuron 3:209 1989). A human neuronal glutamate transporter (EAAT3) was expressed in *Xenopus* oocytes and transport-specific radiotracer flux, electrical currents, and BCECF fluorescence changes were measured under voltage clamp. Forward transport resulted in an intracellular acidification which decreased with increasing pK of the amino acid substrate, suggesting transport of a protonated species. Both influx and efflux were reduced in the presence of kainate, a competitive transport antagonist which does not itself induce a current and is not transported. The reversal potential of the kainate-sensitive transport current was shifted by increasing $[L\text{-}Glu]_o$ (32mV/decade), $[Na^+]_o$ (101mV/decade), $[K^+]_o$ (-31mV/decade), and $[H^+]_o$ (25mV/decade). The transport reversal potential, Ψ, was related to the transmembrane concentration gradients for Glu^-, Na^+, K^+, and H^+ according to the function:

$$\Psi = (RT/2F)\ln\{([Glu_o]/[Glu_i])([Na_o]/[Na_i])^{3.16}([K_i]/[K_o])^{0.97}([H_o]/[H_i]^{0.78})\}$$

The data suggest that permeation of one glutamate molecule is coupled to permeation of three Na^+ ions and one proton, with counterflux of one K^+ ion. This transport cycle results in a free energy change capable of maintaining a transmembrane glutamate concentration gradient exceeding 10^6 in normal ionic conditions and an inwardly directed gradient over the range of ionic conditions typically encountered in pathological conditions such as ischemia.
supported by NIH NS33270

295.10

HIGH K+ AND ENERGY FAILURE INDUCE NON-VESICULAR GLUTAMATE EFFLUX BY DIFFERENT ROUTES: EVIDENCE FOR NON-THERMODYNAMIC CONTROL.
M.C. Longuemare*, K. Farrell, and R.A. Swanson. Dept. of Neurology, Univ. of California and VAMC, San Francisco, CA 94121.

Glutamate may be released from astrocytes via anion channels and by reversal of uptake. The thermodynamic model of Na^+-dependent glutamate transport predicts that increased $[K^+]_o$ will increase $[glutamate]_o$ via reversal of glutamate uptake. Glutamate was measured in media of rat astrocyte cultures. Cultures exposed to isoosmolar elevations in $[K^+]_o$ released far less glutamate than predicted by the thermodynamic model. In contrast, glutamate release during 60 min of energy failure (glycolytic and oxidative blockade) approached the predicted maximal value. Glutamate release also appears to occur by different routes during energy failure and high $[K^+]_o$. Pre-loading cultures with PDC, a competitive inhibitor of Na^+-dependent glutamate transport, reduced glutamate efflux by 70% during energy failure but had no effect on K^+-induced release. The anion channel blocker SITS had opposite effects, causing a 65% reduction in efflux during high $[K^+]_o$ but minimal effect on efflux during energy failure. Taken together, these results suggest that reversal of glutamate uptake can be regulated by factors other than thermodynamic forces. Uptake reversal may be the primary mechanism of efflux during energy-failure, while K^+-induced glutamate release may occur primarily through anion channels. **Supported by NIH RO1 NS31914**

295.11

GLUTAMATE TRANSPORTERS AND UPTAKE IN PLATELETS AS POSSIBLE PERIPHERAL MARKERS IN NEURODEGENERATIVE DISORDERS.
C.Ferrarese*[1], T.Cogliati[1], C. Zoia, G.Bianchi, M.Bugiani, G.Cavaletti, M.Frigo, N.Pecora, and L.Frattola, Department of Neurology, University of Milan - Ospedale San Gerardo, Monza and [1]Scientific Institute "E.Medea", Bosisio Parini- ITALY.

Since modifications of glutamate uptake have been described in brain samples in degenerative disorders and appear related to excitotoxicity, we aimed to investigate these processes in platelets, as possible peripheral markers of excitotoxic mechanisms.

We first used polyclonal anti-peptide antibodies that specifically recognize EAAC1, GLT-1 and GLAST glutamate transporters (provided by Prof. JD Rothstein, Baltimore, MD) to assess, by Western-blot and electron microscopy coupled with the immunogold method, the type of transporter present in platelets. Uptake experiments were then performed using [^{3}H]glutamate; glutamate content was also investigated by reverse-phase HPLC with pre-column derivatization with o-phtaldialdehyde and fluorimetric detection.

Data from platelets of normal volunteers were then compared to those of 34 Parkinson's Disease (PD) patients and 10 age related controls. Studies on Alzheimer's disease and Amyotrophic Lateral Sclerosis patients are now in progress.

Western blot and the immunogold studies revealed that both the neuronal- and the glial-type transporters are present in human platelets.

A significant 35 % reduction of glutamate uptake ($p < 0.05$) was observed in PD patients, respect to controls. The decrease was more consistent and significant ($p < 0.01$) in the subgroup of idiopathic PD and correlated with the severity of PD, measured by the UPDRS ($r = -0.61$; $p < 0.05$).
Source of founding was the University of Milan.

295.12

PORTACAVAL-SHUNTING AND HYPERAMMONEMIA SELECTIVELY STIMULATE THE UPTAKE OF L-ARGININE BUT NOT OF L-NITROARGININE INTO RAT BRAIN SYNAPTOSOMES.
V.L. Raghavendra Rao, R.M. Audet and R.F. Butterworth*, Neuroscience Research Unit, Hôpital Saint-Luc (University of Montreal), Montreal, Quebec, Canada H2X 3J4

Elevated activities of nitric oxide synthase (NOS) have been previously described in the brains of portacaval-shunted (PCS) rats (Raghavendra Rao *et al., J. Neurochem.* **65**, 677-681, 1995). As L-arginine availability for nitric oxide synthesis depends on specific uptake mechanisms in neurons, we studied the kinetics of L-[^{3}H]arginine uptake into synaptosomes prepared from the brains of PCS rats. L-arginine uptake was significantly increased in cerebellum, cortex, hippocampus, striatum and olfactory bulb (+24% to +60%, $p<0.01$) of PCS rats compared to sham-operated controls. Hyperammonemia also stimulated the transport of L-arginine significantly. Kinetic analysis revealed that the elevated uptake is due to an increased Vmax. Incubation of cerebellar synaptosomes with NH_4Ac for 10 min resulted in a dose-dependent stimulation of L-arginine uptake. This stimulation was blocked by the NOS inhibitor, N^G-monomethyl-L-arginine acetate but not by N^G-nitro-L-arginine or N^G-nitro-L-arginine methyl ester. Neither PCS nor hyperammonemia had any significant effect on the uptake of L-nitroarginine into synaptosmes. These results suggest that increased NOS activity observed in HE is due to increased availability of L-arginine due to a direct stimulatory effect of ammonia on L-arginine transport. (Funded by MRC Canada)

PROCESS OUTGROWTH, GROWTH CONES, AND SPROUTING III

296.1

INTRINSIC REGULATION OF THE AXONAL ARBOR OF MESENCEPHALIC DOPAMINERGIC NEURONS.
A. Heller*, H. Choi and L. Won. Dept. of Pharmacol. & Physiol. Sci., The University of Chicago, Chicago, IL 60637.

A variety of studies indicate that both competition and intrinsic regulation play a role in determining the size of specific afferent axonal arbors innervating particular brain nuclei during development. In order to assess the role of these factors in determining dopaminergic (DA) axonal arbor size, mesencephalic DA neurons were grown in three-dimensional reaggregate culture in which striatal target cells were present in excess of that required for quantitative DA neuronal survival. Since a ratio of 1:2 (mesencephalic: striatal cells) provides sufficient target for quantitative survival of DA neurons, higher ratios of up to 1:50 provided excess target to allow for possible expansion of the dopaminergic axonal arbor if it were limited by axonal competition. Determination of dopamine content and axonal arbor size per DA neuron in the aggregates revealed that the size of the DA axonal arbor, over the first three weeks of development, remains constant in the face of increasing target availability. The use of three-dimensional reaggregate culture provides a new approach to the examination of the role of competition for target space in determining axonal arbor size. The findings provide direct evidence that during development, the size of the DA axonal arbor is limited by an intrinsic regulatory program which is independent of the extent of availability of target space.
Supported by NIMH grant #MH 28942.

296.2

NERVE CORD REPAIR IN 3-D COLLAGEN GEL CULTURES.
S.E.Blackshaw *, C. Cameron & J.A.Davies[1]. Institute of Biomedical & Life Sciences, Joseph Black Building, University of Glasgow G12 8QQ, Scotland and [1]Sussex Centre for Neuroscience, Sussex University, Falmer, Brighton BN1 9QG, UK.

Our aim was to establish whether 3-D collagen gels, widely used to reconstruct extracellular matrices for mammalian cells, would support the growth *in vitro* of leech neurons and other cell types involved in neural repair. Collagen was prepared as an acid extract of rat tail tendons in Leibovitz-15 culture medium, and its composition and pH adjusted to give a gel that sets at room temperature. Ganglia or isolated pieces of connectives from the ventral nerve cord of*Hirudo medicinalis* were embedded in the gel and maintained for up to 2 weeks. During this time nerve fibre outgrowth into the gel was consistently seen from the cut ends of both nerve roots and connectives. The gel was also clearly penetrable by cells migrating out from the cut ends of connectives. When the connectives between two ganglia were completely severed, or when two ganglia with shortened connectives were apposed, repair occurred within the gel in such a way that the gap between the cut ends was bridged within 2-5 days in culture. Cells emigrating from the cut ends of the connectives appeared to contribute to the repair process. In summary, 3-D collagen gels provide an *in vitro* system in which we can reliably obtain repair of nerve cords in the dish and visualise cell behaviour underlying regenerative growth at the damage site.
Supported by BBSRC

296.3

PMCA AND SV-2 EXPRESSION IN DEVELOPING SYNAPTIC TERMINALS. I.T. Fujii* and F.T. Su. Dept. of Anatomy & Cell Biology, Wayne State U. Sch. of Med., Detroit, MI 48201

Although the plasma membrane calcium ATPase pump (PMCA) is typically located in the plasma membrane, in recent fine structure studies PMCA immunoreactivity has been found associated with cholinergic synaptic vesicles in the calyciform terminals formed by Edinger-Westphal neurons in the chick ciliary ganglion (Fujii et al., in press). To further study the relationship of PMCA with synaptic vesicles, expression of PMCA immunoreactivity in the ciliary ganglion during development was compared with that of the synaptic vesicle protein SV-2.

SV-2 immunoreactivity appears early in the development of the ciliary ganglion (Hamburger & Hamilton stages 27-29) in a fine, punctate pattern outlining neurons and defining processes. In contrast, patterns of PMCA immunoreactivity are not punctate, appearing instead to coarsely outline the boundaries of neurons. However, as calyciform terminals begin to coalesce and mature morphologically (stages 36-37), patterns of PMCA immunoreactivity come to resemble those of SV-2 very closely, with both heavily labelling newly formed calyciform terminals. This shift in the pattern of PMCA immunoreactivity suggests that PMCA may become associated with synaptic vesicles only as they become part of a mature synaptic terminal, the earlier staining pattern being associated with unrelated PMCA expression in other parts of the cells. Alternatively, it is also possible that PMCA immunoreactivity is associated with a membrane compartment that is distinct from synaptic vesicles and becomes co-localized with them as the synaptic terminals mature. Supported by NIH RO1 EY09768.

296.4

THE NEURAL CELL ADHESION MOLECULE L1 UTILIZES TWO DISTINCT MECHANISMS IN PROMOTING NEURITE OUTGROWTH FROM NEURONAL CELLS. P. Yip, X. Zhao, and C.-H. Siu*. Banting and Best Department of Medical Research, University of Toronto, Toronto, Ontario M5G 1L6, Canada.

L1 is an important neural cell adhesion molecule, which contains six immunoglobulin (Ig)-like domains, five fibronectin type III repeats, a transmembrane domain and a cytoplasmic domain. L1 mediates cell-cell adhesion via homophilic binding and promotes neurite outgrowth from a variety of primary neurons. We have demonstrated that the Ig-like domain 2 (Ig2) of L1 contains both homophilic binding and neuritogenic activities (*J.Biol.Chem.* 270:29413-29421). Using fusion proteins containing different portions of the L1 extracellular domain, we have identified a second neuritogenic site in Ig-like domain 6 (Ig6). Primary neurons were cultured on different substrate-coated proteins for 20 hr and neurite lengths were measured. In addition to Ig2, fusion proteins containing Ig6 were able to promote neurite outgrowth from dorsal root ganglion cells and hippocampal cells. In contrast, neural retinal cells responded to Ig2-containing proteins only. The neuritogenic activity of L1 Ig6 was inhibited when assayed in the presence of soluble fusion protein or domain-specific antibodies. L1 Ig6 contains a RGD sequence, suggesting that it may interact with an integrin. When RGD-containing synthetic peptides were included in the assay, neurite outgrowth was inhibited in a dose-dependent manner. These results indicate that L1 utilizes two distinct mechanisms to induce neurite outgrowth from neuronal cells, one involving L1-L1 homophilic interaction and the other one involving L1-integrin interaction. (Supported by the Medical Research Council of Canada.)

296.5

NITRIC OXIDE ($NO^{\cdot}$) STABILIZES FILOPODIA WHILE NITROSONIUM DONORS (NO^{+}) INDUCE OUTGROWTH BY RAT RETINAL GANGLION CELLS IN VITRO. Wing Cheung, Ishir Bhan, and Stuart A. Lipton*. Dept. of Neurol., Children's Hospital; Progr. in Neuroscience, Harvard Med Sch, Boston, MA 02115.

Recent observations suggest that NO donors can either increase or decrease filopodial and neurite activity (Wang et al., 1993; Hess et al., 1993; Renteria et al., 1994). To explain these seemingly discordant findings, we wondered if different redox-related species of the NO group could display differential effects on outgrowth, analogous to previous findings reporting that NO^{+} donors could be neuroprotective while $NO^{\cdot}$ donors participate in neurodegenerative pathways (Lipton et al., 1993). Here, 450 acutely isolated retinal ganglion cells from P1-P3 rat pups were identified with specific fluorescent tags and monitored over a 5 min period for filopodial outgrowth under computer-enhanced video microscopy (AVEC-DIC) in the presence or absence of various NO donors or membrane permeant cyclic nucleotide analogs. In parallel, digital Ca^{2+} imaging was performed with fluo-3 under confocal microscopy. In the presence of SOD/catalase to prevent peroxynitrite ($ONOO^{-}$) formation from $NO^{\cdot}$ and $O_2^{\cdot-}$, $NO^{\cdot}$ donors (e.g., DEA/NO) or cGMP led to stabilization of filopodia. In contrast, donors capable of transferring NO^{+} to thiol (e.g., S-nitrosocysteine + SOD/catalase) led to filopodial growth. Under conditions in which low concentrations of $ONOO^{-}$ could form (DEA/NO or S-nitrosocysteine, each in the absence of SOD/catalase), outgrowth was markedly enhanced. These results suggest that oxidation of critical thiol (s) of growth-associated proteins may enhance filopodial elongation while cGMP formation via $NO^{\cdot}$ may halt or stabilize filopodia.

Funded by NIH grant R01 EY05477 (to S.A.L.).

296.6

NON-UNIFORM PRESENTATION OF LAMININ CAN DIRECT AXON FORMATION IN CULTURED RAT HIPPOCAMPAL NEURONS. T. Esch* and G. Banker. Department of Neuroscience, University of Virginia School of Medicine, Charlottesville, Virginia, 22908.

Cultured hippocampal neurons growing on polylysine-coated coverslips initially extend several apparently identical neurites, termed minor processes. Previous experiments from this laboratory have suggested that any of the minor processes may become the cell's axon, and that which process *does* become the axon is determined by chance. In the present experiments, we examined the ability of locally presented, substrate-associated molecules to increase the probability that a given minor process will become the axon. We plated neurons on coverslips patterned with alternating stripes of laminin and polylysine and determined whether the minor processes whose growth cones extended on a laminin stripe were more likely to become an axon. Cells were fixed and examined 24 hours after plating. In cells that had at least one process contacting each substrate, 80-90% of the axons formed on laminin, many more than would be expected by chance. This effect was seen most consistently in cells whose somata were on polylysine. To examine this phenomenon more closely, neurons were followed by time-lapse video recording during the first 24 hours in culture. Of particular interest were cells whose minor processes initially developed on a polylysine stripe. In many of these cells, if the growth cone of one minor process crossed onto a laminin stripe, that process immediately entered a prolonged period of growth, becoming the cell's axon. These results indicate that local, substrate-associated cues can influence which of a cell's minor processes becomes its axon. This work was supported by NIH grant NS17112.

296.7

OBSERVATIONS OF THE DEVELOPMENT OF POLARITY IN CULTURED HIPPOCAMPAL NEURONS BY TIME-LAPSE VIDEO MICROSCOPY. J. Cooper and G. Banker*. Department of Neuroscience, University of Virginia School of Medicine, Charlottesville, Virginia, 22908.

Hippocampal neurons developing in culture initially extend several apparently identical neurites, termed minor processes. Previous work from this laboratory suggests that the minor processes grow and retract for several hours until one of them begins to grow much more than the others, becoming the cell's axon. To gain further insight into what triggers this emergence of polarity and how one process is chosen to become the axon, we examined these events by time-lapse video microscopy of individual neurons. Observations were begun 8 hours after plating and continued for several hours after the axon had formed. Our results show that prior to axon formation the minor processes undergo spurts of rapid growth lasting for 30 to 40 minutes followed by periods of quiescence or retraction. The emergence of the axon occurs when one of the processes, which is otherwise indistinguishable from the others, exhibits a novel pattern of growth. It undergoes a spurt of growth that is not significantly faster than other growth spurts, but that persists for a much longer time without interruption. As this occurs, there is also a noticeable decrease in the growth of the cell's other neurites. Thus the first steps in the emergence of neuronal polarity are marked by a change in the pattern of neurite outgrowth that involves all of the cell's processes, not just the emerging axon itself. Supported by NIH grant NS17112.

296.8

PURIFICATION AND MOLECULAR CLONING OF AN INHIBITOR TO CNS REGENERATION

Spillmann*, A.A.; M.S. Chen, M.S.; M. E. Schwab, Brain Research Institute, University of Zurich, CH-8029 Zurich, Switzerland.

We have previously characterized a high molecular-weight (250 kd) protein purified from bovine myelin. This 250 kd protein inhibits neurite outgrowth from NGF treated PC12 cells, it also induces DRG growth cone collapse. These inhibitory activities can be fully neutralized by IN-1, a monoclonal antibody raised against NI250, which enhances regeneration of lesioned nerve fibers in vivo. This 250 kd protein, bNI250, from bovine myelin is therefore likely to be the bovine equivalent of the rat derived NI250.

We have now further demonstrated that this potent inhibitor activity of bovine myelin is specific to bNI250, but not other proteins of similar molecular weight. Treatment with partially purified C6 metalloprotease suppresses the bNI250 inhibitor activity to the similar extend as IN-1 treatment, in contrast to chondroitinase digestion, which does not alter bNI250 activity. When subjected to deglycosylation treatment, the mobility of bNI250 did not change, indicating that bNI250 is not likely to be extensively glycosylated. Microsequencing of gel eluted bNI250 has produced several novel peptide sequences. Antisense oligonucleotides were synthesized from back translation of these peptide sequences, and their effects on the inhibitor activity of cultured oligodendrocytes are studied. Oligonucleotides with sequences derived from bNI250 peptide sequences were also used for both library screening and rapid amplification of cDNA end (RACE). Multiple clones isolated from library screening, as well as 5'and 3'RACE are currently being analyzed.

296.9

A NEUROTRANSMITTER IS RELEASED FROM VARIOUS REGIONS OF NEURITES. H.Soeda†, H.Tatsumi†‡ and Y.Katayama†*. †Department of Autonomic Physiology, Medical Research Institute, Tokyo Medical and Dental University, ‡PRESTRO JRDC, Japan.

Growing neurites of rat dorsal root ganglion (DRG) neurons in culture formed growth cones at their tips. Glutamate released from these growth cones was detected by using a whole cell patch-clamp recording from an acutely dissociated hippocampal neuron containing glutamate receptors; inward currents were recorded from the hippocampal neuron as a biodetector positioned on the growth cones of the DRG neurons in response to the DRG cell body stimulation. Electrophysiological and pharmacological properties of the inward currents indicate glutamate release. Inward currents were also recorded when a biodetector was positioned on flat tips and flat branching regions of neurites. All of these inward currennts were the same in their properties, suggesting the glutamate release from the flat structures of neurites as well as the growth cones. The inward currents were abolished by a Ca^{2+} free external solution or ω-agatoxin IVA (300 nM). Intracellular calcium ion concentration ($[Ca^{2+}]_i$) in the growth cones and the other release-sites of neurites increased in response to DRG cell body stimulation, whereas the elevation of $[Ca^{2+}]_i$ was not observed either in the presence of TTX (1 μM) or in a Ca^{2+} free external solution. These results suggest that growth cones and the flat neurite regions are already endowed with much of the machinery for neurotransmitter release even before making a structure for synaptic transmission.

This study was supported by a Grant-in-Aid for Scienfitic Research from The Ministry of Education, Science and Culture of Japan.

296.10

CATECHOLAMINES AND BASIC FIBROBLAST GROWTH FACTOR DIFFERENTIALLY REGULATE CYTOSKELETAL PROTEINS DURING NEURITE OUTGROWTH IN A BASAL FOREBRAIN CELL LINE. J.Kwon*[1], M. Downen[1], L. Roback[3], E. Eyes[2] and B.H. Wainer[3]. Dept. [1]Path., Albert Einstein Med. College, Bronx, NY 10461; [2]Ben May Inst., Univ. of Chicago, Chicago, IL 60637; [3]Emory Univ., Atlanta, GA, 30329.

Neurite outgrowth in the developing nervous system has been shown to be influenced by growth factors and neurotransmitters. We have previously described the generation of an embryonic day 14-15 rat basal forebrain neuronal cell line (Soc Neurosci. Abs. 17:37, 1991) that undergoes neuritogenesis in response to catecholamines and basic fibroblast growth factor (bFGF). The catecholamines, via activation of ß2-adrenergic receptors, induce a stellate morphology with neurite outgrowth evident within 60 minutes while bFGF induces a bipolar morphology evident within 24 hours. Two-dimensional gel electrophoresis with isoelectric focusing and SDS-PAGE of cytoskeletal preparations and [32-P]-orthophosphate incorporation studies indicate that ß2-adrenergic agonist and bFGF treatments result in the differential regulation of cytoskeletal elements. Further studies indicate that post-translational modifications of tubulin are differentially segregated in response to ß2-adrenergic agonists and bFGF. The distribution of the post-translationally modified tubulins is similar to that seen in primary basal forebrain neurons. These results suggest that neurite outgrowth in the developing basal forebrain is dependent upon both neurotransmitters and growth factors, which differentially regulate cytoskeletal proteins. (Supported by NS 25787 and AECOM BRSG.)

296.11

DIACYLGLYCEROL LIPASE INHIBITION REVERSIBLY SLOWS *XENOPUS* RETINAL GROWTH CONES *IN VITRO* AND *IN VIVO*. B. Lom*, S. McFarlane, & C.E. Holt Biology, UCSD, La Jolla, CA 92037

The *Xenopus* retinotectal projection is a uniquely accesible system to study retinal ganglion cell (RGC) growth and guidance. Fibroblast growth factor-2 (FGF-2) stimulates *Xenopus* RGC axon extension *in vitro*, while inhibiting FGF receptors (FGFR) slows axon extension *in vitro* and *in vivo* (McFarlane et al. *Neuron* 15: 1017; *Soc. Neurosci. Abstr.* 21: 1293). Activated FGFRs are thought to transmit signals by phospholipase C phosphorylation to produce diacylglycerol (DAG) that is converted to arachidonic acid (AA) by DAG lipase, causing Ca2+ influx and neurite extension (Williams et al. *Development* 120: 1685). To determine if *Xenopus* RGC axon extension employs this signaling cascade, DAG lipase activity was blocked by RHC-80267 *in vivo* and *in vitro*.

In vitro application of 100 μM RHC-80267 halted *Xenopus* RGC growth cone extension within minutes. Growth cones then collapsed, withdrawing their lamellipodia and filopodia, and eventually retracted. Thirty minutes after washing out the DAG lipase inhibitor, growth cones reformed active lamellipodia and filopodia, and resumed forward extension. *In vivo*, RGC extension rates were also significantly and reversibly reduced by DAG lipase inhibition. Chronic application of 500 μM RHC-80267 to exposed *Xenopus* brains during the stage when RGCs extend along the optic pathway significantly shortens optic projection lengths. When individual RGC growth cones were observed *in vivo* by time-lapse video microscopy, extension rates were significantly reduced within minutes of 500 μM RHC-80267 application. Growth cones consistently stalled in the optic pathway, occasionally collapsed, but rarely retracted. Extension rates and growth cone morphologies rapidly recovered after inhibitor removal. These results suggest that DAG lipase mediated conversion of DAG to AA is involved in RGC axon extension.

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296.12

THE FUSION PROTEIN, AL-1 IgG, CAUSES COLLAPSE AND RETRACTION OF CORTICAL NEURONS. L.Meima, I. Kljavin, P. Moran, A. Shih, J. Winslow*, and I. W. Caras. Department of Neuroscience, Genentech, South San Francisco, CA 94080.

Previous experiments using cultured cortical neurons suggest that the Eph-related receptor, REK7, and its ligand, AL-1, are involved in axon fasciculation. From this, together with recent findings that the chick homologue of AL-1, RAGS, acts as a repellent axon guidance molecule in the chick visual system, we hypothesized that AL-1 may act as a repellent guidance molecule in the rat cortex as well as in the visual system.

Using video microscopy, we show that an AL-1 IgG fusion protein causes growth cone collapse and axonal retraction when added to cultures of rat retinal ganglion cells and E16-E17 cortical explants. Growth cone morphologies were classified as collapsed, partially collapsed, and normal (did not respond to treatment) for scoring purposes. Experiments with 7.1ug/mL, 1.4ug/mL, and 0.14ug/mL of AL-1 IgG, demonstrate that the growth cone response is dose dependent, and that the ratio of collapsed to partially collapsed growth cones increases with increasing AL-1 IgG concentration. In contrast, the number of growth cones that did not respond to AL-1 IgG treatment was virtually unchanged at the three concentrations, suggesting that a subset of neurons are unresponsive to AL-1 IgG. Immunostaining with an anti-REK7 antibody suggests that responsiveness to AL-1 IgG is mediated by REK7.

Changes in growth cone morphology are thought to be associated with cytoskeletal changes. Consistent with this, AL-1 IgG induces a rapid and dramatic reorganization of the actin cytoskeleton in cortical growth cones, similar to the effects of cytocholasin D. This suggests that AL-1 IgG induces growth cone collapse by perturbing actin polymerization.

296.13

THE INTERACTION OF CORTICAL NEURON EPH RECEPTORS REK7 AND SEK WITH THE ASTROCYTIC LIGAND AL-1. Ai Shih, Janet Valverde-Beck, Paul Moran, Gary Laramee, Robert Soriano*, Klaus Beck, Ingrid Caras, John Winslow. Dept. of Neuroscience, Genentech, Inc. S. San Francisco, CA 94080.

AL-1 is a member of the GPI-linked family of ligands for the eph tyrosine kinase receptors, and is the human homologue of RAGS, the retinal ganglion axonal guidance factor which influences topographical innervation of the chick tectum. We have shown that AL-1 and its receptor REK7 (EHK1 or bsk) is also involved in the axonal fasciculation of rat embryonic cortical neurons. We demonstrate that cortical neuron fasciculation in vitro requires cell contact between cortical neurons and astrocytes, and correlates with increased neuronal REK7 autophosphorylation upon co-culture. The activation of cortical neuron REK7 by astrocytic AL-1 is consistent with the neuronal and astrocytic expression of REK7 and AL-1 mRNA, respectively. A REKIgG fusion protein blocks fasciculation when added at the time that cortical neurons are plated on a astrocyte monolayer, but only partially blocks upon delayed addition, suggesting that neuronal-astrocytic contact results in an early, irreversible reaction supporting fasciculation. A soluble AL-1-IgG fusion protein potently activates the tyrosine kinase of the eph receptor SEK in addition to that of the REK receptor, in both transfected cells and cortical neurons. Together with mRNA expression of REK7 and SEK in cortical neurons, these results suggest that multiple eph receptors may be involved in cortical neuronal function such as axonal fasciculation and guidance.

296.14

BOTULINUM NEUROTOXIN C1 INDUCES GROWTH CONE COLLAPSE. M. Igarashi[1]*, S. Kozaki[2], S. Terakawa[3], S. Kawano[4], C. Ide[4], Y. Komiya[1]. [1]Dept Molec Cell Neurobiol, Gunma Univ Sch Med, Gunma ; [2]Dept Vet Med, Osaka Pref Univ, Osaka; [3]Photon Med Ctr, Hamamatsu Med Sch, Shizuoka; [4]Dept Anat, Univ Kyoto, Kyoto, Japan.

The most likely cause of the membrane expansion for axonal growth is that vesicles in the central domain of growth cones are added to growth cone plasmalemma through exocytosis. A molecular model for vesicular targeting in exocytosis has recently been proposed, referred as the SNARE hypothesis. To test the hypothesis is applicable to membrane expansion for axonal growth, we observed the effect of neurotoxin C1 of Clostridium botulinum, cleaving syntaxin, which is essential to targetin of synaptic vesicles as a t-SNARE, to the growth cone. C1 caused growth cone collapse and inhibition of axonal growth. Video-enhanced microscopic study showed (a) that C1 selectively blocked the central domain at the initial stage, but not the lamellipodia; and (b) that large vacuole formation occurred through fusion of smaller vesicles from the central domain to the most distal segments of normal axonal growth. Our results demonstrate that syntaxin is involved in axonal growth, indicating that syntaxin may participate directly in membrane expansion in the central domain, in a SNARE-like way.

296.15

INTRACELLULAR CALCIUM MODULATES NCAM POLYSIALYLATION IN DEVELOPING NEURONS. Juan L. Brusés* and Urs Rutishauser, Departments of Genetics and Neurosciences, Case Western Reserve University, Cleveland OH 44106.

The expression of NCAM in its high polysialic acid (PSA) form on developing motoneurons is down-regulated by the interaction of neurons with muscle. This regulation appears to occur at the level of PSA synthesis. To study the mechanism of the regulation, the chicken cDNA encoding polysialyltransferase (PST), the enzyme responsible for PSA synthesis, was cloned and the developmental expression of the PST mRNA in the chick ciliary ganglia was studied by northern blot analysis. PST transcript levels were found to remain constant during the period of target innervation and to decrease before hatching (St 41-42). However, no change in PST mRNA level was detected at the time of synaptogenesis, when PSA synthesis is sharply reduced (St 36-37). Therefore, non-transcriptional mechanisms that could control PST activity were investigated. It was found that NCAM polysialylation is suppressed by calcium ionophores (A23187 [1 μM] and ionomycin [10 μM]) after 90 minutes of incubation, and by thapsigargin [10 μM], an intracellular calcium pump inhibitor. However, NCAM polysialylation was not affected by other drugs that increase cytosolic calcium such as caffeine or ryanodine. These results suggest that the ionophore and thapsigargin induced decreases in PST activity were due to a depletion of calcium in intracellular stores. This Ca^{++}-related inhibition of NCAM polysialylation was found to occur specifically at the level of PSA synthesis, in that maturation of the carbohydrate core on which PSA is added was not affected by A23187. These results suggest a direct and rapid mechanism for regulation of NCAM polysialylation via changes in calcium concentration in intracellular compartments. Supported by NIH grants HD 18369 and EY 06107

PROCESS OUTGROWTH, GROWTH CONES, AND SPROUTING IV

297.1

REGULATION OF CEREBELLAR PURKINJE CELL GROWTH BY CERAMIDE GENERATED FROM SPHINGOMYELIN. S. FURUYA*, and Y. HIRABAYASHI, Lab. for Cellular Glycobiology, FRP., The Institute of Physical and Chemical Research (RIKEN), Saitama 351-01, Japan.

There is accumulating evidence that ceramide released by sphingomyelinases in response to various stimuli influences cell growth, differentiation, and programmed cell death in many types of cells. Ceramide has now emerged as a new type of lipid messenger, and is postulated to have a role in CNS by the fact of an abundance of sphingomyelin in brain. Immunohistochemical examination using anti-sphingomyelin monoclonal antibody demonstrated that a particular type of sphingomyelin is present in dendrites and cell bodies of restricted populations of neurons including cerebral pyramidal neurons and cerebellar Purkinje cells. To ascertain whether ceramide-mediated signaling mechanism functions in the growth of brain neurons, we examined effects of in vitro manipulations of ceramide generation on the dendritic development and survival of Purkinje cells using a dissociate cultures prepared from rat embryos. Inhibition of ceramide biosynthesis by a specific inhibitor for serine palmitoyltransferase, ISP-1, reduced the amount of cellular sphingomyelin in cultures. The ISP-1 treatment caused the aberrant growth of Purkinje cells with few dendritic branches and decreased the number of these cells in a dose dependent manner without affecting other cerebellar neurons. Conversely exposure to a bacterial sphingomyelinase promoted the sprouting of dendritic branches and improved the survival. Treatment with cell-permeable ceramide also caused similar effects on Purkinje cell growth. These observations indicate that hydrolysis of sphingomyelin into ceramide play an important role in the morphological development and survival of cerebellar Purkinje cells.

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297.2

cAMP-MEDIATED EFFECTS OF SOMATOSTATIN ON PC12 CELLS DIFFERENTIATION.

G. Traina, C. Petrucci and P. Bagnoli*. Dept. of Physiology and Biochemistry. University of Pisa, 56127 Pisa, Italy.

The peptide somatostatin (SS) has been shown to play a role as a differentiation factor in the developing nervous system. Indeed, SS modulates cell growth by inhibiting proliferation and promoting differentiation. However, the molecular mechanisms underlying SS roles in neuronal differentiation are still to be clarified. In particular, whether SS effects on developing neurons depend on intracellular levels of cAMP still remains unclear. This study was aimed to investigate the effect of SS on neurite outgrowth in PC12 pheochromocytoma cells. Cells were cultured at 37° C in RPMI 1640 medium. They were then plated on glass coverslips coated with poly-L-lysine and examined every 24 hrs for neurite formation. Our results can be summarized as follows: a) SS application (10^{-6} M) does not induce neurite outgrowth; b) neurite outgrowth is promoted by nerve growth factor application (NGF, 100 ng/ml); c) NGF treatment in combination with dibutyril cAMP application (dbcAMP, a membrane permeable cAMP analog, 10^{-3} M) additively promotes cell differentiation; d) forskolin application (an adenylyl cyclase activator, 10^{-5} M) induces neurite outgrowth within 3/4 days from the treatment; e) NGF-, dbcAMP- and forskolin- induced neurite outgrowth is enhanced by SS application; f) application of MDL 12330A (an inhibitor of adenylyl cyclase, 10^{-5} M) does not induce cell differentiation; g) NGF-, dbcAMP- and forskolin-induced neurite outgrowth is inhibited by application of MDL 12330A; h) neurite outgrowth inhibition induced by MDL 12330A is prevented by SS application. Our results demonstrate that SS can influence cell differentiation by enhancing neurite outgrowth as induced by the application of substances which increase intracellular cAMP levels.

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297.3

ECTOPIC ADENOVIRAL VECTOR DIRECTED EXPRESSION OF SEMAPHORIN(D)III/COLLAPSIN-1 IN SPINAL CORD EXPLANTS REPELS GROWING AXONS OF PRIMARY SENSORY NEURONS. R.J. Giger, R.J. Pasterkamp, J. Verhaagen. (SPON: Eur. Neurosci. Ass.) Graduate school Neurosci., Netherlands Inst. for Brain Res., Amsterdam, The Netherlands.

Semaphorin(D)III/collapsin-1 (sema/coll), the first cloned chemorepellent, is a 100 kDa glycoprotein which has been shown to selectively induce growth cone collapse and paralysis of NGF-responsive dorsal root ganglion neurons in vitro. The expression profile of sema/coll mRNA in the embryonic spinal cord suggest a role of this chemorepellent in patterning ingrowing primary sensory neurons. By homologous recombination we constructed a first-generation adenovirus based gene transfer vector AdCMVcol, containing an expression cassette of rat sema/coll in the E1 region of the viral genome. Transgene expression from the recombinant viral genome was confirmed by in situ hybridization and Northern blot analysis of AdCMVcol infected Vero cells. In vitro translation of the corresponding sema/coll cRNA revealed a product of 85 kDa confirming an open reading frame in AdCMVcol encoding full length sema/coll. To test the biological activity of viral vector derived sema/coll we infected dorsal root ganglia explants of E15-E17 rat embryos with AdCMVcol and compared the axonal growth pattern to uninfected and AdCMVlacZ infected cultures. In control cultures neurites did grow in a regular radial orientation and formed only small fascicles. In contrast, in AdCMVcol infected cultures, axonal growth was slower and the regular radial pattern was clearly disturbed. Neurites exhibited a tendency to bend away from non-neuronal cells and formed fascicles in attempt to avoid sema/coll expressing cells visualized by in situ hybridization. Defasciculation apparently occurs when axon bundles have passed sema/coll positive cells. To investigate the function of sema/coll in embryonic spinal cord explants we microinjected AdCMVcol in the dorsal horn of E15 rat spinal cord slices to locally overexpress the recombinant protein. The altered growth pattern of ingrowing sensory fibers was visualized by DiI injection in the dorsal root ganglia. This study shows that recombinant adenoviral vectors are powerful tools to manipulate gene expression in primary cells in neural explants and may be applied in vivo thus, representing an attractive alternative to transgenic mice. *Visitor grant (3440747) from NWO-MW, NL.*

297.4

FUNCTIONAL ANALYSIS OF NEURITE PROMOTING FACTOR(NPF)

H.Nishimune*, A.Uyeda+, M.Nogawa+, Y.Shigeri, K.E.Fujimori++ and T.Taguchi Dept. Organic Materials, Osaka Nat'l. Res.Inst.AIST, Ikeda 563, Japan, +Fac.Eng.Sci., Osaka Univ., Toyonaka 560, Japan, ++Dept.Anat., Fukui Med. Sch., Yoshida 910-11, Japan

Neurite promoting Factor(NPF) was cloned from denervated muscle and telencephalon of chick. Recombinant protein of NPF promoted neurite outgrowth of telencephalic neurons most significantly but not diencephalon, mesencephalon and rhombencephalon, *in vitro*. In the present study, extracellular secretion of NPF was confirmed by expression in COS7 and immunohistochemistry of cultured living neurons from telencephalon, although stereotype signal peptide for secretion was not found at N-terminal. Expression of NPF in other tissues and developmentally regulated expression in telencephalon and crus muscle were analyzed by northernblot and westernblot, respectively. From GenBank data base search, highly homologous gene was found to exist in human. But the product of this gene and it's function have been unknown. We confirmed the expression of this gene product in human brain by PCR amplification of human fetal brain cDNA library and determination of the nucleotide sequence. Biological activity of NPF on other NPF expressing nervous tissues, spinal cord, DRG and sympathetic ganglion was studied *in vitro* . This work was supported in part by grants from AIST, NEDO and JRDC. H.N.is a postdoctoral fellow of JRDC.

297.5

EXTRACELLULAR GTP INCREASES INTRACELLULAR CALCIUM IN PC12 CELLS J.W. Gysbers[1]*, M.A. Mariggiò[2], T. Pietrangelo[2], G. Fanò[2], and M.P. Rathbone[1]. [1]Dept. Biomedical Sci., McMaster University, Hamilton, Canada L8N 3Z5 and [2]Instituto di Fisiopatologia Medica, Universitá di Cheti ,Italy.

Extracellular GTP can enhance the effects of NGF to synergistically increase the proportion of rat pheochromocytoma (PC12) cells that extend neurites to levels significantly greater than that induced in the presence of NGF alone. These cells have specific binding sites for extracellular GTP, which do not bind ATP or UTP. Recently we have determined that the addition of GTP leads to a large and sustained increase of intracellular Ca^{2+} ($[Ca^{2+}]_i$) in these cells. The calcium response to GTP addition is different than that to addition of ATP. This increase can be blocked by the addition of the L-type calcium channel inhibitor, nifedipine. In parallel experiments, the addition of nifedipine or other L-type Ca^{2+} channel inhibitors verapamil and diltiazem were found to inhibit the effects of GTP in enhancing NGF-stimulated neurite outgrowth, but do not affect neurite outgrowth stimulated by NGF alone. Ca^{2+} induced Ca^{2+} release (CICR) from specific stores present in PC12 cells may be involved in neurite outgrowth, as dantrolene treatment (blocks Ca^{2+} release from these stores), or pretreatment of PC12 cells with thapsigargin (an inhibitor of internal store Ca^{2+}-ATPase) also blocks the enhancement of neurite outgrowth by GTP. Another specific CICR blocker, ryanodine, also inhibits the increase of $[Ca^{2+}]_i$ by GTP. Other guanine based nucleotides: GMP, GDP, affect neither neurite outgrowth nor increases in $[Ca^{2+}]_i$. Data also suggest that PKC activation may be involved, and that this response can be inhibited by cGMP. (Supported by Amyotrophic Lateral Sclerosis Society of Canada).

297.6

RHO A REGULATES NEURITE OUTGROWTH IN PC12 CELLS. A.Sebök, D.J. Fischer, N. Nusser, M.F. Santos, Y. Zheng and G.Tigyi* Dept. of Physiology. and Biochem., Univ. of Tenn., Memphis, TN 38163

Clostridial toxins, which selectively modify the Rho-family of small molecular weight GTPases enhance neurite outgrowth in PC12 cells exposed to NGF. C3 toxin from *C.botulinum* , which ADP-ribosylates Rho proteins, induces neuronal differentiation in PC12 cells, characterized by neurite outgrowth and the induction of neuronal marker genes. C3 toxin induces neurite outgrowth in M-M17-27 and A126-1B2 cells expressing dominant negative Ras and protein kinase A, respectively. Toxins A & B from *C. difficile*, which glycosylate CDC42, Rac and Rho, do not induce neurite outgrowth alone, but greatly enhance neurite outgrowth in NGF-stimulated PC12 cells. To study the role of Rho in the neuronal differentiation of PC12 cells, stabile transfectants carrying dominant negative (N19-RhoA) and constitutively active (V14-RhoA) mutants were generated. In cell clones expressing either wild type or the mutant RhoA protein driven by a strong constitutive promoter, neither mutation caused the spontaneous outgrowth of neurites. Similarly, microinjection of recombinant RhoA, and V14RhoA proteins into wild-type PC12 failed to induce neurite outgrowth. Three clones expressing N19-RhoA all extended neurites in response to NGF, dbcAMP+PMA, and the combination of the three. In contrast, neurite outgrowth, in four V14-RhoA clonal cell lines, was greatly diminished following treatment with NGF or dbcAMP+PMA, whereas, the combination of the three, induced neurite outgrowth similar to that found in mock-transfected cells. We propose that the Rho family of GTPases and RhoA in particular, play an essential role in neurite outgrowth in differentiating PC12 cells. Supported by NSF-IBN 9321940, SCRF1293, USPHS-HL0774601 and AHA-TN Chapter.

297.7

SCHWANN CELLS ACCOMPANY NERVE SPROUTS INDUCED IN MUSCLE BY INSULIN-LIKE GROWTH FACTORS. W.J. Thompson* and D.M. Kopp, Dept. Zoology, Univ. Texas at Austin, Austin, Texas 78712.

Partial denervation or muscle paralysis results in sprouting of intact motor neurons. Experiments on rat neuromuscular junctions have shown that Schwann cells at denervated junctions grow extensive processes that induce nearby axons to sprout and then guide their growth. Previous studies also suggest that insulin-like growth factors (IGFs) are involved in the induction of sprouting. IGFs may be released from muscle following injury, and administration of IGFs to normally innervated muscle induces sprouting (Caroni and Grandes, J. Neurosci., 110:1307-1317). We have investigated the involvement of Schwann cells in IGF-induced sprouting. Adult rats were given subcutaneous injections (50 µl of 5µg/ml, twice a day) of IGF-1, IGF-2, or a control solution, directly over one levator auris longus muscle (LAL) for 7 days. LALs were removed and examined by immunocytochemistry for nerve sprouts (antibody to neurofilament) and Schwann cells (antibody to S-100). Terminal sprouts 2-8 µm in length were observed at 13.6% of endplates in muscles treated with IGF-1 (n=2), 13.7% of endplates in IGF-2 muscles (n=2), but only 1.5% of control muscles (n=3). Sprouts emerging from the preterminal axon were observed at 11.2% of endplates after IGF-1 (n=2), 5.4% after IGF-2 (n=2), and 1.5% of control muscles (n=3). In the IGF-2 muscles, 3% of endplates examined had sprouts connecting adjacent endplates, while only 0.08% of controls showed this phenomenon. All sprouts induced in muscles that received either IGF-1 or IGF-2 were accompanied by S-100 immunopositive Schwann cell processes. In a few cases, Schwann cell processes appeared to extend in advance of their associated nerve sprout. We suggest that the induction of nerve sprouting by IGFs involves the extension of Schwann cell processes, similar to that following partial denervation or paralysis. Supported by grants from ALS and Paralyzed Veterans of America.

297.8

EFFECTS OF ASTROCYTES, SCHWANN CELLS AND NEUROTROPHIC FACTORS ON POLARIZATION AND AXON GROWTH OF MOTONEURONS *IN VITRO* H. Hammarberg, F. Piehl, S. Cullheim* Dept. of Neuroscience, Karolinska Institute, Stockholm, Sweden

Neurotrophic factors in muscle and Schwann cells have in recent *in vitro* and *in vivo* studies been shown to support the survival of rat spinal alpha motoneurons. The aim of the present study was to investigate the effects of supporting cells related to motoneurons on polarization and neurite growth of these neurons. Purified embryological (E15) rat motoneurons from the lumbar spinal cord were cocultured with Schwann cells, type 1 astrocytes, leptomeningeal cells and epineural fibroblasts or treated with the neurotrophic factors bFGF, BDNF, NT-3, NT-4/5, GDNF, IGF-1, IGF-2 and CNTF. Axon properties of neurites were established by the presence of immunopositivity for GAP-43 and RT 97, and immunonegativity for MAP 2. For each motoneuron, the cell soma area, the number of neurites and the length of the longest neurite (axon) were determined. The effects on GAP-43 mRNA expression was evaluated with radioactive *in situ* hybridization histochemistry. Astrocytes and in particular Schwann cells showed significant stimulatory effects on polarization, neurite growth and GAP-43 mRNA levels, whereas fibroblasts seemed to have an inhibitory influence on these parameters. All studied neurotrophic factors stimulated neurite growth, but in the case of bFGF, the growth pattern was directed towards a production of many branching neurites, while CNTF induced lengthening of single neurites. (Supported by the Swedish Medical Research Council)

297.9

EFFECTS OF LATERAL AND MEDIAL MIDBRAIN GLIAL CONDITIONED MEDIUM IN MIDBRAIN NEURONS. J. Garcia-Abreu[1,2], F.F. Tovar[1], V. Moura Neto[1] and L.A. Cavalcante*[1] [1]Inst. Biofisica, [2]Depto. Histologia e Embriologia / ICB, UFRJ, 21941-590 Rio de Janeiro, Brazil.

It is now well documented that astrocytes from different brain regions are heterogeneous with respect to morphology, membrane receptors, and interactions with other cells. Lateral (L) and medial (M) midbrain astroglial cells differ in their abbility to suport neuritic growth. The effects of conditioned medium from L (cL) and M (cM) astroglial cells on lateral (l) and medial (m) midbrain neurons were analyzed. Confluent L and M monolayers were washed 3 times with PBS and kept in DMEM/F12 without serum during 48 h. cL and cM were obtained and used as cultivating medium to grow l and m midbrain neurons. After 24 h the neuritic morphology was observed by staining with an anti-β tubulin III antibody. The results show that l or m neurons cultivated in cM present short or no neurites (% of -β tubulin III+ cells without neurites >18%). By contrast, l or m neurons cultivated in cL grow more neurites (% of -β tubulin III+ cells without neurite < 1.5%). The total length of neurites in cells with 1, 2 or 3 or more processes is consistently larger in cL- than in cM-treated cultures. However, cL-treated neurons still have processes 2-3 times shorter than those of neurons in either Ll or Lm cocultures The results show that astroglial secreted factors are partially responsible for differential effects of astroglia upon neurons.

[Support: CNPq, FINEP,CEPG/UFRJ]

297.10

CELL-CELL CONTACT AFTER NEUROTRANSMITTER RELEASE FROM GROWTH CONES OF RAT CENTRAL NEURONS: ESTIMATION OF PROPERTIES OF THE CONTACT USING LASER TWEEZERS. **H. Tatsumi,[+#]* and Y. Katayama[+]. [+]Department of Autonomic Physiology, Medical Research Institute, Tokyo Medical and Dental University, Tokyo, Japan. [#]PRESTO JRDC Japan.**

Neurons protrude growth cones during development and also during axon sprouting after damage. Evoked release of acetylcholine (ACh) from the growth cones of central cholinergic neurons in culture was monitored using whole-cell patch clamp recording from ACh-receptor-rich neuron placed close to the growth cones as a sensor of ACh release. Simultaneous video-enhanced DIC imaging of growth cones showed contacts between growth cones and the sensor neuron after ACh release. Adhesion was also observed between growth cones and latex beads, when growth cones were exposed to high potassium solution. After beads attached on the growth cone membrane, 1 pN of laser optical pressure was required for moving the beads about 0.1 to 1 µm on the growth cone surface. The surface viscosity of the growth cone membrane was estimated by analyzing the video images.

This study was supported by a Grant-in Aid for Scientific Research from The Ministry of Education, Science and Culture of Japan and JRDC.

297.11

AN IMMUNOSUPPRESSANT, FK506, PREVENTS MOSSY FIBER SPROUTING INDUCED BY KINDLING STIMULATION. A. Moriwaki*, Y.-F. Lu, K. Tomizawa, H. Onuma, and H. Matsui, The First Department of Physiology, Okayama University Medical School, Okayama 700, Japan.

Kindling stimulation induces expansive growth of the axons of the dentate granule cells, the mossy fiber, into several areas of the hippocampus including supragranular layer, hilus, and CA3. A relationship between the sprouting and the development kindling is obscure. Intraperitoneal injection (1 mg/kg) of the immunosuppressant drug FK506, which is a specific inhibitor of Ca^{2+}-calmodulin dependent phosphatase, calcineurin, prevented the full development of kindling. The FK506 injected rats developed stage 3 behavioral manifestation but did not develop into stage 4 or 5. Further the FK506 injected rats showed less mossy fiber sprouting evaluated with Timm histochemistry compared with vehicle injected and kindled rats. These inhibitory effects of FK506 were reversible. The results show a correlation between mossy fiber sprouting and the development of kindling. The results suggest also that calcineurin has a promoting role in mossy fiber sprouting and subsequent synaptogenesis.

297.12

FKBP12 BINDING DOMAIN ANALOGUES OF IMMUNOSUPPRESSIVE DRUG FK506 ARE POTENT, NONIMMUNOSUPPRESSIVE NEUROTROPHIC AGENTS IN VITRO G.S. Hamilton,* W. Huang, M.A. Connolly, P.D. Suzdak and J.P. Steiner. Guilford Pharmaceuticals, Inc., 6611 Tributary Street, Baltimore, MD 21224.

The immunophilins FKBP and cyclophilin are "receptors" for the immunosupressant drugs FK506 and cyclosporin A, respectively. Recently it has been discovered that that immunophilins are enriched 10-40 fold more in the brain than in the immune tissues. Within neural tissues, they influence neuronal process extension, and may have potential therapeutic applications in neurodegenerative disorders. Using the X-ray crystal structure of the FKBP-FK506 complex as a basis, we have used the principles of structure-based drug design to prepare novel small molecule neuroimmunophilin ligands which possess neurotrophic actions, but are devoid of immunosuppressive activity. We have evaluated the trophic effects of a number of these structures in cultured sensory neurons from chick dorsal root ganglia. Compounds such as GPI 1080 (ED_{50} = 0.61 nM) and GPI 1080 (ED_{50} = 0.09 nM) potently promote neurite outgrowth in these cultures at picomolar concentration. The response and morphology elicited by these compounds is stikingly similar to the effects of maximal doses of nerve growth factor (NGF); however, the compounds exert their effects on the neuronal cultures without the need for added exogenous NGF. These compounds suggest a novel approach to the development of potent, systemically active orally bioavailable neurotrophic drugs for the treatment of neurodegenerative disorders. This work was supported by Guilford Pharmaceuticals, Inc.

297.13

Nonimmunosuppressive Ligands for Neuroimmunophilins Promote Nerve Extension *In Vitro* and *In Vivo*.
J.P. Steiner[a*], M.A. Connolly[a], H.L. Valentine[a], G.S. Hamilton[a], T.M. Dawson[b], L. Hester[b] and S.H. Snyder[b] Department of Research, Guilford Pharmaceuticals Inc.[a], Baltimore, MD 21224 and Departments of Neurology and Neuroscience[b], The Johns Hopkins University School of Medicine, Baltimore, MD, 21205.

Previously, we have shown that the immunosuppressive drugs FK506 and rapamycin promote neurite outgrowth in PC12 cells and sensory neuronal cultures (Lyons, et.al., Proc. Nat'l Acad. Sci. USA 91, 3191-3195, 1994; Steiner, et. al., Soc. Neurosci. Abstr. 21, 1772a, 1995). Picomolar concentrations of FK506 and rapamycin elicit neurite outgrowth in both systems by increasing sensitivity to nerve growth factor. We have now determined that the nonimmunosuppressive analogs of FK506 and rapamycin, L-685,818 and WAY-124,466 respectively, potently promote neurite outgrowth in both PC12 cells and sensory neuronal cultures of dorsal root ganglia. In the sensory ganglion cultures, the neuroimmunophilin ligands induce nerve extension in the absence of exogenously added nerve growth factor. Likewise, cyclosporin A (CsA) and a nonimmunosuppressive analog of CsA promote neurite outgrowth in PC12 cells and sensory neuronal ganglion cultures. The concentrations of neuroimmunophilin ligands necessary to promote nerve extension correlate with doses that inhibit the rotamase activity of the respective neuroimmunophilin, FKBP-12 and cyclophilin , suggesting that inhibition of rotamase activity is sufficient to elicit neurotrophic effects of these compounds. Furthermore, FK506 and L-685,818 treatment of rats with crushed sciatic nerves induces the functional and morphological recovery of the injured nerve. These studies demonstrate the neurotrophic activities of the neuroimmunophilin ligands, and suggest that these ligands may possess therapeutic implications in treatment of neurodegenerative disorders and peripheral neuropathies.

This research was sponsored by Guilford Pharmaceuticals Inc. and USPHS grant DA-00266 to SHS.

297.14

Novel Neuroimmunophilin Ligands for FKBP-12 Possess Potent Neurotrophic Activity in the Mouse MPTP Model of Parkinson's Disease
D.T. Ross*, H.L. Valentine, H. Guo, G.S. Hamilton, W. Huang, P.D. Suzdak and J.P. Steiner Department of Research, Guilford Pharmaceuticals Inc., Baltimore, MD 21224

We have developed specific and selective inhibitors of the neuroimmunophilin FKBP-12 which possess neurotrophic activity in chick sensory neuronal cultures (See the adjacent poster, Hamilton et. al.), but are devoid of immunosuppressive activity. In order to determine the in vivo efficacy of these compounds, we extended our in vitro studies to determine whether these small molecules could promote nerve regrowth and neuronal repair in an animal model of Parkinson's Disease. Using N-methyl-4-phenyltetrahydropyridine (MPTP) to induce lesioning of dopaminergic neurons, we found that systemic treatment of lesioned mice with the neuroimmunophilin ligand GPI 1125 caused the striking recovery of tyrosine hydroxylase-positive innervation of the striatum. GPI 1125 treatment of lesioned animals spares medial forebrain bundle axons and rescues dopaminergic cell bodies in the substantia nigra, pars compacta. Furthermore, treatment of MPTP-lesioned mice with GPI 1125 did not effect the initial decrease in vertical locomotor activity (or rearing) associated with MPTP administration on day 3, but did lead to increased rearing (back to non-lesioned sham control levels) within 10 days. These data suggest that GPI 1125 may act both to rescue degenerating neurons and increase the plasticity of spared projections. As such, these systemically active small molecules promote neuronal recovery of degenerated neurons and define a novel class of therapeutic agents for the treatment of neurodegenerative disorders, such as Parkinson's Disease.

297.15

NEURONAL GROWTH CONE FILOPODIA ARE REGULATED IN A CELL-TYPE SPECIFIC MANNER. S. Cheng, J. M. Bonsall, V. Rehder*; Department of Biology, Georgia State University, Atlanta, GA 30302.

We have previously demonstrated the importance of the free intracellular calcium concentration ($[Ca^{2+}]_i$) in regulating the length and number of growth cone filopodia on identified neurons of the snail Helisoma trivolvis. The present study investigates the signaling pathways downstream of calcium. The major target of calcium is calmodulin, since fluphenazine (10 mM), a specific inhibitor of calmodulin, blocked the calcium mediated effects on filopodia. We next tested whether calcium's effects are mediated via two prominent targets of calmodulin, namely myosin-light chain kinase (MLCK) and CaM kinase II (CaMK II). Inhibitors of MLCK (ML-7, 5mM), and CaMK II (KN-93, 2μM) decreased filopodial length and number when applied alone at higher concentrations, suggesting that actin-myosin interactions are important in filopodial regulation. At lower concentrations, these inhibitors did not block the calcium-mediated effects on filopodia, indicating that ($[Ca^{2+}]_i$ may not act via MLCK or CaMK II to affect filopodial length and number. Two other potential targets of ($[Ca^{2+}]_i$, protein kinase C (PKC) and calpains, were investigated. Inhibiting the activity of calpain, an intracellular protease, caused significant elongation of filopodia, suggesting that protease activity may play and important role in controlling the actin-based cytoskeleton. We did not find evidence suggesting that PKC is involved in mediating calcium's effects.

Filopodial regulation by calcium does not appear to be a mechanism used by all cell types. Growth cones of dorsal root ganglion neurons (DRG) from E9-E11 chick embryos grown on laminin surfaces did not show significant changes in filopodial disposition when treated with medium containing elevated potassium concentrations (20, 40, 65 mM). These [K+] caused significant elevation in ($[Ca^{2+}]_i$, as evidenced by fura-2 measurements but failed to affect filopodia. In contrast to Helisoma neurons, filopodia on chick DRG growth cones seem to be controlled by PKC. Activation by phorbol esters (PMA, 2.5-5μM) caused a decrease in filopodial length and an increase in filopodial number, while inhibiting PKC with bisindolylmaleimide I (2.5μM) produced the opposite effect. Source of Support: Intramural Funding

297.16

THE ROLE OF INNERVATION IN MUSCLE PATTERNING IN *DROSOPHILA* J.J. Fernandes* and H. Keshishian, Dept. of Biology, Yale University, New Haven, CT 06520.

In *Drosophila* it has been proposed that motoneurons can specify the identity and patterning of adult muscles. We have tested this idea by using a laser to cut the nerve innervating the Dorsal Longitudinal flight Muscles (DLMs). These fibers develop during pupation from three persistent larval muscles that split in two (MFs 9, 10, 19'), and the innervating motoneurons are restructured during this period. We cut the nerve between the persistent larval muscles in early third instars to denervate fibers 9 and 10. The success of the operation was confirmed by dissecting late third instars 2 days later. During early pupation (8-12h) muscles distal to the nerve cut did not show any HRP immunoreactivity, showing that they are completely denervated at this stage. Nevertheless, the formation of a correct muscle fiber pattern is initiated prior to reinnervation, although with a 2-4 hr delay compared to controls. It appears that muscle splitting which takes place soon after myoblast fusion is initiated, can occur in the absence of innervation, suggesting that this process is muscle autonomous. However, the delay in the progression of myogenesis suggests that other steps of myogenesis are likely to be influenced by innervation. Muscle fibers that develop from MF10 are reinnervated earlier than those that develop from the more distally located MF9. Our studies also suggest that attractive cues from the muscle are likely to cause reinnervation. We are currently examining the reinnervation to determine whether the native motor neurons are involved. Supported by NSF and NIH.

297.17

APPLICATION OF SPECIFIC INHIBITORY PEPTIDES TO STUDY THE ROLE OF ENDOGENOUS CaMKII IN THE DEVELOPMENT OF THE XENOPUS RETINOTECTAL PROJECTION. D.J. Zou*, B.J. Burbach and H.T. Cline. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724

We have tested the possibility that a CaMKII pathway could be involved in the activity dependent mechanisms controlling the development of the frog retinotectal projection. When the constitutively active CaMKII is expressed in tectal cells with a recombinant vaccinia virus, elevated postsynaptic CaMKII activity modifies the elaboration of presynaptic retinal axon arbors, suggesting that CaMKII plays a role in the coordinated development of retinotectal system. To further study the role of CaMKII in regulating the formation of retinotectal projection, we plan to decrease endogenous CaMKII activity in the tectal cells by expressing specific inhibitory peptides. We have determined the potency and specificity of two CaMKII inhibitory peptides by using kinase assays in the homogenates of stage 48 Xenopus tadpole brains. We found that a synthesized peptide, AIP (Autocamtide-2-related Inhibitory Peptide) developed and generously provided by Dr. H.Fujisawa, has potent and highly specific inhibitory effects on the CaMKII activity. The IC_{50} of AIP on CaMKII was about 400 nM. AIP was 50 times more potent than the other CaMKII inhibitor [Ala^{286}]$CaMKII_{(281-302)}$ with an IC_{50} around 20 μM. At 10 μM, AIP decreased CaMKII activity to 7% $\pm$ 3% (n=4) of the controls but did not inhibit PKC activity (94% $\pm$ 9% of controls, n=4) in the same homogenate assayed in parallel. The IC_{50} of AIP on PKC was greater than 100 μM. Based on the AIP dose response curves, AIP was about 500 times more potent in blocking CaMKII than PKC *in vitro*. These results indicate that AIP is a potent and highly selective CaMKII inhibitor in frog neurons and is valuable for studying the role of endogenous CaMKII activity in controlling the formation of the retinotectal projections. *We thank Dr. K.P. Giese for bringing AIP to our attention and Dr. H. Fujisawa at Asahikawa Medical College in Japan for the generous gift of AIP. Supported by NIH.*

297.18

NITRIC OXIDE INDUCED RAPID NEURITE ELONGATION AND GROWTHCONE ASYMMETRY OF RAT DRG NEURONS. S. TSUKADA*, and J. FUKUDA.
Department of Physiology, National Defense Medical School, Tokorozawa, Saitama, JAPAN.

Nitric oxide has been implicated as a diffusible second messenger molecule that can act as modification of neuronal differentiation and establishment of neural connections. It has already been known that nitric oxide rapidly inhibits growth of neurites of rat dorsal root ganglion neurons. Here we report that nitric oxide promotes neurite extension and growthcone motility within a limited range of concentrations. NOR3, nitric oxide donors FK409, was focally applied by pulsatile ejection from micropipette that placed 50μm away from the growthcones. Small amount of nitric oxide rapidly enhanced neurite outgrowth and filopodial elongation. Shapes and motility of growthcone also changed in a short period of time. Topical application of NO caused filopodial asymmetry. These chemotropic effects were abolished by methylene blue, an inhibitor of soluble guanylate cyclase. Caged cyclic GMP that was flash photolysised at nerve growthcone also mimicked the effects of nitric oxide. Our results thus indicate that NO-cGMP pathway might play an important role during the establishment of ordered connections by developing neurons.

297.19

GABAERGIC MODULATION OF REGENERATIVE PROCESS OUTGROWTH BY ISOLATED ADULT SALAMANDER RETINAL PHOTORECEPTORS. David M. Sherry[1]* and Ellen Townes-Anderson[2].
[1]College of Optometry, University of Houston, Houston, TX 77204-6052;
[2]Department of Neurosciences, UMDNJ-NJMS, Newark, NJ 07103-2714.

Adult retinal photoreceptors regenerating in culture preferentially establish presynaptic varicosities onto target neurons containing the neurotransmitter GABA, suggesting that GABA may modulate regenerative neurite outgrowth and synapse formation by adult photoreceptors. A similar role for GABA has been postulated in retinal development. To test the role of GABA in modulating regenerative growth by adult photoreceptors, cultured retinal cells were treated with drugs acting at $GABA_A$ receptors, the GABA receptors expressed by salamander photoreceptors, for 1 day, 1 week, or 2 weeks. Cultures were then fixed and the number of neurites and presynaptic varicosities regenerated by photoreceptors in treated and age-matched control cultures from the same animal were compared. Treatment with the $GABA_A$ receptor antagonist bicuculline reduced the number of neurites and varicosities regenerated by photoreceptors in a dose dependent manner, with greatest effect noted at 2 weeks post-isolation. Treatment with muscimol, an agonist at $GABA_A$ receptors, produced only a very slight increase in the number of neurites and varicosities regenerated by photoreceptors, suggesting that endogenous levels of GABA in the cultures produce a maximal response on photoreceptor neurite growth. Pharmacological treatments did not appear to affect photoreceptor survival. These data suggest that GABA modulates regenerative growth and formation of synaptic structures by adult photoreceptors via $GABA_A$ receptors. Supported by College of Optometry, University of Houston (DMS) and EY06135 (ETA).

297.20

GLIAL-DEPENDENT BASAL FOREBRAIN NEURITOGENESIS ELICITED BY NT-3 IS MEDIATED BY A DIFFUSIBLE FACTOR S. Sample*, I.B. Black, and C.F. Dreyfus, Dept. Neuroscience & Cell Biology, Robert Wood Johnson Medical School/UMDNJ, Piscataway, NJ 08854

Our previous observations suggested that NT-3 elicits neurite extension from developing basal forebrain (BF) cholinergic neurons grown in the presence of glia (Sample et al., Soc Neur Abs 1994). To begin defining the glial-neuronal interaction regulated by NT-3, we sought to identify the factors involved and determine the specificity of the interaction. Embryonic day 17 rat BF cultures were treated with NT-3 or vehicle control and grown for 2 days under conditions that foster the establishment of glia. Media conditioned (CM) by these cultures were harvested and applied to freshly dissociated BF cells for 7 days. An anti-NT-3 antibody added to the CM neutralized remaining NT-3 bioactivity. CM generated by NT-3-treated cultures mimicked the NT-3 effect on cholinergic cells. Yet, membranes from cultures treated with NT-3 failed to elicit outgrowth at protein concentrations as great as 80 µg/ml. Similarly, extracellular matrix produced by cells treated with NT-3 did not promote outgrowth. These data suggest that NT-3-elicited outgrowth by BF cholinergic neurons grown in the presence of glia is mediated by a diffusible factor(s).

To determine whether other projection neurons of the basal forebrain exhibit enhanced outgrowth, we treated glial-neuron BF cultures with NT-3 and examined GABAergic neurons. NT-3 also enhanced neurite extension by GABAergic neurons grown in the presence of glia. Thus, glial-neuronal interactions, regulated by NT-3, may provide local cues within the BF that promote outgrowth by different projection neurons.
(Support: NICHD: HD23315)

PROCESS OUTGROWTH, GROWTH CONES, AND SPROUTING V

298.1

RESTRUCTURING OF NEURITES: POST-TRANSCRIPTIONAL CONTROL BY NMDA AND CALCIUM** WL Severt, V. Calabrese*, S. Sombati, C. Gerwin, R. Delorenzo and E. Jakoi
Department of Neurology, Medical College of Virginia, Richmond, VA 23298

Activation of NMDA receptors may direct neuronal plasticity by promoting neurite extension at low levels of receptor activity and retraction at high levels. Molecular mechanisms that follow the influx of calcium through NMDA receptors that can account for alterations in neuronal phenotype remain largely unexplained. Given the importance of calcium in regulating gene expression, and the emerging evidence that polyribosomes and select neuronal mRNAs exist at the synapse, we hypothesize that NMDA receptor activation regulates neuronal gene expression locally by modulating mRNA levels. We have recently demonstrated (Panchision et al, 1995) that NMDA and calcium decrease the stability of the mRNA encoding ligatin, a 10kD, fatty-acylated protein localized to distal dendrites. To explore the contribution of this down-regulation to neuronal restructuring, we used antisense oligodeoxynucleotides (ODNs) to knock-down ligatin protein in cultured hippocampal neurons. An ODN targeted to the coding region suppressed expression of ligatin protein and caused neurites to withdraw or to fail to extend, depending on cell age. These cells also showed decreased EPSPs and decreased dendritic levels of NMDA receptors and CaM kinase II, without decreased total cellular quantities of these proteins. Both IPSPs and GABA-A receptor levels remained unchanged. We also tested a second ODN targeted to a sequence within the 3'-UTR which has potential for directing transport of the mRNA to the synapse. This ODN did not alter ligatin protein levels, but did induce the expression of short neurites terminating in an exuberance of sprouts. These results implicate ligatin in neurite restructuring and suggest that post-transcriptional control of mRNA stability provides a mechanistic link by which synaptic activity could govern synaptic protein synthesis and neuronal remodeling in response to environmental stimuli more rapidly than that governed by nuclear control.
Funding: AD Williams Grant (ERJ), NIH Grants POI 25630 and NS 23350 (RJD)

298.2

LOCALIZATION OF DIFFERENT GLUTAMATE RECEPTORS ON AN IDENTIFIED *LYMNAEA* NEURON, AND THEIR ROLE IN THE GROWTH CONE COLLAPSE. O.Nesic*, N. Syed, K. Lukowiak and A.G.M. Bulloch,
Neuroscience Research Group, HSC, University of Calgary, Calgary, AB, T2N 4N1, Canada.

Glutamate is a putative transmitter in the *Lymnaea* CNS and central neurons possess different types of glutamate receptors. We propose that the same neuron can have various types of glutamate receptors, localized differentially, with possible diverse physiological roles. In this study we examined the effect of glutamate on both the soma and growth cones of the identified neuron cerebral one (C1), *in vitro*.

Pressure application of glutamate to the soma of neuron C1 caused hyperpolarization. Application of glutamate (1 mM, for 15 min) to the growth cone of neuron C1, caused growth cone retraction or collapse. The glutamate response, measured at different membrane potentials, showed a reversal potential of ~-90 mV, indicating the opening of K^+-selective conductance. Further, bath application of TEA (51mM) blocked this response. However, TEA did not prevent glutamate-induced growth cone collapse. Bath application of 0.1 mM kainic acid to neuron C1 caused depolarization and irreversible growth cone collapse. The kainic acid response was reduced by the Ca^{2+} channel blocker, cadmium (Cd^{2+}, 2mM), and by the application of low Ca^{2+} saline. Application of low Ca^{2+} or Cd^{2+}, however, did not affect the hyperpolarizing glutamate response, but Cd^{2+} did indeed prevent glutamate-induced growth collapse.

We propose that neuron C1 possesses different glutamate receptors coupled to different ionic conductances: one, a K^+-selective, TEA-sensitive conductance, and the other a Ca^{2+}-permeable, Cd^{2+}-sensitive conductance. We suggest that both types of receptors are present on the soma, but only the Cd^{2+}-sensitive receptor is present on the growth cone. This receptor could be involved in the navigation of axons extending from neuron C1. Supported by MRC (Canada).

298.3

INJURY-INDUCED CHANGES IN NEURONAL EXCITABILITY ARE INDEPENDENT OF PROCESS OUTGROWTH. M.J. Zoran* and N. L. Achee. Department of Biology, Texas A&M University, College Station, TX 77843.

Helisoma neuron B5, regenerating axons following nerve crush, displays an initial, transient (<24 hrs) reduction in excitability followed by a period of maintained, enhanced excitability (>9 d). Membrane potential of B5 in reduced ganglia was current-clamped to a level of -60 mV and current pulses were applied for 3 seconds. The average number of action potentials (APs) elicited in B5 prior to axotomy was 18.2 ± 3.9 APs (n=12). Maximal spiking was significantly decreased to 5.5 ± 2.0 APs (n=12) several hours after nerve crush ($p<0.01$). Several days later, hyperexcitability of B5 was observed such that maximal spiking on day 9 post-axotomy was 27.0 ± 1.7 APs.

To examine whether these changes in neuronal excitability were governed by intrinsic neuronal mechanisms or extrinsic factors, we examined excitability in axotomized neurons following isolation into cell culture. Neuron B5, *in vitro*, expressed a similar pattern of hypo- and hyperexcitability during neuritic outgrowth. However, elevations in excitability were only present in conditioned medium (CM) containing trophic-factors released from injured neural tissue. In addition, following 5 days post-isolation into cell culture maximal spiking dropped to levels similar to those of newly isolated neurons indicating that sustained hyperexcitability requires support not present in CM.

Neurons were isolated into culture conditions that prevent neurite outgrowth. Changes in electrophysiological properties of spherical B5s were identical to those demonstrated for neurite-bearing neurons. Therefore, B5 hyperexcitability was induced by factors associated with injured neural tissue and was independent of process outgrowth. Whether specific components of conditioned medium govern diferentially changes in excitability and outgrowth remains unknown.

298.4

EFFECT OF NEUROMUSCULAR ACTIVITY ON SPROUTING DEPENDS ON THE EXTENT OF PARTIAL DENERVATION. S.L. Tam, V. Archibald, R.B. Stein*, N. Tyreman, and T. Gordon, Dept. of Pharmacol., and +Dept of Physiol., Div. of Neurosci., Univ. of Alberta, Edmonton, Canada T6G 2S2.

To extend our initial study where moderate exercise was not found to be detrimental to sprouting (Soc. Neurosci. 1994, Abstr.#545.3), we, in the present study, used a rat model of partial denervation to test the hypothesis that high levels of neuromuscular activity compromise the sprouting during the acute phase of motoneuron diseases. Four hindlimb muscles, tibialis anteriar, medial gastrocnemius, soleus and plantaris, were partially denervated (PD) by cutting either the L4 or L5 spinal root and subjected immediately to increased activity: daily functional electrical stimulation (FES, 8hr, 20Hz) or natural stimulation (8hr running on exercise wheels, 1200m/day). Within a month, MU force and number of sprouts in muscles which experienced normal cage activity increased to an upper limit of 3-5 times normal. A one month period of increased neuromuscular activity significantly reduced MU enlargement in extensively denervated muscles (PD>75%). In moderately denervated muscles (PD<75%), the effect was not significant although the same trend was seen. These results show that 1) normal physiological activity is conducive for maximal sprouting during the acute phase of motoneuron diseases, and 2) while high levels of neuromuscular activity can be detrimental to sprouting in extensively denervated muscles, it does not appear to be harmful for sprouting after moderate denervation. Therefore, although exercise generally appears to be helpful, these results suggest that exercise regimes must be used with caution. Supported by AHFMR, Rick Hansen Man in Motion Legacy Fund, MDAC and MRC of Canada.

298.5

EFFECTS OF DEPOLARIZATION ON NEURITE OUTGROWTH IN CULTURED CEREBRAL CORTEX NEURONS. Ger J.A. Ramakers* and J. van Pelt. Neurons and Networks, Netherlands Institute for Brain Research, Meibergdreef 33, 1105 AZ Amsterdam ZO, The Netherlands.

Bioelectric activity has profound effects on neurite outgrowth. In cultured Helisoma neurons electrical stimulation result in a reversible arrest of neurite extension in a calcium-dependent manner, whereas in Xenopus neurons, depolarization with high potassium stimulates neurite extension. DC electric fields moreover have a strong effect on the direction of neurite outgrowth, with growth cones moving towards the cathode. We are investigating the effects of depolarization with elevated potassium chloride on neurite outgrowth of dissociated fetal rat cerebral cortex neurons in primary cultures, using time-lapse video recording and quantitative image analysis. Depolarization was found to increase the surface area of lamellipodia within three min, an effect which lasted at least 20 min. Effects on filopodia were also observed. Continued depolarization resulted in an increased rate of neurite extension for at least 24 hrs. The role of calcium and downstream signal transduction events are currently being investigated to link depolarization to the changes in the growth cone cytoskeleton responsible for alterations in growth cone morphology and neurite outgrowth.

298.6

REGIONAL LASER INACTIVATION OF TYPE 1 INOSITOL 1,4,5-TRISPHOSPHATE RECEPTOR AFFECTS GROWTH CONE MORPHOLOGY AND MOTILITY IN CHICK DRG NEURONS. K. Takei*[1], D. G. Jay[2] and K. Mikoshiba[1]. Lab. of Cell Biol., Calciosignal Net Project, ERATO, Res. Dev. Corp. of Japan, Tokyo 153[1], Dept. of Mol. and Cell. Biol., Harvard Univ., MA 02138[2].

The inositol 1,4,5-trisphosphate receptor ($InsP_3R$) acts as an $InsP_3$-gated Ca^{2+} release channel. Type 1 $InsP_3$ receptor ($InsP_3R1$) is the major neuronal member of the $InsP_3R$ family in the central nervous system. Alteration of intracellular concentration of Ca^{2+} in the growth cone regulates neurite outgrowth but what processes Ca^{2+} regulates and the molecular mechanisms of the regulation are unclear. We asked if $InsP_3R$ is required for neurite outgrowth by observing growth cone motility in response to the acute and localized loss of the $InsP_3R$. This was achieved by applying microscale chromophore-assisted laser inactivation (micro-CALI) to the $InsP_3R1$ in chick dorsal ganglia (DRG) neuronal growth cones cultured on laminin. Micro-CALI directs spatially restricted photogenerated free radical damage to targeted proteins via dye-labeled antibodies that by themselves do not affect growth cone behavior. The neurons were loaded with dye-labeled antibodies by microinjection. Subregions of growth cones were subjected to a laser micro-beam, and subsequent changes in motility and morphology were observed by time lapse video microscopy. Micro-CALI of the $InsP_3R1$ caused lamellipodial collapse, marked change of the growth cone morphology and subsequently growth arrest of neurite. Laser irradiation of neurons loaded with dye-labeled non-specific antibodies or without antibodies did not affect growth cone motility and morphology. These results suggest that $InsP_3R1$ act on growth cone morphology and motility and the $InsP_3$-gated Ca^{2+} release may play important roles in the growth cone behavior and neurite outgrowth.

298.7

INTRACELLULAR CALCIUM INCREASES IN TWO POPULATIONS OF EMBRYONIC NEURONS AS A RESPONSE TO AN APPLICATION OF SOLUBLE LIMBIC SYSTEM-ASSOCIATED MEMBRANE PROTEIN (LAMP). V.Zhukareva*, N.Chernevskaya, M.Nowycky, and P.Levitt. Dept. of Neuroscience and Cell Biology, Robert Wood Johnson Medical School - UMDNJ, Piscataway, NJ 08854 and Dept. Anatomy and Neurobiology, Med.Col. PA. Philadelphia, PA 19129.

Limbic system-associated membrane protein (LAMP) is a GPI-anchored protein, Ig-superfamily member with selective neurite outgrowth promoting effect on fetal neurons. As a GPI-anchored molecule, LAMP potentially could be released from the membrane surface and may interact with its counterpart and/or its receptor as a soluble protein. To investigate a possible role of second messengers in the cascade of events triggered by LAMP, we applied soluble recombinant LAMP on two populations of rat E17 embryonic neurons, from hippocampus that express LAMP (LAMP+) and visual cortex that do not express LAMP (LAMP-). Intracellular calcium levels were measured in cells loaded with fluor-3 using confocal laser microscopy. Bath application of LAMP caused mostly delayed (10 - 20 min.) sustained elevation of $[Ca^{2+}]_i$ (up to 60 min.) in hippocampal neurons. The number of cells responding (from 30% responders at 5-6 DIV to 60% at 12 DIV) and the magnitude of response varied with regard to the neuronal age in culture. In contrast, the number of responders to the LAMP application from the visual cortex (18% responders at 7-8 DIV), the amplitude and the duration of $[Ca^{2+}]_i$ increase (10 - 20 min., with a return to the base line) were smaller. These results suggest that LAMP application triggers an intracellular signaling cascade involving changes in $[Ca^{2+}]_i$ and that the kinetics of the response are specific for different regions of the developing brain. Supported by MH45507 (PL) and NS22281(MN).

298.8

L-TYPE CALCIUM CHANNELS IN GROWTH CONES.

K. Ohbayashi[1,2]*, H. Fukura[1], Y. Komiya[1], M. Igarashi[1]. Departments of [1]Molecular and Cellular Neurobiology and [2]Neurosurgery, Gunma University School of Medicine, Gunma 371, Japan.

Intracellular calcium ($[Ca^{2+}]_i$) in the growth cone is important for neurite elongation, axonal pathfinding, and accurate synaptogenesis. Recent studies have indicated that L-type Ca^{2+} channels in growth cones are closely involved in regulation of $[Ca^{2+}]_i$. To characterize L-type Ca^{2+} channels in growth cones, we isolated growth cone membranes (GCM) from fetal and neonatal rat forebrain, and pharmacologically characterized the channels using [^{3}H]PN200-110. B_{max} values for [^{3}H]PN200-110 in GCM gradually decreased from embryonic day 17 (E17) (0.716 pmol/mg protein) to postnatal 7 (P7) (0.341). To examine the change of $[Ca^{2+}]_i$ through L- or N-type Ca^{2+} channels, we measured $[Ca^{2+}]_i$ of GCP by fluorescence spectrometry using Fura 2/AM. Bay K 8644, an L-type agonist, increased $[Ca^{2+}]_i$ in GCP at all ages. The increase of $[Ca^{2+}]_i$ in embryonic GCP was larger than that of synaptosomes. Diadenosine pentaphosphate (Ap_5A), an agent known to potentiate N-type channels selectively (Panchenko et al., Neuroscience 70:353-360, 1996), increased $[Ca^{2+}]_i$ in postnatal GCP but had no effect on embryonic GCP. Elevation of Ca^{2+} in GCP by Ap_5A was blocked by ω-Conotoxin GVIA. To examine the effect of L-type activation on Ca^{2+}-dependent phosphorylation, we labeled GCP proteins using [^{32}P]orthophosphate. Under these conditions, a 45- and a 80- kDa proteins, identical to GAP-43 and MARCKS, were primarily phosphorylated; Bay K 8644 enhanced phosphorylation of these proteins. Our results suggest that L-type Ca^{2+} channel activity modulates growth cone functions by regulating $[Ca^{2+}]_i$, and that N-type Ca^{2+} channels develop at later stages of synaptogenesis.

299.1

HEPATOCYTE GROWTH FACTOR-SCATTER FACTOR (HGF) EXHIBITS NEUROTROPHIC ACTIVITY AND ACTS ON GABAERGIC AND CALBINDIN D EXPRESSING NEURONS

D. Lindholm[1]*, T. Litzenburger[2], L.Korhonen[1], N. Inagaki[2], and E. Castrén[2] 1.Dept. Dev. Neuroscience, BMC, Box 587, S-75123 Uppsala, Sweden. 2.Dept. Neurochem. Max-Planck-Institute for Psychiatry, Am Klopferspitz 18a, D-82152 Martinsried, Germany.

Hepatocyte growth factor-scatter factor (HGF) and its receptor, c-met are expressed in specific areas of the central nervous system. To study the function of HGF in neurones we transfected pheochromocytoma PC12 nnr5 cells with a chimeric construct consisting of the extracellular part of Trk receptor and the intracellular domain of c-met. Addition of nerve growth factor (NGF) to this cell line, lacking endogenous Trk receptors, activates c-met and induces neurite outgrowth of the cells. Studies using human NT-2 cells which normally express c-met showed that HGF increased survival of serum deprived hNT. These data show that activation of c-met transduces signals promoting both neuronal differentiation and survival. In searching for primary neurons responsive to HGF neuronal cell cultures were made from embryonic rat brain. Addition of HGF specifically enhanced neurite outgrowth of cultured GABAergic neurons from septum and hippocampus. HGF also increased the number of hippocampal neurons expressing the calcium binding protein, calbindin D.The present results show that HGF has potent neurotrophic activities on specific neurons in brain and HGF increases both the survival and differentiation of responsive neurons.

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299.2

SPECIFIC, INCREASED SURVIVAL OF DOPAMINERGIC AND NON-DOPAMINERGIC NEURONS IN A MESENCEPHALIC CELL CULTURE, BY INHIBITORS OF MONOAMINE OXIDASE B .

J. W. Commissiong*, J.P.M. Finberg[1], M.B.H. Youdim[2], Takao Takeshima[3]. LMB, NINDS, NIH, Bldg.36/3D02, Bethesda, MD 20892; [1]Dept. Pharmacol., Fac. Med., Technion-Israel Inst.Tech., Bat-Galim, Haifa 31096, Israel; [2]Div. Neurol., Inst. Neurol. Sci., Tottori Univ. Sch. Med., 86 Nishimachi, Yonago 683, Japan.

Deprenyl, a specific inhibitor of Monoamine Oxidase (MAO) B has been shown to increase the survival of fetal dopaminergic neurons in culture [Roy and Bedard (1993), NeuroReport 4: 1183-1186]. Subsequently, it was shown that deprenyl also increased the survival of axotomized, somatic motoneurons, and that this effect was independent on inhibition of MAO [Ansari et al. (1993), J. Neurosci. 13: 4042-4053]. We have therefore used optimal conditions to test clorgyline, (-) methamphetamine, deprenyl and rasagiline [R(+)-N-propargyl-1-aminoindane mesylate, or TVP-1012], all at 10^{-4} - 10^{-5} M, on rat E14, post-mitotic cells of the ventral mesencephalon [Shimoda et al., (1992), Brain Res. 586: 319-331]. The cells were cultured for 7 days and then double stained to visualize the neuronal marker MAP2 and the specific marker for dopaminergic neurons in this culture, tyrosine hydroxylase (TH). Clorgyline and methamphetamine did not exert a measurable effect at any of the doses tested. Deprenyl, at 10^{-4} and 10^{-5} M, caused a significant ($P<0.01$) increase in dopaminergic neurons only. However, TVP-1012, also at 10^{-4} and 10^{-5} M, increased both neuronal (MAP2-pos) survival as well as the survival of TH-pos neurons ($P<0.05$). Interestingly, GABAergic neurons, which account for about 50% of the neurons in the culture, were not affected by either deprenyl, or rasagiline. We conclude that both MAO B inhibitors, deprenyl and rasagiline are able to increase the survival of dopaminergic neurons in culture, but that additionally, rasagiline can increase the survival of an unknown subset of neurons in the culture. Since the MAO A inhibitor clorgyline was ineffective, the mechanisms involved in both cases are unlikely to be related to inhibition of MAO.

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299.3

PREMARIN INCREASES THE GROWTH OF NEURONS FROM THE CEREBRAL CORTEX.

R.D. Brinton,* M. Montoya, and D. Hsieh. Molecular Pharmacology & Toxicology, The STAR Program, Univ. of Southern California, School of Pharmacy, Los Angeles, CA. 90033.

Premarin is a complex mixture of estrogenic and progesterone steroids and is the leading prescribed pharmaceutical estrogen replacement therapy for postmenopausal women. Recent evidence indicates that postmenopausal women not receiving estrogen replacement therapy are at greater risk for developing cognitive dysfunction, a compromised stress response and Alzheimer's Disease relative to their estrogen replaced contemporaries. Regulation of both the growth of neuronal extensions and the survival of neurons involved in cognitive function can have a significant impact on the function of neural networks involved in memory and other cognitive processes. We therefore investigated the effect of Premarin and select estrogenic steroids contained within Premarin on the growth of neurons derived from the cerebral cortex, a brain region critical to cognitive function. Results of this investigation demonstrated that the complex formulation of Premarin significantly increased the growth of cortical neurons as indicated by a significant increase in the number of neurites ($p<.05$ -.01), number of branches ($p<.05$-.01), branch length ($p<.05$), and in the number of microspikes ($p<.05$-.01). We further investigated the neurotrophic action of individual components of Premarin and found that equilin, a major component of Premarin, induced highly significant increases in the growth of both the macro features of cortical nerve cell morphology as well as the micro features ($p<.05$-.001). Results of a cortical regional selectivity analysis indicated that the regional distribution for both 17 β-estradiol and equilin-induced nerve cell growth exhibited some parallels, most notably the consistent significant induction of occipital nerve cell growth. In addition, equilin induced a significant increase in the growth of frontal and parietal cortical neurons on some morphological measures ($p<.01$-001) whereas 17 β-estradiol exhibited only a trend towards statistically significant increases in these brain regions. We pursued the mechanism of equilin-induced nerve cell growth by investigating the impact of blocking the NMDA glutamate receptor on equilin-induced occipital cortex nerve cell growth. Results of these experiments indicate that the growth promoting effects of equilin (100 nM $p<.01$) were completely abolished in the presence of the NMDA receptor antagonist AP5.

This work was supported by the USC School of Pharmacy.

299.4

THE SEMISYNTHETIC GLYCOSPHINGOLIPID (GSL) LIGA20 POTENTLY PROTECTS NEURONS AGAINST NECROSIS AND APOPTOSIS.

A. Guidotti*[a], M. Saito[b] and E. Costa[a]. [a] The Psychiatric Institute, Department of Psychiatry, University of Illinois at Chicago, Chicago, IL 60612. [b] N.S. Kline Institute for Psychiatric Research, Orangeburg, NY 10962.

LIGA20 (Lyso GMI with N-dichloroacetyl-sphingosine) added to neuronal cultures of newborn rats prevents glutamate-induced necrosis with a potency several-fold higher than that of natural (GM1, GD1B, GD1A, GT1B) or other semisynthetic GSLs (*JPET* **252**: 419, 1990). In the present study the neuroprotective effect of LIGA20 and that of other GSLs on apoptotic neuronal death was examined in cerebellar granule cells. In primary cultures of these cells prepared from newborn rats, apoptotic death was induced by: *a)* serum deprivation and non-depolarizing culture media (5mM K^+); or *b)* adding to the normal medium 10 μM nimodipine, a voltage-sensitive Ca^{2+} channel blocker. We have recently observed (*JPET* in press) that Pb^{2+} in a concentration-dependent manner accelerates apoptosis in granule cell cultures. Thus, we studied the neuroprotective action of GSLs in these three experimental conditions. In all apoptotic conditions LIGA20 (EC_{50} ~ 5 μM) was more potent than GM1 and other GSLs in protecting neurons from death. The protection afforded by GSLs against apoptotic death was confirmed biochemically by the reduction of the typical internucleosomal DNA fragmentation. The data suggest that the neuroprotection against necrosis or apoptosis elicited by LIGA20 and other GSLs depends on a pleiotropic mechanism of action. NS 28130-06.

299.5

EFFECT OF GLIAL GROWTH FACTOR 2 (rhGGF2) ON THE SURVIVAL OF AXOTOMIZED RETINAL GANGLION CELLS IN ADULT RATS.

S.A. Singel[1], M.A. Jarpe[2], G.M. Bray[1]* [1]Centre for Research in Neuroscience, The Montreal General Hospital Research Institute and McGill University, 1650 Cedar Ave., Montréal, QC H3G 1A4; [2]Cambridge NeuroScience Inc., Cambridge, MA 02139.

Retinal ganglion cells (RGCs) in embryonic and adult rats express members of the erbB tyrosine kinase family, which act as receptors for neuregulins. One of the neuregulins, glial growth factor 2 (rhGGF2), enhances the survival and axonal growth of embryonic and neonatal rat retinal cells *in vitro* (Bermingham-McDonogh et al., Development 1996, in press). The purpose of the present study was to determine the effect of rhGGF2 on the survival of retinal ganglion cells (RGCs) *in vivo* after optic nerve (ON) transection. RGCs in adult Sprague-Dawley rats were retrogradely labelled from their main target, the superior colliculus. Seven days later, the left optic nerve was cut close to the eye and a single dose (5 μl) of 250 μg/ml rhGGF2 or 5 μl control acetate buffer (pH 6.5) was injected into the vitreous chamber. RGC densities were determined for groups of retinas 7, 10, and 14 days later. At 7 days, RGC densities in the GGF-treated retinas were not significantly different from those of control retinas. At 10 and 14 days, approximately twice as many RGCs survived in the rhGGF2-treated retinas. This increased RGC survival after rhGGF2 administration is less than that observed with BDNF or NT-4/5 but indicates that GGF can affect the survival of some injured adult RGCs.

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299.6

CEP-1347 PREVENTS EXCITOTOXIN-INDUCED DEATH OF NEURONS IN BASAL FOREBRAIN: A QUANTITATIVE RETROGRADE LABELING STUDY IN ADULT RAT.

F. Haun*, A. DiCamillo, C. Murakata[#], S. Carswell, M. Miller and N. Neff. Cephalon, Inc., West Chester, PA, 19380, and [#]Kyowa-Hakko Kogyo Co., Tokyo, Japan.

The functional loss of basal forebrain cholinergic neurons is thought to be a major contributor to Alzheimer's disease (AD). A number of potential AD therapeutics, including the structurally novel indolocarbazole CEP-1347, have been shown to prevent loss of cholinergic markers in the cortical targets of neurons in nucleus basalis magnocellularis (nbm) following experimental lesion of this basal forebrain region. The present study examined whether this prevention of cortical cholinergic loss by CEP-1347 reflects protection against neuron death. The retrograde tracer Fluoro-Gold (FG) was injected bilaterally into frontal and parietal cortices of adult Sprague-Dawley rats. 7-10 days later these animals received unilateral ibotenic acid lesions of the nbm, followed 18 h later by CEP-1347 (s.c. 0.03 mg/kg, Q.O.D.) for 18 days. FG-labeled neurons were then counted throughout the nbm immediately adjacent to the ventral and medial borders of the globus pallidus. Significantly more neurons remained in the rostral portion of the nbm in animals receiving CEP-1347 (84 ± 4.6 % of the unlesioned side), compared to the vehicle controls (45.5 ± 4.6 %). Counts of ChAT and GAD immunopositive neurons from adjacent sections showed an identical pattern of protection against both cholinergic and GABA-ergic neuron loss in rostral nbm. These results demonstrate directly that CEP-1347 is neuroprotective against excitotoxin-induced cell death in the basal forebrain, and suggest that this compound may be useful in preventing the degeneration of multiple classes of neurons.

299.7

CEP-1347 REDUCES NEURONAL DAMAGE FOLLOWING NMDA LESION OF THE ENTORHINAL CORTEX. T. M. Engber*, S. A. Dennis, C. Murakata#, M. Miller and P. C. Contreras. Cephalon, Inc., West Chester, PA 19380 and #Kyowa-Hakko Kogyo Co. Ltd., Tokyo, Japan.

In Alzheimer's disease, there is a severe loss of glutamatergic neurons in the entorhinal cortex (EC) as well as cholinergic neurons in the basal forebrain. Since the EC provides the major cortical input to the hippocampus and EC lesions in animals cause memory deficits, loss of neurons in this region likely contributes to the cognitive impairments in Alzheimer's disease. The neurotrophic compound CEP-1347, a structurally novel indolocarbazole which lacks excitatory amino acid receptor antagonist activity, has been shown to protect cholinergic neurons in the nbm and septum from experimental lesions in animal models. We have investigated whether CEP-1347 also protects glutamatergic neurons of the EC. Rats were given a unilateral lesion of the EC via stereotaxic injection of NMDA (15 nmols per site at 2 sites). Immediately afterwards and then daily for 14 days, rats were treated with either CEP-1347 (1 mg/kg s.c.) or vehicle (5% Solutol in PBS). The survival of neurons in layer 2 of the EC was assessed in horizontal sections stained with Cresyl violet, while the integrity of their axon terminals in the molecular layer of the dentate gyrus was determined in alternate sections stained with Timm's stain. NMDA lesioning destroyed 62±11% of EC layer 2 and decreased the area of the dentate gyrus middle molecular layer by 19±6%. CEP-1347 reduced the loss of EC layer 2 to 22±10% ($p<0.05$) and prevented the decrease in area of the middle molecular layer ($p<0.05$). Thus, CEP-1347 protected neurons in the EC from an excitotoxic lesion and maintained the integrity of their axon terminals in the dentate gyrus. The efficacy of CEP-1347 in protecting both cholinergic neurons of the basal forebrain and glutamatergic neurons of the EC in animal models suggests that this small neurotrophic molecule may be useful in treating the neurodegeneration associated with Alzheimer's disease.

299.8

EVIDENCE THAT S100β FUNCTIONS TO MAINTAIN SYNAPSES IN THE DENTATE MOLECULAR LAYER OF THE ADULT RAT, K.M. Faber*, C.C. Wilson and J.H. Haring. Dept. of Anatomy and Neurobiology, Saint Louis Univ. Sch. of Med., St. Louis, MO 63104.

S100β is a calcium binding protein produced by astrocytes that has been shown to function as a neurotrophic factor. Furthermore, the synthesis and secretion of S100β is stimulated by 5-HT via astrocytic 5-HT_{1a} receptors, particularly in the developing CNS. We have reported that both 5-HT depletion by PCA or 5-HT_{1a} antagonist treatment of adult rats results in a significant loss of synaptic densities in the molecular layer of the fascia dentata 7 days after drug administration. Moreover, we have also reported that S100β immunoreactivity is significantly reduced after 5-HT depletion in adult rats. The purpose of this study was to determine whether a reduction in available S100β without 5-HT depletion would result in a loss of synaptic densities. Male Sprague-Dawley rats (200-300g) were the subjects of this experiment. Rats were anesthetized with sodium pentobarbital and placed in a stereotaxic instrument. Cannulae were placed in the lateral ventricle and connected to an osmotic pump containing rabbit anti-S100β IgG or normal goat serum diluted 1:250 with PBS. Rats were perfused 7 days later and the area dentata was prepared for EM. Analysis of electron photomicroscopic montages through the molecular layer indicated that the infiltration of anti-S100β for 7 days resulted in a significant reduction in the number of synaptic densities observed in the molecular layer (79% of serum control, $p<0.01$). These data together with previous observations suggest that stimulation of astrocytes by 5-HT produces a basal secretion of S100β that is important for the maintenance of synapses in the adult molecular layer.
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299.9

MORPHOLOGIC CHANGES OF ChAT- OR p75-IMMUNOPOSITIVE NEURONS IN THE AGED RAT FOLLOWING TREATMENT WITH GM1 GANGLIOSIDE. T.G.Fong *, N.H. Neff and M. Hadjiconstantinou. Departments of Pharmacology, Psychiatry, and the Neuroscience Program, The Ohio State University College of Medicine, Columbus, OH 43210

To extend our previous studies which demonstrated that GM1 can correct cholinergic neurochemical and behavioral deficits in the aged rat, we have investigated whether GM1 can also affect neuronal morphology. Aged (21-22 months old) and young (3 months old) Sprague Dawley rats were given GM1, 30 mg/kg ip, or saline for 30 days. Using either antibodies to choline acetyltransferase (ChAT) or to the low-affinity NGF receptor (p75), immunopositive neurons in the medial septum (MS), vertical limb of diagonal band (VDB), nucleus basalis (NB), and the striatum were analyzed for average cross-sectional size and cell number, using a computerized image analysis system. Aged animals treated with saline demonstrated a 15-20% decrease in size of both p75- and ChAT-immunopositive neurons in all regions measured, whereas aged animals treated with GM1 were not different in size from young. Further, GM1 treatment in aged animals reversed decreases in ChAT-immunopositive cell number in the MS and striatum of rats, and the number of p75-immunopositive cells in the MS and NB. GM1 had no effect on the ChAT-positive cells of young animals, but increased the cell size of p75-immunopositive neurons in the MS and VDB. These finding suggest that in addition to its ability to restore neurochemical and behavioral impairment in the aged rat, GM1 treatment will promote recovery of neuronal morphology as well.
These studies were supported in part by NIA grant AG10530, and NIH grant MH 19936.

299.10

NEUREGULINS IN MYELINATING GLIAL CELLS. Timothy D. Raabe and George H. DeVries*. Department of Cell Biology, Neurobiology, and Anatomy, Loyola University Medical Center, Maywood, IL, 60153, and Research Service, Hines VA Hospital, Hines, IL, 60141.

Neuregulins (NRGs) consist of a family of growth factors which include NDF, GGF, ARIA, and HRG. Previous reports suggest that NRGs play an essential role in the development and differentiation of both Schwann cells (SC) and oligodendrocytes (OLG). The NRGs which influence SC and OLG development are presumed to be of neuronal origin since neurons with which these glial cells interact express NRGs. We now report that SC and OLG themselves contain NDF. Immunocytochemical data demonstrated NDF immunoreactivity throughout the cytoplasm of SC and OLG. In addition, NDF immunoreactivity was observed on SC plasma membranes. Western blot analyses of SC and OLG lysates revealed NDF isoforms ranging in molecular weights from 15 kDa to 75 kDa. We have also detected NDF in conditioned media from cultured SC and OLG which corresponds to the mature 45 kDa form of NDF. Furthermore, we have identified mRNA for NDFα and NDFβ in cultured SC and OLG. Our data suggest that NRGs are synthesized and secreted by SC and OLG. The NRGs produced by SC and OLG could possibly function via an autocrine loop during development or following injury.
(Supported by a grant from the National Multiple Sclerosis Society RG2076B6).

299.11

MELATONIN RESCUES DEVELOPING AND/OR MPP+ DAMAGED DOPAMINE NEURONS FROM CELL DEATH IN CULTURE. L. Iacovitti*, K. Johnston and N.D. Stull Dept. of Neurobiology and Anatomy, MCP and Hahnemann University, Philadelphia, PA.

The pineal neurohormone, melatonin, is believed to be a potent endogenous scavenger of hydroxyl radicals. This antioxidant action of melatonin is thought to protect against certain types of cellular damage. In this study, we sought to determine whether melatonin can specifically protect dopamine (DA) neurons from damage or death following the oxidative stress associated with 1) growth factor deprivation or 2) MPP^+ toxin. E15 rat DA neurons were grown in culture at low ($5 \times 10^{3-4}$ cells/80mm^2) or high (5×10^5 cells/20mm^2) density and maintained on serum-free media. In low density cultures, nearly all neurons were dead by day 6, presumably due to growth factor deprivation. However, supplementation with melatonin (0.1-500μM), resulted in the dose dependent survival of robust process-bearing DA neurons. No difference in survival or growth was observed in healthy high density cultures treated with melatonin. In cultures exposed to 2.5 μM MPP^+ for 24hrs, there was a 50% decline in the number of DA neurons surviving 3 days later. Treatment with 500μM melatonin during the post-MPP^+ period, however, restored the number of surviving DA neurons to nearly control levels (86%). We conclude that melatonin can act as a trophic agent to developing DA neurons in low density culture and can rescue from impending death DA neurons treated with the neuortoxin MPP^+. These trophic and neuroprotective properties may reflect melatonin's antioxidant action. (Supported by NIH NS 32519).

299.12

PIGMENT EPITHELIUM-DERIVED FACTOR (PEDF) PROTECTS CEREBELLAR GRANULE CELLS AGAINST APOPTOSIS T. Araki, T. Taniwaki*, S.P. Becerra°, G.J. Chader°, J.P. Schwartz Clinical Neuroscience Branch, NINDS, °Lab. Retinal Cell and Molecular Biology, NEI, NIH, Bethesda MD 20892

Pigment epithelium-derived factor (PEDF) has a neuroprotective effect against glutamate toxicity for cerebellar granule cells (CGCs). In the present study, we have investigated the ability of PEDF to protect against apoptosis in 2 paradigms. Apoptosis was measured by assaying for DNA fragmentation and number of live cells by MTS. Under normal culture conditions(medium containing 25 mM KCl), 55% of CGCs have died of apoptosis after 12 days in vitro (DIV 12). PEDF, when added to CGCs at DIV 1, rescued ~ 40% of those CGCs, whereas addition of PEDF on DIV 5 did not rescue any cells. Apoptosis can also be induced by changing the medium from high potassium (25 mM) to low potassium (5 mM) at DIV 2 or DIV 6. When apoptosis is induced by lowering KCl at DIV 2, the addition of PEDF 24 hrs earlier or simultaneously significantly reduces CGC loss measured 24 hrs later. However, when apoptosis is induced at DIV 6, addition of PEDF up to 24 hrs earlier has little protective effect. These data show that PEDF can protect neurons against low potassium-induced apoptotic cell death at DIV 2, but not at DIV 6. The finding that PEDF has antiapoptotic effects only on immature CGCs (DIV 1 or 2) suggests the possibility that the PEDF receptor or its signal transduction system may undergo changes during this developmental time course.

299.13

EARLY PREGNANCY FACTOR REGULATES BRAIN GROWTH OF THE EARLY POSTIMPLANTATION (E9) MOUSE EMBRYO. S.J. Lee[1]*, S.J. Servoss[1], A.C. Cavanagh[2], P.F. Alewood[2], H. Morton[2], D.E. Brenneman and J.M. Hill[1]. [1]Lab of Developmental Neurobiology, NIH / NICHD, Bethesda, MD. 20892; [2]Dept. Surgery, Univ. Of Queensland, Australia.

During the early post-implantation period of development (E9-E11 in mouse), the regulation of both brain and body growth is influenced by a number of factors which coordinate early morphogenic events. Present in maternal serum during the first half of gestation, early pregnancy factor (EPF), a member of a highly conserved family of heat shock proteins, is an essential component which regulates cell proliferation and is necessary for embryonic well-being (Eur.J.Biochem. 1994, 222:551-60). The current study examines the role of EPF in brain and body growth during the early post-implantation period of ontogenesis. Whole mouse embryos (E9) in culture were treated with EPF (10^{-12}M - 10^{-6} M), anti-EPF (1:100 dilution) or co-treated with EPF (10^{-8}M) and anti-EPF for four hours. Somite growth, body and head growth, DNA and protein contents were measured. The results revealed a significant and dose-related increase in somite number, DNA and protein contents. The dose response curve showed a bimodal distribution with significant growth occurring at 10^{-11}M , 10^{-10}M, 10^{-8}M and 10^{-7}M EPF. EPF also had a proportionately greater effect on brain growth than on body growth. Anti-EPF significantly inhibited growth of both brain and body but had a proportionately greater inhibitory effect on the brain. These data suggest that EPF may play an important role during neurogenesis and may regulate embryonic growth.

299.14

PC12 CELL PHENOTYPE IS ALTERED IN RESPONSE TO ENDOGENOUS EXPRESSION OF THE HUMAN PLEIOTROPHIN (Ptn) GENE, N. Zhang, H.-J. Yeh, R. Zhong, S.P. Han, C.L. Young, M. Shen, and T.F. Deuel* Washington University, Departments of Medicine and Biochemistry and Molecular Biophysics, St. Louis, MO 63110.

Pleiotrophin (PTN) exhibits mitogenic, angiogenic, and neuronal and oligodendrocyte progenitor cell outgrowth promoting activity in vitro. In vivo, Ptn is differentially expressed in both a cell type and temporally regulated manner during development. To further investigate the roles that PTN may have in regulating cell growth and differentiation, we expressed the human Ptn cDNA under control of the SV40 promoter in PC12 cells derived from a rat adrenal pheochromocytoma. PTN dramatically changed the morphology of PC12 cells. Two populations of cells that expressed human Ptn were readily distinguished from homogenous populations of parent PC12 cells. One cell population developed the appearance of sympathetic neurons by neurite outgrowth and branching and retained neuronal markers. The other cell population became larger and the cell body became flat. Importantly, sympathetic neural precursor and chromafin cell precursor markers, including NF200, sympathoadrenal (SA) antigens 1-5 and phenylethanolamine-N-methyltransferase (PNMT) were strikingly reduced, and lowered levels of production of the sympathetic neuronal transmitters including L-dopa, dopamine, and nor-epinephrine were observed in these cells. Both phenotypically altered cell types displayed attenuation of anchorage independent growth and lessened tumor formation in the nude mouse, supporting a role of PTN in driving PC12 cells to a more differentiated state. (Supported by NIH grant CA49712)

299.15

NEUROBASAL MEDIUM PROMOTES GREATER SURVIVAL OF EMBRYONIC CHICK SYMPATHETIC NEURONS THAN HAM'S F12 MEDIUM. D. B. Pettigrew* and K. A. Crutcher, Dept. of Neurosurgery, Univ. of Cincinnati College of Medicine, Cincinnati, OH 45267-0515.

Optimal conditions for culturing primary neurons often require addition of serum and/or neurotrophic factors. For example, the survival-promoting activity of nerve growth factor (NGF) for embryonic sympathetic neurons is well established. However, it is not clear to what extent NGF acts to offset suboptimal conditions of the media used for such cultures. B27-supplemented Neurobasal medium (NBM) (GIBCO/BRL Life Technologies) was developed as a defined medium for cultures of primary neurons of the CNS. The extent to which this medium is suitable for culture of neurons of neural crest origin has not been extensively investigated. The suitability of NBM for cultures of embryonic (E9) chick sympathetic neurons was assessed in this study. Lumbar sympathetic ganglia were dissociated with trypsin and dissociated cells were cultured in either F12 media or NBM with or without exogenous NGF (1 ng/ml) using 96 well plates pretreated with poly-ornithine substrate, and the time course of the effects of these conditions were followed up to five days. Neuronal survival was assessed by labeling the cells with a fluorescent vital dye and subsequent quantification of cell number from digitized images using a cell counting macro with NIH Image software. NBM was found to promote greater survival of sympathetic neurons at all time points when compared with F12 whether or not NGF is present. These results suggest that NBM may be useful for primary cultures of embryonic sympathetic neurons. (Supported by NIH NS17131.)

299.16

ATTEMPT TO PURIFY A NOVEL NEUROTROPHIC FACTOR DERIVED FROM BOVINE BRAIN TISSUE. K. Ishii[1], N. Sahara[1], H. Mori[1], K. Watanabe[2]' [1]Dept. of Mol. Biol., Tokyo Inst. of Psychiatry, Kamikitazawa, Tokyo 156, Japan. [2]Tokyo Metoropolitan Institute of Gerontology, Itabashiku, Tokyo 173, Japan.

Neuronal cells mostly survive, grow and differentiate dependently on neurotrophic factors. Recently, some of the neurotrophic factors are expected to use for one of the therapy of degenerative disorders. We examined activities of growth and survival factors derived from bovine brain tissue. We used a primary culture of rat embryonic neocortical neurons. The activity was measured by MTT assay after 4 days of culture. Bovine brains were homogenated with PBS and fractionated by ammonium sulfate, DEAE sephacel, gel filtration, Resource Q, Heparin affinity chromatography, Phenyl superose and HPLC-gel filtration (Superdex 75). We focused a survival activity in the 0.08-0.1M NaCl fractions of Resorce Q chromatography to avoid the contamination by well-known growth activities such as Annexin V and NSE. The active fraction in the last step was found to still contain several proteins on SDS-PAGE. Of these proteins, two proteins with Mr 28K and 22K were particularly focused because their presence was found to parallel to the activity. These proteins are further purified to be sequenced. We suppose that this fraction contains a new growth and survival factor because of its specific activity and its unique behavior in the chromatographies.

299.17

A FEMTOMOLAR-ACTING NEUROPROTECTIVE PEPTIDE DERIVED FROM ACTIVITY DEPENDANT NEUROTROPHIC FACTOR, Douglas E. Brenneman* and Illana Gozes. SDMP, LDN, NICHD, NIH, Bethesda, MD; Dept. Clin. Biochem., Sackler School of Medicine, Tel Aviv Univ., Israel.

A novel 14-amino acid peptide is revealed that exhibited neuroprotection at unprecedented concentrations. This peptide prevented neuronal cell death associated with the envelope protein from the human immunodeficiency virus (glycoprotein 120), with excitotoxicity (N-methyl D-aspartate), with the beta amyloid peptide (putative cytotoxin in Alzheimer's disease), and with tetrodotoxin (electrical blockade). The peptide is derived from a new neuroprotective protein secreted by astroglial cells in the presence of vasoactive intestinal peptide. The neurotrophic protein was isolated by sequential chromatographic methods combining ion exchange, size separation and hydrophobic interaction. The protein (14,000 Daltons and pI 8.3 $\pm$ 0.25) was named activity dependent neurotrophic factor, as it protected neurons from death associated with electrical blockade. Peptide sequencing led to the synthesis of the novel 14-amino acid peptide that was homologous, but not identical to an intracellular stress protein: heat shock protein 60 (HSP 60). Neutralizing antiserum to HSP 60 produced neuronal cell death that could be prevented by co-treatment with the novel protein, suggesting the existence of extracellular stress-like proteins with neuroprotective properties. Recombinant HSP 60 had no detectable survival-promoting activity in CNS cultures. This is the first demonstration of a neurotrophic factor whose activity was mimicked by a synthesized peptide and whose neuro-protective activity was evident at femtomolar concentrations. These studies identify a potent neuroprotective peptide that provides a basis for developing treatments of currently intractable neurodegenerative diseases.

299.18

A 14-AMINO ACID PEPTIDE PROTECTS AGAINST ALZHEIMER'S-ASSOCIATED NEUROTOXICITY *IN VIVO*. I. Gozes*, A. Davidson, A. Bardea, M. Bechar, E. Giladi and D. E. Brenneman. Clin. Biochem., Sackler Med. Sch., Tel Aviv Univ., Israel and NICHD, NIH, Bethesda, MD 20892.

A potent neuroprotective peptide, ADNF-14, was designed and synthesized to contain a sequence derived from a new neuroprotective protein, activity-dependent neurotrophic factor (ADNF). This protein exhibited structural similarity to a stress protein, heat shock protein 60 (hsp60). Two Alzheimer's related models were assessed for the *in vivo* neuroprotective effects of ADNF-derived peptides: cholinergic blockade and deficiencies in apolipoprotein E (ApoE). Cholinotoxicity was achieved in rats by blockade with ethylcholine aziridium (AF64A) and was associated with impairments in spatial learning and memory. Chronic intranasal delivery of ADNF-14 prevented cognitive impairments associated with cholinergic blockade. Furthermore, we now show that ApoE-deficient mice (Cell 71, 343, 1992) exhibited retardation in the acquisition of developmental milestones. Brains from these mice displayed a marked reduction in the mRNA encoding for the ADNF secretagogue, vasoactive intestinal peptide, as well as reduced cholinergic activity. Daily treatment with ADNF-derived peptides resulted in a significant acceleration in the acquisition of behavioral milestones, in comparison to either control or ApoE-deficient animals. These studies identify a potent neuroprotective hsp-like glial protein and peptides that provide a basis for developing novel treatments for Alzheimer's and other neurodegenerative diseases.

Supported in part by the US-Israel Binational Science Foundation.

299.19

IDENTIFICATION OP AN ACTIVE SITE FOR ACTIVITY-DEPENDENT NEUROTROPHIC FACTOR. A. Davidson,* Y. Gozes, I. Hauser, D. E. Brenneman, and I. Gozes. Clin. Biochem., Sackler Med. Sch., Tel Aviv Univ., Israel; NICHD, NIH, Bethesda, MD 20892.

Activity-dependent neurotrophic factor (ADNF), secreted by astroglial cells in the presence of vasoactive intestinal peptide (VIP), protected neurons from death associated with electrical blockade at concentrations as low as $5x10^{-15}$M. Other known growth factors (insulin-like growth factor-I, platelet-derived growth factor, fibroblast growth factor and nerve growth factor), did not increase neuronal survival in tetrodotoxin-treated spinal cord cultures at concentrations (0.1pM to 10 nM) known to be biologically active in other systems. To further assess ADNF activity, antibodies were elicited in mice following sequential injections of purified ADNF. At a dilution of 1:10,000, anti-ADNF ascites fluid decreased neuronal cell counts to 35-50% of untreated rat cerebral cortical cultures, while control antiserum had no effect. Administration of 1pM purified ADNF prevented the neuronal death associated with the antiserum. Similar results were obtained in dissociated mouse spinal cord cell cultures and in mouse hippocampal cell cultures. ADNF-14 is a 14 amino-peptide with hsp60-like sequences that is derived from ADNF. The antibody-associated neuronal cell death was also prevented by femtomolar concentrations of ADNF-14. *In vitro* association between the antiserum and ADNF-14 was also demonstrated utilizing size exclusion chromatography. These studies identify an active site in ADNF that is also a prominent immunogenic epitope. Furthermore, these data indicate that electrically active CNS cultures contain an endogenous ADNF-like neuronal survival factor.

Supported in part by the US-Israel Binational Science Foundation.

299.20

LIVER DERIVED NEURONAL ACTIVATOR (LDNA) ENHANCES NEURITE REGENERATION FROM AGED HUMAN RETINA. H. Horie*[1], M.Takano[2], T.Kadoya[3], Y.Inagaki[3]. Depts. Physiol.[1] and Ophthalmol.[2], Yokohama City Univ., Sch. of Med., Yokohama 236 and Pharmaceut.,Lab., Kirin Brewery Co.[3], Maebashi 371, JAPAN.

Liver derived neuronal activator (LDNA) enhanced neurite regeneration and their survival from nerve-transected terminals of rat adult dorsal root ganglia (DRG) with associated nerve bundles. This factor is different from known neurotrophic factors (Neurosci. abst., 1995) and promoted neurite regeneration from adult rat retina explants. LDNA was expected to promote neurite regeneration from aged retinal explants. We introduced three-dimensional collagen gel culture system and compared neurite regeneration capability of 24-month-old mouse retina with that of 3-month-old one. The number of regenerating neurites from aged explants was one third smaller than that from young explants 6 days after in culture. This result showed that neurite regeneration capability decreased with aging. When LDNA was applied to the aged retina explant, it enhanced neurite regeneration. This enhancement indicates that LDNA works as a neural activator in aged central nervous tissues. This activity was also proved in an aged human retina. The human retina was obtained at surgery from 70-year-old patient with retrobulber carcinoma. When LDNA was applied to the cultured human retinal explant, it enhanced the neurite regeneration. Since LDNA from rat liver works as a neuronal activator in either mouse or human aged retina, this activity is thought to be reserved among mammalian. (Supported by MESC, Japan)

299.21

CHARACTERIZATION OF A NEUROTROPHIC FACTOR SECRETED BY CULTURED SCIATIC NERVES. R. Villegas*, G.M. Villegas, D. Balbi, B. Maqueira, M. Longart, F. Iribarren, and M. Hernández. Instituto de Estudios Avanzados (IDEA), Apartado 17606, Caracas 1015A, Venezuela.

The present communication deals with further attempts to purify and characterize a previously described neurotrophic factor found in a medium conditioned by rat sciatic nerves (SNCM) (Brain Res., 685: 77-90, 1995). The neurotrophic activity, assayed by its neurite-promoting effect on PC12 cells, was associated with two protein peaks of 65 and 45 kDa. The previous purification procedure, consisting of ultrafiltration, heparin chromatography and size exclusion HPLC, was improved by using a 30,000 MWCO ultrafiltration membrane (Millipore) and by replacing the heparin column by an ionic exchange column (HiTrap Q, Pharmacia). These changes led to an improvement in resolution and relative enrichment of the 65 and 45 kDa peaks, as well as an increase in the activity of the 45 kDa peak. Investigation is continued to identify the active protein(s). We have also reported that the SNCM produced survival and differentiation of 8-day chick embryonic dorsal root and ciliary ganglia neurons. In an attempt to characterize the phenotypic changes induced by the SNCM, RNA from treated-PC12 cells was analyzed by RT-PCR. The relative amount of choline acetyl transferase (ChAT), tyrosine hydroxylase (TH) and ß-actin mRNA seems to remain unmodified after 3 days treatment. However, a sharp increase in the number of cell granules and a two-fold increase in ChAT enzymatic activity were observed. Additional work is needed to establish the mechanisms responsible for this survival and differentiation activity.

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299.22

EFFECTS OF SCIATIC NERVE GRAFTS ON CHOLINE ACETYL TRANSFERASE AND P75 EXPRESSION IN TRANSECTED ADULT HYPOGLOSSAL MOTONEURONS. E. Frezzato, G. Stipa, C. Provenzano and M.Rende* Dept. Exp. Med., Sect. Anatomy, Univ. of Perugia Sch. of Med., Italy

We previously reported that a permanent transection of adult rat sciatic and hypoglossal nerves resulted in distinct temporal changes in both p75 and ChAT immunoreactivity (-Ir). Permanent axotomy of hypoglossal motoneurons induced a progressive loss of ChAT-Ir and a persistant expression of p75-Ir, phenomena which were not observed in spinal motoneurons. These observations indicated that spinal and brainstem motoneurons respond to permanent axotomy with a differential modulation of p75 and ChAT expression. Such differences in expression could be ascribed to specific intrinsic properties of each population of motoneurons or to different factors present in the nerve stump "target" region. The aim of the present study was to test these two possibilities by determining if a segment of sciatic nerve transplanted to a transected hypoglossal nerve counteracts the loss of ChAT and the expression of p75 in injured hypoglossal motoneurons. Prior to grafting, the segments of sciatic nerve were prepared by one of three methods: (i) a *fresh* piece; (ii) a *degenerated* piece; and (iii) a *heated* piece. Seven days following the placement of grafts, hypoglossal motoneurons were analysed for p75 and ChAT. The results revealed that viable sciatic grafts (fresh and degenerated) attenuated the loss of ChAT in the injured hypoglossal motoneurons. In addition, viable sciatic grafts decreased the expression of neuronal p75-Ir, thus inducing these motoneurons to acquire a pattern more characteristic of the uninjured state. These effects indicate that adult hypoglossal motoneurons retain responsiveness to factors released from the sciatic nerve, and that differential expression patterns between these two motoneuron populations is due, at least in part, to different factors released by distal hypoglossal and sciatic nerve segments. Support: Telethon-Italy Grant #582.

299.23

γ-PYRONE-TYPE NOVEL LOW MOLECULAR WEIGHT EXOGENOUS SUBSTANCE WITH NEUROTROPHIC ACTIVITY. N. Nishiyama*[1], T. Moriguchi[1], H. Katsuki[1], H. Matsuura[2], Y. Itakura[2] and H. Saito[1]. 1) Dept. of Chem. Pharmacol., Fac. of Pharmaceut. Sci., The Univ. of Tokyo, Tokyo 113, Japan, 2) Instit. for OTC Res., Wakunaga Pharmaceut. Comp. Ltd., Hiroshima 739-11, Japan.

In our preceding study, aged garlic extract exerted dose-dependent neurotrophic activity on rat hippocampal neurons. On our way to isolate pure chemical substance(s) in the extract, we found for the first time that γ-pyrone type substance allixin (3-hydroxy-5methoxy-6-methyl-2-pentyl-4H-pyrone) enhanced neuronal survival and axonal branching. A disperse neuron cell culture was prepared from embryonic rat and tested chemicals were added in the culture medium a day later. The number of surviving neurons were counted after 3 days exposure to the chemicals. Neuron cell morphology was recorded at 1 and 2 days after exposure. Allixin increased the number of surviving neurons prepared from all brain regions tested concentration-dependently (1-100 ng/ml). It also promoted the branching of the longest neurite in hippocampal neurons. At higher concentrations (>1000 ng/ml), however, allixin started to kill all the cells, indicating its strong cytotoxicity. Thus a number of analogous chemicals were obtained or synthesized, and examined for their neurotrophic activities, aiming to find a substance with less cytotoxicity. Among the numerous chemicals, DHP (2,6-dimethyl-3-hydroxy-4H-pyrone) possessed strong neurotrophic activity at more than 10 ng/ml without any obvious cytotoxicity up to 10 μg/ml. DHP also retained the activity to enhance the neurite branching. These results indicate that allixin and DHP are novel exogenous low molecular weight substances with strong neurotrophic activity. These substances will make helpful leading chemicals for developing therapeutic and/or prophylactic drugs for neurodegenerative disorders.

299.24

TROPHIC INTERACTION BETWEEN BASAL FOREBRAIN NEURONS AND CORTICAL NEURONS IN DISSOCIATED CO-CULTURES. D.H. Ha*, R.T. Robertson, and J.H. Weiss. Depts of Neurology and Anatomy/Neurobiology, U.C. Irvine, Irvine CA 92717.

We have previously shown that cortical neurons are able to enhance survival and morphologic characteristics of basal forebrain cholinergic neurons (BFCNs) when they are co-cultured together (Ha, et al., Neurosci. Abs., 1995). Staining of basal forebrain (BF)-cortical co-cultures with SMI-32, a monoclonal antibody against non-phosphorylated neurofilament epitopes reported to label subsets of pyramidal neurons in cortex, revealed a subset of strongly stained neurons that displayed unusually large cell bodies and long axons. These "large SMI-32(+) neurons" were not present in cultures of either BF or cortical cells alone. Using double labeling techniques, we failed to find evidence of choline acetyltransferase (ChAT), GABA or acetylcholinesterase labeling in large SMI-32(+) neurons, suggesting that they are neither cholinergic nor GABAergic. Furthermore, treating co-cultures with 192-IgG-saporin (a toxin linked to antibody against the low affinity NGF receptor, p75, which has been reported to selectively kill BFCNs in vivo) resulted in the apparent loss of SMI-32(+) neurons as well as ChAT(+) neurons. We are currently preparing BF-cortical co-cultures from wild-type mice and from β-galactosidase expressing transgenic mice, to determine the origin of the large SMI-32(+) neurons. While large SMI-32(+) neurons could be of BF origin, results to date suggest the hypothesis that they are of cortical origin, and that trophic influences from BFCNs in co-cultures enhance their morphologic features. Supported by NIH grants NS 30884 (JHW) and NS 30109 (RTR).

299.25

SCHWANN CELL-DORSAL ROOT GANGLION NEURON CO-CULTURE CAUSES AN INCREASE IN SODIUM CHANNEL mRNA EXPRESSION AND SODIUM CURRENT. Amy A. W. Hinson, Xiang Gu, Sulayman Dib-Hajj, Joel A. Black and Stephen G. Waxman. Dept. of Neurology, Yale Univ. Sch. of Med., New Haven, CT and Neuroscience Res., VA Med. Ctr., West Haven, CT 06516

The pattern of expression of Na channel α and β-subunits in neurons is spatially and developmentally regulated, yet the factors that govern the expression of these subunits are not known. At least seven neural subtypes of the Na channel α-subunit- I, II/IIA, III, NaG, Na6, SNS and hNE-Na- have been described, while only two genes for β1 and β2 subunits have been cloned. Electrophysiological data has shown that dorsal root ganglion neurons (DRGN) display several distinct types of Na currents during development, which may reflect differential expression of Na channel α and/or β-subunits. Previously, this laboratory has demonstrated that the expression of Na channel mRNA in DRGN from E17 rats is influenced by exposure to NGF. In order to study the role of Schwann cells (SC) in modulating Na channel expression in DRGN and to correlate Na current characteristics with molecular structure, we used *in situ* hybridization cytochemistry to examine the expression of Na channel α and β mRNAs in DRGN isolated from E15 rats cultured in the absence or presence of SC. DRGN in culture with SC demonstrated an increase in expression for α-subunits II, NaG, Na6, SNS and for β-subunits. In addition, the increase in sodium channel mRNA expression in DRGN co-cultured with SC coincided with an increase in sodium current and current density. These observations demonstrate that a SC-derived factor differentially up-regulates Na channel α and β-subunit mRNA expression in DRGN isolated from E15 rats. (Supported in part by the VA and NMSS)

299.26

PURIFICATION OF A SURVIVAL-PROMOTING FACTOR FOR CULTURED NEURONS FROM BOVINE SERUM, AND ITS IDENTIFICATION AS A SELENOPROTEIN P-LIKE PROTEIN. J. Yan* and J. N. Barrett. Dept. of Physiology and Biophysics, Univ. of Miami Sch. of Med., Miami, Fl 33101

The neuronal survival-promoting activity in serum is enriched in a 45-80 kD gel filtration fraction (Kaufman and Barrett, 1983 Science 220:1394). We purified from bovine serum a glycoprotein that promotes survival of cultured rat embryonic neurons from several brain regions including septum in a 7 day survival assay. About 10,000 fold purification was achieved using a combination of ammonium sulfate precipitation, metal-chelating chromatography, Cibacron-blue F3GA dye affinity chromatography, ABx ion exchanger and preparative SDS-PAGE. The concentration required for the half maximal survival effect is about 10 ng/ml for the final fraction. This protein has an apparent molecular weight of 56 kD on SDS-PAGE. Amino acid sequencing after cyanogen bromide cleavage yielded two sequences, both of which match with regions of deduced sequence of a bovine selenoprotein P-like protein. The cDNA of this molecule was cloned from the cerebellum (Saijoh et al., 1995 Mol Brain Res 30:301), but the protein had not been purified previously. We generated antibodies against a synthetic peptide matching 16 amino acids of the predicted bovine SPP-like protein sequence. The antibodies neutralized most of the survival-promoting activity in the partially purified fractions. The SPP-like protein may contribute to the neuronal survival-promoting activity of serum by playing a role in selenium homeostasis and antioxidative defense mechanisms.

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299.27

CONDITIONED MEDIUM FROM GLIAL CELLS PREVENTS APOPTOSIS OF CULTURED NEURONS. X. F. Wang* and M. S. Cynader. Dept. of Ophthalmology, Univ. of British Columbia, Vancouver, BC, Canada, V5Z 3N9.

Cultured primary cortical neurons from E18 rat embryo survive poorly at low densities ($5x10^4$ cells/cm^2). However, when co-cultured with a glial cell monolayer in a non-contact method, the survival rate is much increased with over 80% of cultured neurons surviving at DIV (day in vitro) 5. When the glial cell monolayer was removed at DIV 3, the cultured neurons died rapidly (74% within 24 hours, 98% within 48 hours). Cell death occurred by apoptosis, as assessed by morphologic criteria, including blebbing, cell shrinkage, and nuclear condensation. We used this model to test the effects of conditioned medium and other potential survival factors on neuronal apoptosis. At DIV 3, the glial cell monolayer was removed, and conditioned medium or potential survival factors were added. The surviving neurons were counted 24 hours later. Glial-conditioned medium containing 10% Fetal Calf Serum (FCS) resulted in fewer surviving neurons (21%) than a glial-conditioned medium containing albumin (5mg/ml) without FCS (88%). NGF, BDNF, GDNF (100ng/ml and 10ng/ml, respectively) were all tested in albumin-containing medium. None of these neurotrophic factors had any observable effect on neuronal survival (<20% survival rate). These results suggest that glial cells may secret a factor or factors which can improve the survival of cultured neurons, and that these factors are unlikely to be known neurotrophins. The identity of the factor(s) in question is being sought.

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299.28

POSTTRANSLATIONAL REGULATION OF Ca^{2+}-ACTIVATED K^+ CURRENTS BY A TARGET-DERIVED FACTOR IN DEVELOPING CHICK CILIARY GANGLION NEURONS Priya Subramony, Sanja Raucher, +Laurence Dryer & Stuart E. Dryer* *Prog. Neurosci., Florida State University, Tallahassee, FL 32306 and +Dept. Neurobiol., Harvard Medical School, Boston, MA 02115*

Macroscopic $I_{K[Ca]}$ is not expressed at normal levels in chick ciliary ganglion (CG) neurons prior to synapse formation with target tissues or in neurons developing *in vitro* or *in situ* in the absence of target tissues. Here, we have examined the mechanisms underlying target-dependent stimulation of $I_{K[Ca]}$. Two chick CG *slo* partial cDNAs encoding $I_{K[Ca]}$ channels were isolated, cloned and sequenced. Both *slo* transcripts, which appear to be splice variants, were readily detected by RT-PCR in developing CG neurons prior to or in the absence of target tissue interactions. When E9 CG neurons developed *in vitro* for 4 d in the presence of target tissue (iris) extracts, a normal whole-cell $I_{K[Ca]}$ was expressed. The effects of iris extracts did not require protein synthesis and were apparent within 5-7 hr. This activity was detectable throughout the stages of synapse formation in the iris (E9-13) but was not present in E6-7 iris, prior to synapse formation. The active component of iris extract is a heat- and trypsin-sensitive protein with an apparent molecular weight of 40-60 kD. Large-conductance $I_{K[Ca]}$ channels were readily detected in excised inside-out patches from acutely-isolated E13 CG neurons or in E9 neurons cultured for 4 d in the *presence* of iris extracts. These channels were activated by bath application of 1 μM Ca^{2+}. Large-conductance K^+ channels kinetically similar to $I_{K[Ca]}$ were also observed in E9 CG neurons cultured for 4 d in the *absence* of iris extracts. However, those channels had abnormal gating properties as they typically did not increase their activity in the presence of 1 μM Ca^{2+}. RO1-NS-32748 AND AMERICAN HEART ASSOC. (FL AFFILIATE).

NEUROTROPHIC FACTORS: BIOLOGIC EFFECTS—NGF I

300.1

NEUROTROPHINS PREVENT DEGENERATION OF PROXIMAL STUMPS OF TRANSECTED SENSORY AXONS IN ADULT RAT SPINAL CORD

F. Sayer*, M. Oudega #, T. Hagg

Dept. Anatomy & Neurobiology, Dalhousie Univ., Halifax, B3H 4H7, Canada; # The Miami Project to Cure Paralysis, Miami Univ. Sch. Med., FL33136.

We and others have shown that neurotrophic factors can promote axonal regeneration in the CNS. Here, we investigated whether neurotrophins can prevent degeneration of the proximal ends of transected axons, i.e., an event that may contribute to poor regeneration in the adult CNS. Adult Sprague-Dawley rats received a transection of the ascending sensory tract (dorsal column) and descending corticospinal motor tract at thoracic level T9-10. One or 3 days before further analysis, the right sensory and left corticospinal axons were anterogradely labeled with choleratoxin B injected into the right sciatic nerve and left lumbar motor cortex. Sensory and motor axon terminals swelled and formed terminal clubs over the first day after injury and degenerated further over the next few weeks. A mixture of neurotrophins was injected into the lesion site immediately after the injury (nerve growth factor, 1 μg, Cedar Lane Inc ; brain-derived neurotrophic factor and neurotrophin-3, 7 μg each, a gift of Regeneron Pharmaceuticals). After 1 day, the number of terminal clubs of sensory axons in neurotrophin-treated animals was ~25% of that in PBS injected ones and they were much smaller. The effects of the neurotrophin mixture was less clear 7 days after the injection and negligible after 14 days. At a 3-fold higher dose, each factor alone was as effective as the mixture, suggesting that they have additive effects. Surprisingly, the neurotrophins had no clear effects on the degeneration of the motor axons. Support: International Research Institute for Paraplegia and Rick Hansen Man in Motion Foundation.

300.2

REVERSAL OF IMPAIRED CALCIUM INFLUX IN AGING BY NERVE GROWTH FACTOR MAY BE MEDIATED BY DECREASED INHIBITORY G PROTEIN FUNCTION K.E. Hall*, J. M. Spitsbergen+, W. D. Steers+, J. B.Tuttle+, J. W. Wiley. Dept. Internal Medicine, U. of Michigan, Ann Arbor, MI 48109, and +Depts. of Urology and Neuroscience., U. of Virginia, Charlottesville, VA 22908.

The aim of this study was to determine whether nerve growth factor (NGF) could reverse age-associated decreased neuronal calcium influx. Dissociated dorsal root ganglion (DRG) neurons from young (**Y**: 4-6 month), middle-aged (**M**: 12-14 month) and old (**O**: 22-26 month) Fisher 344 x BN F1 hybrid rats were incubated in serum-free medium containing NGF (100 ng/ml), or in control (no NGF) medium for 24 to 96 hours. Medium was changed daily. Mean NGF levels measured by radio-immunoassay were 445±65 pg/ml (n=8) in NGF-treated dishes. NGF treatment was associated with significantly more neurite out-growth and neuronal interconnections at all age levels. Calcium current density (I_{DCa}; pA/pF) was recorded in single, acutely dissociated DRGs using the whole-cell variant of the patch-clamp technique. DRGs from aged animals had a smaller mean amplitude of I_{DCa} (68±43;n=6) than neurons from young and middle-aged animals (**Y**:108.5±13.3;n=10, **M**:93.0±24.0;n=8). NGF treatment increased I_{DCa} in all three age groups by 48 hours (**Y**: 136.8±35;n=4, **M**: 146.0±20; n=8;P<0.05, **O**: 203±54;n=5;P<0.01). Using a two step (pre-pulse/test-pulse) "facilitation" protocol that transiently blocks the predominately inhibitory tonic G protein modulation of calcium channels, we noted that NGF treatment significantly decreased % facilitation (**Y**: 16±2; n=3; P<0.01, **O**: 15±3; n=3, P<0.05) compared to controls (**Y**: 41±6; n=6, **O**: 23±4;n=5). In summary, calcium influx in sensory neurons from aged animals was significantly decreased compared to youthful controls. NGF treatment restored calcium influx in aged neurons to youthful levels, while decreasing the percentage of tonic inhibition of I_{DCa}. These results indicate that NGF may increase I_{DCa} by decreasing tonic inhibition of the G protein-calcium channel complex. Funding by the Claude Pepper Older Americans Independence Center.

300.3

NGF TREATMENT *IN-VIVO* RESCUES DRG THAT ARE PROGRAMMED TO DEGENERATE BY INCREASING PROLIFERATION. R.S. Goldstein. and R.Geffen. Dept. Life Science, Bar-Ilan Univ. 52900 Ramat-Gan, ISRAEL

A striking element of patterning within the meristic series of DRG, is the disappearance of the five or six most rostral ganglia early in the embryonic development of birds and mammals. These transient DRG have been named "Froriep's ganglia" for their nineteenth century discoverer.

We have examined the ontogeny of the longest surviving Froriep's ganglion of the chick embryo, DRG C2, in detail (Rosen et al J. Neurobio., June 1996). At St. 18 (E2.5+), the C2 DRG had the same shape and volume as permanent ganglia C5 and C6. C2's development first diverged from that of normal DRG at St. 19 (E3-), when C2 was observed to be half the size and shaped differently from its neighbors.

Since growth factors modulate both proliferation and apoptosis, we postulated that these molecules and/or their receptors might be responsible for the difference in fate between C2 and C5. We have now treated embryos with NGF *in-ovo* in order to begin to evaluate this possibility. NGF treatments (1μg- St 17 & 21) partially rescued C2, producing a 50% reduction in the normal difference in size between C2 and C5 at St. 23. At least part of this rescue could be accounted for by increased proliferation in the NGF-treated C2 compared to that in control embryos. Proliferation in normal DRG C5 was unaffected by NGF application. NGF treatment rescued DRG cells in both C2 and C5 from cell death. Staining of pycnotic nuclei revealed that NGF dramatically reduced the death in the C2 ganglia, until it became equivalent mental stage. Examination of NGF treated embryos at St.28 revealed that at least some of the cells rescued in the C2 DRG are neurons.

Our finding is the first direct evidence for a role for NGF in control of DRG cell proliferation *in-vivo*. Supported by the Familial Dysautonomia Inc.

300.4

RECEPTIVE PROPERTIES OF CUTANEOUS SENSORY NEURONS IN TRANSGENIC MICE LACKING THE NEUROTROPHIN RECEPTOR p75. M. Koltzenburg* and C.L. Stucky *Dept. of Neurology, University of Würzburg, D-97080 Würzburg, Germany.*

The neurotrophin receptor p75 interacts with all known neurotrophins. Transgenic mice which lack the p75 receptor (p75-/-) exhibit decreased sensitivity to both noxious mechanical and thermal stimuli, reduced innervation density of the skin and approximately 50% loss of sensory neurons. We used neurophysiological techniques to investigate whether p75 has a role in the survival or function of specific subtypes of cutaneous sensory fibers. The sural nerve with innervated skin was dissected from adult p75-/- and wildtype (p75+/+) mice. Standard electrophysiological techniques were used to record from single afferent fibers *in vitro* (p75-/-: n=103; p75+/+: n=70). Fibers were characterized by their conduction velocity and response to controlled mechanical and thermal stimuli. Large myelinated fibers (Aß) were classified as either slowly adapting (SA) or rapidly adapting (RA) low threshold mechanoreceptors. There was no difference in the percentages of SA (38%) and RA (62%) fibers in p75-/- mice compared to controls (SA: 41%; RA: 59%) or in their response properties to mechanical stimuli. Thin myelinated fibers (Aδ) were classified as either nociceptive high threshold mechanoreceptors (AM) or low threshold D-hair receptors. The percentage of AM fibers in p75-/- mice (42%, n=57) was significantly ($p<0.05$, χ^2) reduced compared to p75+/+ mice (68%, n=34). Unmyelinated C-fibers responded to mechanical stimuli or to heat and mechanical stimuli. Whereas the mechanical stimulus response functions of AM fibers in p75-/- mice were normal, the firing frequency of C-fibers to mechanical stimuli was decreased in p75-/- mice compared to controls. These data suggest that the decrease in behavioral sensitivity to noxious stimuli in p75-/- mice may be due to decreased number of AM fibers as well as reduced sensitivity of C-fibers. (Supported by the DFG, SFB 353).

300.5

THE INFLUENCE OF NEUROTROPHIC FACTORS ON NEURITE-FORMATION OF AGING MOUSE DORSAL ROOT GANGLION CELLS. T. Ninomiya*[1], M. Yamauchi[2] and H. Terasawa[1]. Dep. of Anatomy[1] & Anesthesiology[2], Sapporo Med. Univ. Sch. of Med., Sapporo, 060 Japan.

In aging dorsal root ganglion (DRG) cell culture, DRG cells display the multipolar, bipolar or pseudounipolar forms. Some pseudounipolar neurons had many thin neurites elongated from the junction of the stem process or a structure-like swelling body or glomerulus (atypical pseudounipolar cell). These structures were not seen in fetal or newborn mouse DRG cell cultures. In order to study the effect of neurotrophic factor (NTF) on neurite formation of adult mouse DRG cells, dissociated DRG cells from 15-month-old mice were grown on poly-L-lysine coated dishes in alpha MEM serum-free (SF-M) supplemented with nerve growth factor (NGF), neurotrophin-3 (NT-3), neurotrophin-4 (NT-4) or brain derived neurotrophic factor (BDNF). After 6 days, cultures were stained with anti-neurofilament protein (anti-NFP) and microtubule-associated protein 2 (anti-MAP2). Cultured DRG neurons were classified into bipolar, multipolar, typical pseudounipolar or atypical pseudounipolar neuron.

Atypical neurons appeared with an incidence of about 5% in aMEM-SF without NTF or supplemented with NT-3, NT-4 or BDNF. However atypical pseudounipolar neurons increased about 30% in aMEM-SF supplemented with NGF. There is no difference of the number of surviving neurons in each of culture medium. The appearance of this type cell was affected by NGF, not affect other neurotrophic factors.

300.6

EFFECTS OF OVARIECTOMY AND ESTROGEN ON LUMBAR DORSAL ROOT GANGLION NEURONAL NEUROFILAMENT GENE EXPRESSION. S.A. Scoville, S.M. Bufton, M.J. Swierzewski, F.J. Liuzzi and D.E. Scott*. Dept. of Anatomy and Neurobiology, Dept. of Urology and the Molecular Neurobiology Lab., the Diabetes Institutes, Dept. of Internal Medicine, Eastern Virginia Med. Sch., Norfolk, VA 23501.

Recently, estrogen receptor expression by dorsal root ganglion (DRG) of adult female rats was shown by Sohrabji and her colleagues(1994). Previous studies have shown the presence of receptors on the DRG neurons for both low (p75) and high (trkA) affinity nerve growth factor (NGF) receptors. In subpopulations of DRG neurons, these receptors colocalize with the estrogen receptor. More interestingly, endogenous and exogenous estrogen has been shown to up-regulate the message for the NGF receptors and down-regulate the estogen receptor. Previous research has also demonstrated that neurofilament (NF) is regulated by NGF in those DRG neurons with high affinity NGF receptors. The current study investigated the effects of estrogen on 68kD NF gene expression. The four groups of female Sprague-Dawley rats were: normal, ovariectomized, ovariectomized with estrogen replacement and ovariectomized with 10X replacement. A P^{33}-labelled cRNA probe was used to localize 68kD mRNA in DRG neurons by *in situ* hybridization. Ovariectomy reduced NF mRNA levels compared to those in DRGs of normal animals. Estrogen replacement increased DRG neuronal NF mRNA levels. These data suggest that estrogen may act synergistically with NGF to regulate the expression of the 68kD NF gene in DRG neurons.

This work was supported in part by a grant from C.R. Bard to MJS and funds from the Diabetes Institutes Foundation, Norfolk, VA .

300.7

THE EFFECT OF ELECTRICAL STIMULATION ON TRK ACTIVATION AND IMMEDIATE EARLY GENE EXPRESSION IN SENSORY NEURONS. Y. Hao and K.M. Mearow*, Basic Medical Sciences, Memorial University of Newfoundland, St. John's , NF, A1B 3V6.

The collateral sprouting of intact DRG neurons is NGF-dependent, and electrical stimulation of these neurons results in an acceleration of the growth response. In an effort to discern early events which may contribute to this response, the expression of several IEGs, known to be influenced by stimulation or NGF, was examined. In addition, the possibility that alterations in trk and/or its phosphorylation state might be involved was investigated. The dorsal cutaneous nerves of adult rats were subjected to one of the following treatments: 1) electrical stimulation (8V, 20Hz, 1 min) or 2) isolation (adjacent nerves were cut), or 3) isolation + stimulation. Subsequently (1, 4, 8hrs, 1, 2, 4 d), animals were anaesthetized, DRGs were removed and used for RNA or protein extraction or for cryosectioning. IEG expression was examined by immunocytochemistry with antibodies to cJUN, EGR1, OCT2 and pCREB. RT-PCR was carried out using specific primers to detect EGR1, OCT2 and CREB sequences; cyclophilin was used as an internal control. Trk and trk phosphorylation levels were analyzed by immunoprecipitation, and subsequent SDS-PAGE and Western analysis with either trk or phosphotyrosine antibodies.

With respect to the IEGs, about 30% of the DRG neurons displayed basal expression of pCREB, OCT2, EGR1 and cJUN, which was confined to small neurons. Following stimulation, there was a decrease in the % of pCREB and EGR1-positive neurons observed at 1,4 and 8 hrs; RT-PCR analysis provided the same results. However, a significant increase in the % of positive cells was found in the stimulation + isolation condition between d1 and d2, which subsequently returned to the control levels. Preliminary results indicated little or no change in trk protein levels and trk phosphorylation in the DRGs; analysis of potential increases in retrograde transport is underway. Supported by NSERC.

300.8

CONTROL OF AXONAL TRANSPORT BY RECOMBINANT HUMAN NGF IN CULTURED MOUSE DORSAL ROOT GANGLION CELLS.

S.Yuki, T.Takenaka*, T.Kawakami*, I.Sakai* and K-I.Saito*. Pharmaceuticals Lab.1, Yokohama Research Center, Mitsubishi Chemical Corporation, Aoba-ku, Yokohama, 227 Japan.

Department of Physiology, Yokohama City University, Fukuura, Yokohama, 236 Japan.

Control of axonal transport and its related signal transduction mechanism by recombinant human NGF (rhNGF) were studied using cultured mouse dorsal root ganglion cells. The transported particles were measured with a computer-assisted video-enhanced diffrential interference contrast microscope system.

rhNGF increased the number of transported particles with dose-dependent manner in both anterograde and retrograde directions, and also increased the velocity of particles. The effects of rhNGF were continued for 1 hour, and did not decrease immediately after wash out of rhNGF. K252a, a trkA linked tyrosine kinase inhibitor, completely antagonized the effects of rhNGF. This result suggested that the tyrosine kinase signal transduction system took a part in the control of axonal transport.

300.9

TYROSINE HYDROXYLASE-IMMUNOREACTIVE AXONS FORM PLEXUSES AROUND TRIGEMINAL NEURONS OF TRANSGENIC MICE OVEREXPRESSING NERVE GROWTH FACTOR IN GLIAL CELLS. G.S. Walsh and M.D. Kawaja*, Dept. Anatomy and Cell Biology, Queen's University, Kingston, Ontario CANADA K7L 3N6.

Transgenic mice overexpressing NGF under control of the GFAP promoter in glial cells were found to have significantly higher levels of NGF protein in the trigeminal ganglia when compared with control mice (based on ELISA data obtained in Dr. Keith Crutcher's laboratory). Higher levels of NGF mRNA expression were also found in the transgenic ganglia and brainstem. The consequences of such overexpression were examined through immunohistochemical localization of NGF and various phenotypic markers. NGF-immunostained neurons were present in both transgenic and wild-type ganglia, with a higher proportion evident in the former. In transgenic ganglia, however, axons immunostained for tyrosine hydroxylase formed perineuronal plexuses surrounding NGF-positive neurons. Following transection of the infraorbital nerve, NGF mRNA was up-regulated in the ipsilateral transgenic ganglia and brainstem. Neither the proportion of NGF-immunostained neurons nor the neuronal intensity of immunostaining for neurotrophin receptors and neuropeptides was altered between transgenic and wild-type ganglia, with and without nerve transection. From these data, we propose that accumulation and putative release of NGF by adult sensory neurons induce the formation of perineuronal axon plexuses, presumably sympathetic in origin, but do not appear to alter patterns of immunostaining for phenotypic proteins expressed by trigeminal neurons. (Supported by the MRC of Canada and NIH NS17131).

300.10

NEURITE OUTGROWTH AND EXPRESSION OF NERVE GROWTH FACTOR (NGF), NEUROFILAMENT (NF), and CALCITONIN GENE-RELATED PEPTIDE (cGRP) IN NGF-TRANSGENIC AND NORMAL MOUSE DORSAL ROOT GANGLIA CULTURES. **K. Byrd, P. Resig, and B.F. Sisken.* Center for Biomedical Engineering and Dept. of Anatomy and Neurobiology, University of Kentucky, Lexington, Kentucky 40506.**

Dorsal root ganglia (DRG) from fetal transgenic mice that overexpress NGF in the skin (Albers et al, 1994) and nontransgenic mice were used to determine if the presence of additional retrogradely-transported NGF altered neurite outgrowth (length and number, Sisken et al, 1995) and the expression of peptides Nerve Growth Factor (NGF), Neurofilament (NF), and Calcitonin Gene-Related Peptide (cGRP) in explant cultures. DRG were dissected and placed in Matrigel-coated wells and fed with Dulbecco media containing 10% fetal calf serum, glutamine and antibiotics. No exogenous NGF was added to the culture medium. After five days *in vitro* the cultures were fixed with 4% paraformaldehyde. No differences were found in peptide content between transgenic and normal DRG. Neurite outgrowth, defined as neurite length and neurite number, was measured on photographs. Results indicate that there were significantly more neurites ($p > 0.05$) in DRG from transgenics compared to normal DRG but there was no difference in neurite length between transgenics and normal DRG. The enhanced sprouting found *in vitro* parallels skin hyperinnervation found *in vivo* in NGF transgenic mice (Albers et al, 1994). Supported in part by ElectroPharmacology Inc., Pompano Beach, FL and the Lyman T. Johnson Fellowship to KB.

300.11

SCG NEURITE OUTGROWTH MODULATED BY CO-CULTURE WITH SPLEEN. S.L. Carlson* and M. Parrish. Dept. of Anatomy and Neurobiology, University of Kentucky Medical Center, Lexington, KY 40536-0084

Immune tissues normally receive innervation by fibers of the sympathetic nervous system. We have found that immune tissues (spleen and peripheral lymph nodes) of nerve growth factor (NGF)-transgenic mice have a robust hyperinnervation of specific compartments in these tissues compared to controls, including the splenic marginal zone and lymph node medulla and capsule (Carlson, et al., J. Neurosci. 15:5892, 1995). This result was not expected since the NGF transgene was overexpressed in skin, and not in immune tissues. To determine if the immune tissues of the transgenic mice express a factor that promotes sympathetic ingrowth, co-cultures of superior cervical ganglion (SCG) and spleen pieces were established. The tissues were placed in collagen gels with a distance of 1-2mm between the spleen and SCG, cultured for 5 days in the presence or absence of NGF, and sympathetic neurites visualized with anti-tyrosine hydroxylase. SCGs from control animals had extensive neurite outgrowth when cultured with control spleen in the presence of NGF. In contrast, control SCG produced more blunted neurites with greater amounts of fasciculation when cultured with transgenic spleen and NGF. Generally the reverse pattern was observed in cultures with transgenic SCGs: neurite outgrowth was more extensive in the presence of transgenic spleen than control spleen. The possibility that a difference in cytokine production from control vs transgenic spleen is involved in the altered neurite pattern will be investigated. (Supported by MH48644 to SLC).

300.12

NGF SELECTIVELY REGULATES BRADYKININ BINDING SITES IN ADULT SENSORY NEURONS VIA THE NEUROTROPHIN RECEPTOR p75. M. Petersen*, G. Segond von Banchet, B. Heppelmann and M. Koltzenburg* *Institute of Physiology and [1]Department of Neurology, University of Würzburg, 97070 Würzburg, Germany.*

The neurotrophins NGF, BDNF and NT-3 selectively bind to distinct members of the trk receptors, however, all bind to the neurotrophin receptor p75 ($p75^{NTR}$). Since NGF is known to regulate the chemosensitivity of sensory neurons, we asked whether neurotrophins can regulate the expression of bradykinin (BK) binding sites. Dorsal root ganglion cells from adult wild-type mice and p75 (-/-) knockout mice were enzymatically isolated and grown on poly-L-lysine coated cover slips. Neurons were maintained in F12 medium, supplemented with serum, L-glutamine, penicillin and streptomycin and kept for 0.8 or 1.8 days under culture conditions. BK binding sites were visualized by silver enhanced gold (1.4 nm) labeled BK (3 nM) and quantified using an image analyzing system. BK binding sites were constitutively expressed in 32% of neurons from wild-type animals when grown in the presence or absence of NGF for 0.8 days. In p75 (-/-) mice the percentage of neurons expressing BK binding was only 16%, suggesting a developmental loss of this population. The percentage of neurons from wildtype mice expressing BK-binding increased significantly to 72% in the presence of NGF (100 ng/ml) when grown for 1.8 days. However, this upregulation was not observed in neurons isolated from p75 (-/-) mice or from wild-type mice treated with antibodies against $p75^{NTR}$, indicating that binding of NGF to trk receptors alone is insufficient to convey the regulatory signal. Surprisingly, treatment with either BDNF or NT-3 did not result in an upregulation of BK binding sites. These results indicate that the induction of BK binding sites on sensory neurons depends specifically on the interaction of NGF with the $p75^{NTR}$ (Supported by the DFG Pe 299/3-1, He 1919/2-3, M.K. SFB 353).

300.13

OVEREXPRESSION OF NGF IN SKIN OF TRANSGENIC MICE INDUCES SYMPATHETIC PROJECTIONS TO LARGE TRKA NEURONS. B. M. Davis*, A. Soria, K. M. Albers[1], and T. P. Goodness. Depts of Anatomy & Neurobiology and Pathology[1], Univ. of Kentucky College of Medicine, Lexington, KY 40536.

Previous studies in our laboratories have shown that overexpression of NGF in skin of transgenic mice induces novel sympathetic projections to primary sensory neurons similar to that seen following peripheral nerve ligation (Davis et al., 1994; McLachlan et al., 1993). In the ligation model, sympathetic "baskets" form primarily around large neurons typically considered non-trkA expressing and not responsive to NGF. To test the similarities of the two models we used double labeling ICC to analyze the relationship between trkA expressing neurons and TH-positive sympathetic baskets. 39.2% of all neuronal profiles in trigeminal ganglion were trkA positive and 33.6% of all neurons were surrounded by sympathetic projections. Of the cells receiving sympathetic input, 84% were trkA-positive neurons of large to medium diameter. We have previously shown that sensory ganglia of transgenic mice contain supernormal levels of retrogradely transported NGF. That sympathetic neurons project almost exclusively to trkA-positive neurons suggests that sympathetic fibers are responding to the high levels of NGF in these sensory neurons. How NGF induces the sympathetic projection is not clear. However, the observation that similar populations of sensory neurons receive sympathetic projections in ligation and transgenic models suggests a common underlying mechanism. Supported by NS31826 (BMD) and NS33730 (KMA).

300.14

PROPERTIES OF INDIVIDUAL CUTANEOUS AFFERENT FIBERS AND THEIR SPINAL PROJECTIONS IN TRANSGENIC NEONATAL MICE OVEREXPRESSING NGF. K Mimics*, B.M. Davis, K.M. Albers and H.R. Koerber. Dept. of Neurobiology, University of Pittsburgh, Pittsburgh, PA 15261 and Depts. of Anatomy, Neurobiology and Pathology, University of Kentucky, Lexington, KY 40536.

Cold anesthetized pups aged P0-P6 were perfused with cold (4°C) oxygenated artificial cerebrospinal fluid (CSF). Spinal cords (SC) with attached L1-L6 dorsal root ganglia (DRG) and peripheral nerves were isolated and placed in a recording chamber and warmed to 31°C. DRG somata were impaled using electrodes containing 3M K-acetate; or 5% neurobiotin (NB) in 1M K-acetate. Somal action potentials (APs), input resistance, maximum firing frequencies, rheobase and conduction delays from saphenous or sural nerve stimulation were recorded. One cell per DRG was injected with NB and after allowing 4-6 hr. for diffusion the tissue was fixed with 4% paraformaldehyde. Frozen serial sections (50μm) of the SC and DRG were obtained and reacted with HRP-coupled avidin and visualized using DAB with Ni-intensification. To date 87 somata have been impaled at three different stages of development: P0 (37); P2 (26); P6(24). Fibers in transgenic animals showed slower average conduction velocities and longer somal AP durations in all stages compared to controls. The difference in AP duration was most likely due to the fact that BS with inflections were more prominent in transgenic animals (e.g. 24/27 at P0 and 26/28 at P2 *vs.* 8/10 and 12/24 in controls). The rostrocaudal extent and the number of collateral branches entering the dorsal horn in transgenic animals were comparable to those in controls, however, the individual collaterals in transgenic animals exhibited more branching complexity and occupied a greater mediolateral portion of the dorsal horn. Roughly half of the stained transgenic fibers had the normal relationship between somal APs and laminar distribution of terminals, i.e., broad spikes with inflections and projections confined to laminae I & II or narrow spikes supporting terminals in laminae III-V. The rest had broad spikes and terminals throughout laminae I-VI. This latter population represents a phenotype not seen in controls. NS23725 (HRK); NS31826 (BMD, KMA).

300.15

REGULATION BY NGF OF THE EXPRESSION OF P75 LNGFR ON SYMPATHETIC AND SENSORY IRIDIAL NERVES OF YOUNG AND AGED RATS.

L. Gavazzi* and T. Cowen, Dept. of Anatomy and Developmental Biology, Royal Free Hospital School of Medicine, London NW3 2PF, UK

Upregulation of p75 LNGFR accompanies collateral sprouting of sensory and sympathetic neurons in response to NGF or increased target size in mature rats. In the rat iris the density of innervation of both sensory and sympathetic nerves is increased by NGF treatment, but the response of sensory nerves is greater as compared to sympathetic nerves. Furthermore, sensory nerve response is not affected by ageing, whilst the response of sympathetic nerves is impaired in old rats. Differences in the level of expression of p75 on sensory and sympathetic nerves and/or different ability to upregulate the expression of the receptor may explain the difference in responsiveness. We have investigated this issue evaluating p75 expression on mature and aged sympathetic and sensory nerve fibres on the rat iris following NGF treatment. Sprague-Dawley rats, aged 6 weeks or 24 months, received 4 weekly transcleral injections of either 100μg/ml Cytochrome C (Cyt C) or NGF at a concentration of 25, 100 or 200 μ g/ml. One week after the final injection the rats were sacrificed and their irides double immunolabelled with anti-p75 antibodies and either antibodies against TH (a sympathetic marker) or CGRP (a marker for a subpopulation of sensory neurons). The density of p75-positive innervation was measured by computerized image analysis on confocal images. The intensity of p75 staining decreased by approximately 20% on old irises, indicating a decreased density of the receptor on aged nerves. p75 was expressed on over 80% of CGRP-positive untreated nerves in both young and old rats, and p75 expression was upregulated similarly on young and aged sensory nerves following NGF treatment. On the other hand, 50-60% of sympathetic nerves expressed p75, and p75 upregulation was impaired on aged sympathetic axons. These result suggest that p75 expression on sensory and sympathetic nerves, untreated or following NGF treatment, correlates with their ability to respond to NGF with increased innervation density. Supported by BHF project grant n. PG93027.

300.16

NGF, BUT NOT BDNF NOR NT3, ELICITS AXONAL GROWTH FROM ADULT SENSORY NEURONS IN COMPARTMENTED CULTURES.

K. Kimpinski* and K.M. Mearow, Basic Medical Sciences, Memorial University of Newfoundland, St. John's, NF, A1B 3V6

The *in vivo* model of sensory axon collateral sprouting has shown that the sprouting response requires NGF, while the regeneration following injury does not. While this model has been invaluable in demonstrating the role of NGF in these phenomena, it cannot be easily manipulated to study, for example, signal transduction mechanisms, or the contribution of the different receptor types to the response. We have thus taken an *in vitro* approach to study the regulation of adult sensory neurite growth by neurotrophins.

Using the Campenot compartmented culture system we have examined the effects of the neurotrophins NGF, NT3 and BDNF on neurite growth from both neonatal and adult rat DRG neurons. DRG neurons were dissociated using standard techniques and plated in the centre chambers in the absence of neurotrophin (NT); the side chambers contained either no NT or varying concentrations of NT (1, 10 or 100 ng/ml). While the adult neurons survived in the absence of any NT in the centre and side compartments, the neonatal neurons required access to a side chamber containing NGF for prolonged survival. Our results demonstrated that only NGF elicited neurite growth into the side chambers and that the density of growth was increased at higher NGF concentrations. No neurite growth was observed into chambers with BDNF or NT3. We then asked whether the regrowth (subsequent to *in vitro* axotomy) of neurites that had originally grown into NGF still required NGF. The results demonstrated that, unlike the *in vivo* regeneration, the *in vitro* regrowth did require NGF, and that neither BDNF nor NT3 was able to substitute for NGF. We are currently examining the differential distribution of p75 and trkA between the cell body and distal neurite compartments, using immunocytochemistry, Western blotting and RT-PCR. Supported by the NeuroScience Network.

300.17

THE MORPHOMETRIC AND IMMUNOHISTOCHEMICAL STUDY OF THE EFFECT OF NERVE GROWTH FACTOR ON DORSAL ROOT GANGLIA IN STREPTOZOTOCIN INDUCED DIABETIC RAT.

S. H. Park, E. J. Baik, H. C. Han*, K. A. Park. *Dept. of Anatomy, Kon-Kuk Univ. College of Med. Chung Ju and Yonsei Univ. Seoul, Dept. of Physiology, Ajou Univ. College of Med. Suwon and Korea Univ*. Seoul, Korea.*

This experiment was performed to identified the effect of nerve growth factor (NGF) on L5 dorsal root ganglia (DRG) in streptozotocin induced diabetic rat morphometrically and immunohistochemically. Thirty diabetic Sprague Dawley rats (200-250 gm) were prepared by a single intraperitonial injection of 65 mg/kg streptozotocin in saline. 4 weeks later, L5 DRG were serially sectioned (6 μm) and each section was stained for thionin and immunostained for NGF(1:1000), SP(1:1000) and CGRP(1:2000). The total number of Nissl and immunostained L5 dorsal root ganglia(DRG) cells were counted and cross sectional area was measured with image analyzer.

The results obtained are as follows;

1. In control group, total number of type A and B cells were 1268 and 8479 in L5 DRG, respectively. In diabetic group, total number of type A and B cells were 68.8 % and 44.6 % reduced in L5 DRG, respectively. In NGF administered diabetic group, total number of type A and B cells were recovered to normal control group level.
2. In control group, total number of immunoreactive for NGF, SP and CGRP cells were 2056, 1858 and 2776 in L5 DRG, respectively. In diabetic group, total number of immunoreactive for NGF, SP and CGRP cells were 49.7 %, 78.7 % and 81.5 % decreased in L5 DRG, respectively. In NGF administered diabetic group, total number of immunoreactive for NGF, SP and CGRP cells were recovered to the number of control group.

300.18

THE ACTIVATION OF PHOSPHATIDYLINOSITOL-3 KINASE (PI3-KINASE) IS MORE IMPORTANT FOR THE SURVIVAL OF SYMPATHETIC THAN SENSORY NEURONS.

S. E. Bartlett, A. J. Reynolds and I. A. Hendry* Division of Neuroscience, John Curtin School of Medical Research, Australian National University, Canberra, Australia.

The survival of developing sympathetic and sensory neurons depends on nerve growth factor (NGF) released by the target tissues they are innervating. The signalling pathway mediating this survival message is unknown. Yao and Cooper, 1995 have shown that PI3-kinase is required for the survival of PC12 cells; these cells are also dependent on NGF for their survival. Therefore this study aimed to determine if this enzyme was important for the survival of sympathetic and sensory neurons. We grew dissociated chick 8 day sensory neurons or 12 day sympathetic neurons in cell culture maintained with a dose response to either NGF and in the presence of increasing concentrations of Wortmannin (0.01 nM-10 μM), an inhibitor of PI3-kinase. We found that Wortmannin (10 nM) inhibited the survival of sympathetic neurons by ~50-60% compared to control cells. This concentration has been reported to inhibit the activity of PI3-kinase by ~60% in vitro (Yao and Cooper, 1995). However, in sensory neurons the concentration of Wortmannin required to cause a 50% inhibition of survival was a 100 fold greater, 1μM which completely inhibits PI3-kinase. This suggests that the full activation of PI3-kinase is essential for the survival of sympathetic neurons. However, the survival of sensory neurons requires either a much lower level of PI3-kinase activation or some other pathway is utilised. Thus these experiments demonstrate a difference in the second messenger pathways required for the survival of sympathetic and sensory neurons.

Yao, R. and G. M. Cooper. Science, 267, 2003-2005.

300.19

IN VIVO EFFECTS OF NGF AND NT3 ON DEVELOPING SYMPATHETIC CHICK GANGLIA.

David von Schack, Georg Dechant*, Yves- Alain Barde, Max-Planck-Institute for Psychiatry, Department of Neurobiochemistry, D-81252 Martinsried, Germany.

In vitro studies with chick sympathetic neurons have shown that both NT3 and NGF support the survival of these neurons. In order to investigate if the effects of these factors can also be observed *in vivo*, chick embryos were treated *in ovo* at E3 with hybridoma cells secreting functionally blocking antibodies against NT3 or with NGF or NT3 protein. Cell numbers were determined using a method based on the stochiometric incorporation of a fluorescent dye (Hoechst dye H33258) into dsDNA. Anti NT3 treatments of chick embryos lead to a small reduction in sympathetic cell numbers (-20%) with a significant decrease at E11-E13, a time when these neurons become dependent on target derived factors.
When either NGF or NT3 were injected daily between E3 and E5 cell numbers per ganglion were increased by about 2 fold. Several marker-genes for sympathetic neurons such as tyrosine hydroxylase, choline acetyl transferase, trk A, trk C, p75, were assayed for differential regulation by NGF and NT3. Tyrosine hydroxylase protein was upregulated only after treatment with NGF, detected by Western blot experiments, trk A message was downregulated after the same treatment as determined by RT-PCR.

300.20

P75 EXPRESSION AFFECTS THE NGF DEPENDENCY OF SYMPATHETIC NEURON SURVIVAL IN TRANSGENIC MICE.

C. Brennan*† H.S. Phillips# and S.C. Landis†. †Dept. of Neuroscience, Case Western Reserve University, Cleveland, OH 44118; # Dept. of Neuroscience, Genentech, Inc. San Francisco, CA 94080

Nerve growth factor (NGF), which is required for the survival of sympathetic neurons, binds to two receptors, trkA and p75, the low affinity neurotrophin receptor. TrkA is responsible for signal transduction and few sympathetic neurons survive in trkA deficient mice. The role of p75, however, in mediating NGF effects is unclear. p75 has been suggested to increase the sensitivity of trkA bearing cells to NGF. To determine how p75 and NGF interact to influence sympathetic neuron survival in vivo, we crossed two transgenic lines in which the coding regions of p75 or NGF were disrupted to obtain mice which lacked p75 and were homozygous wild-type or heterozygous for the NGF deletion. At 8 weeks, there was no significant difference in the number of sympathetic neurons in the superior cervical ganglia (SCG) of mice lacking p75 (NGF+/+ p75-/-, 11942±1058 n=3) and of wild-type mice (NGF+/+ p75+/+, 15760±1119 n=3). As expected, the SCG of mice which were NGF+/- p75+/+ had significantly fewer neurons (7585±518 n=3; p>0.01) than wild-type, consistent with the notion that the NGF content of target tissues is limiting. Surprisingly, in the absence of p75 (p75-/-), the number of SCG neurons in mice which were NGF+/- (12594±1573 n=3) was indistinguishable from wild-type. Similar results were obtained when the effect of genotype on neuron number was examined in stellate, thoracic, and lumbar ganglia. In contrast to cell number, neuron area was unaffected by genotype. The increase in neuron number in NGF+/- mice which lack p75 suggests that p75 in vivo, at low NGF concentrations, does not act to enhance the efficiency of NGF binding or trkA signal transduction. Rather, our results are consistent with two other roles that have been put forward for p75. p75 may participate in a signaling pathway involving sphingomyelin that leads to neuronal cell death when NGF levels are reduced. Alternatively or additionally, p75 may decrease the ability of neurotrophin-3 to bind trkA and compensate for the lack of NGF. Supported by NS023678 (SCL) and T32NS07118 (CB).

300.21

MECHANISM OF REGULATION OF Ca^{2+} CHANNELS IN AN ADULT SYMPATHETIC NEURONE BY NERVE GROWTH FACTOR. S. Lei, W.F Dryden* & P.A. Smith. Dept. Pharmacol., Univ. Alberta, Edmonton, T6G 2H7 Canada.

Although nerve growth factor (NGF)-induced differentiation of PC12 cells into sympathetic-like neurones is accompanied by increases in Ca^{2+} channel currents (I_{Ca}), it is not known whether NGF maintains the integrity of ion channels in sympathetic neurones throughout adulthood. We have been using standard whole-cell recording methods to study the effects of NGF on I_{Ca} in identified B-cells of bullfrog sympathetic ganglia maintained for 12d in a neurone-enriched, serum-free, defined medium culture (Lei *et al., Biophys. J.* 68 A209 1995). Although *total* current increased as the cells extended neurites, current *density* was unchanged. The neurotropin may therefore be involved in adjusting the size of I_{Ca} to match the increase in size that occurs during growth. NGF-induced increases in I_{Ca} were mediated via a tyrosine kinase (presumably *TrkA*) as they were inhibited by 20μM genistein or by 10μM lavendustin A. The inactive isomer of genistein (daidzein, 20μM) failed to prevent the effect of NGF. Higher concentrations of genistein (100μM) suppressed I_{Ca} recorded in the absence of NGF. This may reflect non-specific actions or may suggest that I_{Ca} is maintained by tonic tyrosine kinase activity. Downstream effects of NGF do not seem to be mediated via the phosphatidyl inositol-3-kinase pathway as they were not antagonised by wortmannin (100nM) or by LY 294002 (30μM). The possible involvement of other downstream transduction mechanisms from *TrkA* remains to be investigated.

Supported by the Alberta Paraplegic Association, Rick Hansen Man in Motion Foundation and MRC of Canada

300.22

MODULATION OF SYMPATHETIC SYNAPSES BY NGF. S.T. Lockhart*, G.G. Turrigiano and S.J. Birren, Department of Biology and Volen Center, Brandeis University, Waltham, MA 02254.

Neurotrophins modulate synaptic strengths in the CNS and at the neuromuscular junction. We have shown that Nerve Growth Factor (NGF) modulates noradrenergic synaptic connections between cultured sympathetic superior cervical ganglion (SCG) neurons and cardiac myocytes from neonatal rat. Intracellular stimulation of a presynaptic neuron results in an increase in myocyte beat frequency. This increase in beat rate is rapidly and reversibly potentiated by 50 ng/ml NGF (200% of control, $p< 0.02$). Preliminary data indicate that this effect is presynaptic, since the increase in beat rate produced by puffing norepinephrine onto myocytes is not potentiated by NGF. Potentiation of synaptic transmission by NGF is blocked by the tyrosine kinase inhibitor K-252A (200 nM). Although K-252A alone has no effect, in the presence of this drug, NGF depresses synaptic transmission to 0% of control ($p< 0.05$). This suggests that in the absence of receptor tyrosine kinase (trk) signaling, NGF signals via an alternate pathway. The low affinity neurotrophin receptor, P75, is expressed both in SCG neurons and in cardiac myocytes, as measured by RT/PCR. Together, these data support the hypothesis that NGF potentiates synaptic transmission via trk signaling. Further, these data raise the possibility that NGF modulates synaptic transmission by activating distinct and antagonistic signal transduction pathways through the p75 and trk receptors. Supported by research grants from the Whitehall Institute (SJB) and NSF and the Whitehall Institute (GGT) and NIH training grant NS 07292 to Brandeis University (STL).

300.23

ALTERED STRUCTURE AND NEUROCHEMISTRY OF SUPERIOR CERVICAL GANGLION NEURONS IN ADULT TRANSGENIC MICE OVEREXPRESSING NERVE GROWTH FACTOR. G.E.A. Coome* and M.D. Kawaja, Dept. Anatomy and Cell Biology, Queen's University, Kingston, Ontario CANADA K7L 3N6.

This investigation examines the effects of elevated endogenous levels of nerve growth factor (NGF) on the structural and neurochemical features of neuronal cell bodies of the superior cervical ganglion (SCG). Transgenic mice, which overexpress this neurotrophin in GFAP-producing astrocytes of the brain, appear normal at birth and into adulthood. These transgenic mice differ from their age-matched wild-type counterparts, in that they have a dense plexus of SCG axons in their cerebellum; this aberrant sprouting of new sympathetic axons was attributed to the markedly increased levels of NGF mRNA and protein. The adult transgenic mice also had larger SCG than control mice, due to increases in both neuron size and number (30% more). NGF-immunostaining in SCG neurons of postnatal transgenic mice was more intense compared to wild-type mice, but comparable between adults. EM analysis of transgenic neurons revealed more lysosomes and disrupted perinuclear Golgi apparati. *In situ* hybridization for the high-affinity NGF receptor (trkA) showed that the ratio of grain density per neuron did not differ between transgenic and wild-type mice, despite the increase in size of transgenic neurons. It remains to be determined whether levels of mRNA for the low-affinity receptor are likewise unaltered in transgenic neurons. These data provide new evidence that elevated levels of endogenous NGF produced in the intact mammalian brain can induce structural and neurochemical remodelling of SCG neurons. (Supported by MRC of Canada)

300.24

CONCENTRATION-DEPENDENT RESPONSE OF CEREBROVASCULAR AXONS TO INTRACRANIAL INFUSION OF NGF. W.L. Nixdorf, M. Mareska, J.T. Oris, R.G. Sherman*, and L.G. Isaacson, Center for Neurosci, Dept Zoology, Miami Univ, Oxford, OH 45056

Following a 2 week intracerebroventricular infusion of nerve growth factor (NGF; 15 µg) into the young adult rat, we observed a sprouting response by perivascular axons associated with intradural internal carotid artery (ICA), most of which originate in the superior cervical ganglia. In those studies we used electron microscopy (EM) to evaluate axonal growth and found that axons associated with the ICA were increased by 150%. Our present goal was to examine the relationship between the amount of NGF infused and the sprouting response by cerebrovascular axons. Young adult rats received 2 wk infusions of cytochrome C (VEH) or NGF at concentrations of 100 µg/ml (NGF:n = 6; VEH:n = 6), 50 µg/ml (NGF:n=4; VEH:n=2), 20 µg/ml (NGF: n=6; VEH:n=2), and 10 µg/ml (NGF:n=2; VEH: n=1). Approximately 150 µl infusate entered the ventricular system, resulting in infusions of 15 µg, 7.5 µg, 3.0 µg and 1.5 µg. respectively. Analysis of EM data revealed a linear relationship between the number of perivascular axons and the µg NGF infused. No change in axonal number was observed following infusion of 1.5 µg NGF, though infusions of 3.0 µg NGF or higher resulted in a significant increase in axons when compared with VEH of similar concentration. No change in axons was observed following VEH infusions. These findings reveal a positive relationship between the response of perivascular axons and the amount of NGF infused during the 2 wk infusion period. (Supported by NS32876 to LGI)

300.25

NERVE GROWTH FACTOR (NGF) INDUCES APOPTOSIS IN HUMAN MEDULLOBLASTOMA CELL LINES THAT EXPRESS TRKA RECEPTORS. Y. Muragaki [1]*, T. T. Chou[2], D. R. Kaplan[1], J. Q. Trojanowski[2] and V. M. -Y. Lee[2]. 1 Dep. of Neurosurgery, Tokyo Women's Medical College, Tokyo, Japan 162. 2 Dep.of Pathology and Laboratory Medicine, Univ. of Pennsylvania School of Medicine, Philadelphia, PA 19104. 3 ABL-Basic Research Program, National Cancer Institute, Frederic Cancer Research and Development Center, Frederic, MD 21702.

Neurotrophins act through their cognate receptors to promote the differentiation and /or survival of neuronal progenitor cells, immature neurons and other cells. Here we examined the effects of NGF and its cognate receptor (Trk or TrkA) on the survival of a common childhood brain tumor, i.e. medulloblastoma, a tumor that resembles CNS neuroepithelial progenitor cells. To do this, we engineered two human medulloblastoma cell lines (i.e. D283 MED and DAOY cells) to express human TrkA using a retroviral expression vector.

Surprisingly, NGF treated medulloblastoma cells expressing the TrkA receptor underwent massive apoptosis which could be prevented by K252a or anti-NGF antibodies. Similar treatment did not induce apoptosis in wild type uninfected cells, cells expressing an empty vector or cells engineered to express the human p75 NGF receptor.

NGF/TrkA signal transduction pathway could activate apoptotic cell death programs in CNS neuroepithelial progenitor cells and childhood brain tumors.

Supported in part by grants from the National Institutes of Health and by a Zenith Award from the Alzheimer's Assosiation.

300.26

A STUDY OF SALIVARY NERVE GROWTH FACTOR (NGF) ACTIVITY IN FEMALE HOUSE MICE (*Mus domesticus*) OF VARYING ESTRUS STAGES. B. M. Jubilan*, P. Leonard and K. A. Hild. Behavioral Neuroscience Program, Allentown College of St. Francis de Sales, Center Valley, PA 18034.

The saliva of mice has been demonstrated to contain significant concentration of nerve growth factor (NGF). The functional significance of this observation has been the subject of speculation. The present study predicts that NGF concentration fluctuates in accordance with hormone levels during different phases of the estrus cycle in female mice.

Rat pheochromocytoma cell line (PC-12) was used for NGF bioassay. The number of neurite bearing cells was used as the dependent measure. Saliva was collected by placing a sterile cotton ball of known weight under the tongue of the anesthetized female mouse and left to soak for 45 min. The cotton soaked with saliva was then weighed to measure the amount of saliva collected. Female estrus cycles were identified as either "estrus" or "diestrus" based on the histological composition of vaginal smears stained with methylene blue solution. The cotton ball containing the saliva was then dropped in plates containing known concentration of PC-12 cells. The salivary bioassay was run simultaneously with purified NGF bioassay.

Initial results show more neurite bearing cells in PC-12 cultures with saliva from estrus female mice compared to PC-12 cultures with saliva from diestrus females. Further analysis is being done to consider the rate of saliva secretion and interpolated NGF concentration in interpreting these results.

300.27

EXPRESSION OF A BIOLOGICALLY ACTIVE ANTI-NGF MONOCLONAL ANTIBODY BY TRANSFECTION INTO CELLS OF NEURONAL LINEAGE. Dao-Chao Huang*, Marc Pinard, Moshe Szyf and A. Claudio Cuello. Department of Pharmacology and Therapeutics, McGill University, 3655 Drummond Street, Montreal, Quebec, Canada, H3G 1Y6.

To develop a transgenic model expressing neutralizing anti-nerve growth factor (NGF) antibodies within the nervous system at specific sites and specific times in development, we cloned the cDNAs encoding the monoclonal anti-NGF30 antibody which neutralizes the biological activity of NGF. Nucleotide sequence analysis revealed that these cDNAs differ from other known variable regions. The heavy (H)- and light (L)-chains variable and constant regions of the gene were obtained by RT-PCR amplification from the mRNA of αNGF30 hybridoma cells. To secure antibody expression in neuronal cell types, cDNAs were cloned into plasmid expression vectors under control of a neuron-specific enolase (NSE) promoter. The recombinant αNGF30 have been successfully expressed in the neuroblastoma cell line N18TG2 under NSE promoter control and examined by ELISA, immunofluorescence and immunocytochemistry. Transfected cell supernatants containing recombinant mABs were tested for their ability to block the NGF-induced stimulation of neurite outgrowth in PC12 cells. These experiments demonstrate the feasibility of applying recombinant antibodies, expressed by cells of neuronal lineage, as tools for specific inactivation of specific molecules in the CNS and PNS. The relevance of these cDNA constructs towards the generation of transgenic animals is discussed. *Initially supported by the Neuroscience Network (Canadian NCE) and completed with support from MRC (Canada).*

300.28

AMYLOID PRECURSOR PROTEIN ALTERS NGF BINDING IN PC12 CELLS

C.A. AKAR, H.Y.T. YANG* AND W.C. WALLACE GRC,NIA Baltimore, MD and LBG, NIMH Neuroscience Center St. Elizabeth's Hospital, Washington, D.C.

Amyloid precursor protein (APP) is expressed throughout the brain. However, physiological function of the protein has not yet been well characterized. We have previously observed that APP induces neurite outgrowth and potentiates neurotrophic activity of NGF on PC12 cells.This potentiation is also reflected in the tyrosine phosphorylation of several proteins in the NGF signal transduction pathway (e.g. ERKs 1and 2). In order to determine the molecular basis for this potentiation, we have examined the effect of APP on NGF receptor binding in PC12 cells. The binding assay was performed on suspended whole cells using ^{125}I-NGF (40pM-1nM) in the presence and absence of APP (2nM). Nonspecific binding was determined in the presence of NGF (400nM). Free ligand was seperated from the bound by cenrifuging through 0.15 M sucrose.The K_D for NGF binding was reduced in the presence of APP from 7.7 nM to 1.2 nM. This increase in NGF binding suggests that APP may potentiate the effects of NGF by increasing the affinity of NGF binding sites. (Supported by IRP, NIA)

300.29

NGF INCREASES THE TRANSCRIPTION OF THE NA CHANNEL GENE EXPRESSED BY PANCREATIC ß CELLS. Vidaltamayo, R., Rosenbaum, T. and Hiriart, M*. Dept. Neurosciences, Inst. Cell. Physiol., UNAM, MEXICO.

We have previously shown that NGF induces morphological and physiological changes in pancreatic ß-cells. After 5-7 days in culture, approximately 50% of ß cells treated with 50 ng/mL of 2.5SNGF extend neurite-like processes (*Endocrine* 4:19-26); a similar effect was also observed in the ß-cell line RINm5F(Polak et al., *PNAS* 90:5781-5785). Moreover, in ß cells treated with NGF we observed a 30% increase on peak Na-current density, with no change on the steady state inactivation of Na current nor on the conductance-voltage relationship. These data suggest that NGF induces an increase on the number of functional Na channels in ß-cell membrane.

In this study we used the RT-PCR technique to asses if NGF could increase the transcription of the Na-channel gene expressed by ß cells. We found that ß-cells express a product similar to the brain Na-channel type III. After 5 days in culture in the presence of NGF, we observed a 3-4 fold increase in Na channel gene transcription of RINm5F cells. In primary cultures of ß-cells this effect was not observed. However, NGF did not induce the expression of a different Na-channel gene. We are evaluating if this increase could be observed earlier. These data suggest that NGF could elicit in ß-cells a cellular response similar to the one observed in chromaffin and PC12 cells.

This work was supported by DGAPA UNAM through grant IN212194, and by scholarships to R. Vidaltamayo and T. Rosenbaum

300.30

NGF REGULATES VOLTAGE-GATED POTASSIUM CHANNELS THROUGH A CNTF-LIKE AUTOCRINE LOOP IN NEUROBLASTOMA CELLS. T.M. Holmes, S.S. Lesser, and D.C. Lo*. Dept. of Neurobiology, Duke University Medical Center, Durham, NC 27710.

Neurotrophic factors strongly influence neuronal function through their regulation of electrical excitability. We have previously shown that prolonged NGF treatment increases levels of voltage-gated Na, Ca, and K currents in SK-N-SH neuroblastoma cells, while CNTF increases K currents only. Moreover, we showed that the K channels regulated by NGF and CNTF are functionally indistinguishable. We thus hypothesized that NGF and CNTF regulate K channel levels through a common pathway, perhaps involving an autocrine or paracrine substance.

We first tested if NGF induces the secretion of a substance into conditioned medium (CM) that can increase K channel levels. SK-N-SH cells were treated with NGF for several days and CM transferred to naive cells; NGF in the CM was neutralized by addition of excess anti-NGF antibody. Using whole-cell patch clamp recording, we found that NGF-depleted CM was still able to induce K currents. Thus, NGF induces the secretion of a substance into CM that is *sufficient* to induce K currents in the absence of NGF.

To test if a CNTF-like molecule could be this autocrine substance, we treated SK-N-SH cells with NGF together with a neutralizing anti-CNTF antibody--this antibody completely blocked the ability of NGF to induce K currents. In contrast, NGF could still induce Na and Ca currents in the presence of the anti-CNTF antibody, indicating that the effects of this antibody were specific upon K channel regulation. Thus, a secreted, CNTF-like activity is *necessary* for induction of K currents by NGF. Together, these findings suggest that NGF regulates Na and Ca channels directly while its induction of K currents occurs via a CNTF-like autocrine/paracrine loop.

We thank Regeneron Pharmaceuticals for their generous provision of CNTF; this work was supported by grants from the Alfred P. Sloan and Ruth K. Broad Foundations, the NIH (NS32742), and the Duke Mechanisms of Behavior Program.

300.31

DOWN-REGULATION OF PROTEIN KINASE C PREVENTS THE ACTION OF NERVE GROWTH FACTOR ON CALCIUM UPTAKE INTO PC12 CELLS, G. Dickens, M. Lavarreda, W-H. Zheng', and G. Guroff Section on Growth Factors, NICHD, NIH, Bethesda, MD 20892.

Nerve growth factor (NGF) treatment of PC12 cells produces a modest, transient increase in the uptake of $^{45}Ca^{2+}$ into PC12 cells. Pre-treatment of the cells for 24 hours with phorbol 12-myristate 13-acetate prevented this action of NGF. Pre-treatment with PMA was specific in that it did not prevent the stimulation of $^{45}Ca^{2+}$ uptake by the L-channel agonist, Bay K 8644, nor by ATP. The action of NGF was prevented by either calphostin C or staurosporine, inhibitors of kinase C. It was also prevented by GO 6976, an inhibitor specific for the α, β, and γ forms of kinase C. Since previous work from this laboratory has shown that our PC12 cells are deficient in the β- and γ-forms of kinase C, these data implicate protein kinase α in the stimulation of $^{45}Ca^{2+}$ uptake by NGF.

301.1

EFFECT OF SYSTEMICALLY-INJECTED NGF ON SP AND CGRP RELEASE FROM RAT SPINAL CORD IN VITRO. M. Malcangio, N.E. Garrett, D.R. Tomlinson and N.G. Bowery* Depts of Pharmacology, Queen Mary and Westfield College, Mile End Road, London E1 4NS and Medical School, Edgbaston, Birmingham B15 2TT, U.K.

Nerve growth factor (NGF), which is a classical member of the polypeptide growth factor family, is synthesized in target tissues and then retrogradely transported by sensory neurons to dorsal root ganglia (DRG) neuronal cell bodies. Systemically injected NGF is also transported to DRG where it regulates substance P (SP) and calcitonin gene-related peptide (CGRP) expression.

In the present study we have investigated whether 2-week treatment of rats with human recombinant NGF (1 mg/kg s.c.) could influence basal outflow and electrically-evoked release of endogenous SP and CGRP from spinal cord slices *in vitro*.

Dorsal horn slices (350 μm thick) were obtained from the lumbar enlargement and release of neuropeptides induced by electrical stimulation of dorsal roots attached to the tissue. Peptide content in collected fractions was determined by radioimmunoassay. Sub-chronic treatment with NGF induced a significant 2 fold-increase in SP- and CGRP- basal outflow (in controls: 10.0±2.0 and 4.8±0.6 fmol/8ml-fraction, respectively). The evoked release of SP was increased by 2 fold over control evoked release (22.8±3.2 fmol/8ml-fraction) and that of CGRP by 5 fold over evoked release in controls (21.2±0.9 fmol/8 ml-fraction).

These findings whilst giving the first evidence that NGF can increase SP and CGRP pools which are available within the spinal cord to be tonically and electrically released from primary afferent fibres, supports a role for NGF in modulation of pain perception at segmental level.

Thanks to Genentech for NGF supply and British Diabetic association for support

301.2

ELEVATION OF BRAIN mRNA FOR NEUROTROPHINS BY ORAL AIT-082 IN MICE. A. J. Glasky*[1], R. F. Ritzmann[1], I. Prisecaru[1], S. Santos[1], K. Kafi[1], M. P. Rathbone[2], P.J. Middlemiss[2] and C. Crocker[2] [1]ERI, Olive View UCLA Medical Center, Sylmar, CA 91342 and Dept. Biomed. Sci., McMaster Univ., Hamilton, ONT, Canada L8N 3Z5

The object of this study was to determine whether AIT-082, a analog of hypoxanthine, modified the levels of neurotrophins in the brains of aged mice. Previous studies have shown that AIT-082 increased the levels of mRNA for NGF, NT-3 and bFGF but not BDNF in cultures of astrocytes. Thirteen month old C57BL/6 mice received AIT-082 in their drinking water (equiv. to 30 mg/kg/day) for ten months. A control group received no drug in their drinking water. During this period, mice were tested for working memory performance in the win-shift paradigm, a delayed alternation positive reinforcement task which measures the duration of the memory trace. At 23 months of age, there was a clear difference between controls (no memory) and AIT-082 treated (in which 50% of the mice exhibited no deficit). Mice were sacrificed and brain tissue analyzed for neurotrophin mRNA using RT-PCR technology. There was a significant increase in the mRNA for NGF and NT-3 in the frontal cortex and hippocampus but not the cerebellum of animals that had functionally intact memory when compared to animals that could not perform the memory task. AIT-082 is orally active, rapidly penetrates the blood brain barrier and induces the production of multiple growth factors at the appropriate target site in the central nervous system.

Supported by grants from National Institute on Aging (AG09911), ALS Society of Canada, Ontario Mental Health Foundation and Advanced ImmunoTherapeutics.

301.3

BDNF, aFGF, bFGF, IGF-I, AND VARIOUS ANTI-OXIDANTS DO NOT PREVENT THE APOPTOTIC DEATH OF DEVELOPING BASAL FOREBRAIN CHOLINERGIC NEURONS FOLLOWING NGF WITHDRAWAL *IN VITRO*. J.N.C. Kew and M.V. Sofroniew* MRC Cambridge Centre for Brain Repair and Department of Anatomy, University of Cambridge, Cambridge CB2 2PY, U.K.

We investigated whether multiple growth factors might interact to regulate the survival of developing basal forebrain cholinergic neurons *in vitro*. Septal cholinergic neurons grown in 'sandwich cultures' in serum-free, completely defined medium are partially dependent on NGF during a critical period of their development, such that NGF withdrawal during this period results in the protein synthesis-dependent, apoptotic death of most, but not all, of these neurons. Here we report that BDNF, acidic and basic FGF, and IGF-I applied in this culture system were all not able either to support the development of septal cholinergic neurons from plating, or to prevent the cell death of these neurons induced by NGF withdrawal. Various antioxidants also did not prevent the apoptotic death of developing septal cholinergic neurons induced by NGF withdrawal. These findings suggest that in the absence of serum and other additives, BDNF, acidic and basic FGF, and IGF-I do not interact with NGF to regulate the survival of developing basal forebrain cholinergic neurons *in vitro*. The findings also suggest that the apoptotic death of septal cholinergic neurons induced by NGF withdrawal is not mediated by oxidative stress or free radical generation.

Supported by the MRC.

301.4

NERVE GROWTH FACTOR INDUCES GALANIN EXPRESSION IN RAT BASAL FOREBRAIN: IMPLICATIONS FOR TREATMENT OF CHOLINERGIC DYSFUNCTION. B. Planas*, P.E. Kolb, M.A. Raskind and M.A. Miller, Dept. of Psychiatry & Behav. Sci., University of Washington, Seattle, WA 98195.

Galanin (GAL) messenger RNA is coexpressed by a limited number of cholinergic neurons in the basal forebrain (BF) of adult male rats. GAL, co-secreted with ACh, may inhibit cholinergic-based memory functions since GAL infusion (icv) has been shown to impair performance on memory tasks. Nerve growth factor (NGF) is a potent stimulator of choline acetyltransferase (ChAT) in the BF and has been proposed as a therapeutic agent for the treatment of human age- and disease-related cholinergic dysfunction. However, the effects of chronic NGF administration on GAL gene expression within the BF have not been studied. We used in situ hybridization and quantitative autoradiography to assess GAL and ChAT gene expression within the BF of young adult male rats following chronic infusion (icv) of NGF or cytochrome c. Confirming the effectiveness of NGF infusion, the number of ChAT mRNA-expressing neurons ($p \leq 0.0002$) and their intensity of labeling ($p \leq 0.0001$) were enhanced in all regions of the BF. NGF infusion also induced a significant increase in the number of GAL mRNA-expressing neurons ($p \leq 0.0005$) in the BF and in their average labeling intensity ($p \leq 0.0013$). The effects of NGF on GAL gene expression were restricted to the BF since the number of GAL expressing neurons and the cellular GAL mRNA content in another forebrain region, the bed nucleus of the stria terminalis, were unaffected. NGF induction of GAL in the BF was specific, since the expression of another neuropeptide, neurotensin, present within non-cholinergic BF neurons was unchanged. These data provide the first evidence that in vivo infusion of NGF enhances GAL gene expression in the cholinergic BF. These results suggest that the ameliorating effects of NGF treatment on cholinergic dysfunction could be antagonized by the concomitant induction of the inhibitory neuropeptide GAL in the cholinergic BF.

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301.5

Effects of NGF-Secreting Cells on Cholinergic Neurons in Aged Primates. MH Tuszynski[1,2,*], D Smith[1], FH Gage[3], J Roberts[4]. [1]Dept Neurosci., Univ Calif-San Diego, La Jolla, CA; [2]VA Med Ctr, San Diego, CA.; [3]Salk Institute, La Jolla, CA; [4]UC-Davis, Davis, CA.

Spontaneous degeneration of basal forebrain cholinergic (BFC) neurons occurs in Alzheimer's disease and in aged rats and monkeys. Deficits on selected cognitive tasks also occur in aged rats and primates. Nerve growth factor (NGF) reduces age-related cognitive decline in rats and prevents injury-induced BFC degeneration in primates. In the present study, we examined the effects of NGF, delivered by gene transfer, on spontaneous BFC neuronal atrophy in aged rhesus monkeys.

BFC neuron numbers and size were quantified throughout the Ch4 region in 16 aged rhesus monkeys, 8 of whom received NGF-secreting autologous cell grafts as previously described. Non-stereological methods were used since comparisons between NGF- and control- grafted subjects were sought rather than estimates of total neuron numbers. Results were compared to young control monkeys.

Preliminary results from quantification of neurons in the Ch4-Intermediate division reveal that aged monkeys (n=2) show a 16±10% decline in p75-immunoreactive neuron numbers compared to young adult monkeys (n=4), but NGF-grafted monkeys (n=5) show a decline of only 1.4±5%. Both aged groups show hypertrophy in neuronal area compared to young subjects, however. Results from additional subjects across Ch4 subregions will be presented.

301.6

C-FOS INDUCTION IN A RAT MODEL OF CYSTITIS: ROLE OF NGF. N. Dmitrieva*, R. Iqbal, D.Shelton[1] ad S.B. McMahon, Dept. Physiol., UMDS, London, UK and [1]Dept. Neuroscience, Genentech, South San Francisco, USA.

Fos protein expression was studied by immunohistochemistry in the dorsal horn after experimental inflammation of the urinary bladder and after intraluminal instillation of putative inflammatory mediators.

Experimental inflammation with turpentine (50% in olive oil - a model of Interstitial Cystitis, McMahon et al., 1995, Brit J Aneasth. 75:132-144) and intravesical treatments with murine TNF (20 ng/ml in saline) or human recombinant NGF (10 μg/ml, in 10% DMSO in saline) for 3 hours in urethane aneasthetised rats (1.25 g/kg, i.p.) all resulted in a high levels of expression of c-fos immunoreactivity in spinal segments L5 to S1. The pattern of induction was remarkably constant with all stimuli: most immunoreactive cells were seen in laminae I/II and lamina X, with fewer cells in the deep dorsal horn and the intermediolateral cell column. The total number of c-fos expressing cells (estimated from serial sections) with these treatments was 5780 to 6120 significantly greater than saline (460) or DMSO (4140), $P<0.05$ in all cases, t-test.

The involvement of endogenously produced NGF in the response to turpentine was also examined by pre-treating some animals with an NGF sequestering molecule, trkA-IgG (1mg/kg, i.v., 1 hour before turpentine). Here c-fos expression was significantly reduced ($P<0.005$, ANOVA) compared to turpentine alone.

The results suggest that c-fos induction after turpentine-induced inflammation depends critically upon the up-regulation of NGF that is known to occur.

Supported by the MRC

301.7

COGNITIVELY IMPAIRED AGED RATS DO NOT DISPLAY REDUCED NGF-IMMUNOREACTIVITY WITHIN THE MEDIAL SEPTUM. V. Charles*, M. Say, E.J. Mufson, T.J. Collier and J.H. Kordower Research Center for Brain Repair and Dept. Neurological Sciences, Rush Presbyterian Med. Ctr., Chicago Ill. 60612.

Recently we reported that remaining cholinergic basal forebrain neurons in Alzheimer's disease express diminished immunoreactivity for retrogradely transported nerve growth factor (NGF; Mufson et al., 1995). Similarly, NGF-immunoreactivity is reported to be decreased within cholinergic basal forebrain neurons of senescently accelerated mice (Ohnishi et al., 1995). These data suggest that the degeneration of cholinergic neurons and resulting cognitive deficits may result from impaired NGF-related trophic support. To test this hypothesis, young (3 month) and aged (27 month) rats were examined for cognitive abilities using the Morris water maze. Aged rats were cognitively impaired relative to young controls. Following behavioral testing, rats were sacrificed by perfusion with 2% paraformaldehyde and 0.2% paraquinone. Sections through the forebrain were then immunostained for NGF using a polyclonal antibody. Relative optical density measurements for NGF-immunoreactivity were quantified using the NIH image analysis system on three sections matched for level through the medial septum of each rat by an investigator blinded to the animals age or behavioral performance. All neurons on each section were measured. No differences in the relative optical density measurements were observed between the two groups ($p>.05$). The data suggest that reduced NGF-immunoreactivity is not associated with age-related changes in medial septal neurons. (Supported by AG09466)

301.8

ANTIOXIDATIVE MECHANISM OF NGF-MEDIATED NEUROPROTECTION IN A 3-NP MODEL OF HUNTINGTON'S DISEASE W.R. Galpern,*[1,3] D.M. Frim,[1] R. Matthews,[2] M.F. Beal,[2] and O. Isacson[1,2] [1]Neuroregeneration Laboratory, McLean Hospital and Program in Neuroscience, Harvard Medical School, Belmont, MA; [2]Neurology Service, Massachusetts General Hospital, Boston, MA; [3]University of Massachusetts Medical Center, Worcester, MA

Systemic administration of 3-nitropropionic acid (3-NP) produces striatal lesions characteristic of the neurodegeneration of Huntington's disease and is associated with increased production of hydroxyl radicals and the oxidant peroxynitrite. Inhibition of neuronal nitric oxide synthase (NOS) protects against 3-NP induced lesions and attenuates hydroxyl radical and peroxynitrite formation. We have previously shown that NGF-secreting fibroblasts protect against the striatal neurodegeneration induced by 3-NP. In the present study, we have investigated the mechanism of NGF-mediated neuroprotection. Rats were grafted in the corpus callosum with NGF[+] or NGF[-] fibroblasts. In the first series of experiments, rats were sacrificed 7 days after implantation and brains were evaluated for expression of catalase as well as striatal levels of ATP. In the second series of experiments, rats received a series of 3 systemic injections of 3-NP 7 days after grafting, and the generation of peroxynitrite was evaluated by measuring the striatal levels of 3-nitrotyrosine by HPLC. Implantation of NGF-secreting fibroblasts was associated with an increase in catalase expression at the graft site and a concomitant decrease in striatal ATP levels. In addition, NGF grafts significantly decreased the 3-NP induced generation of 3-nitrotyrosine, presumably by decreasing peroxynitrite formation. These findings suggest that NGF may protect against neuronal death by enhancing the levels of antioxidant enzymes as well as by decreasing the production of oxidative agents such as peroxynitrite. Furthermore, the attenuation of peroxynitrite formation suggests NGF neuroprotection may be mediated in part by increased scavenging of superoxide radicals. (Supported in part by the Milton Fund/Harvard Medical School and NIH grants NS 16367 and NS 31579)

301.9

ELECTROPHYSIOLOGICAL EFFECTS OF OX-26-NGF ON MEDIAL SEPTAL NEURONS. D. Albeck*[1], G. M. Rose[2,3], L. Veng[2], R. Bartus[4], and A-C. Granholm[1,2]. Depts. of Basic Science[1] and Pharmacology[2], Univ of Colorado HSC, and VAMC[3], Denver, CO 80262, Alkermes Inc[4], Cambridge MA 02139

Nerve growth factor (NGF) is a neurotrophin which has actions on cholinergic cells in the medial septum. Recent reports have shown that NGF affects the electrophysiological characteristics of these cells; both acute and long term effects have been described. Conjugation of NGF to a transferrin receptor antibody (OX-26-NGF) allows peripherally administered NGF to cross the blood-brain barrier and exert effects in the brain. The present study was undertaken to characterize the action of OX-26-NGF upon medial septal neurons *in vivo*. Both untreated young (3-6 months) and aged (24 months) male Fischer 344 rats were studied. Aged rats were given long term OX-26-NGF treatment using one of two different treatment protocols. Rats received i.v. injections either weekly (50μg) for 3 months (long-term, LT), or weekly for the first month followed by no injections for 2 months (short-term, ST). Rats were anesthetized with urethane and prepared for recording. Following the isolation of a medial septal area neuron, baseline activity was recorded before and after i.v. injection of either OX-26-NGF (50μg) or unconjugated NGF and antibody (control solution). Neither solution had consistent effects upon neuronal activity in the young rats. In contrast, in cells recorded in aged ST rats, OX-26-NGF (but not control solution) injections caused a prolonged elevation of firing. This effect was not observed in aged LT rats. Taken together, these data suggest that NGF affects medial septal neuron electrophysiology in aged rats which have not received NGF for a prolonged period but not in young rats, and that the effect seen in the aged rats is normalized by long term treatment with OX-26-NGF. Supported by USPHS grant AG12122 to A-C Granholm, and grant AG-10755 to G. M. Rose

301.10

ELECTROPHYSIOLOGICAL STUDIES OF NGF EFFECTS ON MEDIAL SEPTAL TRANSPLANTS. C. Bäckman[1*], C. Lupica[1], R. Bartus[4], A-C. Granholm[1,2,3]. Neuroscience Program[1], Depts. of Basic Science[2] and Pharmacology[3], University of Colorado HSC, Denver, CO80262. Alkermes Inc.[4], Cambridge, MA.

Cholinergic neurons of the medial septal nucleus in the basal forebrain are sensitive to nerve growth factor (NGF). It has been demonstrated that an anti-transferrin receptor antibody (OX-26) conjugated to NGF (OX-26-NGF) crosses the blood-brain barrier, stimulating survival and growth of these neurons in isolated transplants *in oculo*. In the present study we investigated the effects of chronic OX-26-NGF treatment on single neuronal activity stimulated by acute local NGF application in intraocular grafts of septal tissue. Fetal septal tissue containing cholinergic neurons was grafted into the anterior chamber of the eye of adult Fischer 344 albino rats. A mixture of OX-26 and NGF (co-mixture) or OX-26-NGF was injected (10μg/injection) into the tail vein of recipient rats once every two weeks for a period of 3 months following transplantation. Preliminary results of extracellular recordings showed that acute administration of NGF has a slow-onset, long lasting excitatory effect on the spontaneous firing of neurons in septal grafts treated chronically with the co-mixture. In contrast, acute administration of NGF does not seem to have such an excitatory effect on the spontaneous activity of grafted septal neurons chronically treated with the OX-26-NGF conjugate. Intracellular recordings will also be undertaken on the septal grafts, and intracellular labeling with biocytin in conjunction with ChAT immuno-staining will determine the identity of recorded neurons. These data suggest that chronic treatment with exogenous NGF gives rise to a desensitization of acute NGF effects on individual neurons in the septal area. Supported by USPHS grant AG12122.

301.11

EXPRESSION OF THE IMMEDIATE EARLY GENE ZIF268 IN MONOCULARLY DEPRIVED RATS: EFFECTS OF NGF. M. Caleo[1], T. Pizzorusso[1], C. Lodovichi[2] and L. Maffei*[1,3]. (1) Scuola Normale Superiore, (2) Scuola Superiore S. Anna, (3) Istituto Neurofisiologia CNR, 56127 Pisa (Italy).

We have tested the effects of NGF administration in monocularly deprived rats by using immunodetection for zif268 as an assay of cortical activity with single-cell resolution. Animals were monocularly deprived for 10 days during the critical period and provided every other day with intraventricular injections of NGF (3-4 μg) or cytochrome C as control. At the end of the deprivation period we tested the ability of the deprived eye to drive the response of cortical neurons. The deprived eye was re-opened and the normal eye silenced by means of intraocular TTX injection, 5 hours later the animals were sacrificed and the brains processed for immunohistochemistry. In cytochrome C-treated animals staining for zif268 was restricted to the monocular visual cortex contralateral to the re-opened eye and no staining at all was observed in the binocular segment, where virtually all cells had become dominated by the TTX-eye. In contrast, in NGF-treated animals many activated cells were found in the binocular region and the overall pattern of staining resembled that observed in normally-reared, TTX-injected animals. This result is consistent with previous electrophysiological reports showing that exogenous supply of NGF prevents the effects of monocular deprivation on the ocular dominance distribution. One possible explanation of the result is that NGF potentiates the electrical activity of the deprived pathway. To address this issue, immunodetection for zif268 was performed during the period of monocular deprivation. We found a considerable reduction of zif268 immunostaining in the monocular visual cortex contralateral to the deprived eye. In monocularly-deprived, NGF-treated animals zif268 staining was still reduced in the monocular region, suggesting that NGF does not interfere with neural activity in the visual cortex.

Supported by Italian CNR and HFSP grant RG93/93.

301.12

CHRONIC NERVE GROWTH FACTOR ADMINISTRATION ENHANCES HIPPOCAMPAL PRIMED BURST POTENTIATION IN AGED, BUT NOT YOUNG, MALE FISCHER 344 RATS. G.M. Rose[1,2*], K.L. Heman[1] and L.R. Williams[3]. [1]Dept. of Pharmacology, UCHSC and [2]Medical Research, VAMC, Denver CO 80262; [3]Amgen Neuroscience, Thousand Oaks, CA 91320

In aged rats, chronic administration of nerve growth factor (NGF) has been shown to enhance spatial learning and memory. In contrast, similar treatment of young rats has sometimes resulted in learning impairments. The goal of the present study was to evaluate the effect of i.c.v. NGF administration on hippocampal primed burst (PB) potentiation, a measure of long-term synaptic plasticity. Male F344 rats at either 3 or 23 months of age were given four weeks of i.c.v. NGF (6 μg/day) or vehicle infusion via Alzet osmotic minipump. During the fourth week, the rats were anesthetized with pentobarbital and responses were recorded from the CA1 pyramidal cell layer in response to electrical stimulation of commissural afferents. Hippocampal plasticity was assessed using PB potentiation (5 stimuli) and conventional LTP (200 stimuli). Consistent with our previous *in vitro* studies, PB potentiation, but not LTP, was significantly reduced in the hippocampus of aged rats. NGF, but not vehicle, administration enhanced PB potentiation in the aged rats. In contrast, NGF administration to young animals caused a severe attenuation of PB potentiation. Taken together, these results support the idea that alterations in hippocampal plasticity induced by aging or NGF treatment could have functional consequences, and support the involvement of cholinergic mechanisms in these processes. Supported by AG10755, the VAMRS and Amgen, Inc.

301.13

NGF-INDUCED IMPROVEMENTS IN SPATIAL WORKING MEMORY PERSIST AFTER DISCONTINUATION OF NGF INFUSION IN AGED RATS. K.M. Frick*, R.R. Sukhov, D.L. Price, V.E. Koliatsos, & A.L. Markowska. Depts. of Psychology, Pathology, Neurology, and Neuroscience, The Johns Hopkins University, Baltimore, MD 21218.

Nerve growth factor (NGF) infusion significantly reduces spatial working memory deficits in aged rats. However, the persistence of this NGF-induced memory improvement after discontinuation of infusion has not previously been assessed and is of crucial importance for the potential clinical use of this compound. The present experiment measured the duration of the NGF-induced improvement of spatial working memory, a type of memory that is significantly deteriorated in Alzheimer's disease. Spatial working memory was tested in a Morris water maze delayed nonmatch-to-position task. In addition to memory, sensorimotor (SM) skills were also examined with a battery of tasks. Four and 22 month-old rats were tested preoperatively, infused intraventricularly with recombinant human NGF or vehicle, and tested both during the 4-week infusion period and during the 4 weeks after discontinuation of infusion. NGF significantly improved working memory during the fourth week of infusion and for up to four weeks after discontinuation of infusion in 22 month-old rats only. Although NGF did not affect SM skills during infusion in either age group, SM skills were significantly improved 2 and 4 weeks after discontinuation of infusion in 22 month-old rats. These findings demonstrate that the beneficial effects of NGF on spatial working memory and SM skills can persist for up to 1 month after discontinuation of infusion and suggest that NGF can be used intermittently for the treatment of age-associated memory dysfunction and Alzheimer's disease. (Supported by NIA Program Project P5020417 and USPHS grant NS AG05146)

301.14

AUTOCRINE/PARACRINE VS TARGET-DERIVED RESPONSIVENESS OF CHOLINERGIC NEURONS OF THE BASAL FOREBRAIN TO TROPHIC STIMULATION L. Hu*, K. McLarren, S. Cote, A. Ribeiro-da-Silva and A. C. Cuello. Department of Pharmacology and Therapeutics, McGill University, Montreal, Quebec, Canada H3G 1Y6

It is well known that exogenous NGF can dramatically affect the phenotype of cholinergic neurons of the basal forebrain in normal, aged and lesioned animals. However, it is not clear whether NGF acts at the distal or the proximal ends of these neurons *in vivo*. To clarify this issue, we delivered NGF locally into the cortex or corpus striatum adjacent to the nucleus basalis magnocellularis (NBM) in naive or cortically devascularized mature rats, respectively. NGF delivered into either the cortex or the corpus striatum for two weeks caused ipsilateral hypertrophy of the cholinergic neurons of the NBM in naive rats and rescued the NBM cholinergic neurons from retrograde degeneration in lesioned animals. Thus, exogenous NGF can exert biological effects at the distal fields (terminals and distal axons) or at the proximal fields (dendrites, proximal axons and cell bodies) when offered at different regions. These findings suggest a possible paracrine or autocrine role for NGF in these NBM cholinergic neurons. This idea is further strengthened by the detection of immunoreactivity to NGF associated with the rough endoplasmic reticulum and the Golgi apparatus in ChAT-immunoreactive neurons. *In situ* hybridization studies indicate that NGF mRNA expression is abundant in the cerebral cortex (pyramidal neurons and glia), which is consistent with the well characterized target-derived role of NGF in this system. The presence of this message in the NBM area is currently under investigation . Finally, the distribution of *TrkA* antigenic sites in the basalo-cortical cholinergic pathway would indicate that besides the target-derived function of NGF, a paracrine/autocrine role of this neurotrophin is conceivable as E.M. investigation revealed *TrkA* sites in cell bodies and dendrites, which were internalized from these sites after the administration of NGF to adjacent tissue.
(Research supported by the Canadian MRC)

301.15

TRKA ACTIVATION IN THE RAT VISUAL CORTEX BY ANTI-RAT TRKA IGG PREVENTS THE EFFECT OF MONOCULAR DEPRIVATION ON OCULAR DOMINANCE DISTRIBUTION.
T. Pizzorusso, N. Berardi, K. Venstrom, L.F. Reichardt, L. Maffei. Scuola Normale Superiore; Istituto Neurofisiologia CNR, I-56127 Pisa, ITALY; Howard Hughes Medical Institute, Dept. Physiology UCSF, CA U.S.A. (SPON: European Brain and Behaviour Society).

It has been recently shown that intraventricular injection of NGF prevents the effects of monocular deprivation. We have tested the molecular nature of the NGF receptor responsible for this effect by infusing anti-rat trkA IgG polyclonal antibodies that has been shown to act as specific agonist of trkA (Clary et al. Mol. Biol. Cell, 5:549-563, 1994) into the visual cortex of monocularly deprived (MD) rats. Rats were monocularly deprived for one week at the peak of cortical plasticity (P22-P29). Anti-rat trkA IgG (1.4 mg/ml) was delivered either by osmotic minipumps (Alzet 1007D, pumping rate 0.5 µl/h) connected via a PE tubing to a cannula implanted (L 1 mm, AP 0 mm from λ) into, or by topic application every other day of fibrin soaked with 5 µl of anti-rat trkA IgG onto, the visual cortex contralateral to the deprived eye. Control rats consisted of MD rats treated with saline by either local application or minipump implant. At the end of the deprivation period ocular dominance distribution (ODD) was assessed by extracellular single cell recordings from the binocular primary visual cortex. We found that the shift in ODD towards the ipsilateral, non deprived eye was largely prevented by anti-rat trkA IgG treatment (N=5, Ipsilateral Index I.I. 0.085±0.06). Strong ODD shift was found in control animals (N=3, I.I. 0.64±0.04) with respect to normals (N=4, I.I. 0.07±0.01). These results suggest that NGF action in visual cortical plasticity is mediated by TrkA receptors.
Supported by HFSP grant RG 93/93.

301.16

CHRONIC INTRAVENTRICULAR INFUSION, BUT NOT ACUTE APPLICATION OF NGF REVERSES THE DEFICIT IN HIPPOCAMPAL SYNAPTIC PLASTICITY OF THE *ngf+/-* MICE. N. Shinsky*, M. C. Nishimura, K. S. Chen, H. S. Phillips and W.-Q. Gao. Department of Neuroscience, Genentech, Inc., South San Francisco, CA 94080.

We have previously shown that there is a significant deficit in maintenance of long-term potentiation (LTP) or late phase of LTP (L-LTP, 3 hours after tetanic stimulation), recorded from hippocampal slices prepared from adult mice, heterozygous for NGF gene deletion (*ngf+/-*) as compared to the wild-type animals (*ngf+/+*) (*Soc. Neurosci. Abstr.*, **21:** 548, 1995). In addition, the *ngf+/-* mutant mice showed a significant decrease in the magnitude of paired-pulse facilitation (PPF) obtained by giving 2 pulses with an interval of 50-250 msec. In the present experiments, we are interested in determining whether acute or chronic administration of NGF can reverse the deficits in the L-LTP and prevent the decrease in the magnitude of PPF in the *ngf+/-* mice.

While acute perfusion (2 hours) of NGF to the slices prepared from *ngf+/-* mice had no effect, intraventricular infusion of NGF by osmotic minipump (50 *ug*/ml, 0.25 *ul*/hr) for 2 weeks completely rescued the L-LTP, and partially reversed the decrease in the magnitude of PPF in the *ngf+/-* mice. Application of BDNF to the slice chamber for 2 hours elicited no effect on slices prepared from *ngf+/-* animals, although this enhanced short-term post tetanic potentiation and resulted in a rapid decay of L-LTP in slices from *ngf+/+* mice. These findings suggest that the mechanisms for actions of NGF and BDNF on synaptic plasticity are different. Most likely, NGF does not directly act on synaptic transmission in the hippocampus, but rather works indirectly by rescuing the deficit of cholinergic input to the hippocampus from the septum in the *ngf+/-* mice.

301.17

BASAL FOREBRAIN CHOLINERGIC NEURONS ARE MAXIMALLY SENSITIVE TO NGF DEPRIVATION DURING EARLY POSTNATAL DEVELOPMENT M. Molnar*, E. Tongiorgi, F. Ruberti, L. Domenici and A. Cattaneo. International School for Advanced Studies (ISAS), 34014 Trieste, Italy; Istituto di Neurofisiologia, CNR, 56127 Pisa, Italy.

Cholinergic neurons of basal forebrain (BF) present receptors for NGF and are sensitive to NGF. In many structures of CNS, including the BF region, the expression of NGF and its receptors is developmentally regulated. To test whether BF neurons depend on NGF during restricted time windows of postnatal development, we antagonized the endogenous NGF by implanting in the lateral ventricle of the rat hybridoma cells producing blocking antibodies. These antibodies do not recognize other neurotrophins. Hybridoma cells were implanted under anaesthesia at three different postnatal ages: postnatal day 2 (P2), P8 and P15. Myeloma implanted rats were used as controls. One week after implants the brain of rats was fixed by perfusion and immunohistochemistry for choline acetyltransferase developed. To assess the presence of antibodies we used an enzyme-immunoassay. We found that cholinergic neurons of BF drastically reduced in number when endogenous NGF was antagonized during the first postnatal week. Hybridoma cells implanted at P8 and P15 did not result in any significant reduction of cholinergic neurons. In parallel experiments performed in normal rats by using double immuno-in situ histochemistry we found that after the first postnatal week, virtually all BF neurons presenting trkA co-express also trkB, while at P2-4 the co-expression was not complete. We conclude that BF cholinergic neurons are sensitive to NGF deprivation during early postnatal development. We hypothesize that BF cholinergic neurons at later developmental stages, when they widely coexpress TrkA and TrkB, are sustained by multiple neurotrophic factors and therefore loose the sensitivity to a single neurotrophin, namely the NGF.
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301.18

TRANSGENIC MICE EXPRESSING NGF ANTIBODIES DURING POSTNATAL LIFE. A. Cattaneo*, F. Ruberti. Int School for Adv. Studies (SISSA) 34013 Trieste ITALY

NGF plays a role in neural plasticity both in developing and adults animals. To study the actions of NGF in postnatal life, we exploited an experimental approach based on the ectopic expression of recombinant antibodies. The monoclonal antibody aD11 was chosen as it is very efficient in neutralizing the actions of NGF, but not of other known neurotrophins, both in vitro and in vivo. The variable regions of mAb aD11 were cloned, joined to human constant regions, and placed under the transcriptional control of the cytomegalovirus early promoter. Transgenic mice were produced by microinjection of plasmid DNA into fertilized eggs. Both single and double transgenic founder mice were obtained, but the latter did not produce offspring, over a two years time period. Therefore we crossed mice transgenic for aD11 heavy chain with mice transgenic for the light chain. The resulting offspring express a functional aD11 antibody. The expression of the antibody chains was studied by ribonuclease protection analysis, immunohistochemistry and ELISA. The results show that the transgenic antibody chains are expressed in different tissues, brain, kidney, heart and spleen. The transgenic antibody chains are widely expressed in almost every CNS region of adult mice, with highest levels found in the hippocampus, cerebellum and cortex. The expression appears to be developmentally regulated, being very low during embryonic and early postnatal life, and reaching high levels only after one month. Functional circulating aD11 antibodies are undetectable in the serum of p8 mice, while their concentration reaches 50-100 ng/mL in the serum of adult mice. Also in the brain the levels of antibodies at p8 are much lower than those found in adult mice. As a consequence, classical NGF target cells (cholinergic neurons of the basal forebrain, sensory neurons in dorsal root ganglia) are spared in these mice, with the exception of a 30% reduction of neurons in superior cervical ganglia. Thus aD11 transgenic mice provide a useful model to study the role of NGF in adult mice, after a normal development has occurred.
Supported by HFSP (RG93/93)

301.19

CHOLINERGIC FUNCTION IN THE HIPPOCAMPUS OF JUVENILE RATS CHRONICALLY DEPRIVED OF NGF: AN ELECTROPHYSIOLOGICAL STUDY. E. Avignone, M. Molnar, A. Cattaneo, M. Sciancalepore* and E. Cherubini. Biophys Lab, Int Sch Adv Studies (SISSA), 34013 Trieste, Italy.

In the hippocampus, nerve growth factor (NGF) and NGF mRNA are highly expressed in regions innervated by cholinergic fibres, suggesting that this neurotrophin is a potential maintenance factor for the survival of cholinergic neurons. Responses to muscarinic receptor stimulation were studied with intracellular recordings from CA1 hippocampal neurons in slices from P15-P18 rats. In these animals endogenous NGF was antagonized by blocking antibodies, produced by hybridoma cells (αD11) implanted (at P2) in the lateral ventricle. As controls, P3U myeloma implanted rats of the same age were used. The presence of antibodies in the cerebral cortex and controlateral hippocampus was assessed with an enzyme-immunoassay (ELISA). Resting membrane potential, input resistance or time constant were similar in the two experimental groups. Stimulation of cholinergic fibres in the alveus (at 15-30 Hz, for 2 s), in the presence of the AChE inhibitor eserine (3 μM) induced slow ACh-mediated EPSPs whose amplitude and duration were similar both in the αD11- or P3U-treated animals. When the muscarinic agonist carbachol was applied directly to the postsynaptic cells in the presence of tetrodotoxin (TTX, 1μM), differences between the two experimental groups were observed. In comparison with P3U, the dose-response curve to carbachol of the αD11-treated animals was shifted to the left. The EC_{50} for carbachol was 2.6 μM in P3U- and 0.6 μM in αD11-treated animals, respectively. The increase in sensitivity of CA1 neurons to ACh might reflect a compensatory mechanism for the loss of cholinergic innervation in animals deprived of endogenous NGF during development.

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301.20

THE EFFECTS OF NERVE GROWTH FACTOR ON THE DISTRIBUTION OF TRANSGANGLIONICALLY TRANSPORTED CHOLERAGENOID AND SUBSTANCE P FOLLOWING TRANSECTION OF THE SCIATIC NERVE IN THE ADULT RAT.

N.P. Eriksson*, H. Aldskogius, G. Grant and C. Rivero-Melián
Department of Neuroscience, Karolinska Institutet, Stockholm, Sweden

In the intact rat spinal cord transganglionically transported choleragenoid does not label primary afferents in the outer part of lamina II (II_O) and only weakly in lamina I. Following transection of the sciatic nerve, however, the distribution of transganglionically transported choleragenoid occurs also in lamina II_O and increases in lamina I. The mechanism underlying this expansion is unknown buf it is believed to involve myelinated afferents. Since nerve growth factor (NGF) seems to be important as a survival factor for small-sized trkA-positive dorsal root ganglion (DRG) neurons, out of which the majority contain substance P (SP), it is conceivable that it may also affect the axotomy-induced expansion of the spinal cord projection. The aim of the present study was therefore to investigate the effect of NGF in this system with the tracer choleragenoid. The efficacy of the NGF administration was assessed by analyzing the extent of SP-like immunoreactivity.

The sciatic nerve was transected bilaterally, at which time NGF or cytochrome C was applied unilaterally during the total postoperative survival time ranging from eight to 32 days. NGF was applied in one lower and one higher dose. Two days before sacrifice, the animals were injected bilaterally with choleragenoid.

In the early postoperative period, laminae I, II_O and the zone of Lissauer on the transected, non-treated side showed choleragenoid-like immunoreactivity, while on the NGF-treated side, regardless of dosage, these regions were almost totally devoid of labelled profiles. At longer postoperative survival times this effect gradually declined in a dose-dependent fashion and after 32 days survival there was no effect. SP-like immunoreactivity was markedly reduced in the dorsal root ganglia and in the superficial part of the dorsal horn on the transected, non-treated side. On the transected, NGF-treated side this decrease was counteracted in a dose-dependent fashion.

The conclusion from this study is that peripheral nerve injury induced expansion of primary afferent choleragenoid labelling in the spinal cord dorsal horn is the result of insufficient supply of NGF to axotomized NGF-responsive dorsal root ganglion neurons. NGF can reverse these changes including the downregulation of SP in a dose-dependent fashion. This study was supported by the Swedish Medical Research Council.

301.21

ROLE OF c-*fos* IN TRANSACTIVATION OF THE CHOLINE ACETYLTRANSFERASE GENE IN NERVE GROWTH FACTOR-TREATED CULTURED BASAL FOREBRAIN NEURONS. Julie Pongrac* and R. Jane Rylett. Depts. Pharm./Toxicol. and Physiology, Univ. Western Ontario, London, Canada.

Basal forebrain neurons respond to nerve growth factor (NGF) by enhancing the cholinergic phenotype. This occurs through increases in choline acetyltransferase (ChAT) activity, high-affinity choline transport and acetylcholine (ACh) synthesis, content and release. Increases steady-state levels of ChAT mRNA following NGF administration have been measured in basal forebrain *in vivo* and *in vitro*, thus a plausable mechanism for NGF-mediated increases in cholinergic phenotypic markers involves transcription. This hypothesis is further advanced by the presence of NGF-responsive elements in the promoter region of ChAT gene sequences containing AP-1 sites. Immediate early genes (IEGs) known to be induced by NGF in PC12 cells include c-*fos*, c-*jun* and *jun*-B of which the protein products combine to form AP-1 transcription factors. We determined the role of transcriptional events, in particular, the induction of c-*fos*, in NGF-mediated increases in ChAT activity in non-transformed cholinergic neurons. Using rat basal forebrain neurons cultured in chemically defined medium, blockade of c-*fos* with antisense oligonucleotides and assays of ChAT activity, we show that c-*fos* participates in the NGF-mediated changes in ChAT activity. NGF (100 ng/ml) increased ChAT activity of cultures greater than 2 fold above basal levels as measured by radioenzyme assay. In sister cultures, 20 μM of antisense oligonucleotides targeting the initiation codon of c-*fos* were administered 6 and 12 h prior to NGF treatment. This resulted in a 34% and 94% reduction in the NGF-mediated ChAT activity, respectively. Similar treatment with 20 μM of a mismatch oligonucleotide control did not block increases in ChAT activity mediated by NGF. These experiments suggest that c-*fos* transactivation of the ChAT gene is involved in NGF-mediated changes in the cholinergic phenotype. (Sponsored by MRC Canada and Ontario Mental Health Foundation)

301.22

INTRASEPTAL INJECTION OF NGF LOCALLY EFFECTS EDEMA FORMATION AND MAST CELLS FOLLOWING SEPTAL INJURY IN RATS.

L.S. Janis*[1], J.W. Heyburn[1], Z. Fulop[2], and D.G. Stein[2]. [1]Inst of Animal Behav, Rutgers University, Newark, NJ 07102 and [2]Dept of Neurology, Emory Univ Sch of Med, Atlanta, GA 30322.

Following injury to the central nervous system, NGF is generally thought to induce recovery through cholinergic neurons. However in models of a single administration of NGF, behavioral recovery is often seen without the sparing of cholinergic neurons. Alternatively, NGF may induce recovery by mediating immune reaction. In order to examine this possibility, the formation of edema and the presence of mast cells were examined. Rats with electrolytic lesions of the medial septum received a single intraseptal injection of either NGF or saline immediately following surgery. The formation of edema was then assessed at 6, 24, 48, 72, and 120 hr's following the lesion. Edema formation was detected as early as 6 hr's in both groups. However, by 48 hr's following surgery, the NGF-treated rats had significantly less edema formation than their lesion alone counterparts. We also examined the septum for the presence of mast cells. Mast cells were identified near the damaged tissue and vascular walls in both groups. Initially, by 2 hr's the NGF-treated rats had more cells than the lesion alone rats. However, by 72 hr's fewer mast cells were seen in the NGF rats compared to the lesion alone rats. These results can be taken to indicate that NGF may alter locally mediating events that limit the effects of reactions associated with the inflammation of damaged tissue.

Supported by GenRe Corp. research grant.

301.23

NERVE GROWTH FACTOR (NGF) ENHANCES TRANSMISSION AT A DOPAMINERGIC SYNAPSE IN THE MOLLUSC, *LYMNAEA STAGNALIS*.

N.S.Magoski* and A.G.M.Bulloch. Neuroscience Research Group, The University of Calgary, Calgary, Alberta, T2N 4N1, Canada.

We examined an excitatory synapse from interneuron Right Pedal Dorsal one (**RPeD1**) to neurons Visceral Dorsal two and three (**VD2/3**) in the *Lymnaea* CNS. The synaptic latency was relatively rapid, at 24.1 ± 5.2 ms (n=27), and the synapse appeared to be dopaminergic. The RPeD1 → VD2/3 EPSP and the VD2/3 bath-applied dopamine (100 μM) response displayed a similar decrease in input resistance (n=5 & 7) and a similar predicted reversal potential (-31.1 mV vs -25.5 mV; n=7 & 7), suggesting that the synapse and exogenous dopamine activate the same conductance. In addition, as previously shown (J Neurophysiol 74:1287), the synapse and the dopamine response were blocked by the D-2 antagonist, (±) sulpiride (100 μM; n=11 & 6). There is evidence for the involvement of neurotrophic factors in the plasticity of *Lymnaea* neurons (Neth J Zool 44:317). To explore this further, the effect of 1 day organ culture in either defined medium (**DM**), brain-conditioned medium (**CM**), or defined medium supplemented with 400 ng/ml of 2.5s murine Nerve Growth Factor (**NGF**), on the magnitude of the EPSP, was tested. The EPSP was measured at the same synapse before, and after, 1 day of organ culture. Following culture in CM, the EPSP increased by 100.0% (n=9), while in parallel controls, the EPSP showed no significant change following culture in DM (n=12). NGF mimicked the effect of CM, with the EPSP showing an increase of 46.2% following culture in NGF (n=18). Again, in parallel DM controls the EPSP showed no significant change (n=15). None of the culture conditions had a significant effect on the membrane potential or input resistance of VD2/3. The ability of NGF to mimic the effects of CM indicates that the component of CM responsible for enhancement of synaptic efficacy is likely an NGF-like molecule. This provides further evidence for the presence of neurotrophins in *Lymnaea*, and suggests that an NGF-like molecule may regulate synaptic transmission in the adult CNS.

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301.24

SHORT TERM ACTIONS OF NGF AND EXTRACELLULAR MATRIX PROTEINS ON HIGH VOLTAGE ACTIVATED CALCIUM CURRENTS OF MOLLUSCAN MOTONEURONS. W.C. Wildering* and A.G.M. Bulloch. Neuroscience Research Group, HSC, University of Calgary, Calgary, Alberta, T2N 4N1, Canada.

Previously, we demonstrated that mouse 2.5S Nerve Growth Factor (NGF) modulates high-voltage activated Ca^{2+} currents in molluscan motoneurons within minutes (Wildering et al. 1995, J. Neurophysiol. 74: 2778). More recently we extended our studies to the short term actions of extracellular matrix (ECM) proteins on neuronal physiology and their possible interaction with neurotrophin signalling. Motoneurons taken from the brain of the gastropod mollusc *Lymnaea stagnalis* were plated in defined medium on untreated plastic culture dishes. One day after plating HVA Ca^{2+} currents were recorded using standard gigaseal whole cell voltage clamp techniques. Addition of fibronectin (0.1 μg/ml) to the superfusing saline induced a change in the amplitude and inactivation properties of the HVA Ca^{2+} current within a few minutes. The effect of fibronectin appeared to be specific since it could be mimicked by superfusion of synthetic analogous of the integrin binding motive (RGD-peptides). These results raise the possibility that in addition to neurotrophins, ECM proteins may also be involved in short term modulation of neuronal physiological properties.

Supported by MRC, AHFMR and the NCE Neuroscience Network (Canada).

301.25

TRK RELATED TYROSINE KINASE RECEPTORS CLONED FROM THE *LYMNAEA* CNS. A.G.M.Bulloch*, R.E.van Kesteren, G.Hauser, M.Fainzilber, C.Ibanez, J.van Minnen, A.B.Smit, W.P.M.Geraerts and E.Vreugdenhil. Neuroscience Research Group, Univ. Calgary, HSC, Calgary, AB, T2N 4N1 Canada; Molecular Neurobiology, Berzelius Laboratoriet, Plan 3, Karolinska Institute, Doktorsringen 12A, S 17177 Stockholm, Sweden; Faculty of Biology, Vrije Universiteit, 1081 HV, Amsterdam, The Netherlands; The University of Leiden, Leiden, The Netherlands.

Neurons from the CNS of the mollusc *Lymnaea* have been shown to respond to the mammalian neurotrophic factors, NGF and CNTF. In order to analyze the transduction mechanism involved in the response of these neurons to NGF, a PCR strategy was employed to search for receptors related to the trk family of tyrosine kinase neurotrophin receptors. Primers based on conserved sequences in the tyrosine kinase domain were used to amplify cDNA templates from the CNS. A number of tyrosine kinase products were obtained and sequenced, and homology searches suggest that the products obtained are similar to a number of tyrosine kinase receptors. A full length sequence from a cDNA clone representing one of the trk-like products was obtained revealing the entire intracellular and extracellular domains of this receptor, which is designated as Ltrk. The intracellular domain of Ltrk shows highest homology to trkA; strikingly this homology is much closer than the homology between Ltrk and the non-neurotrophin receptor-like gene from *Drosophila*, Dtrk. The extracellular domain of the Ltrk protein is amino-terminally extended by ~100 amino acids, the existence of which was confirmed by *in vitro* translation. Northern blot analysis revealed that one Ltrk transcript is expressed in the CNS, which is not up-regulated following injury. *In situ* hybridization showed expression of this receptor by a number of neurons and a group of endocrine cells. Efforts are underway to express these clones for functional expression studies. This is the first molecular data supporting the existence of the neurotrophin receptor family in the invertebrates, supporting previous indications that these molecules emerged early in evolution. Supported by MRC (Canada)

HORMONES AND DEVELOPMENT: SEX STEROIDS

302.1

IMPLICATIONS OF FOS IMMUNOREACTIVITY IN THE PREOPTIC AREA OF NEONATAL RATS. H.R. Besmer[1]*, G.M.O. Keidan[2], R.B. Gibbs[3], and M.M.McCarthy[1], [1]Dept. of Physiol., Univ. of MD, Baltimore, MD, [2]Dept. of Biol., Coll. of Wooster, Wooster, OH, and [3]Univ. of Pitt. Sch. of Pharm., Pittsburgh, PA.

The sexually dimorphic nucleus of the preoptic area (SDN-POA) is 5-7 times larger in male rats than in females. The mechanisms that establish and maintain this sex difference are largely unknown. During the postnatal critical period for sexual differentiation, the dam grooms the anogenital region of pups to stimulate urination and defecation. Male pups are groomed significantly more often than females and this is critical for expression of male sexual behavior in adulthood. To test the hypothesis that anogenital grooming is important to brain sexual differentiation, we stimulated the anogenital region of 3-day-old pups (PN3) with a stiff-bristle paint brush. Control animals were handled in the same manner but not stimulated. One hour later brains were removed and processed for Fos-like immunoreactivity (Fos-ir) as an indicator of neuronal activation. Significantly more Fos-ir cells were detected in the POA of stimulated animals than in controls (2-way ANOVA, $p<.05$). There was no sex difference in Fos-ir cells in either control or stimulated animals. The majority of Fos-ir cells detected was found in the ventral POA.

Sex differences in the volume of SDN-POA are likely related to differential cell death during postnatal development. In a separate study, brains from 3-, 5-, 7-, and 12-day-old animals were examined for correlations between Fos expression and cell death. No Fos-ir cells were detected in SDN-POA of either sex. In addition, there was no sex difference in the number of pyknotic cells detected in the SDN-POA. However, when the area surrounding the SDN-POA of females was also included so as to equal the survey area of males, significantly more pyknotic cells were detected in 5-,7-, and 12-day-old females than in the SDN-POA of males (2-way ANOVA, $p<.01$), with a maximum 3-fold difference detected on PN7.

These data suggest that maternal anogenital grooming activates neurons in the POA, possibly decreasing cell death and thereby contributing to masculinization of this region of the brain. *Supported by R29MH52716 (MMM) and R01NS28896 (RBG).*

302.2

SEX DIFFERENCES IN HORMONAL RESPONSIVENESS OF ARCUATE ASTROGLIA IN NEONATAL RAT BRAIN. J.A. Mong*, A.M. Davis, R.L. Kurzweil, and M.M. McCarthy. Departments of Pharmacology and Physiology University of MD Baltimore, MD, 21201.

It is established that estrogen mediates sexual differentiation of the brain via direct effects on neurons. However, *in vitro* studies indicate that estrogen induces astroglial differentiation. The present study investigates the effect of estrogen on astroglia *in vivo* during the critical developmental period.

Based on glial fibrillary acidic protein immunoreactivity (GFAP-ir), the surface area of astroglial cells in the arcuate nucleus of the hypothalamus was measured and compared between five hormonally different groups; 1) castrate males injected with testosterone propionate (TP, 50µg), or 2) oil vehicle, 3) gonadally intact males injected with oil vehicle, 4) gonadally intact females injected with TP, or 5) oil vehicle. All gonadally intact animals received sham operations. The treatments were performed on the day of birth (PN 0), and the animals were sacrificed on PN 1. The surface area of GFAP-ir cells was evaluated with computerized video densitometry. The data demonstrate an increased GFAP-ir surface area in castrate males compared to gonadally intact females and gonadally intact males. Moreover, intact females have an increased surface area compared to intact males (ANOVA $p<0.01$) The increase in GFAP-ir surface area seen in castrate males was attenuated by TP administration and resulted in GFAP-ir surface area comparable to intact males. Finally, TP administered to intact females was without effect in this paradigm. There was no difference in astroglial cell number between groups.

It is hypothesized that the absence of testosterone during a critical period in the developing male brain results in a loss of astroglial differentiation. The cells have a stellate morphology but their processes are short and thick. In contrast, the glial processes are thin and extended in intact males and castrate males injected with TP. We further hypothesize that in intact females, glial cells remain partially undifferentiated, which may be important to the glial plasticity seen in the adult female arcuate. *Supported by NSF grant 1BN-9511328 to MMM.*

302.3

EFFECTS OF NEONATAL ADMINISTRATION OF THE NEUROSTEROID ALLOPREGNANOLONE ON THE VOLUME OF THE SDN-POA IN RATS. M.J. Sickel*, and M.M. McCarthy, Dept. of Physiology, Univ. of Maryland at Baltimore School of Medicine, Baltimore, MD

The SDN-POA, or sexually dimorphic nucleus of the preoptic area, is an intensely staining nucleus in the hypothalamus that is 2-8 times greater in volume in male rats than in female rats, and has a dense concentration of $GABA_A$ receptors. These receptors are known to have sexual dimorphic modulation in the adult rat. Allopregnanolone, a neurosteroid metabolite of progesterone, is a positive allosteric modulator at the $GABA_A$ receptor. Because of the high density of $GABA_A$ receptors in the SDN-POA, we decided to investigate the neuroanatomical effects of neonatal allopregnanolone administration on the volume of the SDN-POA.

Once the rats were anesthetized with cold, we subcutaneously administered 5 µg or 200 µg doses of allopregnanolone in a .05 ml sesame oil vehicle, or just the sesame oil vehicle, daily from PN2 through PN5. Injections were at 2 hours after the start of the dark phase of the light cycle. After sacrificing the rats at PN22, we collected and sectioned the brains, and stained the sections with cresyl violet. We measured the volume of the SDN-POA using camera lucida drawings and NIH Image software. The data indicate a strong trend that allopregnanolone decreases the volume of the SDN-POA in both males and females (t-test, $p<.08$). There was evidence for a dose response in males, but not in females. In males, the 5 µg dose of allopregnanolone caused a 67% decrease in volume while the 200 µg dose caused an almost 80% decrease in volume. We hypothesize that the SDN-POA is modulated by changes in GABAergic neurotransmission during the critical period for sexual differentiation. Additional studies will replicate and extend these findings. This research was supported by grant R29MH52716 to MMM.

302.4

LONG-TERM SURVIVAL OF SONG CIRCUIT ELEMENTS IN CULTURED SLICES OF ZEBRA FINCH BRAIN. C. C. Holloway and D. F. Clayton* Dept. of Cell and Structural Biology and Beckman Institute, Univ. of Illinois, Urbana, IL 61801.

The avian song control circuit is an attractive model for study of how specific brain circuits are formed in development. The organization of the circuit involves discrete nuclei rather than cortical areas or lamina, and some of these nuclei become connected only after the rest of the brain has developed (25 to 35 days post-hatch). In the zebra finch, a critical component of the circuit is formed only in males but not in females: the projection from nucleus HVC to RA. The goal of the present study is to define conditions for the *in vitro* maintenance of brain slices containing this projection, in order to determine the molecular signals and mechanisms responsible for its selective formation in juvenile males. Brain slices are made by cutting the brain parasagitally every 400 µm on a MacIlwain tissue chopper. The slices are maintained in a static culture system (Stoppini et al., J. Neurosci. Meth. 37: 173, 1991), in media developed for zebra finch primary cultures (Goldman et al., J. Neurosci. 12: 2532, 1992) in a 5% CO2 incubator at 37°C. ^{35}S-methionine incorporation studies show that cells in the slice stay alive for at least six weeks *in vitro*. Axon-tracing studies using the lipophilic dye DiI show that the HVC-RA connections in an adult male brain are maintained during this time in culture as well. Long-term survival of neurons in these slices is further supported by sustained immunoreactivity against the neuron-specific antigens neurofilament (an intermediate filament of the cytoskeleton), MAP-2 (a protein associated with stabilization of microtubules), and synelfin (a pre-synaptic marker). Supported by NS25742

302.5

PRE-HATCHING INHIBITION OF AROMATASE ACTIVITY MASCULINIZES SYRINGEAL AND GONADAL TISSUE BUT NOT THE SONG SYSTEM IN ZEBRA FINCH FEMALES.
A. Gong* and A. P. Arnold. UCLA Dept. of Physiological Science and Brain Research Institute, Los Angeles, CA 90095-1527

To test how gonadal secretions affect sexual differentiation of the Zebra Finch song system, we injected Fadrozole (CGS 16949A), an aromatase inhibitor, into eggs on day 3 of incubation; controls received saline. Since ovarian development is estrogen-dependent, fadrozole injection on day 5 induced the formation of large amounts of testicular tissue in addition to ovarian tissue in genetic females (Wade & Arnold 1996). Therefore, we chose the earlier treatment date of day 3 in the hope of completely preventing development of ovarian tissue, in order to determine whether genetic females with completely sex-reversed gonads will develop a masculine song system. We examined the syrinx, gonads and song system nuclei in 100 days-old birds. Treated females possessed significantly larger syringes, a left ovotestis and a right testis. Despite the large amount of testicular tissue, the volumes of song system nuclei (HVC, RA) did not differ from control females. These results suggest that testicular secretions masculinize the syrinx, but are not sufficient to masculinize the song system in Zebra Finches. The results are compatible with the hypothesis that ovarian secretions prevent masculinization of the song system.

Supported by NIH grant DC00217.

302.6

IN OVO ANTIANDROGEN OR ANTIESTROGEN DOES NOT ALTER THE DEVELOPMENT OF THE SONG SYSTEM IN MALE ZEBRA FINCHES.
W. Grisham*, & A.P. Arnold. Dept. of Physiological Science & Brain Research Institute, UCLA, Los Angeles, CA 90095-1527.

Although exogenous estrogen has been shown to masculinize the development of the song system when administered to hatchling females, few studies have examined which hormones, if any, are responsible for normal masculine development in male zebra finches. In an effort to answer this question, we injected 20 microgram doses of an antiandrogen (flutamide), or an antiestrogen (ICI 182,780--ICI Pharmaceuticals), or the steroid suspension vehicle alone (SSV--10 microl) into eggs on day 10 of incubation and examined these birds in adulthood (94-124 days old).

In all birds, plumage was consistent with gonadal sex, and the gonads appeared to be macroscopically normal. The syringes were dissected and weighed in flutamide-treated (11 male and 7 female), ICI 182,780-treated (17 male and 7 female), and SSV-treated (5 male and 8 female) birds. Males had significantly heavier syringes, but there was no effect of treatment or sex X treatment interaction. The brains of males that were flutamide treated (n=6), ICI 182,780-treated (n=7), or SSV-treated (n=6) were embedded, sectioned at 40 microns, and stained with thionin. There was no effect of treatment on the volumes of Area X, lMAN, HVC, or RA; soma size in lMAN, HVC, or RA; or number of neurons in RA. These results offer no support for the importance of androgen or estrogen in organizing normal male development of gonadal, syringeal, or neural tissue in this species at this time of incubation. Supported by USPHS DC00217 to A.P.A.

302.7

INFLUENCE OF SEX STEROIDS ON THE DEVELOPMENT OF A SEXUALLY DIMORPHIC PROJECTION FROM THE BED NUCLEUS OF THE STRIA TERMINALIS TO THE ANTEROVENTRAL PERIVENTRICULAR NUCLEUS OF THE RAT.
L.A. Hutton*, G. Gu and R.B. Simerly. Division of Neuroscience, Oregon Regional Primate Research Center, Beaverton, OR 97006, and Department of Cell and Developmental Biology, Oregon Health Sciences University, Portland, OR 97201.

In addition to modulating the activity of neural circuits in the limbic system and hypothalamus, sex steroid hormones also influence the development of sexually dimorphic pathways. The principal nucleus of the bed nuclei of the stria terminalis (BSTp) is larger in male rats, and contains a high density of neurons that express sex steroid receptors throughout postnatal development. In males, the BSTp provides a massive projection to the anteroventral periventricular nucleus of the hypothalamus (AVPV), which in contrast to most sexually dimorphic nuclei contains more neurons in female rats. Although the sexual differentiation of the BSTp and AVPV has been shown to be dependent on the neonatal hormone environment, little is known about the impact of sex steroids on the development of the sexually dimorphic pathway between these nuclei. DiI was used to study the ontogeny of the projection from the BSTp to the AVPV in fixed tissue derived from rats perfused on postnatal days 0-10 (P0-P10). In male rats the projection from the BSTp to the AVPV is established between P7 and P10 and is maintained during the juvenile period (P22) and into adulthood. A similar connection is not apparent in female rats at any of the ages studied. Surprisingly, treatment of female rats with high doses of testosterone from P0 to P10 did not induce a connection between the BSTp and the AVPV suggesting that this sexual dimorphism is refractory to postnatal steroids, or is determined prenatally. Supported by NIH grants MH49236 and RR00163.

302.8

POSTPUBERTAL GONADECTOMY AND HORMONAL TREATMENT ON EEG OF RATS.
I. del Río-Portilla, E. Ugalde, J. Juárez, A. Roldán, C. Arce and M. Corsi-Cabrera*. Facultad de Psicología. Universidad Nacional Autónoma de México, México, D.F. 04510, Instituto de Neurociencias Universidad de Guadalajara.

When compared to females, males show higher theta relative power (RP), lower delta and beta RP, higher interparietal correlation (IPC) and similar absolute power (AP). These sex differences are dependent on hormonal oscillations during estrous and on organizing actions of testosterone. Fetal androgen exposure masculinize the female EEG abolishing sex differences.

This study was conducted to determine if EEG sex differences are influenced by postpubertal gonadal steroids. EEG was recorded at 100 days of age in males and females gonadectomized (GNX) at 80 days without and with either vehicle, testosterone propionate (TP) or 5α-DHT in males and vehicle, progesterone (P) or estradiol (E) in females.

Sex differences in RP and IPC were abolished after GNX, however, GNX induced higher AP in females than males. TP induced higher AP abolishing sex differences. Isolated TP, 5α-DHT, E or P did not reestablish sex differences in RP and IPC. Sexual dimorphism in EEG need, in addition to organizing effects, the activation actions of gonadal steroids.

302.9

ESTRADIOL ACTIVATES IMMEDIATE EARLY GENES IN CULTURED RAT HIPPOCAMPAL NEURONS.
D.D. Murphy* and M. Segal. LN, NINDS, NIH, Bethesda MD 20892, and The Weizmann Institute, 76100 Israel

We have previously shown that 17-b-estradiol causes a two-fold increase in dendritic spine density in cultured rat hippocampal neurons, which is accompanied by a concomitant increase in synaptophysin immunoreactivity (Murphy and Segal, J. Neurosci. in press, 1996). Presumably, the formation of new spines and synapses requires the addition of new plasma membrane and synaptic proteins. We began studying the chain of events leading from the estrogen receptors to the formation of new spines by examining estradiol-treated hippocampal cultures for indicators of immediate early gene activation, phosphorylated cAMP response element binding protein (pCREB) and CREB binding protein (CBP). Cells were fixed and stained for CBP and p-CREB at various time points during a 24 hour exposure to estradiol. The proportion of stained neurons and glia, as well as the intensity of staining were assessed in confocal-reconstructed cells. Only a low proportion of cells (about 15% of neurons and 30% of glial cells) were stained for CBP in control, non-drugged conditions. Glial cells displayed a significant increase in CBP staining at 16 hours of estradiol treatment (80% of cells stained) while neurons displayed the greatest proportion of staining at 24 hours (83% of the cells stained, staining intensity was 228% of that in control conditions). At this time, only 42% of glial cells stained for CBP. As seen before for the effects of estradiol on spine formation, the N-methyl D-aspartate antagonist, 2-APV, partially blocked the CBP response to estradiol, while the AMPA/KA antagonist, DNQX was ineffective. p-CREB staining was similar to that of CBP, although the magnitude of estradiol-induced staining was lower than that of CBP. Taken together, these results indicate that p-CREB and CBP may be intermediate steps in the formation of new spines in response to estradiol.

Supported by NIH Intramural Research Program.

302.10

NEONATAL ANDROGENS AFFECT ADULT SPATIAL BEHAVIOR AND CA3 PYRAMIDAL CELL MORPHOLOGY IN RATS: A GOLGI STUDY.
C. Isgor* and D.R. Sengelaub. Department of Psychology, Indiana University, Bloomington, IN 47405.

We have previously reported androgen-sensitive sex differences in adult spatial learning and hippocampal (CA3) volume (male>female; androgen-treated female>female; male>castrates), which were partly due to increases in pyramidal soma size and density. In this study we examined changes in spatial learning and dendritic morphology of CA3 pyramidal cells after neonatal hormonal manipulations.

Spatial learning (using a water-maze) and CA3 pyramidal cell morphology were assessed in adulthood (90+ days of age) in normal males and females, androgen-treated females (testosterone propionate, 1 mg/day, postnatal (P) day 3 and 5), gonadectomized males and females (P2), as well as castrated males with replacement androgen (n=6 per group). Consistent with our previous results, acquisition of water-maze performance was observed only in high-androgen groups. After behavioral testing, brains were stained using the Golgi technique, and dendritic morphology of CA3 pyramidal cells was reconstructed in three dimensions (Eutectic NTS). Pyramidal cells of high-androgen groups had significantly greater total dendritic lengths and number of branches. No effects were observed in spine density, but total number of spines per neuron was greater in high-androgen groups due to increases in dendritic length. Similar findings were found in both apical and basilar dendrites. Thus, neonatal androgens establish the sex difference in adult spatial learning and CA3 volume through regulating neuronal soma size, density, and dendritic morphology. Supported by Sigma Xi and NSF DIR-901427.

302.11

LOCAL INHIBITORY CIRCUITS UNDERGO AGE- AND SEXUAL HORMONE DEPENDENT CHANGES. R.W.Greene[1], D.G.Rainnie[1*], M. Berger[2], R.W.McCarley[1*] and H.C.R. Grunze[1,2]. [1]Harvard Medical School & VAMC Brockton, Brockton MA, [2]Psychiatrische Universitätsklinik, 79104 Freiburg, Germany

We have previously reported an NMDA-dependent modulation of local inhibitory circuits in the rat hippocampal CA1 layer, and its potential impact on information processing (Grunze et al., 1996). As these data were obtained from prepubescent rats, aged 20-30 d, we now compared these results with those obtained from 9-12 month old rats of both gender, and with 9-12 month old rats who were castrated prior to puberty. Whole cell patch clamp recordings were obtained from CA1 pyramidal neurones, manually held at -60 mV, and the IPSP amplitude in response to alvear stimulation was measured. Recordings from prepubescent rats (n=72) showed an IPSP with a mean maximal amplitude (± SE) of 4.5 ± 2.8 mV. In contrast, the IPSP of older rats was observed with a reduced amplitude of 1.1 ± 0.9 mV. All neurones showed an EPSP of equal size compared to prepubescent controls. A statistically significant gender difference was not observed in the old rats, although there was a tendency toward IPSPs with higher amplitudes in females. However, age matched castrated rats (n=14), showed an IPSP with a mean amplitude of 2.4 ± 1.3% which was significantly larger than that of the control old rats ($p < 0.25$, Mann-Whitney test). Furthermoré, the metabotropic glutamate receptor agonist, trans-ACPD (5μm, n=12), applied to the superfusate increased the IPSP amplitude in castrated rats by 35 ± 27%, an effect not seen in young control animals. On the other hand, the reduction of the IPSP amplitude by APV (25μm) was comparable to young rats, (49 ± 19% vs. 31± 9%). Data for these chemical modulations of the IPSP have yet to be obtained from old, non castrated rats. In conclusion, these data suggest that hippocampal inhibitory local circuits undergo age dependent changes, possibly with an important modulatory role of sexual steroids.
Supported by the Department of Veterans' Affairs

302.12

Rapid and transient downregulation of a cerebral cortical gene by estrogen identified by differential display PCR. G.M. Berbary, K.W. Peebles, & F. Sohrabji* Dept. of Human Anatomy & Medical Neurobiology, Texas A&M University Health Science Center, College Station, TX 77843.

We are interested in the molecular actions of estrogen on forebrain cholinergic circuits that mediate cognitive functions. Our current strategy is based on the analysis of novel estrogen-responsive genes, with a view to identifying early events in a hormone-stimulated cellular cascade. Here we describe a cerebral cortical gene rapidly and transiently downregulated by estrogen. Adult ovariectomized (OVX) females were exposed to either estradiol (10 ugs) or vehicle (sesame oil) and sacrificed 4 (4h) or 52 (52h) hours later. DNase-treated cerebral cortical RNA was reverse transcribed and amplified using a modification of the differential display technique. Gel autoradiograms revealed a ~650bp cDNA strongly expressed in OVX animals and to a lesser extent in the 52h group. However, this cDNA was conspicuously absent in the 4h group. Northern blot analysis, using this cDNA as probe, revealed hybridization to 18S rRNA as well as a ~4.2 kb transcript. While hybridization to rRNA was unchanged by estrogen treatment, hybridization to the 4.2 kb transcript was almost absent in the 4h group. Restriction mapping of this cDNA based on sequence analysis of a single clone indicated the presence of two separate cDNAs each of 650bp. One cDNA was found to be identical to 18 rRNA. Sequence analysis of the other cDNA (called C650β) indicated significant (65%) homology to the human gene coding for iduronate sulfatase (IDS). IDS has been linked to Hunter's syndrome, a genetic disorder characterized by short stature, mental retardation and deafness. Cerebral cortex-specific cDNA libraries are currently being screened to identify the full length clone related to C650β, and to determine its relatedness to IDS.
Supported by funds from the Texas A&M University Health Science Center.

302.13

ESTROGEN REGULATION OF FAS/APO-1 mRNA EXPRESSION IN EXPLANT CULTURES OF THE DEVELOPING CEREBRAL CORTEX. Z.F. Cheema* and R.C. Miranda, Dept. Human Anatomy and Medical Neurobiology, Texas A&M University College of Medicine, College Station, TX 77843-1114

The cerebral cortex expresses high levels of the estrogen receptor transiently during development. Estrogen receptor levels peak during the first postnatal week and decline thereafter. Peak estrogen receptor levels correspond to the occurrence of apoptosis in the cerebral cortex. We therefore, examined the role of estrogen in the regulation of a key cell suicide receptor, the Fas/Apo[apoptosis]-1 receptor. Fas initiates apoptotic cell death in lymphoid tissue without *de novo* mRNA or protein synthesis, suggesting that this receptor is mechanistically proximate to the initiation of cell death. Organotypic explants of the peri-septal cerebral cortex, cultured from postnatal day one rats, were maintained in a humidified roller tube assembly for 6 days. Estrogen was administered on days 2, 4 and 6. Fas mRNA-expressing cells were identified by *in situ* hybridization. Estrogen administration significantly ($p<.05$) increased (by 360%) the number of cells expressing Fas mRNA in the cingulate cortex. In contrast, retinoic acid administration did not yield a significant increase in the numbers of Fas mRNA-expressing cells. These results suggest that estrogen may rescue Fas mRNA-expressing cells that ordinarily die in control cultures. Our current research is focused on the elucidation of the intracellular signaling pathways mediated by Fas in the developing cerebral cortex. *Supported by funds from Texas A&M Health Science Center.*

302.14

SEX STEROID RECEPTORS AND NITRIC OXIDE SYNTHASE IN THE PREMAMMILLARY NUCLEUS OF THE NEWBORN MALE AND FEMALE RAT Makoto Yokosuka* and Shinji Hayashi Dept. Anat. Embryol, Tokyo Metropol. Inst. Neurosci, Tokyo 183.

The distribution of the estrogen- and androgen receptors (ER and AR), aromatase (AROM) and neuronal nitric oxide (NO) synthase (nNOS) was studied in dorsal and ventral premammillary nuclei (PMd and PMv) of the newborn (the day of birth to postnatal day 7) rat by immunohistochemistry. In the intact male, nNOS immunoreactivity (-IR) was present both in the PMd and PMv, while AR-IR was detected only in the PMv. On the other hand, ER-IR and AROM-IR were scarcely encountered in the both PMd and PMv. By double immunostaining of AR and nNOS, all the nNOS-IR cells in the PMv contained AR-IR. In the intact female, the nNOS-IR was also present in the both PMd and PMv, but neither ER-, AR-, nor AROM-IR were detected. When the male pup was castrated neonatally, no AR-IR was detected in the PMv. Subcutaneous injections of 5-alpha-dihydrotestosterone induced strong AR-IR in the castrated male and the intact female pups. On the contrary, the intensity of nNOS-IR stayed unchanged among these animals.

Neonatal androgen and NO has been considered important to brain development. Moreover, in male rodents, involvement of PMv in aggression and mating behavior has been reported. Together with the fact that the AR-IR and nNOS-IR were found in the same neurons in the PMv, involvement of this nucleus in masculinization of the brain by non-aromataizable androgen is suggested.

302.15

DISTRIBUTION AND ONTOGENY OF NADPH-DIAPHORASE POSITIVE NEURONS IN THE RAT BRAINSTEM J.X.Zhu, L.Lin, M.F.Zhou, H.Zhang, X.T.Zhou and Z.P.Sun*. Departments of Physiology and Anatomy, Henan Medical University, Zhengzhou 450052, P.R.C

The experiment studied the distribution of NADPH-diaphorase(NADPH-D) positive neurons of brainstem in adult rats and the ontogeny of them in rats of the first postnatal month with NADPH-D histochemistry and computer graphic analysis system. Adult rats of 60 days and rats at the ages of 1,3,5,7,9,13,20 and 30 days were used. The rats were treated with routine method, and the frozen sections of the brainstem were stained for NADPH-D activity. In the adult rats the NADPH-D positive neurons mainly distributed densely in the pedunculopotine tegmental nucleus (PPTg), laterodorsal tegmental nucleus (LDTg), dorsal raphe nucleus (NDR), laterodorsal central gray(LDCG) and superficial gray layer of superior colliculus (SuG). A few of positive neurons were found in the interpeduncular nucleus (NIPC). On the first day after the rats' birth, the NADPH-D positive neurons were found only in the LDTg. From the second day after birth the positive neurons also appeared in the PPTg,NDR,LDCGand SuG. In the first 9 postnatal days the numbers and density of the neurons increased with the age, reaching a peak at the 9th day after birth. There was no obvious difference between the adult rats and the rats at the ages of 9,13,20 or30 days. the results indicated that NO may take part in the functional regulation and developing process of the brainstem.

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303.1

AFFERENT ARRIVAL AND THE ONSET OF FUNCTIONAL ACTIVITY IN THE TRIGEMINOTHALAMIC PATHWAY OF THE RAT

C.A. Leamey, S. M. Ho and R.F. Mark*, Developmental Neurobiology, RSBS, ANU, Canberra, 2601, ACT Australia

An *in vitro* slice preparation has been developed which permits the study of the anatomical and functional development of the trigeminothalamic pathway. Wistar rats ranging between embryonic day (E)15 and postnatal day (P)2 were used. The anatomical development of the trigeminothalamic pathway was traced with 3% biocytin. Tracing studies revealed that the most rostral trigeminal fibres had reached the cephalic flexure by E15, and by E16 were in the diencephalon, ventral to the thalamus. By E17 a few fibres had entered the thalamus where they terminated in unbranched growth cones. Fibres had begun branching by E18 and arbors were more elaborate by E19. Functional development was studied by recording evoked potentials from VPM following stimulation of the trigeminal nerve (5n) and principal sensory nucleus (Pr5). Postsynaptic responses to stimulation of 5n and Pr5 could first be recorded at E17. Reliable responses to stimulation of 5n were recorded from E18 onwards. Stimulation of Pr5 permitted both axonal and postsynaptic responses to be recorded in VPM. The postsynaptic response was reversibly blocked by the addition of 3mM cobalt. The addition of 50μM bicuculline increased the amplitude and duration of the response at all ages studied. Much of the excitatory response was blocked by application of 50μM AP5 (NMDA receptor antagonist) in animals P0 and younger. By P1, a significant component of the response was insensitive to AP5. This latter component was blocked with DNQX (10μM; non-NMDA antagonist). In conclusion, these results demonstrate that the capacity for axonal conduction in the trigeminothalamic fibres and synaptic transmission in the thalamus are present from the time when anatomical connections are made at E17.

303.2

EFFECT OF NITRIC OXIDE INHIBITION ON PLASTICITY IN THE RAT FIRST SOMATOSENSORY (S1) CORTEX.

N.W. Sohn[3], P.J. Hand*[2], J.H. Greenberg[1], [1]Cerebrovascular Research Ctr., School of Medicine, [2]Dept. of Animal Biology, School of Veterinary Medicine, Univ. of Pennsylvania, Phila., PA 19104, & [3]Dept. of Anatomy, College of Oriental Medicine, Kyung Hee Univ., Seoul, Korea

It has been previously shown that deafferentation of the vibrissae, sparing a single whisker, in neonatal rats leads to a significantly larger metabolic representation of the spared vibrissa in the S1 "barrel" cortex. Nitric oxide has been implicated in development of long-term potentiation and in learning. Its possible role in plasticity has not been examined. We chose to examine the effect of chronic nitric oxide inhibition on the deafferentation-spared whisker metabolic representation in S1. Ten Sprague-Dawley rats received vibrissae deafferentation on postnatal day (PND) 1 (birthdate=PND0) sparing C3 (SC3) unilaterally. Half of the animals received daily intraperitoneal injections of the nitric oxide synthase (NOS) inhibitor L-nitroarginine (20 mg/kg), while the remaining animals received saline. Six weeks post deafferentation, the treatment was terminated, and 3 days later the rats underwent bilateral C3 stimulation with a yoked mechanical stimulator (5 Hz). Local glucose utilization was measured with [^{14}C]2-deoxyglucose during stimulation. Glucose metabolic activity was localized in S1 barrel field using cytochrome oxidase staining. Treated animals showed a 97% inhibition of NOS catalytic activity within 3 days of treatment, and inhibition greater than 80% at six weeks. In both treated and control animals the metabolic representation of SC3 was significantly enlarged with respect to the contralateral control C3. Animals receiving NOS inhibition exhibited a 23% smaller increase in areal extent of the metabolic representation of SC3 than saline treated animals ($p<0.05$). Although chronic NOS inhibition does significantly attenuate the metabolic expansion of the representation of SC3 in S1 cortex, the effect is not large, suggesting that nitric oxide does not play a major role in somatosensory functional plasticity. (Supported by NIH grant NS33785)

303.3

EFFECTS OF $GABA_A$ AGONIST APPLICATION ON DEVELOPMENT AND PLASTICITY OF RAT BARREL CORTEX

P. Golshani, H. Truong, S. Akbarian*, and E.G. Jones, Department of Anatomy and Neurobiology, University of California, Irvine, CA 92717.

Several studies have shown that GABA may play an important role in the development and plasticity of the mammalian CNS, either by modulating synaptic activity as a neurotransmitter, or by acting as a neurotrophin. $GABA_A$ receptor subunits are expressed at high levels during the early postnatal days in the rodent barrel cortex, indicating that early in development GABA may act to direct and confine thalamic afferents to individual barrels, or through an activity dependent manner modulate the maintanance or elimination of synapses. To examine the role of GABA in the formation of cortical barrels , Elvax loaded with the $GABA_A$ agonist muscimol was implanted over the left primary somatosensory cortex of P1 rats. Tangential sections of barrel cortex were either processed for cytochrome oxidase histochemistry or for in-situ hybridization histochemistry using riboprobes directed against the $\alpha1$, $\beta2$, and $\gamma2$ subunits of the $GABA_A$ receptor. Infusion of muscimol during the first 12 days of life did not alter the morphology of cortical barrels as determined by CO histochemistry or alter the expression of $GABA_A$ receptor subunits $\alpha1$, $\beta2$, and $\gamma2$. To examine the effects of GABA on plasticity of barrel cortex, the C row of whiskers of P1 rats was ablated by cauterization and muscimol loaded Elvax was implanted over the corresponding primary somatosensory cortex. In tangential section processed for cytochrome oxidase histochemistry no change was found in the ratio of cortical area representing the ablated whiskers to the cortical area representing the non-ablated whiskers compared with non-Elvax implanted controls. Higher resolution studies of afferent and efferent axon terminal morphology of muscimol treated barrel cortex will be necessary to delineate a role for GABA during cortical development. Supported by NS21377.

303.4

PATTERNS OF 2DG LABELING EVOKED BY SINGLE WHISKER DEFLECTION IN THE TRIGEMINAL SYSTEM OF MICE AT DEFINED POSTNATAL AGES.

T.M. Mosconi*, J.J. Christensen, M.F. Jacquin & T.A. Woolsey, Neurology & Neurological Surgery, Washington University School of Medicine, St. Louis, MO 63110.

We previously demonstrated that passive whisker stimulation in 7-day old mice evokes functional activation in restricted and somatotopic foci principally in the barrel cortex revealed by high-resolution 2DG autoradiography and cytochrome oxidase histochemistry, respectively (Mosconi et al. '95). These results have been extended caudally along the trigeminal neuraxis and are being further compared to patterns of activation in actively whisking adults. 7-day old Swiss-Webster mice were injected with 0.5 mCi/5g body weight of (^{3}H)2DG (for detailed methods see McCasland and Woolsey '88a) and, under gentle restraint, a whisker on the left mystacial pad was repeatedly stroked at ~2 Hz for 35 minutes. There was increased 2DG uptake in the appropriate barrel in the right SI cortex, in the appropriate barreloid in the right VPM thalamus, and in the appropriate barrelettes in the left trigeminal brainstem subnuclei principalis, interpolaris and caudalis. Elevated levels were noted when the activated barrels, barreloids and barrelettes were compared to representations of adjacent whiskers. These data show that passive whisker stimulation in young mice effectively activates the somatotopically appropriate "cylinder" (Jacquin et al '93) throughout the rostral-caudal extent of the trigeminal neuraxis. Experiments are in progress to evaluate the pattern resulting from "passive" whisker stimulation in later perinatal life (e.g., when whisking begins, ~P14) and in adults.

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303.5

ORAL SOMATOSENSORY INNERVATION IN *trk*A, *trk*B, OR *trk*C KNOCKOUT MICE.

S. Matsuo*, T.A. Henderson, T.M. Mosconi, I. Silos-Santiago, M. Barbacid & M.F. Jacquin, Neurology and Center for the Study of Nervous System Injury, Washington Univ. Sch. of Med., St. Louis, MO 63110; Molecular Biology, Bristol-Myers Squibb, Princeton, NJ 08543.

The development of the mammalian peripheral nervous system is dependent upon access to specific neurotrophic factors. Prior studies of spinal systems suggest that nociceptive, tactile and proprioceptive endings fail to develop in mice homozygous for gene deletion of the high-affinity neurotrophin receptors *trk*A, *trk*B, or *trk*C, respectively. Little is known of the neurotrophic requirements of developing oral somatosensory afferents. Innervation patterns were assessed in *trk* knockout mice by immunohistochemistry for CCRP, substance P, and neurofilament protein-200. *trk*A homozygotes lacked stained fibers in the incisor tooth pulp and had only sparse innervation of the periodontal ligament, whereas heterozygotes and wild-type controls displayed many stained fibers in these same structures. Thin fibers in the palate were also diminished. *trk*A deletion had no obvious effect on the Ruffini endings in the periodontal ligament or the Meissner corpuscles in the palate. Thus, the *trk*A receptor may mediate the neurotrophic signal required for the development of dental innervation. Moreover, in every *trk* knockout preparation examined to date, masseter muscles contained spindle-like structures. The presence of jaw muscle spindles in *trk*C knockouts was surprising, given prior reports of a complete absence of skeletal muscle spindles and afferent fibers in these animals. Perhaps muscle spindle afferents in the trigeminal and spinal systems have differing neurotrophic requirements and associated receptors, as suggested by the data presented in the accompanying abstract (Arends et al.).

Supported by NIH DE07734, DE07662, NS17763.

303.6

NEONATAL CAPSAICIN EFFECTS ON INFRAORBITAL NERVE, PLASMA EXTRAVASATION & PRINCIPALIS RECEPTIVE FIELDS.

J.W. Hu*, J.A. DeMaro, C.L. Kwan, B.J. Sessle & M.F. Jacquin, Fac. of Dentistry, Univ. Toronto, Ontario M5G1G6; Neurol. & Center for Study of Nervous System Injury, Washington Univ. Sch. Med., St. Louis, MO 63110.

Prior reports (Nussbaumer & Wall '85; Kwan et al. '96), based upon capsaicin treatment, suggest that unmyelinated primary afferents contribute to the development of single whisker-dominant receptive fields (RFs) in the barrel cortex and trigeminal nucleus principalis. Insofar as capsaicin toxicity may not be limited to unmyelinated afferents, it is necessary to assess the properties of the whisker afferent nerve in animals displaying higher-order RF alterations. To begin to address this, we assessed neonatal capsaicin effects on the infraorbital nerve (by electron microscopic analysis of fiber spectra) and on plasma extravasation induced in ipsilateral skin by the inflammatory irritant and small fiber excitant mustard oil in adult rats displaying principalis RF changes. Two rats received 50 mg/kg capsaicin (in ethanol, Tween 80, saline) within 48 hr of birth, another vehicle only, and the other was untreated. The untreated and vehicle-treated rats averaged 19,111 myelinated and 9,697 unmyelinated fibers; they also showed 1-whisker principalis RFs, and ipsilateral plasma extravasation that was 5 times that in contralateral control skin. The capsaicin cases averaged 20,373 myelinated and 1,473 unmyelinated fibers and, consistent with our recent data (Kwan et al. '96), showed multi-whisker RFs and much reduced (2.3 times control) plasma extravasation. Thus, unmyelinated fibers were selectively and significantly reduced in the infraorbital nerves of cases with reduced plasma extravasation and higher-order RF alterations. These data add further evidence that unmyelinated fibers may be necessary for normal RF development in the whisker-barrel neuraxis. Supported by NIH DE04786, DE07734, DE07662.

303.7

SEQUENTIAL TIMING OF GAP-43-IR IN DEVELOPING CUTANEOUS INNERVATION OF THE MOUSE MYSTACIAL PAD

F.L. Rice*, B.T.Fundin. Dept. of Pharmacology and Neuroscience, Albany Medical College, Albany, NY 12208; Dept. of Neuroscience, Karolinska Institute, Stockholm, Sweden S171 77.

Prior studies using reduced silver techniques have shown sequential development of cutaneous endings in mammals. However, recent immunocytochemical techniques have revealed far more innervation than was seen previously. Also, questions about the sequential differentiation of the sensory endings have become increasingly important in light of investigations on the impact of neurotrophins during development. To further assess this issue, the developing innervation in mystacial pads from newborn (P0), P6, P16 and P25 mice was analyzed with antibodies against growth-associated protein GAP-43 that is present in axonal growth cones during development. The total innervation was colabeled with antibodies against a ubiquitous neuronal cytoplasmatic protein (PGP 9.5) which labels all known peripheral innervation. Large caliber axons that supply Merkel and lanceolate endings to the whisker follicles showed GAP-43-LIR at all ages studied. However, during the first postnatal week the intensity was high and gradually declined to a fairly low intensity by P25. In contrast, the Merkel terminals showed high intensity GAP-43-LIR at all ages which increased at P25, suggesting that these endings might be undergoing a continuous turnover at least during the first four weeks postnatally. Medium caliber axons that supply reticular and circular lanceolate endings showed a different timing in the expression of GAP-43-LIR. Only a weak GAP-43-LIR was evident at birth in the reticular afferents. GAP-43-LIR increased in intensity within the reticular endings over the next two weeks then declined. Circular lanceolate endings, which terminate in the inner conical body of the whisker follicle were not present until P2. These endings showed intense GAP-43-LIR only at P6. The epidermis receives several types of unmyelinated as well as small caliber myelinated innervation. At birth, the epidermal innervation was intensely labeled with GAP-43. Within a week, the intensity dropped in the epidermis but remained high in the superficial dermis. At P16 and P25, no profiles in the epidermis were GAP-43 positive. These observations indicate the various sensory endings in the mystacial pad have different developmental and maturational dynamics with regards to GAP-43 expression.

303.8

CELL SURVIVAL IN TRIGEMINAL BRAINSTEM NUCLEI OF *trk*A, *trk*B, OR *trk*C KNOCKOUT MICE. L.L.A. Arends*, T.A. Henderson, J. Silos-Santiago, M. Barbacid & M.F. Jacquin. Neurology and Center for the Study of Nervous System Injury, Washington Univ. Sch. of Med., St. Louis, MO 63110; Molecular Biology, Bristol-Myers Squibb, Princeton, NJ 08543.

Nociceptive, proprioceptive, and tactile afferents in dorsal root and trigeminal ganglia require NGF, BDNF, NT-3, and/or NT-4 for their survival. In mice homozygous for gene deletion of the high-affinity neurotrophin receptors *trk*A, *trk*B, or *trk*C, corresponding cell losses occur. Perhaps most impressive is the complete absence of proprioceptive afferents innervating stretch and tension receptors in skeletal muscle of *trk*C knockout mice. However, in these preparations, little is known of the fate of jaw muscle afferents whose distinct pseudounipolar cell bodies constitute the mesencephalic trigeminal nucleus (MesV). Nor is there information on the transsynaptic effects of these gene deletions upon trigeminal brainstem neurons. Nissl-stained profiles of cell bodies with nucleoli were counted blind to genotype in complete series of paraffin-embedded "thin" sections from 11 mice. 6 wild-type or heterozygote controls averaged 638 MesV cell profiles (range: 529-813). 1 *trk*A knockout had 612 MesV cells. 1 *trk*B knockout had 424 MesV cells. 3 *trk*C knockouts had 255, 301, and 310 MesV cells. Cell numbers in the principal and spinal trigeminal nuclei will be reported. These preliminary data suggest that while MesV cells are significantly reduced in number by *trk*C deletion, they are certainly not absent. Therefore, unlike skeletal muscle afferents, many jaw muscle afferents survive the *trk*C knockout (see accompanying Matsuo et al. for stained afferent fibers and spindles in the masseter muscles of these mice). Perhaps *trk*B receptors mediate neurotrophic actions on some jaw muscle afferents, as suggested above. Supported by NIH DE07734, DE07662, NS17763.

303.9

TRIGEMINAL GANGLION NEURONAL ACTIVITY AND SATELLITE CELL GFAP RESPONSES TO INFERIOR ALVEOLAR NERVE CRUSH. E.H. Chudler[1], L.C. Anderson[2,3] and M.R. Byers[1,2,3]*. Depts. of Anesthesiology[1], Oral Biology[2] and Biological Structure[3], Univ. Washington, Seattle, WA 98195.

Injury to peripheral branches of the trigeminal nerve may cause abnormal sensory phenomena such as paresthesia and pain. The present study investigates the time course of electrophysiological and glial fibrillary acidic protein immunoreactivity (GFAP-IR) alterations within the trigeminal ganglion (TG) after crush injury to the inferior alveolar nerve (IAN). After recovery periods of 3, 10 or 59 days, rats were anesthetized and prepared for acute, extracellular recording from the TG. Following acute recording, the TG were fixed and removed for GFAP-IR processing. By 10d after IAN crush, regenerating neurons responded to mechanical and electrical stimuli of their innervated tissues, but with slower conduction velocities and higher thresholds than normal. These abnormal properties persisted at 59 days. In all groups 3-4% of the neurons had spontaneous activity. There was intense GFAP-IR in satellite cells of almost all neurons in the mandibular division at 3d, 30-50% at 10d, and 10-30% at 59d. At 3 and 10 days GFAP-IR was also found in satellite cells of 2-10% of maxillary neurons despite normal neuronal electrophysiological properties. Although altered neuronal physiology was recorded in the same region as the greatest GFAP-IR response, the satellite cell reaction spread further into areas of apparently normal electrophysiology.
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303.10

EXPRESSION OF GUANOSINE 3',5'-CYCLIC MONOPHOSPHATE-DEPENDENT PROTEIN KINASE Iα AND NITRIC OXIDE SYNTHASE IN THE DEVELOPING CHICK EMBRYO. Bonnie L. Firestein* and David S. Bredt. Department of Physiology, UCSF, San Francisco, CA 94143-0444.

We have studied the distribution of guanosine 3',5'-cyclic monophosphate-dependent protein kinase type Iα (cGKIα) and nitric oxide synthase (NOS) in the embryonic chick. In order to identify the onset of cGKIα expression, embryonic day 3-12 chicks were fixed with 4% paraformaldehyde for 4 hours and cryoprotected with 20% sucrose in PBS for 16-18 hours. Embryos were sectioned at 20 μm (sagitally or horizontally) using a cryostat. Serial sections were stained using an antibody raised against cGKIα (polyclonal rabbit) and were visualized using an avidin-horseradish peroxidase conjugate and diaminobenzadine tetrahydrochloride as a chromagen. These immunohistochemical studies suggest that cGKIα expression is restricted to embryonic chick trigeminal (TG) and dorsal root ganglia (DRG) as early as E5 and expression continues to be present up to E12. Sections were also assayed for the presence of NOS using ß-NADPH diaphorase histochemistry. Unlike cGKIα, NOS is absent from TG and DRG E7 and earlier, but it is present on E10. Since sensory ganglia begin to condense at E3, and apoptosis of neurons commences at E4.5 and continues to E12, this pattern of expression may suggest a role for cGKIα and NOS in TG and DRG development. (Supported by grants from NSF, NARSAD)

303.11

CAN DORSAL ROOT AXONS SURVIVE AFTER BEING DEPRIVED OF CENTRAL AND PERIPHERAL TROPHIC FACTORS. R.E.Coggeshall[1]*, G. Kendall[2], and C.J. Woolf[2]. Dept. of Anat. and Neurosci., Univ. Tex. Med. Br., Galveston, Tx. 77555, USA[1] and Dept. of Anat. and Dev. Biol., Univ. College London, WC1E 6BT, UK[2].

In previous work, we showed no loss of doral root (DR) axons within 16 weeks after peripheral nerve section. This raised the question as to whether survival of these axons, as well as the cells that give rise to them, could be due to trophic factors from the spinal cord. Accordingly, we determined mean numbers of L4 and L5 DR axons 4 and 8 weeks following sciatic N (PN) section + L4 and L5 DR lesions or dorsal root lesions alone in adult rats:

	Naive	4 week DR cuts	4 week PN and DR cuts	8 week DR cuts	8 week PN and DR cuts
MY	5048	4702	5317	4735	5385
UN	9756	8517	13099	9779	10925

Thus there is no evidence for loss of dorsal root axons 4 and 8 weeks following either DR section alone or PN + DR section. Our preliminary conclusion is that adult dorsal root axons and presumably the cells that give rise to them do not depend for their survival on either peripheral or central trophic factors within the time frame of this experiment. We have measured BDNF, NGF and NT3 mRNAs in the DRG, and this upregulation induced by the lesions may promote cell survival. Supported by NIH grants NS10161, NS11255 and the Medical Research Council, UK.

303.12

GALANIN AND GALANIN MESSAGE ASSOCIATED PEPTIDE (GMAP) IN SENSORY GANGLIA DURING RAT EMBRYOGENESIS. Z.-Q. Xu, T.-J. Shi, P. Wallén*, and T. Hökfelt. Dept. of Neuroscience, Karolinska Institute, S-171 77 Stockholm, Sweden.

Galanin, a 29 amino acid peptide, has a wide spread distribution in the nervous system. However, little is known about expression of galanin during ontogenesis. In this study the distribution of galanin/GMAP-like immunoreactivities was investigated in the fetal rat at embryonic days 12, 15, 17, 19 and 21 using immunohistochemistry, mainly focusing on sensory ganglia. We found that galanin/GMAP immunoreactivities were expressed in dorsal root ganglia and the trigeminal ganglia at embryonic days 15, 17 and 19. Galanin/GMAP were also present in the intestine, in the retina as well as in several other regions of the central nervous system, in some areas as early as embryonic day 12. Galanin binding sites were observed in the dorsal horn of the spinal cord at embryonic day 19. Our results suggest that galanin/GMAP may exert developmental roles in sensory neurons and other neuronal systems. (Supported by the Swedish MRC 04X-2887.)

303.13

THE NERVE REGENERATION OF PIG FREE SKIN GRAFT C.L. He*, W.R. Kennedy, G. Wendelschafer-Crabb Dept. of Neurology, Sch. of Med., Univ. of Minnesota, Minneapolis, MN 55455.

The regeneration of nerves in free skin flaps with 3 mm in diameter in the pig was investigated by indirect immunohistochemical technique, using antisera to protein gene product 9.5(panneural), to calcitonin gene-related peptide(sensory neurons), to substance P(sensory neurons), and to vasoactive intestinal peptide-(sympathetic neurons). Digitized images were taken with laser scanning confocol microscope(LSCM). Using these techniques, the three-dimensional distribution of regenerating nerves within a thick section was studied. The normal pig skin are innervated with big bundles of nerves in the deep dermis which give out small nerve bundles in the superficial dermis. The nerves in the superficial dermis are running into the epidermis more or less perpendicularly to the surface. Hair follicle, big blood vessels in the deep dermis and erector pili muscles innervated with nerves are observed. No sweat gland is observed in the pig skin. No regenerating nerves in the graft were observed in the first week. However, the nerve have started to regenerate from the normal edge toward the graft at first week. At the second, third and forth week, regenerating nerves are gradually covering more area of the graft. The regenerating nerves around the basement membrane in the epidermis and superficial dermis grow more fast than the nerves in the deep dermis. Most regenerating nerves come from the normal margin. Only a few regenerating nerves are from the base of the graft. At five and six week, some nerves in the epidermis and superficial dermis start to retract but nerves in regenerating are still can be encountered. The architecture of regenerating nerves are much more near normal. But the reinnervation remain patchy and inadequate in some area of the graft. Supported by NS 26348, NS 31397 and Toray Industries Inc..

303.14

GLIAL GROWTH FACTOR RESCUES MECHANORECEPTOR SCHWANN CELLS FROM DENERVATION-INDUCED APOPTOSIS. D.M. Kopp*, J.T. Tractenberg and W.J. Thompson, Dept. Zoology, Univ. Texas at Austin, Austin, Texas 78712.

Golgi tendon organs and Pacinian corpuscles are encapsulated mechanoreceptors whose sensory endings are closely associated with S-100 immunoreactive cells presumed to be Schwann cells. Previous studies have shown that both types of receptors disappear and do not reform if denervated during a critical period in early postnatal development. We have investigated the response to denervation of Schwann cells in Golgi tendon organs and Pacinian corpuscles in the lower hindlimb of neonatal rats. Within 24 hours following sciatic nerve resection, the S-100 positive Schwann cells of these mechanoreceptors undergo apoptosis: the cells and their nuclei fragment, as detected by the TUNEL technique. 3 days later the cells have completely disappeared. Subcutaneous administration of the neuregulin glial growth factor-2 (GGF-2) into the hindlimb after denervation rescues many of these cells from apoptosis. This rescue suggests that neuregulins derived from the afferent axons are necessary for the survival of these Schwann cells, just as terminal Schwann cells at the neuromuscular junction appear to be dependent on motor axon-derived neuregulin (Nature, 1996, 379:174). Our studies also suggest that the inability of these mechanoreceptors to reform following neonatal denervation may be related to the death of their associated Schwann cells. Supported by NIH. We acknowledge Cambridge Neuroscience for GGF-2.

303.15

CALCIUM-BINDING PROTEIN IMMUNOLOCALIZATION IN FOETAL AND ADULT HUMAN SPINAL CORD. P. Rizzonelli [1], P. Liberini [1], G. Vezzoli [1], L. Novelli [1], G. Moretto [2], M. Memo [1], and P.F. Spano *[1]. [1] Div. of Pharmacol., Dept. Biomed. Sci. and Biotech., Brescia University School of Medicine, 25123 Brescia; [2] Section Neurology, Dept. of Neurol. and Visual Science, Verona University School of Medicine, Verona, Italy.

Calcium-binding proteins are widely distributed in the central nervous system and are considerated to play important roles in the modulation of transmembrane and intracellular signaling. Monoclonal antibodies raised against Parvalbumin (PV), Calmodulin (CM) and Calbindin D-28K (CB-D28K) have been used to determine and compare the immunohistochemical expression of these calcium-binding proteins in foetal (6, 10, 12, and 22 weeks of gestation) and adult human spinal cord. In foetal spinal cord PV immunoreactivity was observed in regions containing primary sensory afferents: the dorsal root ganglia neurons, the dorsal and dorsolateral funiculi and restricted regions of dorsal horn. At this stage, immunoreactive fibers with a dorsoventral orientation through the middle of the dorsal horn were observed. In adult spinal cord a pattern of PV fibre immunoreactivity was localized in the superficial layer of dorsal horn (lamine I, II) and lamina X but not in dorsal and dorsolateral funiculi and in the dorsal root ganglion. CM immunoreactivity was found in the dorsal and dorsolateral funiculi in the mantle layer and in ventral horn but not in dorsal root ganglia. In adult spinal cord CM immunoreactivity appared in lamina II and in neurons of lamina VII. In foetal stages CB-D28K cellular immunoreactivity was localized in sub-ependimal position. In all this areas the maximun level of immunostaining was found at the 12th weeks of gestation. Overall, our data revealed a specific pattern of expression with different distribution and overlappings. The transient selective pattern of PV, CM and CB-D28K immunoreactivity may disclose some relevant functions of these calcium-binding proteins during somatosensory pathway development. *This research was supported by the Italian Study Group on Paraplegia.*

303.16

MATURATION OF THE BIPHASIC BEHAVIORAL AND HEART RATE RESPONSES IN THE FORMALIN TEST IN THE RAT G. A. Barr* Hunter College and NY State Psychiatric Institute 722 W. 168th St. New York, NY 10032.

Injection of formalin into the hindpaw of the adult rat produces a biphasic nociceptive response. There is an initial reaction that lasts approximately 10-15 minutes, a subsequent decrease in the response, and a second phase of increased nociceptive behavior. This pattern is seen when either behavior, heart rate or blood pressure is measured. The neural mechanisms that underlie the two phases of nociceptive responding, which have been studied intensively in the adult, are not likely to be fully functional in the infant. We examined the maturation of the nociceptive response, over a 60 minute period, after injection of formalin into the hindpaw of 7, 14 or 21 day old rats. Both behavior and heart acceleration were dependent measures. At all ages formalin injection elicited an initial behavioral response that was constant during development. At 7 and 14 days of age, however, there was no evidence of the second nociceptive stage. At 21 days of age both the early and late phases were present, and the time course of the response was similar to that reported for the adult. Formalin injection also induced a tachycardia that declined linearly with time at all ages; there was no evidence of a biphasic cardiac response at any age. These data are consistent with the known development of excitatory amino acid receptors in the spinal cord, and of the late maturation of autonomic control of phasic heart rate.
Supported by grant DA07646 and funds from PSC-CUNY.

SUBCORTICAL VISUAL DEVELOPMENT

304.1

ALTERED EXPRESSION OF C-FOS IN THE LATERAL GENICULATE NUCLEUS OF RED-LIGHT-REARED TREE SHREWS. J.L. D'Arcy and H.M. Petry*. Departments of Psychology and Ophthalmology & Visual Science, University of Louisville, Kentucky 40292

Objectives were to: 1) clarify the functional organization of chromatic and achromatic channels in tree shrew (T. belangeri) LGN, and 2) study permanent effects of early post-natal rearing in deep red light that does not stimulate short-wavelength-sensitive (SWS) cone photoreceptors. Due to differential input of the tree shrew's 2 cone types (SWS and LWS) to its chromatic and achromatic channels, red-light-rearing (RLR) produces biased stimulation of the LWS-cone-driven achromatic channel. Prior work showed permanent effects of RLR in retina, cortex and in visual abilities (Petry & Kelly,'91; Petry, '93; Petry & Murphy, '95).

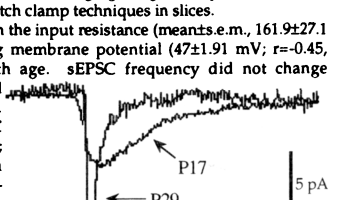
Expression of c-fos is elevated (from basal levels) following novel visual stimulation. We assessed changes in c-fos levels following 1 hr. of alternating blue/yellow (B/Y; 455nm/555nm) stimulation using immunocytochemical methods (c-fos (Ab-2), Oncogene). Brain tissue from 9 adult animals represented 4 groups: WL (normally-reared, white-light housed); RL (normally reared, red-light housed for 4-43 days); RL-B/Y (normally-reared, red-light housed for 4-7 days, B/Y stimulated prior to perfusion); and RLR-B/Y (red-light-reared, B/Y stimulated prior to perfusion). Results in control animals (groups WL & RL) revealed very light and uniform basal c-fos immunoreactivity (c-fos IR) across all layers of the dLGN and vLGN. RL-B/Y shrews showed consistently dark c-fos IR in layers 1, 2, 4, and 5 of the dLGN (and occasional dark cells in layer 6) as well as a band of cells in the parvocellular lamina of vLGN (likely due to rod stimulation). RLR-B/Y shrews revealed light, uniform staining like controls. These results suggest: 1) color-opponent information is likely to be processed in layers 1, 2, 4, 5, and not konio layer 3; and 2) lack of SWS cone stimulation during early post-natal development, and the corresponding competitive advantage of the achromatic channel, permanently changes c-fos expression in central visual structures. Supported by NSF grant #OSR-9452895 and Univ. of Louisville.

304.2

DEVELOPMENTAL CHANGES IN SPONTANEOUS EPSCs DURING ON/OFF SUBLAMINATION IN THE FERRET LATERAL GENICULATE NUCLEUS, C. D. Hohnke* and M. Sur, Dept. of Brain and Cognitive Sciences, MIT, Cambridge, MA 02139.

In the lateral geniculate nucleus (LGN) of the ferret, retinal afferents segregate into ON and OFF sublaminae during the third and fourth postnatal weeks; this segregation depends on neuronal activity. To better understand the development of synaptic transmission during this period of remodeling, we have recorded spontaneous excitatory post-synaptic currents (sEPSCs) from LGN relay cells (n=15) in animals ranging in age from postnatal day 15 (P15) to P29 using whole-cell patch clamp techniques in slices.

No change was observed in the input resistance (mean±s.e.m., 161.9±27.1 MΩ; r=0.28, p=0.38) or resting membrane potential (47±1.91 mV; r=-0.45, p=0.09) of these neurons with age. sEPSC frequency did not change significantly during this period (2.2±0.5 Hz; r=-0.17, p=0.55). However, the mean sEPSC amplitude increased (by ~40%; r=0.62, p<0.05) and the mean width decreased(by ~40%; r=-0.52, p<0.05; see figure: each trace is an average of 50 consecutive sEPSCs in 1 cell) with age. Additionally, the standard deviation of sEPSC width decreased (by ~60%; r=-0.54, p<0.05) during this period. These changes may reflect a process of synaptic reorganization accompanying retinogeniculate pattern formation: retinal axon synapses might withdraw from distal locations on LGN cell dendrites and concentrate near the soma, where they are known to cluster in the adult. *Supported by EY07023.*



304.3

ON/OFF SUBLAMINATION IN THE FERRET LGN IS INDEPENDENT OF SYSTEMIC CHANGES IN BLOOD PRESSURE AND REQUIRES NEURONAL NOS. K. S. Cramer* and M. Sur, Department of Brain & Cognitive Sciences, M.I.T., Cambridge, MA 02139.

Blockade of nitric oxide synthase (NOS) with N-Nitro-L-Arginine (L-NoArg) during the third and fourth postnatal weeks in the ferret disrupts the formation of ON/OFF sublaminae (Cramer and Sur, 1994). Because NO is a potent vasodilator, we examined the role of systemic changes in blood pressure on sublamination during systemic treatment with L-NoArg. Mean arterial blood pressure (MAP) was measured during the fourth postnatal week in four animals treated with 40 mg/kg/day i.p. L-NoArg from postnatal day 14 (P14) and in four normal, age-matched controls. MAP (± s.e.m.) increased from 57.6 ± 4.6 mmHg to 83.1 ± 4.8 mmHg following L-NoArg treatment. When animals received the antihypertensive calcium channel blocker verapamil, 5 mg/kg/day i.p., together with L-NoArg, blood pressure was normal (60.0 ± 1.6; n = 4). In these animals, sublamination was assessed in sections of LGN contralateral to an intraocular injection of WGA-HRP; disruption of sublamination was similar to that seen in animals treated with L-NoArg alone, suggesting that NOS blockade-induced changes in retinogeniculate projections occur independently of changes in MAP.

Sublamination in the LGN relies at least in part on the neuronal form of NOS. Neuronal NOS immunohistochemistry was similar to NADPH-diaphorase histochemistry in its transient expression during retinogeniculate segregation. In addition, ON/OFF sublamination was disrupted by blockade of the neuronal form of NOS from P14 to P26 with 7-nitroindazole, which appears not to produce hypertension. While systemic changes in blood pressure are not involved in the segregation of sublaminae, further experiments are necessary to examine the role of local changes in cerebral blood flow produced by NO release. *Supported by EY07023*

304.4

ROLE OF SPONTANEOUS RETINAL ACTIVITY IN REORGANIZATION OF RETINOGENICULATE CONNECTIONS DURING DEVELOPMENT. P.M. Cook*, G. Prusky and A.S. Ramoa Dept. of Anatomy, Virginia Commonwealth Un., Richmond, VA 23298 and Dept. of Psychology, Un. of Lethbridge, Lethbridge AB T1K3M4.

The adult form and function of the lateral geniculate nucleus (LGN) arise after extensive modifications in circuit organization that include segregation of axonal arbors from the two eyes into eye-specific layers. Previous studies have shown that intracranial infusion of tetrodotoxin (TTX) in fetal cats prevents segregation (Shatz and Stryker, 1988), suggesting that spontaneous action potential activity contributes to remodeling of the retinogeniculate pathway. We have examined the activity-dependent mechanisms of eye-specific segregation using a technique which allows direct inferences about the role of spontaneous retinal activity. Our method uses continuous intraocular application of TTX to block spontaneous discharge of retinal ganglion cells in newborn ferrets, which display retinogeniculate remodeling during the first 2 weeks of postnatal life. After a 2 week infusion, intraocular injection of horseradish peroxidase (HRP) revealed the projection pattern contralateral and ipsilateral to the HRP injected eye. Eye-specific segregation of retinogeniculate projections was observed at postnatal day 14 in controls as well as in ferrets that received monocular (n=3) or binocular (n=1) injection of TTX. However, segregation was aberrant in the monocularly injected animals. First, projections from the untreated eye occupied an approximately 50% larger volume of both LGNs than those from the TTX-treated eye. The total size of the LGN, however, remained unaltered and this suggests that the normal eye invaded territory normally occupied by the TTX treated eye. Moreover, a low density of afferents from the contralateral eye were observed to terminate in layer A1, which normally receives only ipsilateral eye input. These results suggest that binocular competition modulates rearrangements in retinogeniculate projections. Additionally, they are consistent with the hypothesis that spontaneous retinal activity may fine-tune segregation of retinal afferents into eye-specific layers. (Supported by NSF IBN-9421983)

304.5

POSTNATAL DEVELOPMENT OF SYNAPTIC PLASTICITY IN THE RAT SUPERIOR COLLICULUS: LONG-TERM DEPRESSION IN THE SUPERFICIAL LAYERS. R. J. Cork*, F-S. Lo and R. R. Mize. Anatomy Department. and Neuroscience Center of Excellence, LSU Medical Center, New Orleans, LA 70112.

We have been studying the development of the synaptic circuitry in the rat superior colliculus (SC) between ages P1 and P10. Using an in-vitro brainstem preparation, we have measured extracellular field potentials, and made whole-cell recordings of postsynaptic potentials, from the superficial layers of SC. We have also used immunocytochemistry and NADPH-histochemistry to map the development of various molecules thought to be involved in modulating synaptic transmission (i.e. NMDAR1, and NOS). Field potentials or post-synaptic potentials were measured following stimulation of optic tract (OT) fibers. Whole cell recordings showed EPSPs with two components. The later component was blocked by APV, suggesting that it was mediated by the NMDA receptor. NMDAR1 antibody also intensely labeled the neuropil of the superficial layers at P1. From P3 some neurons also showed an IPSP following the EPSP. The IPSP could be blocked by bicuculline, an antagonist of $GABA_A$ receptors. Between ages P1 and P8, tetanic stimulation (50 Hz, 20 s, submaximal intensity) induced a long-term (>90 min.) depression (LTD) of the field potential and the EPSP. Neither APV (10 μM or 50 μM) nor bicuculline (10 μM) could prevent LTD, suggesting that it is independent of either NMDA or $GABA_A$ receptor activation. Nitrendipine (5 μM), an L-type Ca^{2+}-channel blocker that blocks induction of LTD in the hippocampus, also failed to prevent LTD induction in the SC. The magnitude of the LTD decreased steadily from P1 to P8 and at P9/P10 tetanic OT stimulation induced a mixture of depression and long-term potentiation (LTP). Consistent with the idea that NOS promotes LTP, significant NOS expression was first observed in cells of the superficial layers at P9.

Supported by DOD cooperative agreement DAMD 17-93-V-3013, NIH grant EY02973, and the LSUMC Neuroscience Center.

304.6

INHIBITION OF NITRIC OXIDE SYNTHASE FAILS TO DISRUPT THE DEVELOPMENT OF CHOLINERGIC FIBER PATCHES IN THE RAT SUPERIOR COLLICULUS. C.A. Scheiner*, R.J. Cork and R.R. Mize. Dept. of Anatomy and the Neuroscience Center, Louisiana State University Medical Center, New Orleans, LA 70112

Nitric oxide (NO) has been proposed to be a retrograde messenger involved in synaptic refinement during development. The patch-cluster system in the intermediate gray layer (IGL) of the superior colliculus (SC) is an excellent model for studying the role of NO because both the clustered neurons and one of the fiber systems (ACh) that form the patches contain NOS during development. We have used Nω-nitro-L-arginine, an inhibitor of nitric oxide synthase (NOS), to determine if inhibition of NOS disrupts the formation of the ACh patches. Sprague-Dawley rats received 1-100 μM of NoArg from birth until sacrifice at P10, P14, P18, P21-22, or P28. Control animals were litter mates of the experimental animals plus other younger and older animals. ACh fibers were identified using choline acetyltransferase (ChAT) immunocytochemistry and NOS containing cells were labelled with nicotinamide adenine dinucleotide phosphate diaphorase (NADPH-d) histochemistry. NADPH-d was expressed in cells within the periaquaductal gray (PAG) and the deep gray layer (DGL) of the SC by P4, the earliest age examined. By P9, many cells in the IGL expressed NADPH-d, while few in the superficial layers were labeled. Some of these neurons in the IGL formed obvious clusters, while others were loosely scattered throughout the layer. NADPH-d labeling in IGL cells declined by P14-P18 and virtually absent in the adult. ChAT labeled fibers first appeared in the IGL at P10 and were readily visualized by P14. At this age, the ChAT fibers were fairly uniformly distributed through the IGL, but by P18 they had a patch like distribution with as many as five patches spaced at 250-300 μM intervals. Inhibition of NOS from birth produced no qualitative differences in the distribution or density of ChAT labeled fibers at any of the ages examined. We therefore conclude that NO does not contribute to the refinement of cholinergic fiber patches in the rat SC, probably because the fiber system is not glutamatergic. Supported by NIH NEI-02973, DOD cooperative agreement DAMD 17-93-V-3013,a Louisiana State Board of Regents Graduate Student scholarship, and the LSUMC Neuroscience Center.

304.7

ROLE OF RETINAL ACTIVITY IN MATURATION OF ELECTROPHYSIOLOGICAL MEMBRANE PROPERTIES AND SYNAPTIC RESPONSES OF FERRET LGN A.S. Ramoa* and G. Prusky Dept. of Anatomy, Virginia Commonwealth University, Richmond, VA 23298 and Dept. of Psychology, University of Lethbridge, AB T1K3M4.

The adult form and function of the lateral geniculate nucleus (LGN) arise after extensive modifications in circuit organization that, in the ferret, occur during the first postnatal month. We have shown that electrophysiological membrane properties and synaptic responses in LGN neurons of the ferret change markedly during this critical period of development (J. Neurosci. 14:2089, 1994; J. Neurosci. 14:2098, 1994; J. Neurosci. 15:5739, 1995). These changes, which appear to be coordinated to facilitate circuit remodeling of LGN neurons, include: late maturation of low-threshold Ca++ spikes and inhibitory potentials as well as developmental switches in the expression of NMDA receptor isotypes. We examined whether these changes are regulated by retinal activity. Our method uses continuous intraocular application of tetrodotoxin starting as early as the day of birth to block spontaneous discharge of retinal ganglion cells. Whole-cell recordings in the LGN slice preparation revealed that maturation of low-threshold calcium spikes and hyperpolarization-activated currents was unaltered by a 3 to 4-week binocular infusion of tetrodotoxin. In contrast, synaptic properties were markedly affected. Treated animals at P40 were found to display long duration NMDA-EPSCs that resembled those present in normal newborn animals rather than the shorter-duration EPSCs seen in normal animals at similar age ($P<0.01$). Additionally, application of ifenprodil, which binds with higher affinity to heteromeric receptors containing the NR-2B subunit, blocked more potently NMDA-EPSCs in newborn and TTX-treated animals animals at P40 than in normal P40 animals ($p<0.01$), suggesting that a developmental switch in the subunit composition of the NMDA receptor is prevented by intraocular TTX. In conclusion, retinal activity may regulate developmental changes in synaptic properties of LGN neurons (NSF IBN-9421983).

304.8

CHOLINERGIC PROCESSES IN XENOPUS TECTUM. M.J. Titmus*, L. Pleban, R. Lima & S.B. Udin, Dept. of Physiology & Biophysics, SUNY, Buffalo, NY 14214.

The ipsilateral visual input to Xenopus tectum comes into register with the contralateral map during development. The major transmitter for the contralateral (retinotectal) input is glutamate, and the major transmitter for ipsilateral input, relayed via the nucleus isthmi, is probably acetylcholine. The role of NMDA receptors in the activity-dependent process of organization of the ipsilateral map is demonstrated by the ability of NMDA receptor blockers to prevent matching of the ipsilateral map to the contralateral map during the critical period, but little is known about the role of acetylcholine.

Immunohistochemistry indicates that nicotinic receptors are located in the layers of the tectum that receive binocular inputs; unilateral eye enucleation indicates that most of those receptors are located on retinotectal axons. Receptor binding using tritiated cytisine also indicates the presence of nicotinic receptors in the tectum. Muscarinic receptors are located on cells and dendrites located appropriately to receive isthmotectal input.

Calcium imaging using Fura-2 in tectal slices demonstrates no measurable response to nicotinic agonists alone, but shows significant synergism when nicotine or cytisine are applied with NMDA. In contrast, muscarinic agonists decrease calcium influxes elicited with NMDA. These results suggest that activity of isthmotectal axons can significantly modulate the effects of glutamate released from retinotectal axons.

Supported by USPHS Grant EY-10690 to M.J.T and S.B.U.

304.9

SP-RECEPTOR DISTRIBUTION PATTERNS IN THE SUPERIOR COLLICULUS OF DEVELOPING RATS, WITH SPECIAL REFERENCE TO ENUCLEATION EFFECTS. R. Meguro and M. Norita. Dept. Neurobiology and Anatomy, Niigata Univ. Sch. Med., Niigata, 951, Japan.

We have reported that many neurons in the superior colliculus (SC) of the adult rat contain substance P-receptor (SPR), and most of those send fibers outside the SC (Meguro et al., 1993). Neurons with SPR in the SC are likely to receive fibers with SP which originate within the SC. Neurons with SPR, as well as those with SP, are also shown to receive retinal fibers, suggesting SPR-containing tectal outputs would be modulated by retinal afferents.

To clarify the influence of retinal afferents on SPR-containing tectal neurons, we examined developmental changes of the distribution patterns of SP and SPR-containing neurons in the SC with special reference to eye opening time or enucleation effects. The SP-containing neural elements began to be detected by postnatal day 8 and there were no marked changes after enucleation. On the other hand, many of the SC neurons containing SPR in their somata appeared around postnatal day 14, when eyes opened. In rats enucleated at birth, the SC neurons with SPR in their somata decreased in number and changed their morphological polarity. Thus, SP-activating/modifying efferent pathways from the SC were influenced by visual stimulation or visual deprivation.
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304.10

IN RAT SUPERIOR COLLICULUS, DARK REARING DELAYS MATURATION OF VISUAL RESPONSES AND MODULATES THE ROLE OF NMDA RECEPTORS IN VISUAL TRANSMISSION. K.E. Binns & T.E. Salt. Institute of Ophthalmology, University College London, Bath Street, London, EC1V 9EL, UK.

The development of visual responses in rat superficial SC extends beyond eye opening (P14). The role of NMDA receptors in visual transmission is age dependent. Thus visual experience may influence late maturation and the role of NMDA receptors. We studied the effects of visual deprivation (by dark rearing) on visual responses and NMDA mediated transmission, by recording extracellularly from single neurones in urethane anaesthetized rats. The responses to visual stimulation and iontophoretically ejected agonists were challenged with NMDA receptor selective currents of AP5.

In normal rats there is a progressive change in the response profile t visual stimuli from bi-phasic to mono-phasic between P14 and P21. At the same time, the proportion of neurones with AP5 sensitive visual responses increased from 33% in P14-19 rats to a peak of 85% (of sample, n=20) at P20-21, and then reduced to 60% in adults. Similar, but delayed, maturational changes occurred in dark reared rats and there was a corresponding time lag in the rise in the proportion of neurones with AP5 sensitive visual responses. A maximum was reached at P22-23, but, the proportion did not decline. Dark rearing also enhanced the degree by which AP5 reduced individual visual responses. The differences between dark reared rats and normal rats were most pronounced in older subjects: In normal P23-P40 rats, NMDA receptors partially mediate 66% [n=9] of the visual responses, whereas in dark reared P25-40 rats, all [n=8] of the visual responses had NMDA mediated components. Also, the mean reduction with AP5 was significantly increased ($0.025 < P < 0.05$, Mann-Whitney 'U' test) from 45±7 in normal rats to 65±6 in dark reared rats. These data indicate that visual experience influences maturational changes in the profile of the visual responses in SC. In addition, during development, the role of synaptic NMDA receptors in visual transmisson is dependent on environmental factors. **Supported by the Wellcome Trust.**

304.11

EFFECTS OF NEONATAL AND ADULT ENUCLEATION ON THE DISTRIBUTION OF NITRIC OXIDE SYNTHASE IN THE SUPERFICIAL LAYERS OF THE RAT SUPERIOR COLLICULUS. R. Mendez-Otero*, F. Tenório, A. Giraldi-Guimarães, W. M. Cintra, H. R. Santos, M. S. Soares. Instituto de Biofisica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro, Rio de Janeiro, RJ 21941-590, Brazil.

Recent results indicate that nitric oxide (NO) can play an important role in neuronal excitability by modifying the strength of activated synapses. We sought to determine whether the level of nitric oxide synthase (NOS) could, in turn, be regulated by neural activity. In this work we have investigated the role of retinotectal projections on the distribution and light-microscopy features of nitric oxide synthase (NOS)-positive cells in the retinoceptive layers of the rat superior colliculus (SC) using histochemical methods. Newborn and adult Lister rats had one or both eyes removed and perfused after different survival times. Histochemical results from rats enucleated at birth indicate that the expression of NOS, as revealed by the NADPH-diaphorase method, is not dependent on the presence of retinal projections during development. In the early enucleated animals the temporal pattern of NOS expression did not change as compared with normal animals. In both groups, NOS-positive cells were found in the SC starting at postnatal day 7. However, the morphology of NOS-positive cells was greatly disturbed in animals enucleated at early ages as well as in animals enucleated at adulthood when compared to the normal pattern. We concluded that retinotectal projections can regulate the subcellular distribution of NOS in SC cells in developing and adult animals.
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304.12

CULTURED RAT RETINOCOLLICULUR SYSTEM ON PLANAR ELECTRODE ARRAYS. A. Kawana*, H. Kamioka and Y. Jimbo. NTT Basic Research Laboratories, Atsugi-shi, Kanagawa, 243-01 Japan

In order to study the roles of electrical activity in development of the visual system, we have cultured components of the rat visual system on substrates with planar electrode arrays (PEA) (A.Kawana et al ; Soc. for Neurosci., 1995 517.11). However, the retina (RE) formed hardly any functional synaptic connections with lateral geniculate neuclius(LGN). Here we describe the organotypic coculture of rat RE with superior colliculus (SC) which is considered to be the major target region of the RE in rat on the PEA.

Slices (300 um) of SC, RE and the LGN, which is a major target of RE in other many mammals, were isolated from E16 rat, and fixed by gentle centrifugation onto the substrate. 7 days after initiating the culture, electrical activity was observed from the RE, SC and LGN simultaneously in culture conditions. SC usually showed synchronized bursting and the retina showed tonic firing with long quiecent period. LGN showed long lasting bursting. Electrical stimulation of the retina did not evoke any electrical activity in other areas. Around 14 days in vitro, the spontaneous electrical activity in RE was observed to propagate to SC, but not to LGN. Electrical activity of LGN could not be evoked by electrical stimulation at RE. When crystals of dil were placed into the retinal explant, anterogadely labeled retinal cells follwed into both SC and LGN. These results suggest that the major target of the RE is SC in this system as shown *in vivo* and that spontaneous activity of retina which can evoke the electrical activity in SC has the potential to modify the retinocollicular connection.

304.13

FAST FOURIER TRANSFORM ANALYSIS (FFT) AND ORIGIN OF ON AND OFF EVOKED FIELD POTENTIALS IN THE RAT SUPERIOR COLLICULUS DURING DEVELOPMENT. S. Fortin[1], S. Itaya*[2], I. Dumont[1], S. Chemtob[1], and S. Molotchnikoff[1]. 1: Dept. Biology, Univ. of Montreal, Que., Canada. 2: Dept. Biomed. Sci., Univ. South Alabama, Mobile, AL 36688.

ON and OFF light evoked field potentials were recorded in the superior colliculus of anaesthetized rats during development. The results presented last year (Soc. Neurosci. Abstract 21:816) showed that OFF evoked field potentials exhibited a strong oscillatory pattern.

Recent simultaneous multi-electrode recordings demonstrated that these oscillatory potentials appear at P15 at a frequency of 20 Hz, and as the rats grow older, the oscillatory frequency increases to reach 35-40 Hz in the adult. The form and the amplitude of ON and OFF field potentials vary considerably depending on the position of the electrode tip in the colliculus. Experiments show that OFF oscillatory potentials are circumscribed in a small zone of the superior colliculus. It appears that oscillations are most prominent in the ventral and medial part of the stratum griseum superficiale. Finally, local cobalt injection provided evidence that these oscillations are produced within the superior colliculus itself rather than in an extra-collicular structure.
Supp. by C.R.S.N.G. and F.C.A.R.

305.1

EARLY EFFECTS OF GONADAL STEROIDS ON NUCLEOLAR MORPHOLOGY IN AXOTOMIZED HAMSTER FACIAL MOTOR NEURONS (FMN). N.B.Kinderman* and K.J. Jones, Dept. of Cell Biology, Neurobiology & Anatomy, Loyola Univ. Chicago, Maywood, IL 60153, and Rehab. Res. & Dev., Hines VA Hospital, Hines, IL 60141.

Transcriptional activation of the ribosomal gene occurs within 30' after facial nerve transection at the stylomastoid foramen in gonadectomized (gdx) male hamsters, with or without the addition of exogenous testosterone propionate (TP). However, the subsequent processing steps necessary to form mature ribosomes are substantially accelerated by the addition of TP at the time of axotomy. To explore these findings further, we examined nucleolar morphology in axotomized FMN. Gdx male hamsters received right facial nerve axotomies, with the left side serving as internal control. Half the axotomized animals were immediately implanted with 1 TP Silastic capsule., with 3 animals per group. At postoperative (po) times of 12 and 24 h, the animals were killed by intracardiac perfusion-fixation. Right and left facial nuclei were dissected and processed for routine electron microscopy. Fifteen- 20 EMs (mag =15,000x) of FMN from each group were collected for analysis. Stereological analysis using a point counting system was accomplished for the various subcomponents of the nucleolus, including granular region, nucleolonema, vacuolar space, and fibrillar centers. The data were expressed as a ratio of nucleolar subcomponent area/nucleolar area. The results indicate that, at these early po times, only the granular material was affected. The addition of TP at the time of axotomy resulted in an initial mobilization of the granular portion of the nucleolus at 12 h that was not observed with injury alone. By 24 h, however, the effects of axotomy with or without TP were evident, with there being an increase in granular material. The other subcomponents of the nucleolus were unaffected by either axotomy or TP treatment. These data support the idea that gonadal steroids accelerate, or somehow alter, the process of ribosome buildup after axotomy, probably at the level of processing, not transcription activation, of the rRNA gene. Supported by NIH grants NS28238 (KJJ).

305.2

DETECTION OF RETROGRADELY TRANSPORTED WGA-HRP IN AXOTOMIZED HAMSTER FACIAL MOTONEURONS (HFMN) OCCURS AFTER INITATION OF THE CELL BODY RESPONSE. Christopher Huppenbauer[1,2], Lisa Tanzer[1,2], Talat Khan*[2] and K.J. Jones[1,2], [1]Dept. of Cell Biology, Neurobiology & Anatomy, Loyola Univ. Chicago, Maywood, IL 60153 and [2]Rehab. Res. & Dev., Hines VA Hospital, Hines, IL 60141.

We have previously demonstrated, in adult male hamsters, that axotomy of the facial nerve at the stylomastoid foramen activates ribosomal RNA transcription within 30' postoperative (po). The signal for initiation of the axon reaction in motoneurons subsequent to a peripheral nerve lesion is unknown. One hypothesis put forth is that a factor at the periphery is retrogradely transported to the cell body and initiates the injury response. To examine this in view of our rapid effects of axotomy on rRNA transcription, male and female hamsters were subjected to right facial axotomies, with the left side serving as internal control. WGA-HRP was applied at the proximal stump. At 30', 2,3,4, 6 and 24 h po, the animals were killed by intracardiac perfusion. The brains were removed, blocked to include the facial nuclei, and 25 µm cryostat sections collected. The sections were processed for WGA-HRP, using the TMB method. Four stages of labelling intensity, ranging from no labeling to dark labelling, were defined. Quantitative analysis was accomplished and the percentage of sections labeled at the 4 different levels determined for experimental and control groups at all timepoints. No labelling was detected in any of the contralateral control sides. In males, the earliest timepoint at which detection was observed in the axotomized facial nucleus occurred at 3 h. In females, the earliest timepoint occurred at 4 h. Significant labelling was observed in axotomized facial neurons of both sexes by 6h po. These results do not support the hypothesis that a retrogradely transported factor is the signal for the axon reaction, but do support our previous observations of sex differences in facial nerve regeneration in the hamster. Supported by NIH grant NS28238 (KJJ).

305.3

ANDROGEN RECEPTOR (AR) REGULATION IN ADULT HAMSTER FACIAL MOTOR NEURONS (FMN): EFFECTS OF AXOTOMY AND/ OR TESTOSTERONE PROPIONATE (TP). S.M. Drengler*[1,2], R.J. Handa[1], L.L. DonCarlos[1], K.J. Jones[1,2], [1]Loyola University Chicago, Maywood, IL, 60153 and [2]Hines VA Hospital, Hines, IL,60141.

Exogenous TP accelerates recovery from facial paralysis induced by facial nerve crush axotomy, through an androgen-receptor mediated mechanism. In this study, we examined the regulatory effects of axotomy, with and without exogenous TP, on AR mRNA levels in FMN of adult, gonadectomized (gdx) male hamsters. Following right facial nerve axotomy, half the axotomized animals were subcutaneously implanted with one 10-mm Silastic capsule containing 100% crystalline TP, with the other half sham implanted. Postoperative times were 1,2,&7 d. *In situ* hybridization with an AR riboprobe, encoding the DNA binding domain of the AR, demonstrated a down-regulation of AR mRNA after AX, relative to the uninjured side. Surprisingly, TP treatment did not reverse the axotomy-induced down-regulation of AR mRNA levels, even though TP treatment has been shown to upregulate AR mRNA in FMN of uninjured gdx animals. These findings suggest that axon injury regulates the expression of androgen receptor mRNA in motoneurons. It is concluded that accelerative effects of TP on FMN regeneration are mediated by existing, rather than newly synthesized, AR. We are currently using ICC and the PG-21 antibody (gift of Dr. G. Prins) to AR to test this hypothesis. NIH NS28238 (KJJ),NIMH 48794 LLDC), and NSF IBN 9408890 (RJH).

305.4

GONADAL STEROID ENHANCEMENT OF HAMSTER FACIAL NERVE REGENERATION: EFFECTS OF DIHYDROTESTOSTERONE PROPIONATE (DHTP) AND ESTRADIOL (E). Lisa Tanzer[1,2], Jane M. Jacob*[3] and K.J. Jones[1,2], [1]Dept. of Cell Biology, Neurobiology & Anatomy, Loyola Univ. Chicago, Maywood, IL 60153, [2]Rehab. Res. & Dev., Hines VA Hospital, Hines, IL 60141, and [3]Dept. of Anat. Sci., Univ. Oklahoma, Oklahoma City, OK 73190.

In adult male hamsters, systemic administration of testosterone propionate (T) at the time of facial nerve crush axotomy of the facial nerve accelerates functional recovery and the rate of axonal regeneration by 30%. While further work indicates that these effects are, at least in part, androgen receptor (AR)-mediated, T is capable of being converted, by aromatase enzymatic activity, into E. Hence, a role for E in the enhancement of facial nerve regeneration could not be ruled out. Given recent reports of synergistic actions of T and E, as well as our findings of a role for E in AR mRNA regulation in hamster facial motoneurons (FMN), we explored the question of estrogenic regulation of facial nerve regeneration in this study. Adult male hamsters were gonadectomized, and subjected to right facial nerve crush axotomies. The axotomized animals were implanted with 1 subcutaneous capsule containing either 100% crystalline DHTP, a nonaromatizable androgen, 100% crystalline E, or nothing. The radioisotopic procedure to label fast axonally tranported proteins was used to assess outgrowth distances of the fastest regrowing axons at postoperative times of 4 and 7 days. The results indicate that, as expected, DHTP accelerated the rate of facial nerve regeneration by about 40%. E also accelerated the rate of facial nerve regeneration, albeit to a lesser degree (30%). Interestingly, DHTP prolonged the delay time before sprout formation occured, whereas E did not. These results indicate that E may also act to affect facial nerve regeneration, but through a different pathway than androgens. This conclusion is further supported by the knowledge that the facial nucleus of the hamster does not appear to contain E receptors, but does contain androgen receptors. Supported by NIH grant NS28238 (KJJ).

305.5

REVERSE TRANSCRIPTION - POLYMERASE CHAIN REACTION IDENTIFICATION OF GROWTH FACTORS AND GROWTH FACTOR RECEPTORS EXPRESSED BY HUMAN NEUROBLASTOMA SH-SY5Y CELLS. D.L. Hynds, A.A Rampersaud, D.A. Kniss and A.J. Yates*, Departments of Cell Biology, Neurobiology and Anatomy and Pathology, The Ohio State University College of Medicine, Columbus, OH 43210.

SH-SY5Y human neuroblastoma cells respond both mitogenically and neuritogenically to some growth factors, such as insulin, insulin-like growth factor-I (IGF-I) and platelet-derived growth factor (PDGF), that signal through protein tyrosine kinase receptors. Our results indicate PDGF increase average neurite length in SH-SY5Y cells by 2.5 fold and over unstimulated controls. Insulin and IGF-I also increase average neurite length by approximately 2.0 fold over controls. PDGF, insulin and IGF-I increase [^{3}H]-thymidine incorporation by 2.5, 1.2 and 1.3 fold, respectively. SH-SY5Y cells have a high basal level of mitosis in serum-free conditions. Therefore, it appears that SH-SY5Y cells are stimulated in an autocrine fashion to promote their own proliferation. Using a reverse transcription - polymerase chain reaction (RT-PCR) of the total RNA fraction we identify mRNA expression of growth factors and growth factor receptors. Co-expression of a growth factor and its receptor may indicate routes of autocrine stimulation in these cells. SH-SY5Y cells express the mRNA for insulin receptor, IGF-I receptor, both α and β type PDGF receptors, insulin-like growth factor-II (IGF-II) and PDGF-A. From these data, it is concluded that PDGF-A and IGF-II may be an autocrine stimulators of mitogenesis or neuritogenesis in SH-SY5Y cells. Supported by NINDS grant NS10165.

305.6

TROPHIC EFFECTS ON CULTURES OF IDENTIFIED BRAINSTEM-SPINAL NEURONS. D.M. Pataky* and J.D. Steeves, Departments of Zoology, Surgery and Anatomy, UBC., Vancouver, Canada.

Damage to brainstem-spinal neurons as a result of spinal cord injury results in sensorimotor deficits. In order to promote effective repair of these projections it is desirable to specifically study these neurons. Using a retrograde pre-labelling paradigm we have developed an *in vitro* system for studying brainstem-spinal projection neurons. This allows the *in vitro* identification of neurons that are typically disrupted after a spinal cord injury. Fertilized eggs from White Leghorn chickens were windowed on embryonic day (E)5 and a small crystal of DiI implanted into the cervical spinal cord. This procedure labeled many of the neurons that are actively extending axons from the brainstem into the spinal cord. Three days later (E8), the hindbrain was dissected from the embryos, the cells dissociated and plated at low density. Previously, we have shown that FGF-2 (10ng/ml) can enhance the survival of identified brainstem-spinal neurons (82% more than serum-free control) using this assay system. We are following up this finding with an examination of FGF receptor expression in the assay system and *in vivo* during the normal development of the chick. We have also begun to address the possibility that non-neuronal cell types present in the culture (ie. astrocytes) may mediate the FGF-2 survival effect. We prepared astrocyte conditioned medium (ACM) from 99% pure human fetal astrocyte cultures (gift from Dr. Seung Kim, UBC). In a dose-dependent manner, ACM can support the survival of up to 43% more identified brainstem-spinal neurons compared to serum-free control medium (DMEM). This raises the possibility that astrocytes can contribute to the survival of axotomized long projection CNS neurons. Supported by the Canadian Neuroscience Network.

305.7

AXOTOMY-INDUCED GLIAL CELL DEATH IN THE DEVELOPING CHICK SPINAL CORD. Christopher B. McBride*, John McGraw and John D. Steeves, Depts of Zoology, Surgery, and Anatomy, UBC, Vancouver, BC.

Studies of programmed cell death (PCD) in the nervous system have focused primarily on central and peripheral neurons. However, the role of glial PCD is becoming recognized as an important feature of nervous system development and perhaps degenerative disorders such as multiple sclerosis. In this study, we investigated the role of one form of PCD, apoptosis, in spinal glia following transection of embryonic day (E) 12, E14 and mature (P2) chick spinal cord. Within 24-72 hrs, TUNEL and Hoescht 33258 staining revealed large numbers of apoptotic cells confined to the white matter tracts over several segments rostral and caudal to the transection site in embryonic chicks. By contrast, few apoptotic cells were detected in sham treated embryonic or transected hatchling spinal cords. Determination of the cell type undergoing apoptosis was accomplished by double-labeling spinal cord sections for apoptosis and oligodendrocyte-specific (carbonic anhydrase II, myelin basic protein) or astrocyte-specific (GFAP) markers. Many apoptotic cells were positively labeled for carbonic anhydrase II and MBP, but not GFAP, indicating apoptosis in the spinal white matter was restricted primarily to dying oligodendrocytes as opposed to astrocytes. Previous studies have demonstrated that oligodendrocytes begin to differentiate on E9, with myelination occurring between E13 and E17. This period appears to coincide with an increased susceptibility to injury-induced apoptosis. These results indicate that axotomy-induced PCD of oligodendrocytes is a feature of the injury response following axotomy of the developing CNS. Thus, this model may be useful in elucidating the mechanisms of oligodendrocyte cell death. Supported by Rick Hansen Man in Motion Foundation, Canadian Neuroscieence Network and the Medical Research Council of Canada.

305.8

REGENERATION OF DESCENDING BRAINSTEM-SPINAL & CORTICOSPINAL AXONS AFTER IMMUNOLOGICAL MYELIN DISRUPTION OF THE ADULT RAT SPINAL CORD. J.K. Dyer* and J.D. Steeves. Depts. Zoology, Anatomy and Surgery, UBC, 6270 University Boulevard, Vancouver, BC, V6T 1Z4, CANADA.

The regenerative ability of the adult vertebrate CNS is limited. During development the loss of CNS axonal regeneration coincides with the onset of myelination.

Demyelination of the injured nervous system has been postulated as a possible way of overcoming these inhibitory influences. Most demyelination protocols are irreversible, as well as having adverse effects on other CNS cell phenotypes. We have previously described a transient immunological, myelin-specific, antibody-mediated (anti-GalC), complement-dependent protocol that: (1) suppresses the developmental onset of spinal cord myelination, and (2) disrupts myelin structure in the mature avian CNS; promoting some axonal regeneration and functional recovery after complete transection of the avian spinal cord.

We now describe the effects of injection/infusion of the immunological reagents on adult rat CNS myelin and the possible application to facilitate the regeneration of specific brainstem-spinal tracts. Myelin disruption is evident in the adult rat spinal cord shortly (<1d) after infusion. There is a down-regulation of myelin-associated proteins and a concomitant unraveling of the myelin lamellae. Long term infusion results in local demyelination, with distal myelin-disruption.

Our previous studies in the hatching chick indicated that this change in the myelin is sufficient for some axonal regeneration after injury. We are currently investigating whether this approach facilitates regeneration of descending spinal axons (e.g. corticospinal, rubrospinal and vestibulospinal) in the adult rat.

JKD is a Fellow of The Rick Hansen Man in Motion Foundation and a trainee of the Canadian NeuroScience Network, supported by the Canadian Neuroscience Network.

305.9

TRANSPLANTS, NEUROTROPHIC FACTORS AND MYELIN-ASSOCIATED NEURITE GROWTH INHIBITORS: EFFECTS ON RECOVERY OF LOCOMOTOR FUNCTION AFTER SPINAL CORD INJURY IN ADULT RATS B.S. Bregman*, H.N. Dai, T. Lin, C.V. James, M.McAtee, M.E. Schwab, L. Schnell, Georgetown University Medical Center, Washington, D.C. 20007 and University of Zurich, Zurich, Switzerland

The extent of axonal growth after CNS injury in the adult rat can be increased by any one of a variety of interventions. Transplants of fetal spinal cord tissue, exogenous neurotrophic support and antibodies to myelin-associated neurite growth inhibitors (IN-1) each lead to an increase in growth of mature CNS axons after injury. We sought to determine whether the anatomical plasticity elicited by these interventions **in combination** increases the degree of recovery of function after spinal cord injury. Spinal cord over-hemisection lesions were made at T6 in adult rats. Transplants of E14 spinal cord tissue were placed into the lesion site. At the time of the lesion IN-1 was applied intraventricularly and neurotrophin (NT-3) was applied at the site of the transplant. Recovery of locomotor function was examined for 6 weeks after injury in overground and treadmill conditions. Locomotor function was assessed both directly and from videotapes; the Peak Performance System was used for kinematic analysis. There is a dramatic loss of locomotor function in both hindlimbs immediately after the injury. The early recovery (1-7 days after injury) is identical in all treatment groups. By 10-14 days after injury and thereafter, however, the extent of recovery in all of the IN-1 treated groups was significantly better than that in groups receiving control antibodies. In addition, some aspects of accurate locomotion (number of errors on grid runway) show greater recovery in the rats receiving combined treatment (HX+TP+IN-1+NT-3) than any of the individual interventions (HX+TP or HX+IN-1). The greater recovery was accompanied by increases in the anatomical plasticity of corticospinal, raphespinal and coeruleospinal pathways both within the transplant and within the host spinal cord caudal to the lesion. Supported by NIH-NINDS NS 27054; T32 HD07459, ISRT; neurotrophins supplied by Regeneron.

305.10

NT3 ENHANCES DORSAL ROOT REGENERATION INTO SPINAL CORD. Y.Itoh[2], K.Iwaya[1], F.Mori[1], T.Sugawara[1], K.Mineura[1], M.Kowada[1], A.Tessler[2]. [1]Dept. of Neurosurg., Akita Univ. Sch. of Med., Akita City, Akita 010, Japan; [2]Dept. of Neurobiol. and Anat., Med. Col. of PA / Hahnemann Univ. Philadelphia, PA 19129, Philadelphia VA Hospital, Philadelphia, PA 19104.

We have shown previously that embryonic CNS transplants allow adult dorsal roots to regenerate into host spinal cord and form permanent synapses with host neurons, suggesting that transplants release factors permissive for dorsal root regeneration. In the present study we examined whether neurotrophin-3 (NT3) is one of these factors. We used immunocytochemical methods to label regenerated dorsal roots that contain calcitonin gene-related peptide (CGRP). Adult Sprague-Dawley rats received NT3 (provided by Regeneron) by release from a fibrin glue ball placed into a dorsal quadrant cavity in the lumbar enlargement. The cut L4 or L5 dorsal root stump was sandwiched between the fibrin glue ball and host spinal cord. One month later the grafts were processed for CGRP immunocytochemistry, and regenerated axons analyzed quantitatively. CGRP-labeled axons regenerated into host spinal cord; some of them extended into the motoneuron pool. The area fraction occupied by regenerated axons in rats that received NT3 was significantly larger than in control rats that did not receive NT3. NT3 is one factor that contributes to regeneration of dorsal root axons into host spinal cord and may therefore promote reconstruction of interrupted spinal reflex arcs.

305.11

PROMOTION OF THE SURVIVAL OF DEVELOPING RETINAL GANGLION CELLS AFTER AXOTOMY. S. Shen* and B. A. Barres, Stanford University School of Medicine, Department of Neurobiology, Stanford, CA 94305.

We have recently proposed that the signaling mechanisms that promote the survival and growth of developing CNS and PNS neurons may differ: whereas many types of PNS neurons respond to a single peptide trophic factor, CNS neurons require a combination of peptide trophic factors as well as a coincident signal, elevation of intracellular cAMP, that enhances responsiveness to the peptide trophic factors. Could this difference help to explain why CNS and PNS neurons differ in their response to injury?; whereas PNS neurons survive and regenerate their axons after an axotomy, most types of CNS neurons die and do not regenerate. For instance, CNS neurons might die after axotomy not only because they lose their retrograde supply of trophic factors but because their cAMP levels decrease.

We previously showed that highly purified postnatal day 8 (P8) retinal ganglion cells (RGCs) rapidly undergo apoptosis in serum-free medium in vitro; the majority of RGCs survive in culture when the medium also contains the combination of peptide trophic factors BDNF, CNTF, IGF-1, as well as forskolin to active adenylate cyclase (Meyer-Franke et al., Neuron 15:805-919, 1995). Others have found that P8 RGCs in vivo after an axotomy, but that their survival is not significantly promoted by intraocular injection of BDNF or CNTF. Here we show that the survival of P8 RGCs in vivo is significantly decreased by pharmacological inhibition of protein kinase A and that the survival of axotomized P8 RGCs is significantly increased by intraocular injection of peptide trophic factors (BDNF and CNTF) together with forskolin. These findings demonstrate that axotomized RGCs in vivo have similar survival requirements as RGCs in vitro and raise the question of whether regeneration of their axons can be achieved by activating the same signaling pathways.

This work was funded by the National Eye Institute (1RO1-EY11310).

305.12

Effects of Calcium, Trophic Factors, and Conditioning Lesions on Neuronal Morphogenesis. K.L. Lankford*, S.G. Waxman and J.D. Kocsis Dept. of Neurology, Yale Univ. School of Med., New Haven CT 06510

In order to identify possible contributions of trophic factors and calcium signals to conditioning lesion enhancement of peripheral nerve regeneration we subjected adult rats to a unilateral midthigh sciatic nerve crush or ligation and compared the timing of neurite initiation, rate of outgrowth and branching frequency in cultures of conditioning lesioned neurons to control neurons exposed to NGF, BDNF, NT-3, ligated DRG-conditioned media, Schwann cell-conditioned media, 200 nM thapsigargin, 3 mM caffeine or 50 mM KCl. Our results indicate that conditioning lesioned neurons are developmentally advanced compared to controls. Both crushed and ligated neurons initiate processes earlier than controls and branch less, appearing similar to cultures 1-2 days older. However, conditioning lesioned neurons do not extend processes in culture at significantly greater rates than controls. No single factor appeared to account for all of the conditioning lesion-induced effects. KCl and caffeine both mimicked conditioning lesion-induced advancement of neurite initiation, while thapsigargin inhibited initiation, but branching frequency was unaffected by these Ca^{2+} manipulations. NGF and NT-3 increased neurite lengths in at least some classes of neurons and their production by denervated Schwann cells could account for the greater rate of neurite outgrowth in crushed as compared with ligated neurons, but neither agent affected branching frequency and effects on neurite initiation were marginal. Schwann cell-conditioned media reduced neurite branching and increased outgrowth rates, but had no effect on initiation. Our result suggest that: (1) prior injury promotes subsequent neurite initiation through a possibly Ca^{2+} dependent mechanism, (2) exposure to neurotrophins during regeneration stimulates faster outgrowth, and (3) an unidentified mechanism reduces neurite branching. Together, these changes result in more rapid recovery after a second nerve injury. Supported by NIH grant NS10174.

305.13

ACTIVATION OF THE EGF-RECEPTOR STIMULATES CHONDROITIN SULFATE PROTEOGLYCAN PRODUCTION IN PRIMARY ASTROCYTES. G.M. Smith* Dept. Anesthesiology and Pain Management, U.T. Southwestern Medical Center, Dallas, TX 75235-9068

The up-regulation of chondroitin sulfate proteoglycan (CSPG) after injury may play an important role in regenerative failure. To reduce the deleterious effects of these molecules it is important to understand which factors regulate their expression. ELISA assays of the conditioned medium from astrocytes treated with cytokines and growth factors, known to be expressed after injury, reveal that in the presence of epithelial growth factor (EGF) CSPG is increased 6 to 8 fold. Treatment of astrocytes with other EGF-receptor ligands, such as TGF-alpha and heparin-binding EGF, produced similar increases in CSPG. In addition, these effects could be reversed by AG 1478, a selective EGF-receptor antagonist. Interestingly, the amount of CSPG bound to the surface of astrocytes remained unchanged with all treatments. Western blot analysis of supernatants pre-digested with chondroitinase ABC indicates the presence of multiple isoforms of both the 4-sulfated and 6-sulfated chondroitin as detected by the antibodies 2B6 and 3B3, respectively. To determine if EGF-induced CSPG inhibits neurite outgrowth, we grew dorsal root ganglion neurons on dishes blotted with EGF-treated or non-treated astrocyte-conditioned medium. Neurites did not grow over the area containing the supernatant from astrocytes treated with EGF. However, they grew well over the area containing the supernatant from control astrocytes (not treated with EGF). The inhibitory effect on neurite growth could also be reduced when the supernatant from EGF-treated astrocytes was incubated with chondroitinase ABC. These data show that EGF-receptor ligands up-regulate CSPG production by astrocytes and that this increase in CSPG inhibits neurite outgrowth. Supported by the Sid W. Richardson Foundation and NIH (NS 33776).

305.14

ANTIBODIES AGAINST BASIC FIBROBLAST GROWTH FACTOR (bFGF) SUPPRESS THE FORMATION OF THE LESION INTERFACE AFTER STAB WOUNDS TO THE ADULT RAT CEREBRAL CORTEX. Xia Wang and Samuel David*, Centre for Research in Neuroscience, The MGH Research Institute & McGill University, 1650 Cedar Ave., Montreal, Que., Canada, H3G 1A4.

A lesion interface consisting of a glia limitans, a laminin-rich basal lamina, and a fibrotic scar comprising of leptomeningeal cells, forms within 2-3 weeks after stab wounds to the adult rat cerebral cortex. Such an interface prevents the growth of axons across CNS lesions. The expression of bFGF increases after cortical stab wounds, and peaks at 7 days post-lesion. In this study, we have examined the effects of anti-bFGF in suppressing the formation of this interface. Anti-bFGF (50μg/ml) was infused for 14 days over the site of cortical stab wounds in adult rats using Alzet osmotic mini-pumps. Animals were then perfused with 4% paraformaldehyde in 0.1M phosphate buffer, and cryostat sections through the area of the lesion were double-labeled by immunofluorescence with either anti-laminin/anti-GFAP, or anti-fibronectin/anti-GFAP antibodies. There was a marked reduction in the length of the laminin$^+$ and fibronectin$^+$ areas along the lesion, as compared with lesioned controls. The percentage of the total length of the lesion that was laminin$^+$ was 7.6 ± 7.5% as compared with 69.5 ± 8.5% for lesioned controls. Similarly, the percentage of the total length of the lesion that was fibronectin$^+$ was 22 ± 6% as compared with 60 ± 11% for controls. In addition, the width of the fibronectin$^+$ areas comprising the fibrotic scar was also markedly thinner and weakly labeled in anti-bFGF treated lesions. These results indicate that the fibrotic scar that forms within the lesion cavity, and the basal lamina that lines the glia limitans after cerebral cortical stab wounds in adult rats, can be diminished by blocking bFGF.

Supported by grants from NSERC and MRC.

305.15

MOLECULAR CLONING AND REGULATION OF RAT AXL TYROSINE KINASE RECEPTOR COMPARED WITH ITS PUTATIVE LIGANDS GAS6 /PROTEIN S IN THE NERVOUS SYSTEM Hiroshi FUAKOSHI[1*], Tomoko YONEMASU[1,2], and Toshikazu NAKAMURA[1] [1]Division of Biochemisrty, Biomedical Research Center, Osaka University Medical School & [2]Protein Research Institute, Osaka University, Osaka 565, Japan

Axl/UFO tyrosine kinase receptor is a member of tyrosine kinase receptor "Sky/Rse/Tyro3" family, which also includes c-Mer. The family exhibits a unique extracellular structure characterized by the immunoglobulin-like (IgL) repeats and fibronectin III (FNIII) repeats with intracellular tyrosine kinase. IgL repeat is observed in the receptor for neurotrophic factor and FNIII repeat is observed in cell adhesion molecule, suggesting that Axl could have important function such as trophic and guidance.

To assess the role of Axl tyrosine kinase receptor, we have cloned a rat axl by PCR. Cloned rat axl had two forms, with and without 27 nuleotides in the juxtamembrane domain. Expression of mRNAs for rat axl, gas6 and proteinS were analyzed quantitatively by RNase protection assay using specific cRNA probes. All these genes were detected in the brain, spinal cord, DRG, and sciatic nerve. Moreover gas6 and proteinS mRNAs were regulated in the lesioned nerve, suggesting that Axl and its putative ligands have an important role during nerve regeneration. axl mRNA was detected in glial cells, C6 / U138MG, but not in neuronal cells, PC12 / GH3, suggesting that Axl mainly works in non-neuronal cells like glial cells in the nervous system.

305.16

REGENERATION OF CEREBELLAR PURKINJE CELL AXONS IS AN AGE-DEPENDENT PROCESS: AN IN VITRO STUDY AFTER AXOTOMY, I. Dusart* and C. Sotelo, INSERM U106, Hôpital de la Salpêtrière, 75013 Paris, France.

In vivo adult Purkinje cells are among the most resistant neurons to axotomy but their axons do not regenerate. Either a role of environmental factors or a loss of the program for axonal growth could explain this lack of regeneration. We have developed an *in vitro* set up of axotomy to study age and environment related factors potentially implicated in Purkinje cell axonal regeneration. Purkinje cells from 10 days old rats have been axotomized after one week in culture and have been confronted with slices of sciatic nerves or slices of embryonic cerebellum (from E19 to E22). These Purkinje cells survive *in vitro* axotomy, and their axons, although unretracted, do not regenerate in the presence of slices of sciatic nerves or embryonic cerebellum slices. In contrast, E19 to P0 axons of Purkinje cells are able to regenerate on slices of mature-like cerebellum. Most of the Purkinje cells from 1 to 5 day old rats die when seeded in organotypic culture. Purkinje cells from rats older than 7 days survive but do not regenerate. In all cases, few postnatal deep nuclei neurons, the target neurons of Purkinje cells, survive in organotypic culture. Thus, we demonstrate that between P1 and P5, Purkinje cells depend on their target neurons for their survival. It is interesting to note that this developmental period (P1 to P5) corresponds to the synaptogenic period of these neurons. Furthermore, the failure of Purkinje cell regeneration is age-related since embryonic Purkinje cells regenerate on postnatal cerebellum whereas postnatal Purkinje cells do not.

305.17

DO ALL SPINOCEREBELLAR AXONS GROW THROUGH A LESION OF THEIR SPINAL PATHWAY DURING THE SAME CRITICAL PERIOD?, J.R. Terman*, X.M. Wang and G.F. Martin, Dept. of Cell Biology, Neurobiology & Anatomy and Neuroscience Program, The Ohio State University, Columbus, OH 43210.

Using the developing opossum, Didelphis virginiana, we have shown that axons which originate within Clarke's nucleus (axons of the dorsal spinocerebellar tract [DSCT]) grow through a lesion of their spinal pathway and reach the cerebellum when the lesion is made on postnatal day (PD) 5 but not when it is made on PD12. Spinocerebellar fibers originate throughout the spinal cord, however, and differ in their developmental history, laterality, funicular position, course, and terminal distribution. We have asked, therefore, whether spinocerebellar fibers which do not originate within Clarke's nucleus (CN) also grow through a spinal lesion and, if so, whether the critical period for such growth is the same as that for axons of the DSCT. In the present study, we focused on spinocerebellar axons supported by spinal border cells (SBC), neurons within Stilling's nucleus (SN), and neurons within the sacral/coocygeal ventrolateral nucleus (VLN). Pouch-young opossums were anesthetized and subjected to a right hemisection of the mid-thoracic cord on PD5. One to nine months later, bilateral injections of Fast Blue (FB) or Fluoro-Gold were made into the anterior lobe of the cerebellum, the major target of spinocerebellar axons. After a 7 day survival, the animals were sacrificed so that their spinal cord and brain could be processed for fluorescence photomicroscopy. In all cases, neurons were labeled bilaterally within SBC, SN and VLN. Since the cerebellar projection from each nucleus is contralateral (unpublished results), labeled neurons contralateral to the hemisection must have supported axons which grew past the lesion and reached the cerebellum. When similar lesions were made on PD12, and the animals were allowed to survive for 1-4 months prior to injections of FB, labeled neurons were not present in any of the above mentioned nuclei contralateral to the lesion although they were present ipsilateral to it. These results suggest that spinocerebellar axons supported by SBC, neurons within SN, and neurons within the VLN grow rostral to a lesion of their spinal pathway during early development and that the critical period for such growth is comparable to that for axons which originate within CN. (Supp. by NS-25095 and 10165).

305.18

PLASTICITY OF GALANINERGIC FIBERS FOLLOWING NEUROTOXIC DAMAGE WITHIN THE RAT BASAL FOREBRAIN. S. de Lacalle*, R.G. Wiley and E.J. Mufson. Depts. of Neurology, Beth Israel Hosp., Boston, MA 02115; VAMC, Nashville, TN 3 7212, Rush Med. Ctr, Chicago, IL 60612.

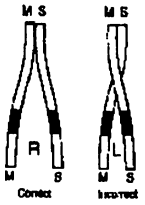
Galanin (GAL)-immunoreactive (-ir) fibers hyperinnervate remaining cholinergic basal forebrain (CBF) neurons within the medial septum (MS) and diagonal band of Broca (DBB) in AD. The present investigation determines whether an hyperinnervation of GAL-ir fibers occur following unilateral intraparenchimal injections of ibotenic acid or 192 IgG-saporin within the MS or DBB in young adult F344 rats. Sections through the MS and DBB were concurrently stained for GAL and the p75 receptor, an excellent marker of CBF neurons. We found a moderate to intense increase in the density of galanin immunoreactivity within the MS on the lesion, as opposed to contralateral side. In contrast, in DBB-lesioned rats the increase was low to moderate as compared to the contralateral side, and also surrounded the lesion site. There was no apparent correlation with the amount of cell loss in either lesion paradigm. GAL hypertrophy occurred independent of lesion type. In both models, GAL-ir fibers appeared coarse and thicker on the lesioned side as compared to the non-lesioned side. Preliminary experiments indicate that the GAL hypertrophy is not triggered by the injection itself since it was not seen in animals surviving less than 7 days. The plastic response was documented in animals surviving 2 weeks post-lesion. Since longer survival (>8 weeks) returned galanin innervation to normal levels, we hypothesize that GAL hyperinnervation of surviving neurons may have a transient protective effect by down-regulating neurotransmitter release. Supported by NIH AG12401, AG10161, AG10668 and AG9466.

305.19

MOTOR AND SENSORY SPECIFICITY OF HOST NERVE AXONS INFLUENCE NERVE ALLOGRAFT REJECTION RESPONSE, R. Midha*, C. Munro, S.E. Mackinnon and L. Ang. Sunnybrook Health Sciences Centre, Univ. of Toronto, Toronto, Ontario and Washington University, St. Louis, MO.

Previous studies have shown both demyelination and Wallerian degeneration within nerve allograft segments after withdrawal of Cyclosporin A (CsA) immunosuppression (J Neurosurg 78:90, 1993; Trans Proc 25:532, 1993). We hypothesized that the nature of end-organ reinnervation may influence the response of the axon, with demyelination for appropriate innervation versus degeneration for inappropriate innervation. The Lewis rat femoral nerve model was chosen. Four ACI rat peroneal nerve allografts (2 cm length) were sutured in correct (right leg) or incorrect (left leg) orientation with regard to motor (M) and sensory (S) specificity (see diagram) in each Lewis rat. Rats received 5 mg/kg s.c. CsA daily for 8 weeks to allow end-organ reinnervation and were sacrificed at 8 (n=5), 10 (n=5), 12 (n=20), 14 (n=10) and 20 (n=5) weeks. Wallerian degeneration, then regeneration, predominated in graft segments. At week 12 and 14, demyelination of the axons was a dominant response in at least one graft in 13/30 rats, and was significantly more common in the motor to motor and motor to sensory orientations ($p<0.05$). We conclude that the nature of the host axon, rather than the end-organ, may influence whether the axon undergoes demyelination or degeneration after immunosuppression cessation.

Supported by Physicians' Service Incorporated and Sunnybrook Trust Fund.



TRANSPLANTATION II

306.1

A GENE THERAPY MODEL FOR MOTOR NEURON INJURY.

A. Blesch*, K. Farhoomand and M.H. Tuszynski.
Dept. of Neurosciences-0608, Univ. of California at San Diego, La Jolla, CA 92093 and VA Medical Center, San Diego, CA 92165

The hypoglossal nucleus is a useful model for the study of motor neuronal responses to injury. Axotomy of hypoglossal axons results in downregulation of their cholinergic neuronal phenotype, which we and others have previously shown is preventable by intracerebroventricular infusions of the trkB agonists NT-4/5 and BDNF, but not by NT-3 or NGF. To test whether cells genetically modified to produce neurotrophins are also able to prevent this change in phenotype, MLV based retroviral vectors for NT-4/5, GDNF and LIF/CDF were constructed. Bioactivity and secretion rates were tested by survival of E-14 ventral mesencephalon cultures (for NT-4/5 and GDNF), proliferation of TF-1 cells (for LIF/CDF) and ELISA (for GDNF). Autologous transfected neurotrophin-producing fibroblasts were then grafted adjacent to the hypoglossal nucleus after unilateral nerve transection. Size and number of ChAT positive neurons were determined using NIH Image software. Results will be discussed at the meeting.

Supported by grants from Veterans Affairs, International Spinal Research Trust, and the Deutsche Forschungsgemeinschaft.

306.2

TREATMENT OF C6 GLIOMA BY IN SITU RETROVIRUS TRANSFER OF A "SUICIDE" GENE USING AN ENCAPSULATED PACKAGING CELL LINE. V. Padrun*[1], O. Martinet[1], N. Schreyer[1], J.M. Joseph[1], X.O. Breakefield[2], P. Aebischer[1]. [1]Gene Therapy Center, CHUV, Lausanne University, Switzerland, [2]Department of Neurology, Massachussets General Hospital, Charlestown, MA 02129

The goal of this project is the development of a gene therapy approach for the treatment of glioblastoma based on the introduction of a "suicide" gene in tumor cells that confers a drug sensitivity. The gene is transmitted by replication-incompetent retroviral vectors that will only integrate into dividing cells allowing targeting of the malignant glial cells. The retroviral vectors are continuously produced by a packaging cell line. The use of microporous membrane to encapsulate the packaging cell line should prolong the life span of the xenotransplanted cells therefore enhancing the probability of infecting a large number of tumor cells. In the present study, we investigated the efficacy and dynamics of *in vivo* transduction of growing malignant brain tumors (C6) with the herpes simplex thymidine kinase gene (HSV-tk) followed by administration of the antiviral drug ganciclovir (GCV). Rats inoculated with $4x10^4$ C6 malignant glioma cells into the striatum were treated 7 days later with an intratumoral stereotaxic injection of encapsulated murine fibroblasts (psi2-VIK) producing retroviral vectors bearing the HSV-tk gene. The capsule was composed of a microporous membrane allowing the diffusion of retroviral particles. Controls received either an empty capsule, $5x10^6$ unencapsulated psi2-VIK, 50 µl of psi2-VIK cells supernatant or 50 µl of sterile cell culture medium. The animals were treated 7 days later with GCV 15 mg/kg ip twice daily for 14 days. In the group treated with encapsulated psi2-VIK cells, the amount of tumor necrosis was significantly higher than in the other control groups. No complete tumor regression was however observed. These results suggest the potential use of an encapsulated packaging cell line for infecting dividing cells in the nervous system.

Supported in part by CytoTherapeutics, Inc.

306.3

INTRAVENTRICULAR ENCAPSULATED CAC CELLS: VIABLE FOR AT LEAST 500 DAYS *IN VIVO* WITHOUT DETECTABLE HOST IMMUNE SENSITIZATION OR ADVERSE EFFECTS ON BEHAVIORAL/COGNITIVE FUNCTION, M.D. Lindner*, M.A. Plone, B. Frydel, F. Kaplan, P.M. Krueger, W.J. Bell, T.J. Blaney, S.R. Winn, S.S. Sherman, E.J. Doherty, and D.F. Emerich, CytoTherapeutics Inc., Two Richmond Sq., Providence, RI, 02906.

Numerous studies have reported that adrenal chromaffin cell transplants, including encapsulated xenogeneic adrenal chromaffin cells, have analgesic effects. However, in addition to efficacy, the clinical utility of encapsulated xenogeneic adrenal chromaffin cells for treatment of chronic pain is dependent on the duration of cell viability *in vivo*, and their relative safety. The objectives of the present study in rats were to: (1) examine encapsulated call adrenal chromaffin (CAC) cells for evidence of viable cells and continued release of analgesic agents after an extended period *in vivo*, (2) determine if intraventricular encapsulated CAC cells produce detectable adverse effects on behavioral/cognitive function, and (3) test for evidence of host immune sensitization after an extended period of exposure to encapsulated xenogeneic adrenal chromaffin cells. Results of the present study suggest that some encapsulated CAC cells remain viable for well over one year *in vivo* and continue to produce catecholamines and met-enkephalin. Post-explant device NE output was equivalent to amounts previously shown to produce analgesic effects with intrathecal implants. Encapsulated adrenal chromaffin cells also appeared relatively safe, even when implanted in the cerebral ventricals, with a lower side-effect profile than systemic morphine (4 mg/kg). There was no evidence that encapsulated CAC-cells implanted in the ventricles affected body weight, spontaneous activity levels, or performance in the delayed matching to position operant task which is sensitive to deficits in learning, memory, attention, motivation, and motor function. Finally, encapsulated CAC cells produced no detectable evidence of host immune sensitization after 16.7 months *in vivo*, although unencapsulated CAC cells produced a robust immune response even in aged rats. The results of the present study suggest that adrenal chromaffin cells remain viable *in vivo* for long periods of time, and that long-term exposure to encapsulated xenogeneic adrenal chromaffin cell implants appears relatively safe.

306.4

GRAFTING OF FIBROBLASTS IMMORTALIZED WITH THERMOLABILE LARGE T ANTIGEN INTO RAT BRAIN

Timothy C. Ryken*, Toby G. Bush, Parmjit S. Jat, Michael V. Sofroniew. Department of Neurosurgery, SUNY-HSC, Syracuse, New York, Center for Brain Repair, Cambridge, U.K., Ludwig Institute for Cancer Research, London, U.K.

Conditionally immortalized cells may provide a renewable source of tissue for CNS grafting studies. Cell lines immortalized with the thermolabile large T antigen proliferate at 33°C but cease dividing at 39°C. We investigated the properties of these conditionally immortal fibroblasts grafted syngeneically into rat brain. Clonal cell lines established by transfecting Sprague-Dawley (tsa129) and Fisher (tsa8) embryonic rat fibroblasts with the thermolabile large T antigen encoded by the simian virus 40 early region mutant tsA58 were grafted stereotactically into the forebrain of adult male rats using varying cell concentration (10,000 - 100,000 cells per µl) and volume (0.5 - 10 µl). After survival times from 3 days to 3 months, the implant sites were studied histologically. Grafted cells could be identified at all time points studied using cresyl-violet and immunohistochemistry with an antibody to the large T antigen (pab101), indicating that these cells survive grafting for a minimum of three months. No adverse neurological deficits were noted. No evidence of intracranial tumor formation or invasion of the adjacent parenchyma was observed. There was a general retraction of graft size noted over time and some indication of inflammatory response, particularly noted at the three week time point. These findings suggest that thermolabile donor cells may be useful in central nervous system grafting models, allowing unlimited propagation in vitro but limited expansion of cell number following implantation.

Supported by the AANS VanWagenen Fellowship, the Wellcome Trust and the MRC

306.5

MYOBLAST TRANSFER CLINICAL TRIALS. J.A. Florendo*, T. Quinley, G. Vastagh, T.G. Goodwin, Q. Fang, M.B. Deering, V. Duggirala, C. Larkin, L.M. Li, T.J. Yoo, N. Chase, M.D. Neel, T. Krahn, R.L. Holcomb, and P.K. Law. Cell Therapy Research Foundation, 1770 Moriah Woods Blvd. Suite 18, Memphis, TN 38117

Myoblast Transfer is a potential cell/gene therapy in which normal immature muscle cells repair genetically defective cells by integration of the normal genome through natural cell fusion. Myoblast Transfer Therapy (MTT, U.S. patent #5130141) is completing Phase II clinical trials (IND 5108) on Duchenne muscular dystrophy (DMD). The whole body trial (WBT) consists of injecting 25 billion myoblasts in two MTT procedures separated by 3 to 9 mo. Each procedure delivers up to 200 injections or 12.5 billion myoblasts to either 28 muscles in the upper body (UBT) or to 36 muscles in the lower body (LBT). A randomized double-blind portion of the study is being conducted on the biceps brachii or quadriceps. Subjects take oral cyclosporine for 3 mo after each MTT. One infantile facioscapulohumeral dystrophy and 27 DMD boys aged 6 to 16 have received WBT and 18 more DMD boys have received UBT or LBT in the past 32 mo with no adverse reaction. Nine months after MTT, immunocytochemical evidence of dystrophin has been demonstrated in 18 of the 20 subjects biopsied. Forced vital capacity increases by 33.3%, and maximum voluntary ventilation increases by 28% at 12 months after UBT. Plantar flexion increases by 52% in force in 6 mo and elbow extension increases by 28.6% in 12 mo in the ambulatory subjects. Behavioral improvements in walking, balancing, and climbing stairs have been reported by subjects. Additional analyses comparing myoblast-treated vs placebo muscles will be available upon completion of the trial, FDA review, and removal of blinding. Six years after MTT, the world's first MTT patient continues to show dystrophin in a myoblast-injected muscle with the absence of dystrophin in the contralateral sham-injected muscle. This would indicate donor cell survival, development, and function six years after transplant. MTT also has potential application to treating other genetic diseases. (Supported by public donations with FDA approval to charge for direct costs.)

306.6

XENOGRAFTS OF CLONAL MOUSE MULTIPOTENT NEURAL PROGENITORS INTO INTACT & IBOTENIC ACID (IA)-LESIONED RAT BRAIN, R.A. Fricker*[1], C. Winkler[1], E.Y. Snyder[2], A. Bjorklund[1]. [1]Lund University, Wallendberg Neuroscience Center, Solvegatan 17, S-223 62 Lund, Sweden; [2]Depts. of Neurology & Pediatrics, Harvard Medical School, Children's Hospital, Boston, MA 02115, USA

It has previously been demonstrated that the C17-2 murine multipotent, clonal, neural progenitor cell line can engraft & differentiate appropriately throughout the neural axis & across developmental stages (from embryo to adult) of intact (Neurosci. Abstr. 19:613, 1993; 20:1672, 1994; Nature 374:367, 1995; Nature Med 4:424, 1996) & injured (Exp. Neurol. 127:9, 1994; Neurosci. Abstr. 21:2028,1995; 21:2027, 1995) mice. We wished to explore whether these cells would similarly be useful as xenografts in adult rat striatum subjected to an IA lesion, a model for Huntingtons Disease. We investigated the engraftment of C17-2 in intact newborn & adult rats in order to compare their behavior in these hosts with that observed in mice. C17-2 cells, which express lacZ, were first transplanted as a suspension into the striatum or lateral ventricles of neonatal rat pups without immunosuppression. Analysis 4 wks. after grafting revealed that lacZ-expressing cells were detectable at a lower frequency in neonatal rats than in murine controls (20% vs. 60%, respectively). In addition, striatal grafts showed limited migration throughout the striatum & cortex, while intraventricular implants yielded more extensive integration in areas such as cortex, cerebellum & hippocampus, as previously reported for mice. Cells with morphologies resembling astrocytes, oligodendrocytes, & neurons were observed in varying proportions, depending on the site of integration. In the 2nd phase of the study, intact adult rat recipients, which were maintained on immunosuppression, were sacrificed 2 wks. after receiving transplants into the striatum. These results were compared, with adult immunosuppressed rats who received similar transplants into an IA-lesioned striatum. There was ~80% survival in all adult rat recipients. In intact adult rats, donor cells were confined to the injection site, whereas in lesioned hosts, the cells showed a greater degree of migration within the striatum, primarily within the lesioned area. *Supported by the Wellcome Trust and the Swedish MRC.*

306.7

TEMPERATURE-SENSITIVE INDUCTION OF P53 AND DIFFERENTIATION BY ANTICANCER AGENTS IN M213-20 CELLS. C. Conejero*, R. Wright, C. Tornatore[1] and W. Freed. *NIMH Neuroscience Center at St. Elizabeths, Washington, D.C., 20032, and [1]Laboratory of Molecular Medicine and Neuroscience, NINDS, Bethesda, Md., 20892.

M213-20 cells, a rat striatal cell line inmortalized by the temperature-sensitive tsA58 mutant of SV40 large T, expresses an immortal phenotype at the permissive temperature (33°C). At 39.5°C, T protein is thought to be inactivated, releasing p53. We have studied the temperature-sensitive induction of p53 by adriamycin, cytosine arabinoside (Ara-C), and glutamate, and the relationship between p53 activation and cellular differentiation. P53 activity was measured by a transient transfection assay, using the PG13-Luc reporter plasmid (El-Deiry et al., Cell 75: 817, 1993). The PG13-Luc plasmid contains 13 copies of the p53 binding site upstream from the polyoma promoter and a luciferase reporter construct. Increases in cellular levels of wild-type p53 activate the p53 enhancer, thereby resulting in an increase in luciferase activity. The anticancer drugs, adriamycin and Ara-C, increased p53 at both temperatures. At 33°C p53 activity was increased more markedly than at 39.5°C, showing a peak 8 hr after the initial exposure for both drugs. Glutamate also increased p53 levels, with the increase at 33°C being more sustained, and was more effective in stimulating cellular differentiation, as measured by process extension, at 33°C than at 39.5°C. In contrast, both anticancer agents, adriamycin and Ara-C, were about twice as effective in stimulating process formation at 39.5°C as compared to 33°C. These data are consistent with the possibility that adriamycin and Ara-C compared to glutamate, stimulate cellular differentiation through separate mechanisms. Induction of differentiation by adriamycin and Ara-C may require inactivation of SV40 large T tsA58 at the non-permissive temperature, and activation of p53 may contribute to differentiation under conditions of cellular damage.

306.8

SV40 LARGE T ANTIGEN IMPAIRS DIFFERENTIATION OF N18-RE-105 CELLS. **O.Dillon-Carter*, C.Conejero, C.Tornatore and W.Freed.** NIMH Neuroscience Center at St. Elizabeths, Washington, D.C. 20032*; LMMN, NINDS, NIH, Bethesda, MD 20892

N18-RE-105 cells differentiate in response to adriamycin and glutamate, both of which increase p53 activity. In N18-RE-105 cells, glutamate toxicity is thought to involve inhibition of the glutamate-cystine antiporter and consequent depletion of glutathione (Murphy et al. Neuron 2:1547, 1989). Adriamycin is an anticancer agent which increases p53 activity in many cell types (El-Deiry et al. Cancer Res. 54:1669, 1993). SV40 Large T antigen, which is frequently employed to immortalize cells, has p53 binding activity. The present study tested the possibility that SV40 Large T antigen would impair the ability of N18-RE-105 cells to differentiate. N18-RE-105 cells were incubated with filtered supernatant from Ψ-2 cells which produce defective recombinant retrovirus containing the SV40 Large T tsA58 and neomycin resistance genes. The alkaline phosphatase gene was used as a control. After selection in G418, clones were isolated and examined for differences in growth properties and differentiation. Control clones expressing alkaline phosphatase showed normal growth patterns and differentiation in response to glutamate and adriamycin. In contrast, clones expressing SV40 Large T grew as isolated colonies with a rounded morphology and no processes. In addition to binding p53, SV40 Large T antigen blocks other cellular proteins which are important for growth control. Expression of SV40 Large T antigen may induce a state of continuous growth which is incompatible with differentiation.

306.9

NEW BIOERODIBLE COPOLYMER AS IMPLANTS IN THE RAT BRAIN S. Lahtivirta, W. Lu, P. Törmälä", J. Seppälä"" and A. Hervonen* Lab of Gerontol. School of Public Health, Univ. of Tampere, Finland, "Biomaterial Res. Instit. of Plastic Technol., Tampere Univ. of Technology, ""Dept. of Chemical Engineering, Helsinki Univ. of Technology, Finland

Extensive research has been carried out into the use of nontoxic and tissue compatible biodegradable polymers in surgery. To develope a new drug delivery system for CNS,we have tested a new soft polymer as implant up to 32 weeks. Cylinders of mixtures of oligo(L-lactate) and caprolactone were placed surgically in rat parietal cortex. The fate of polymer and the tissue compatibility were followed using several histochemical markes.

Traces of the polymer could be discovered still after 24-32 weeks. Some degree of surface erosion and degradation of the material was visible after 2-4 weeks and it proceeded with time. The implants showed ingrowth of glial elements but only weak surrounding gliosis. Capillaries were found inside the defrading implants after 4 weeks. Mixing drugs into the polymer did not change the degradation properties essentially.

The new soft copolymer tested in this study may provide a way to introduce neuroactive drugs into the brain. A sustained release may last as long as 8 months. The high tissue compatibility suggests that the polymer may serve as implantable scaffold for living cells as well.

Sources: Univ. of Tampere, Tampere Univ. of Technology

306.10

EX VIVO ADENOVIRUS-MEDIATED GENE TRANSFER INTO SUPRACHIASMATIC NUCLEUS (SCN) TRANSPLANTS. K.E. van Esseveldt, W.T.M.C. Hermens, R. Liu, R.W.H. Verwer*, J. Verhaagen and G.J. Boer. Netherlands Institute for Brain Research, Meibergdreef 33, 1105 AZ Amsterdam ZO, The Netherlands.

Solid pieces of fetal anterior hypothalamus tissue containing the SCN were infected with an adenoviral vector containing the marker gene LacZ under control of the CMV promoter (Ad-LacZ). Thereafter, explants were either cultured in vitro or grafted into the third ventricle of adult Wistar rats. Using immunocytochemistry, expression of ß-galactosidase (ß-gal), the product of the LacZ gene, was evaluated after 8, 21 and 35 days in vitro (DIV) and 8, 21 and 70 days postgrafting (DPG). Infection of the explants for 18 h with $2x10^8$ pfu/ml Ad-LacZ resulted in high numbers of ß-gal-positive neurons and glial cells at 8, 21 and 35 DIV. Upon transplantation, expression of ß-gal persisted up to 70 DPG. In transplants, the number of ß-gal-expressing cells was initially high (DPG 8), but at DPG 21 and DPG 70 only a limited number of cells expressing the transgene was present. This decline in ß-gal-positive cells is probably not related to an immune response directed towards the viral capsid since immunocytochemical stainings for macrophages (ED-1 positive) and T-lymphocytes (OX-8 positive) revealed a staining pattern as seen in sham-infected transplants. The procedure described for ex-vivo Ad-LacZ-mediated gene transfer will be used in the future to introduce expression of neurotrophic factors in SCN grafts.

This study was supported by a grant from NWO-MW, The Hague, The Netherlands (903-47-006) to K.E. van Esseveldt.

306.11

A NOVEL HERPES VIRUS BASED AMPLICON VECTOR FOR GENE DELIVERY INTO THE CNS. S. Di[1]*, S. Wang[2], C. D. Jacobson[1] and C. Link[2] [1]Dept. of Vet. Anatomy, Iowa State University, Ames, Iowa 50011 & [2]Human Gene Therapy Institute, Iowa Methodist Medical Center, Des Moines, Iowa 50309.

We have developed a Herpes Simplex Virus (HSV) / Epstein Barr Virus (EBV) based amplicon system for high transgene packaging titer and efficient transgene delivery both *in vitro* and *in vivo*. The viral vector can carry at least a 17 kb insert and show stable replication and packaging. Stereotactic injection of this viral vector with a reporter lac Z gene (pHE-lac) into the adult rat brain resulted in limited, transient viral expression. A moderate β-gal expression was present around the injection site within the caudate nucleus, whereas a substantial population of positively stained neurons was detected in the dentate gyrus following hippocampal injection. The facial nucleus showed maximum expression after injection at that site. In addition, the viral vectors appeared to have been transported along the facial nerve to its genu, and also reached the ipsilateral and contralateral locus coeruleus. Our data demonstrate a novel HSV/EBV amplicon vector that can reliably deliver transgenes into the rat CNS. Interestingly, the HSV/EBV viral vector may possess a preference for specific neuronal pathways with distinct expression thresholds and distribution patterns. This study suggests the interaction between viral vectors and target neurons is one of the key factors which may determine the success of transgene delivery and expression.

Supported by: The Whitehall Foundation, NSF and IMMC.

306.12

ADENO-ASSOCIATED (AAV) VIRAL VECTOR-MEDIATED GENE DELIVERY IN NON-HUMAN PRIMATES K.S. Bankiewicz*, R. Snyder, S.Z. Zhou, M. Morton, J. Conway and D. Nagy. Somatix Therapy Corporation. Alameda, CA 94501.

In vivo gene therapy consists of introducing genetic material into dividing and non-dividing cells in situ. AAV vectors engineered to express β-galactosidase (lacZ) gene were introduced into monkey brains to determine the extent of infection and the cell types infected by the vector at 2 and 16 weeks. AAV-lacZ vector was infused into the striatum of normal monkeys (n=5) at 5, 10, 30 and 162/μl using a bulk flow delivery method. There were no clinical side effects observed at 2 (n=4) or 16 weeks (n=1) post implantation. 40 μm whole mount frozen sections were stained with hematoxylin-eosin (H&E), and with antibodies to β-galactosidase (β-gal), neurofilament (NFT) and glial fibrillary acidic protein (GFAP). Injection sites were found in the caudate nucleus, putamen and in one case the midbrain. Moderate infiltration was observed in the needle tracks and in close proximity to the injection site. In one infusion site, where the largest volume (162 μl) was injected, some evidence of necrosis in the center was observed. β-gal -IR cells were found at the site of infusion. The spread of the infected cells appear to be volume-dependent reaching 5-7 mm from the needle track in the 162 μl infusion sites. β-gal-immunoreactive (IR) cells appear to have neuronal morphology with long processes extending over 2 mm from the cell bodies. Double-labeling technique with NTF/ β-gal immunoreactivity confirmed further the neuronal character of the majority of the infected cells. Only a small fraction of the cells presented GFAP/ β-gal IR, and were recognized as astrocytes. The number of AAV infected β-gal-IR cells also appeared volume-dependent, and ranged from 1K cells at 5 μl infusion to 31K in 162 μl at 2 weeks. Some β-gal-IR cells were also present at 16 weeks. These findings suggest that the AAV vector administration is a potential tool for gene delivery in the CNS.

AGING PROCESSES: NEURONAL ALTERATIONS

307.1

Sex Differences in Aging of the Human Striatum: An In Vivo MRI Study.

F.M. Gunning*, D. Head, J.D. Acker, & N. Raz

Age and gender differences as well as lateral asymmetries have been observed in motor behavior across many species. These differences may be related to the morphology of the different nuclei in the basal ganglia. However, the evidence of morphological asymmetries and sex differences in the basal ganglia of healthy adults is sparse, especially in regards to the paleostriatum. To examine age, sex, and hemispheric differences in the basal ganglia we measured the volumes of these nuclei in healthy right-handed adults (N=144, 18-77 years old) using MRI-based morphometry. Previously (Soc. Neurosci. Abstr. 21, p 1564, 1995) we reported bilateral age-related changes in both the head of the caudate nucleus (Cd) and the putamen (Pt). In this study, we examined the globus pallidus (Gp) and observed bilateral age-related shrinkage of that structure in males but not in females. Leftward asymmetry of the Cd and rightward asymmetry of the Pt were observed; no hemispheric asymmetry was found in the Gp. Supported by the grant AG11230 to N.R.

307.2

AGE-RELATED DENDRITIC AND SPINE CHANGES IN HUMAN OCCIPITAL AND PREFRONTAL CORTICES: A QUANTITATIVE GOLGI STUDY. K. Courns[1], J. Ferguson[1], L. Larsen[1], B. Addleson[1], M. Schall[2], and B. Jacobs[1]* [1]Laboratory of Quantitative Neuromorphology, Psychology, The Colorado College, Colorado Springs, CO 80903. [2]Psychology, Tulane University, New Orleans, LA 70130.

The present study quantitatively examines age-related dendritic/spine changes in human prefrontal (area 10) and occipital (area 18) cortices. Tissue was removed from the left hemisphere of 10 neurologically normal subjects, ranging in age from 23 to 81 years. These individuals were grouped into a younger (<50 years) and older (>50 years) age group for subsequent analyses. After staining with a modified rapid Golgi technique, the basilar dendritic systems of 10 supragranular pyramidal cells per tissue block (N=200) were quantified on a Neurolucida system (Microbrightfield, Inc.). Dependent measures were total dendritic length (TDL), mean dendritic length (MDL), dendritic segment count (DSC), dendritic spine number (DSN), and dendritic spine density (DSD).

With the exception of DSC, all dependent measures tended to decrease with age. Most pronounced were significant declines of approximately 50% in DSN (F (1,2) = 118.15, $p < 0.0001$) and DSD (F (1,2) = 210.12, $p < 0.0001$) from the younger to the older age group. Dendritic differences in Brodmann's areas tended to be greater in the younger group than in the older group, with area 10 exhibiting higher dependent values than area 18, especially for DSN (F (2,36) = 11.11, $p < 0.0001$) and DSC (F (2,36) = 6.81, $p < 0.0015$). Across the represented adult lifespan, dendritic declines were slightly more pronounced in area 10 than in area 18. The present results quantitatively document the ongoing, dynamic refinement of dendritic neuropil across the human life span, and suggest that higher order cortical areas (e.g., area 10) may be more susceptible to age-related changes than lower order cortical areas (e.g., area 18). (Tissue generously provided by Dr. D. Bowerman, El Paso County Coroner, and Dr. R. Sherwin, Penrose Hospital)

307.3

AGE-RELATED ALTERATIONS IN NEUROFILAMENT PROTEIN IMMUNOREACTIVITY IN MOTONEURONS IN THE BRAINSTEM AND SPINAL CORD IN CATS. J-H. Zhang*, S. Sampogna, F.R. Morales and M.H. Chase. Department of Physiology and Brain Research Institute, UCLA School of Medicine, Los Angeles, CA 90024.

Abnormalities in neurofilament expression occur in a variety of human neurodegenerative diseases that progress with age such as Alzheimer's Disease, Parkinson's Disease and amyotrophic lateral sclerosis. However, little is known about the alteration of neurofilament expression during normal aging. Accordingly, using immunohistochemical techniques, we compared neurofilament protein immunoreactivity in motoneurons of the brainstem and spinal cord in two adult (1-3 years of age) and an old (18 years of age) cat which did not exhibit signs of human neurodegenerative diseases. We examined these neurons because in previous studies we have found age-related changes in the electrophysiological properties and morphological characteristics of motoneurons and their axons (Morales et al., J of Neurophysiology, 58:180-194, 1987; Chase et al., Brain Res., 586:279-288, 1992), which could be the result of alterations of neuronal neurofilament expression. Three kinds of antibodies were used to identify different neurofilament subunits of low-, medium- and high-molecular weight, respectively. In order to compare changes in the immunoreactivity of these three neurofilament subunits, sets of sections from adult and old cats were treated simultaneously under identical conditions. The results demonstrated a dramatic decrease in the density of neurofilament immunoreactivity involving the three different subunits in motoneurons of the 18 year old cat. These data indicate that neurofilament abnormalities occur not only in a variety of human neurodegenerative diseases, but also in another mammal and that they may play a role in the electrophysiological and morphological changes observed in aged cat motoneurons. Supported by USPHS Grant AG 04307.

307.4

DECREASED NEUROFILAMENT (NF-L) GENE EXPRESSION IS AN INDEX OF SELECTIVE AXONAL HYPOTROPHY IN AGING.

G.A. Kuchel*[1], T. Poon[1], K. Irshad[1], C. Richard[1], J-P. Julien[2] and T. Cowen[3] [1]Geriatrics and [2]Neuroscience, Montreal General Hospital, Montreal, Canada H3G 1A4; [3]Royal Free Hospital School of Medicine, London, UK.

The ability of neurons to sustain an innervation to their targets is necessary for the maintenance of normal function in aging. Axonal atrophy and degeneration by vulnerable projection neurons may be earlier and more readily reversible events in neurodegeneration and aging than are regressive somatic changes. Sympathetic innervation to the middle cerebral artery (MCA) and pineal, both targets of the rat superior cervical ganglion (SCG), decreases by over 50% in old age, while sympathetic innervation density to the iris is unchanged.

Young and aged male SD rats underwent retrograde labeling of neurons projecting either to MCA (Fast Blue) or to iris (Fluorogold). A minority of SCG neurons (~2%) exhibited either FB or FG (but never both). *In situ* hybridization for neurofilament light chain (NF-L) mRNA revealed that a moderate 22-25% decrease in the mean grain density for this mRNA was present in many aged SCG neurons. Using retrograde tracers, there was evidence for a 40% decline with aging in the grain density for NFL mRNA in MCA-projecting neurons ($p<0.05$), while this parameter was unchanged in SCG neurons innervating the iris. Our results suggest that selective regressive changes in the axonal compartment of aged neurons are associated with significant decreases in neurofilament (NF-L) mRNA expression. (funding: MRC Canada; FRSQ Quebec & British Heart Foundation)

307.5

Protein Profiling: Use of Immuno-aRNA. Jim Eberwine* and Lori Rogers. Depts. of Pharmacology and Neuroscience, University of Pennsylvania Medical School, Philadelphia, PA. 19104.

Information about mRNA levels is critical to understanding the functioning of the cell and provides a molecular fingerprint of physiological state; yet, in general, it is the resultant proteins that are translated from these mRNAs which act as receptors, channels, enzymes and structural proteins which facilitate cellular functioning. Electrophysiological techniques at the single cell level will permit the monitoring of ion channel functioning as well as the functioning of other receptors or proteins which are coupled to channels. It is however difficult to detect the small amounts of other proteins that do not directly couple to ion channels, which the expression profiling and immunohistochemsitry show are present and potentially regulated within an individual cell. To make this connection between coordinated mRNA level changes and presence of protein, a new technique called immuno-aRNA has been developed for use in the single cell. This technique permits the detection of small amounts of antigen-antibody complex by taking advantage of the sensitivity of aRNA amplification. Briefly, the technique provides increased sensitivity over standard protein detection methodologies by covalently coupling an T7 promoter-driven cDNA sequence to the secondary antibody so that the nucleotide sequence can be amplified using T7 RNA polymerase. We have generated data showing that we can detect TAU protein (an intermediate filament) utilizing this technology. Currently we are attempting to determine the relative ratios of phosphorylated-Tau to nonphosphorylated Tau in single cells during the ageing process. This work was supported in part by AG9900.

307.6

CHARACTERIZATION OF INCREASED TYROSINE HYDROXYLASE mRNA IN AGED SYMPATHETIC NEURONS.

M. Salas-Prato* and G.A. Kuchel Geriatric Medicine, Montreal General Hospital, Montreal, Quebec, Canada H3G 1A4

It appears that declines in gene expression in the aging nervous system are not generalized, but rather modest, with considerable specificity in terms of the mRNA species and neuronal populations affected. Examples of increased gene expression in aging have often involved non-neuronal cells. Several studies have reported moderately increased tyrosine hydroxylase (TH) activity and mRNA levels with aging in extracts of rodent sympathetic superior cervical ganglia (SCG). It has been assumed that these changes are due to increased sympathetic nerve activity.

Quantitative *in situ* hybridization studies were performed using sections from mature and aged male F-344 rats mounted on the same slide. We observed no significant difference in the mean Labeling Index (LI) for cyclophilin mRNA. In contrast, the LI for TH mRNA exhibited a mean increase of 74% ($p<0.0001$) in aged as compared to mature SCG neurons. The majority of neurons demonstrated only modest (~10-20%) increases. Concomitantly, a subset (~11%) of intermediate-sized (300-800 μ^2) aged neurons appeared to be responsible for the greatest increase in TH mRNA LI with 4-10 fold increases above the mean found in mature neurons. Glyoxylic acid fluorescence, cell size, numbers and distribution indicate that these are not SIF cells. Tracer studies combined with *in situ* hybridization suggest that these neurons do not overlap with those projecting to the middle cerebral artery, a target exhibiting considerable axonal sympathetic hypotrophy in aging. Further tracer and lesion studies are underway to establish the nature of the anterograde and/or retrograde stimuli underlying this marked increase in neuronal gene expression with aging. (funding: MRC Canada & FRSQ)

307.7

SYNAPTOSOMAL AND MITOCHONDRIAL MEMBRANE ALTERATIONS IN BRAIN AGING.

M. Sugawa, G. Schulze, F. Krause, C. Sajak and N.A. Dencher (SPON: European Neuroscience Association). HMI, BENSC-NE, Glienicker Str. 100, D-14109 Berlin, Germany (M.S); Neuropsychopharmacology, FU Berlin (G.S); Physical Biochemistry, TH, D-64287 Darmstadt (F.K, C.S, N.A.D).

Age-associated alterations in the energetic state of the cell as well as in the cAMP and PI signal pathways could be due to changes in the physical and chemical properties of the membranes. To support this hypothesis, age-dependent changes in the physical state and the biochemical composition of synaptosomal and mitochondrial membranes from the cortex, cerebellum, hippocampus, hypothalamus, and striatum of female Wistar rats were examined by measuring the steady-state fluorescence anisotropy of the membrane probes 1,6-diphenyl-1,3,5-hexatriene (DPH) and TMAP-DPH. Significant differences in the properties of the synaptosomal membranes existed between young (3.5 months) and very aged rats, expressed by a higher anisotropy (i.e., decreased membrane "fluidity") and an increase in cholesterol content of the 40-month-old rat brain tissue. For the various brain regions investigated, the magnitude of the age-dependent differences in anisotropy was similar and similar differences were observed in the hydrophobic core of the membranes (DPH) and in the regions closer to the lipid headgroups (TMAP-DPH). Furthermore, protein and lipid oxidative damage in synaptosomal and mitochondrial membranes was analyzed.

(Supported by the German government)

307.8

DISTRIBUTION OF NEUROPEPTIDE Y-IMMUNOREACTIVE NEURONS IN THE CAUDAL PART OF SPINAL TRIGEMINAL NUCLEUS OF RAT WITH AGE.

M. K. Kim*, K. S. Paik, S. P. Lee. Dept. of Oral Anatomy, College of Dentistry, Seoul National University, Seoul 110-749.

The purpose of this study was to undertaken the distribution of neuropeptide Y(NPY)-immunoreactive (IR) neurons in the caudal part of spinal trigeminal nucleus of rat with age by means of immunohistochemistry. In the medulla oblongata of rat, a large group of NPY-immunoreactive neurons was seen in spinal trigeminal nucleus. (Yamazoe et al., Brain Research, 335: 109-120, 1985). For this study, Sprague-Dawley rats of 3, 12, 24, 36 months of age were used. Animals were perfused transcardially with 4% paraformaldehyde in 0.1M phosphate buffer(PH7.4). Brains were taken out immediately. Serial frozen 30μm thick sections were made in the coronal plane. Sections were incubated in primary rabbit antiserum to NPY with a dilution of 1:2000, placed into goat rabbit IgG with a dilution of 1:500 and incubated in rabbit PAP(peroxidase- anti-peroxidase). Then, sections were developed for peroxidase reactivity with 3,3'-diaminobenzidine (DAB). After visualization with DAB, sections mounted in a glycergel and coverslipped. The some sections were stained with toluidine blue and cresyl violet for couterstain. The numbers of NPY-IR perikarya and NPY-IR fibers in the caudal part of spinal trigeminal nucleus were gradually decreased with aging. These results indicate that decrease of the NPY-IR neurons and fibers with aging may concerned with their hypofunction.

307.9

Ontogenic changes of $GABA_A$ function of the rat Meynert neuron.

M. Uki*, J. S. Rhee, Y. H. Jin and N. Akaike Department of Physiology, Kyushu University Faculty of Medicine, Fukuoka 812-82, Japan.

The functional change of $GABA_A$ receptor with aging was studied in the freshly dissociated rat Meynert neurons by using nystatin perforated patch recording configurations under voltage-clamp condition. Kinetic fits of GABA concentration-response relationships revealed clear developmental changes in the potency of GABA response. The current density decreased from 111.1 pA/pF at 0-3 days after birth (p0 - p3 days) to 74 pA/pF at p6 months, indicating that the current amplitude decreased to almost half of immature rats in adult rats. However, the ligand binding affinities increased with aging, where EC_{50} values were 3.46×10^{-5} M for p0-p3 days rats and 1.16×10^{-5} M for p6 months. Study for benzodiazepine (BZ) receptor modulated GABA response using diazepam and zolpidem revealed that BZ action is mediated mainly by BZ1 receptor in p2 weeks and p6 months, whereas in p1 day rat, the most of BZ receptors were BZ2. We obtained spontaneous GABAergic inhibitory post synaptic current (sIPSC) in acutely dissociated neurons attached presynaptic bouton. The 'off' relaxation kinetics of sIPSCs also have fitted biexponential which is τ_f and τ_s for p0-2 days and p2 weeks rats. The sIPSCs of p2 week rat were faster twice relatively than p0-2 days rat. These changing of GABA response with aging might correspond to functionally distance postsynaptic receptors in inhibitory synapses.

307.10

AGE-RELATED CHANGES IN THE RHESUS MONKEY: MORPHOLOGY OF NEURONS IN SUBSTANTIA NIGRA-PARS COMPACTA

Z. A. Siddiqi, A. Peters, T. L. Kemper and M. L. Feldman* Dept. of Anatomy and Neurobiology, Boston Univ. School of Med., 80 East Concord St., Boston, MA 02118.

Our studies in rhesus monkey have shown that the dopaminergic pars compacta undergoes a significant age-related neuronal loss. This reduction in the total neuronal number is about 30 per cent when the oldest subject is compared to the youngest animal. The aim of present study is to examine the morphological changes that occur in the neurons of this nucleus during the aging process. Three types of neurons, small, medium and large, have been identified in the pars compacta in younger monkeys (<10 years) on the basis of the size and shape of their soma, dendritic pattern and arrangement of rough endoplasmic reticulum. All three types of neurons show age-related degenerative changes in older animals (>25 years), with the small type being most affected. Although most neurons of the pars compacta accumulate lipofuscin with progressing age, it is the small neurons that show the most extensive accumulation of this pigment. In the electron microscope all types of neurons show membrane bound, clear vacuole in their cytoplasm and a variety of inclusions have been observed in the neuronal nuclei of old animals. In the neuropil there is a large number of degenerating dendritic profiles in which the cytoplasm is vacuolated, and frequently these dendrites contain dark inclusions whose origin is not known. In the pars compacta of older monkeys there are round to oval shaped, darkly staining bodies of the same size as small neurons some of which appear to have a process radiating out from them. In the electron microscope these bodies are seen to be membrane bound, have irregular contours and have a pale homogenous matrix in which a dark granular material is embedded. These bodies are surrounded by astrocytic processes. Whether these represent degenerating neurons or large inclusions in astrocytes has yet to be determined. (Supported by NIH grant P01- AG00001)

307.11

AGE-RELATED SYNAPTIC CHANGES IN SENSORIMOTOR CORTEX OF THE F344 X BROWN NORWAY RAT. M.C.Linville*, J.K.Brunso-Bechtold, and W.E.Sonntag. Depts. Neurobio./Anat. & Physio./Pharm., Prog. In Neuroscience, Bowman Gray School of Med., Wake Forest Univ., Winston-Salem, NC 27157.

In the present study, the focus was to assess age-related synaptic changes in sensorimotor cortex. Five F344 X Brown Norway rats were cardiac perfused at each of the following ages: young (10 mos), middle age (15 mos), and old (32 mos). The brains were coronally vibratomed at 200 μm. The sensorimotor cortex was blocked by examining the sections using a dissecting microscope. The blocks were processed for electron microscopy and embedded in plastic. Three different sensorimotor cortex blocks from each animal were selected and semithin (1μm) sections were cut and collected on glass slides. Thin sections were cut and examined using a Zeiss 10-CA electron microscope. Using the semithin section as a map for locating layers II and IV, twelve electron micrographs were taken in each of these layers. From the electron micrographs, sample area size, percent neuropil, and total number of synapses were determined. Synapses were also characterized by vesicle shape and postsynaptic element. Preliminary analysis shows that synaptic density increases significantly in old compared to young rats in layer II but not in layer IV. Of these synaptic terminals, the number of round vesicle containing terminals per unit volume increases in old compared to young rats in layer II but not in layer IV whereas the number of nonround vesicle containing terminals increases in both layers. Interestingly, the percent of synaptic terminals that contain round vesicles (presumptive excitatory terminals) decreases significantly between young and old in both layers II and IV. Taken together, these data suggest 1) that age-related changes in synaptic input may affect cortical layers differentially and 2) the balance of presumptive excitatory input to cortical layers in relation to presumptive inhibitory input may decrease with age. Supported by 1 P01 AG 11370.

307.12

EVIDENCE FOR DECLINE IN SMOOTH ENDOPLASMIC RETICULUM CALCIUM BUFFERING WITH AGE IN RAT ADRENERGIC NERVES. H. Tsai, B.J. Pottorf, S. Foucart, J. Buchholz* and S.P. Duckles. Depts. Pharmacology, Coll. Medi. Univ. Calif., Irvine, CA 92717 and Loma Linda Univ. Sch. Medi. Loma Linda, CA 92350.

Stimulation-evoked norepinephrine (NE) release from tail artery adrenergic nerves increases with advancing age in the Fischer-344 rat with no corresponding changes in function of uptake mechanisms, metabolism or prejunctional alpha-2 adrenoceptors. To examine this at the cellular level, intracellular calcium concentration was measured in acutely dissociated superior cervical ganglion neurons using fura-2/AM. When smooth endoplasmic reticulum (SER) calcium uptake was blocked with thapsigargin (1 μM), stimulation-evoked NE release from tail arteries and depolarization-evoked calcium transients in isolated cells significantly increased in 6 month-old tissues with no effect in 20 months. The rate of rise of calcium with K^+-depolarization increased after thapsigargin treatment but only in young cells. In contrast, we have previously shown that blockade of mitochondrial calcium uptake with dinitrophenol increased stimulation-evoked NE release from tail arteries and depolarization-evoked calcium transients in isolated superior cervical ganglion cells in 20 months with no effect in 6 months. Therefore, older nerves appear to be more reliant on mitochondria for intracellular calcium buffering due to a decline in function of SER calcium buffering systems. These data suggest that age-related increases in NE release are due to a decline in SER calcium buffering mechanisms.
Supported by Amer. Heart Assoc., Grant in Aid #95015640.

307.13

SPINAL CORD EXPLANT CULTURES ARE USED TO STUDY NERVE REGENERATION IN MATURE AND OLD F344 RATS. JM Jacob, AL Aulthouse and DL O'Donoghue*. Dept of Anat Sci., Univ. of Oklahoma HSC, OKC, OK 73190.

The use of tissue and organ cultures to examine growth and repair processes in injured neurons is not new. However, until now, most studies have focused on dissociated cells and tissues derived from embryonic and neonatal animals because they are undifferentiated and still mitotic. To examine the regulatory pathways involved in initiating and sustaining the cell body response to injury in mature and old animals, we have developed an organ culture model that is not subject to the "age limitations" of dissociated cell and tissue culture. Using lumbar spinal cord explants taken from 6 and 24 mo F344 rats, we tested several culture conditions to assess neuronal viability. Here we demonstrate that both poly-L-lysine and laminin permit attachment of the explant to the substratum while an agarose overlay is necessary to secure the organ culture in place. Dulbecco's Modified Eagle's Medium (DMEM) *without* serum provides sufficient nutrient support for neurite outgrowth, which was observed within 24 hours. Histochemical (H&E, cresyl violet) and immunocytochemical (GFAP, CGRP, NSE) analysis of cultures was used to demonstrate cell viability as well as demonstrate that this model system is, in some respects, comparable to a lesion *in vivo*.
Supported in part by a grant from AFAR (JMJ).

307.14

DECREASE IN THE CONDUCTION VELOCITY OF PYRAMIDAL TRACT NEURONS IN THE AGED CAT. R.-H. Liu*, M.-C. Xi, F.R. Morales, J.K. Engelhardt and M.H. Chase. Department of Physiology and the Brain Research Institute, UCLA School of Medicine, University of California, Los Angeles, CA 90024.

In the present study we wished to determine whether age-dependent changes in conduction velocity occur in pyramidal tract neurons. A total of 260 and 254 pyramidal tract cells were recorded extracellularly in the left pericruciate gyrus from 2 adult control and 2 aged cats, respectively. These cells were antidromically activated by stimulation of the medullary pyramidal tract. In order to calculate their conduction velocities, the straight-line distances between stimulating and recording sites were divided by the antidromic conduction times of the spikes. Fast and slow conducting pyramidal tract cells were recorded in both control and aged cats. However, 50% of the pyramidal tract cells recorded in the control cats were fast conducting, while only 26% in the aged cats were fast conducting. As a consequence, there was a 27% decrease in the conduction velocity of pyramidal tract neurons in aged cats when compared with neurons in the control cats (22.5 ± 0.7 vs. 16.4 ± 0.7 m/s, for control and aged cats, respectively, $P<0.0001$). The bases for this decrease in conduction velocity remain to be determined; possible contributing factors are demyelination, reduction in axon diameter and/or death of the fast conducting cells. We have previously reported a reduction in the conduction velocity of spinal cord motoneurons in aged cats (*Morales et al., J. Neurophysiol., 1987, 58:180*); these cells have long axons which are located principally outside the CNS and receive trophic support from Schwann cells and the muscle fibers which they innervate. The finding of a reduction in pyramidal tract conduction velocity in aged cats is noteworthy because it shows that this phenomenon is present in neurons that, in contrast to motoneurons, are contained entirely within the CNS, are supported by different types of glial cells and innervate neuronal targets, not muscle fibers. Therefore, we postulate that during the process of aging there are specific alterations inherent to neurons that do not depend on changes in non-neuronal tissue. Supported by USPHS Grant AG 04307.

307.15

AGE-RELATED CRITICAL PERIODS IN MATURATION OF CEREBELLAR PURKINJE CELLS IN MATURE AND ALTRICIAL BORN ANIMALS. R.Grigorian, *E.Prigarina. Institute of Evol. Physiology and Biochemistry, St.Petersburg, Russia.

The maturation of synapses from principal cerebellar afferents - mossy and climbing fibers afferent systems onto Purkinje cells (PC) dendrites in guinea pigs and kittens takes place mainly after birth and goes on irregularly in the early stages of postnatal ontogenesis. The present study aimed to determine age-associated critical periods of functional maturation of cerebellar PC in mature (guinea pig) and altricial (kitten) born animals. Experiments were performed on anaesthetized animals aging from newborn up to 2 months old. Critical periods were evaluated by the significant changes in PC activity and by the animal motor status before the experiment. In guinea pigs which are born capable of standing, only one critical period of change in PC discharge frequency occured (from 12.9 ± 1.8 to 23 ± 2.2 imp/s at 12-14 days of ontogenesis). Three critical periods were found in kittens, however: 1/ before eye opening time, 7-8 days of postnatal life (PL); 2/ after eye opening time on 18-22 days of PL, when discharge frequency of PC increased dramatically by 10 times; 3/ on the border of the first and second month of postnatal life, when play-like movements begin to elaborate. The received data suggest that critical periods in maturation of PC discharge frequency reflect the development of motor skills.

307.16

NEURON NUMBERS IN THE MYENTERIC PLEXUS OF AGED RATS: EFFECTS OF DIETARY RESTRICTION

R.J.R.Johnson, R.M.Santer[1] & T.Cowen*, Dept. of Anatomy and Developmental Biology, Royal Free Hospital School of Medicine, London NW3 2PF, UK and [1]MOMED, University of Wales College of Cardiff, P.O. Box 911, Cardiff CF1 3US, UK.

A reduction in neuronal numbers of up to 60% has been reported in the enteric nervous system of aged rats fed on an *ad lib* diet, offering at least a partial explanation for the age related functional deficits apparent in enteric neuromuscular transmission. Our study has investigated whether this loss occurs when a 60% restriction of diet is imposed from 10 weeks of age, other studies having shown this regime to enhance the longevity and health of laboratory animals. We have evaluated total neuronal numbers within the myenteric plexus of ileal whole mount preparations from Sprague Dawley rats, aged 4 and 24 months, using the NADH-diaphorase method and the pan-neuronal marker, PGP 9.5. Neuronal sub-population numbers were assessed using antibodies to ChAT, SP, ENK, SOM, NPY, VIP and neurofilament, as well as the NADPH-diaphorase method to demonstrate nitrergic neurons. Positive cell bodies were counted using light or fluorescence microscopy and corrected for age related growth of the small intestine. Differences between age groups were assessed with students t-test. Whilst a small decline of 15% ($p<0.05$) was evident in the numbers of NADH-positive neurons with age, PGP not only stained a substantially larger numbers of neurons in young and old preparations, but showed no evidence of neuronal loss in old age. Neurofilament immunoreactive neuron numbers increased significantly with age ($p<0.05$), whilst the cholinergic, peptidergic and nitrergic markers failed to demonstrate any significant cell loss, confirming that neuron numbers remain relatively unaffected by age. We speculate that dietary restriction may prevent the cell loss apparent in the myenteric plexus of aged *ad lib* fed rats. This work was supported by the Anatomical Society of Great Britain & Ireland.

307.17

ALTERATIONS IN MYSTACIAL PAD INNERVATION IN THE AGED RAT B.T.Fundin*, E.Bergman and B.Ulfhake Dept. of Neuroscience, Karolinska Institute, Stockholm, Sweden S171 77.

In the aging rat, changes in the content of neuropeptides have been observed in sensory ganglia. Primary sensory neurons in the aged rat showed an increase in the expression of neuropeptide Y (NPY) mRNA and galanin mRNA whereas a decrease was evident in the expression of calcitonin-gene related peptide (CGRP) mRNA and substance P (SP) mRNA. The changes in expression resemble many of those induced by nerve lesions in young adult rats, suggesting that aging-related lesions are involved. Dystrophy and degeneration of axons have been reported to occur in both central and peripheral sensory pathways.

The mystacial pad was used to assess the impact of aging on cutaneous innervation. The mystacial pad has been shown to receive a large variety of sensory nerve endings organized in a highly predictable pattern. Seven aged (30 months old) male Sprague-Dawley rats and six young adult male rats were used. Immunofluorescence analyses were performed using antibodies against human neuronal cytoplasmic protein (PGP 9.5), neurofilaments and several neuropeptides. The Merkel endings and lanceolate endings that eminate from large caliber afferents in the whisker follicles showed signs of degeneration as well as a substantial decrease in number, whereas most medium caliber mechanoreceptors appeared normal. No obvious change in the CGRP, SP or galanin-IR could be determined in terms of intensity and localization. However, NPY was upregulated but only in a specific population of CGRP-IR small caliber axons which also coexpressed galanin-IR. Normally, NPY-IR is not observed in this set of axons. Interestingly, two additional networks of fine caliber axons which are not present normally were located in the outer and inner root sheaths of the whisker follicles. The epidermis was thinner in the aged rat and showed a loss of unmyelinated components that arise from the subepidermal plexus located just under the basement membrane. Perivascular innervation was present but showed a great loss in the centrifugally distributed sensory components.

BLOOD-BRAIN BARRIER II

308.1

COMPARISON OF THE LIGAND BINDING SITES OF THE BLOOD-BRAIN BARRIER, NEURONAL AND ERYTHROCYTE CHOLINE TRANSPORTERS. DD Allen*, JRS Matharu, PA Crooks, QR Smith. School of Pharmacy, Texas Tech HSC, Amarillo TX; College of Pharmacy, Univ. of Kentucky, Lexington KY; LNS, NIA, Bethesda MD.

Choline uptake into cells is mediated by one or more transport systems that differ in substrate affinity and sodium dependence. In the present study, the substrate specificity of neuronal, erythrocyte and brain capillary choline transporters was assessed to obtain insight into binding site structure and to identify selective ligands. These carriers exhibit similar 'affinity' ratios (K_m/K_i) with some differences. Hemicholinium-3 exhibited strong preference for the neuronal high affinity choline transporter, with an inhibition constant (K_i) 10-100 times lower than that at the brain capillary or red cell membranes. In contrast, deanol exhibited 6-fold higher affinity for the brain capillary transporter compared to the erythrocyte and N-*n*-butyl choline demonstrated ~25 fold range in affinity ratios among the BBB, neuronal and erythrocyte transporters, suggesting these compounds may have utility in distinguishing the carriers. Two very high affinity analogs were identified for the brain capillary transporter, N-*n*-octyl- and N-*n*-hexyl-choline, which showed 20-fold greater affinity at the brain capillary than choline itself. Using affinity data for a range of compounds, the structure of the binding sites was modeled with the computer program SYBYL. Comparison of highly active, active, and inactive compounds allowed for prediction of ligand available, ligand excluded and ligand high affinity zones of the binding sites. Comparative molecular field analysis (CoMFA) was used to develop a 3-D model which incorporated steric, hydrophobic and electrostatic factors. The models predict different binding sites that may have utility in differentiating affinity and selectivity among these carrier systems. (NIH Intramural Program)

308.2

DISTINCT EXPRESSION OF RECEPTORS LINKED TO Ca^{2+} SIGNALING IN RAT AND HUMAN CEREBROMICROVASCULAR ENDOTHELIAL CELLS. R.Monette, D.Stanimirovic, R.Ball, [1]E.Hamel, K.Chaundy* & P.Morley National Research Council, Ottawa & [1]Montreal Neurological Institute, Montreal, Canada

Cerebromicrovascular endothelial cells, an *in vitro* model of the blood brain barrier (BBB), express a variety of receptors for neurotransmitters and vasoactive mediators known to regulate local cerebral blood flow and/or BBB permeability *in vivo*. In this study we compare the expression of receptors for vasoactive mediators coupled to Ca^{2+}-signaling in rat (RBEC) and human (HBEC) cerebromicrovascular endothelial cells. RBEC and HBEC were isolated by cloning capillary endothelium from dissociated microvessels derived from adult rat brain cortex and from samples of human temporal lobe surgically excised for the treatment of idiopathic epilepsy, respectively. Both RBEC and HBEC expressed Factor VIII-related antigen and high activities of alkaline phosphatase. HBEC were highly enriched in the BBB-specific enzyme, γ-glutamyl-transpeptidase (γ-GTP), relative to RBEC. Vasoactive peptides, angiotensin II (200 μM), bradykinin (20 μM) and endothelin-1 (10 nM) induced stimultaneous increases in IP_3 and $[Ca^{2+}]_i$ (measured by fluorescence techniques) in both RBEC and HBEC. The $[Ca^{2+}]_i$ responses for all three peptides were due to the release of Ca^{2+} from internal stores since they were not affected by incubating the cells in Ca^{2+}-free medium or by pretreating the cells with Ca^{2+} channel blockers or the phospholipase C inhibitors, neomycin or U-73122. In contrast, IP_3 and $[Ca^{2+}]_i$ increases were observed in RBEC, but not HBEC, in response to ATP (100 μM), and in HBEC only, in response to histamine (100 μM). Neither RBEC nor HBEC responded to carbachol (1 mM), serotonin (100 μM), or glutamate (1 mM). The distinct profiles of γ-GTP expression and receptor-linked IP_3/Ca^{2+} signaling in RBEC vs. HBEC, demonstrate significant species differences in the properties of brain endothelial cells, which may translate into dissimilar responses of brain microvasculature to vasoactive mediators in different species *in vivo*. *Supported by the National Research Council of Canada*

308.3

DIFFERENTIAL AGRIN AND ZO-1 EXPRESSION ON PERMEABLE AND IMPERMEABLE BLOOD VESSELS OF THE RAT RETINA. E. Lieth*[1], A.J. Barber[2], S. Khin[2] and T.W. Gardner[2]. Depts. of [1]Neuroscience & Anatomy, and [2]Ophthalmology, Penn State University College of Medicine, Hershey, PA 17033.

Agrin is expressed in the basal lamina (BL) of brain capillaries, where it accumulates during the time when the blood-brain barrier forms. The microvascular distribution of agrin is selective to tissues with blood-tissue barriers. We have now looked also at the retina, which has both permeable and impermeable capillaries. We used indirect immunofluorescence to determine the expression of agrin and the tight junction protein ZO-1 on the barrier vessels in the neural retina compared to the relatively permeable choroid vasculature.

ZO-1 expression was determined by immunofluorescence staining either with monoclonal antibody R-40-76 or with a rabbit polyclonal antiserum. R-40-76 selectively stains vessels in the neural retina and choroid vasculature, but not the RPE in a pattern consistent with localization to tight junctions. The antiserum also stains intercellular junctions of the RPE. Agrin immunofluorescence is very bright on vessels of the neural retina, much dimmer on the choroid vessels, and undetectable in the BL of the RPE. In the ciliary body epithelial tight junctions are immunoreactive with anti-ZO-1 and the epithelial BL contains agrin, consistent with our previous findings in the choroid plexus of the brain. These results suggest that high microvascular agrin expression correlates with the presence of blood-retinal barrier in capillaries of the neural retina while tight junctions are present on all vessels. (Funding NIH K11 EY00331 and PA Lions).

308.4

DEHYDRATION INCREASES THE GLUT1 GLUCOSE TRANSPORTER BUT NOT CAPILLARY DENSITY OR FILLING IN THE HYPOTHALAMO-PITUITARY AXIS OF THE RAT. R.B. Page*, T.M. Rutherford, R.M. Bryan, Jr., I.A. Simpson, and S.J. Vannucci. Div. of Neurosurgery, Milton S. Hershey Medical Center, Hershey, PA 17033.

We investigated the hypothesis that increased delivery of substrate to magnocellular neurons in the *supraoptic* and *paraventricular nuclei* during periods of increased functional demands could be secondary to increased capillary filling or an increase in the glucose transport capacity of microvessels. We measured the capillary volume fraction and the percent capillary filling in the *supraoptic* and *paraventricular nuclei* of adult male rats by the point counting technique under control conditions and following two days of dehydration. The percent capillary filling did not significantly change in either of these neurosecretory nuclei with dehydration. We measured the concentration of the GLUT1 transporter in the same neurosecretory nuclei under the same conditions by in situ hybridization and autoradiography. The optical density in the *paraventricular nucleus* increased 18.7% and that in the *supraoptic nucleus* increased by 27.1 % with dehydration. These increases were statistically significant. Capillary volume fraction did not change during this period of dehydration. Because the concentration of capillaries in the *supraoptic nucleus* and in the *paraventricular nucleus* did not increase and the expression of glucose transporters present in capillaries did increase, these results support the hypothesis that the concentration of vascular glucose transporters increases in response to increased regional functional demand.

Division of Neurosurgery

308.5

ENDONEURIAL HYDROSTATIC PRESSURE IN RAT SCIATIC NERVE DURING DEVELOPMENT. N. R. Crowley, S. L. Nelson, A. Weerasuriya and A. C. Black*. Mercer University Schools of Medicine and Engineering, Macon, GA 31207.

In the endoneurial microenvironment of mammalian sciatic nerve, during development, endoneurial water content, endoneurial blood flow, and blood-nerve exchange of solutes decreases. On the other hand, endoneurial albumin content increases. A more comprehensive and quantitative description of endoneurial fluid dynamics during development requires additional information on endoneurial hydrostatic pressure and endoneurial fluid flow. In pursuance of this information, the objective of this study was to monitor endoneurial hydrostatic pressure in the sciatic nerve during ontogenic development. In anesthetized Sprague-Dawley rats, ranging in age from 1 to 12 weeks, endoneurial hydrostatic pressure (EHP) was measured in the mid-thigh portion of the sciatic nerve with a servo-null micropressure system. The results (body weight in gms, and EHP in mm Hg) are presented as mean ± SEM in the following table.

	1 wk	2 wks	3 wks	4 wks	6 wks	8 wks	10 wks	12 wks
n	7	7	5	5	9	9	4	9
weight	16±1	29±1	60±3	78±3	164±6	238±16	352±7	384±6
EHP	2.0±0.1	2.5±0.1	2.6±0.2	2.7±0.2	2.8±0.2	2.8±0.2	3.3±0.4	3.2±0.3

EHP increases rapidly during the first two weeks of development and then increases slowly over the next ten weeks. Thus, unlike in tissue edema, it is clear that the higher endoneurial water content seen within the first few weeks after birth is not accompanied by an elevated EHP. On the other hand, the ontogenetic increase of EHP parallels a similar increase in cerebrospinal fluid pressure (Deane & Jones, 1984). This supports the hypothesis that CSF is a major contributor to the endoneurial microenvironment. (NIH NS 30197).

308.6

GLYCOSPHINGOLIPID COMPOSITION OF BOVINE BRAIN MICROVASCULAR PERICYTES. **T.Iwasaki, M.Yamawaki and T.Kanda* Department of Neurology, Tokyo Medical and Dental University, Tokyo 113, Japan.**

Although the brain microvascular pericytes (BMPs) are one of the cardinal constituents of blood-brain barrier (BBB), their biological or chemical properties are almost unknown so far. Recently glycosphingolipid (GSL) composition of brain microvascular endothelial cells (BMECs) was determined and sulfoglucuronosyl paragloboside (SGPG) was shown to play important function as a ligand for L-selectin (Kanda *et al.*,Proc.Natl.Acad.Sci.,1995). Here we report GSL composition of BMPs, which may participate in important immunological functions in BBB. BMPs were isolated using a modified method for BMECs (Kanda *et al.*,J.Cell Biol.,1994). BMPs were identified by their polymorphic morphology, immunoreactivity against alpha-smooth muscle actin and the lack of von Willebrand factor. After confluency, cultured BMPs were harvested and GSLs were purified with Sephadex LH20 and DEAE-Toyopeal column, and GSL composition was analyzed using high-performance thin layer chromatography. BMECs and BMPs shared GM3 as major acidic GSL and GM1 as minor species. Differed from BMECs, BMPs did not express SGPG but were shown to contain a substantial amount of some polysialic GSLs (GD1a,GT1b) and sulfoglucuronosyl lactosaminyl paragloboside (SGLPG); this unique, HNK1 epitope-bearing GSL may play a key role in the physiological function of BBB.

308.7

DEVELOPMENT OF A STABLE HUMAN CEREBROMICROVASCULAR ENDOTHELIAL CELL LINE AS AN *IN VITRO* MODEL OF THE HUMAN BLOOD-BRAIN BARRIER. A. Muruganandam, J. Durkin*, and D. Stanimirovic. IBS, National Research Council, Ottawa, Canada K1A 0R6

Our objective was to generate an immortal human brain microvascular endothelial cell line which would provide an adequate model for assessing properties of the human blood-brain barrier (BBB) *in vitro*. To this effect, human capillary and microvascular endothelial cell cultures (passage 7), derived from samples of temporal lobe surgically removed for the treatment of idiopathic epilepsy, were transfected with the plasmid pSV3-neo coding for the SV40 large T antigen. The selection of the successful transformants was enabled by the coexpression of the neomycin resistance gene from the same plasmid. SDS-PAGE followed by immunoblot analysis positively identified the presence of SV40 large T antigen. Further immunofluorescence studies confirmed the uniform and predominantly nuclear expression of the SV40 large T antigen in all of the transfected cells. The transfected cells were contact-inhibited, maintained the characteristic cobblestone morphology of primary endothelial cells in culture, and serum- and anchorage-dependent proliferation. The transfected cells also maintained the phenotypic properties of the parental cells, in that they expressed similar levels of the BBB specific enzymes, alkaline phosphatase and γ-glutamyl transpeptidase, and binding profile of fluorecently labelled lectins relative to naive cells. Moreover, the transfected cells responded to IL-1β and TNFα by up-regulating ICAM-1 in a manner similar to that of their naive counterparts. However, the transfected cells overcame proliferative senescence, commonly observed at passage 10-12 in naive cultures, and their life span was extended until they entered crisis (passage 24). Survivors of this crisis were cloned and propagated further. These prospective immortalized endothelial cell lines of the human brain microvasculature may serve as a representative and readily available *in vitro* model for studying the properties of the BBB.

Supported by the National Research Council of Canada

308.8

CNS PERICYTES AND INTEGRINS MEDIATE INDUCTION OF ANGIOGENESIS. R. Balabanov, S. Malek-Hedayat, and P. Dore-Duffy*. Department of Neurology, Division of Neuroimmunology, Wayne State Univ. Sch. of Med., Detroit, MI 48201.

Angiogenesis or neovascularization is the formation of new vessels from established microcirculation. The mechanisms involved in regulation of angiogenesis are not well understood. It is likely that they involve complex fine tuning between the cellular compartments of the endothelium [endothelial cells (EC) and pericytes (PC)] and the structural components (basal lamina). We have established a model of angiogenesis which mimics the multicellular nature of the blood brain barrier (BBB). Primary CNS EC and PC are simultaneously co-cultured on gelatin coated dishes. A second layer containing an integrin ligand is then added. When EC are cultured alone, cells grow to confluency without tube formation. Upon simultaneous co-culture with primary PC, EC form a few elongated tube-like structures after two weeks in culture. However, if collagen I is used in a liquid overlay, EC form true capillary structures classically associated with angiogenesis. Maximum capillary formation is seen by within 24-48 hrs. Addition of collagen I (col-I) to the EC cultures alone had no effect. PC were found to induce EC activation and angiogenesis as well as upregulation of uPAR protein and gene expression. The PC effect on EC is contact dependent. This primes the EC for a second signal mediated by col-I. EC activation also results in increased expression of β_1 integrins. Thus engagement of col-I with β_1 integrins initiates rapid formation of capillary structures in CNS EC-PC cultures.

308.9

POTENTIAL ROLE OF PLEIOTROPHIN IN ANGIOGENESIS IN CEREBRAL ENDOTHELIAL CELLS. T.F. Deuel, J. Xu, H.-J. Yeh, W. Zhang, Y.-L. Lu, R. Hu*, C.Y. Hsu. Departments of Medicine and Neurology, Washington University School of Medicine, St. Louis, MO 63110.

Pleiotrophin (PTN), a 17 kD heparin-binding cytokine, has been reported to promote mitogenic, transforming, angiogenic and neurite promoting outgrowth activities. We employed bovine cerebral endothelial cells (BCECs) in culture to investigate potential biological functions of PTN. We found that PTN at 50 ng/ml promoted BCEC proliferation which increased by two-fold 48 hours after exposure of BCEC to PTN. PTN increased DNA synthesis by 2.4-fold in BCECs based on measurement of the incorporation of a nuclear analog BrdU. When BCECs were cultured on matrigel, PTN-treated BCECs morphologically mimicked the appearance of the vascular endothelial cells in vivo. Treatment of BCEC with PTN also significantly reduced monolayer permeability of macromolecule. Together, these results indicate that PTN promotes a weak mitogenic response in BCECs and may be involved in the regulation of blood brain barrier function. (supported by an NIH grant NS28995 and an Office of Naval Research grant #4114503-01)

308.10

THE PRESENCE OF ARGININE VASOPRESSIN (AVP) AND ITS mRNA IN RAT CHOROID PLEXUS. A. Chodobski*[1], Y.P. Loh[2], L.-P. Pu[2], C.E. Johanson[1], S. Corsetti[1], and J. Szmydynger-Chodobska[1]. [1]Prog. Neurosurg., Dept. Clin. Neurosci., Brown. Univ./R.I. Hosp., Providence, RI 02903 and [2]Lab. Dev. Neurobiol., NICHD, NIH, Bethesda, MD 20892.

AVP is a potent regulator of secretory function of choroid plexus, the primary site of cerebrospinal fluid (CSF) formation. This AVP action requires, however, high peptide concentrations. In the present experiments, the hypothesis was tested that AVP is produced locally within the choroid plexus, which would result in sufficiently high peptide levels to alter CSF secretion. The presence of AVP in choroidal tissue was demonstrated using rabbit anti-lysine VP antibody (Incstar) that cross-reacts with AVP. AVP-immunopositive product was detected using biotinylated donkey anti-rabbit IgG, streptavidin-biotin-peroxidase complex, and DAB. *In situ* hybridization histochemistry was employed, to show the presence of AVP mRNA in choroid plexus. Plasmid, pGrVP (a gift from Dr. W.S. Young), containing a 232-bp fragment of rat AVP cDNA encoding the C-terminus of proAVP, was used as a probe. To synthesize ^{35}S-labeled antisense or sense cRNA probe, pGrVP was linearized and transcribed *in vitro* with T7 or T3 RNA polymerase in the presence of ^{35}S-UTP. Choroidal tissue sections following hybridization and post-hybridization washes were coated with nuclear track emulsion and developed 3 wk later. AVP-immunoreactive product was predominantly localized close to the apical membrane and silver grains representing AVP mRNA were distributed throughout the cytoplasm of epithelial cells. Our findings indicate that AVP is synthesized in choroid plexus, and may exert autocrine and/or paracrine effects. Supported by RIH RD Grant 7512-01-95 and NIH Grant NS27601.

308.11

THE CHARACTERIZATION OF A UNIQUE FORM OF VASOPRESSIN-NEUROPHYSIN (VP-NP) GENE PRODUCT FOUND IN RAT CHOROID PLEXUS. D.M. Demers, J. Szmydynger-Chodobska, C.E. Johanson, S.M. Cascio*, and A. Chodobski. Prog. Neurosurg., Dept. Clin. Neurosci., Brown. Univ./R.I. Hosp., Providence, RI 02903.

In an accompanying abstract (by A. Chodobski et al.) we report the presence of VP and its mRNA in choroid plexus epithelium. In the present study, western blot and reverse-transcription polymerase chain reaction (RT-PCR) analyses were employed to characterize NP-immunoreactive products in rat choroid plexus. For western blot analysis, two different polyclonal anti-NP antibodies (ICN and gift of Dr. A.G. Robinson) were used to probe protein extracts of choroid plexus and hypothalamus. As expected, NP was detected as a ~10 kD polypeptide in hypothalamic extracts. This band was absent in choroid plexus; however, a ~19 kD protein was found to cross-react with the antibodies instead. The difference in molecular weight suggests a variance in the primary structure of the choroid plexus-derived VP-NP gene product. To confirm that choroid plexus expresses the VP-NP gene, RT-PCR was performed on RNA extracted from choroid plexus. Primers designed to amplify a 103-bp fragment corresponding to the 3' end of the NP and the first 75 nucleotides of the glycopeptide coding domains were employed for PCR following reverse transcription. RT-PCR products were then fractionated via agarose gel electrophoresis and stained with ethidium bromide. A single PCR product of the predicted size was obtained from choroid plexus RNA samples. DNA sequencing confirmed that this PCR product was derived from VP-NP transcripts. Our observations demonstrate that NP-immunoreactive protein products and their respective mRNAs are present in choroidal tissue; however, western blot analysis suggests unique post-translational processing of proAVP in the choroid plexus compared to hypothalamus. Supported by RIH RD Grant 7512-01-95 (A. Chodobski) and NIH Grants NS27601 (C.E. Johanson) and AM16166 (A.G. Robinson).

308.12

EFFECT OF CHRONIC FGF-2 INFUSION ON THE CHOROID PLEXUS. E. G. Stopa*, M. A. Mittler, M. H. Lebow, P. N. MacMillan, V. Kuo-LeBlanc, A. Chodobski, J. Chodobski, A. Baird, and C. E. Johanson. Brown U. Sch. of Med./RIH, Providence, RI and PRIZM Pharmaceuticals, San Diego, CA.

Growing evidence suggests that the choroid plexus can synthesize various growth factors normally found within the extracellular milieu of the central nervous system (CNS). FGF-2 mRNA, receptor and protein have all been localized within the choroid plexus.

To assess the possible effects of FGF-2 on choroid plexus epithelium, we examined the ultrastructural changes following continuous infusion of FGF-2 into the right lateral ventricle. Sprague-Dawley rats (250 g) received a dose of 41.7 ng/hr of FGF-2 (n=6) or sterile saline (n=6) for a 2 week period using an Alzet pump. Samples of choroid plexus were then processed for electron microscopy. Separate groups of FGF-2 infused (n=3) and control (n=3) animals were used for immunocytochemical studies of FGF-2, GFAP, Ki-67 and vimentin.

Rats treated with FGF-2 showed a striking increase in the percentage of dark epithelial cells when compared to controls (20% vs 2%). Villous surface area was also increased. Immunocytochemical stains showed increased FGF-2, GFAP and vimentin within the brain parenchyma of FGF-2 treated animals. The choroid plexus Ki-67 proliferative index was unchanged.

Our findings support the hypothesis that FGF-2 may enhance CSF secretion and influence the osmotic regulation of the extracellular fluid volume within the CNS. (Supported by AG 10682, NS 27601 and the Rhode Island Hospital Research Fund)

308.13

PROSTAGLANDIN CATABOLISM IN THE FETAL CHOROID PLEXUS: A SPECIAL ARRANGEMENT. N. Krunic[1,2], S.L. Adamson[2], I. Bishai[1] and F. Coceani[1]*. [1]Div. of Neurosciences, Hosp. for Sick Children, Toronto, On., Canada M5G 1X8. [2]Samuel Lunenfeld Res. Inst., Mount Sinai Hospital, Toronto, On., Canada M5G 1X5.

In the adult, eicosanoids, such as prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), are actively removed from the cerebrospinal fluid (CSF) by the choroid plexus for catabolism in the periphery. Catabolism to 13,14-dihydro-15-keto-$PGF_{2\alpha}$ (15KD-$PGF_{2\alpha}$) occurs primarily in the lung via cytosolic enzymes. We showed previously that the choroid plexus of the fetal sheep catabolizes $PGF_{2\alpha}$ to 15KD-$PGF_{2\alpha}$, whereas newborn and adult were unable to do so. In the current study, we determined the functional relationship between transport and catabolism in the choroid plexus. The intact choroid plexus (0.9 gestation) was incubated in Krebs buffer with ^{3}H-$PGF_{2\alpha}$. Transport was expressed as the tissue-to-medium ratio for radioactivity (T/M). Catabolism was expressed as the % radioactivity migrating with authentic 15KD-$PGF_{2\alpha}$ on thin-layer radiochromatography (%15KD). Catabolism was also measured in the 2,000g pellet and supernatant from minced choroid plexus, and in the 100,000g supernatant from homogenized choroid plexus. Lung was used as a reference. Probenecid (1 mM) significantly inhibited uptake in the intact choroid plexus (Control T/M: 5.6 ± 0.6, n=16; Probenecid T/M: 2.5 ± 0.2, n=10) but did not affect catabolism (Control %15KD: 55 ± 4%, n=9; Probenecid %15KD: 60 ± 3%, n=10). Catabolism was not detected in the 100,000g supernatant from choroid plexus, while it was high (%15KD about 80%) in the same fraction from lung. Catabolism was not restored by NAD^+ supplementation or treatment with indomethacin. Conversely, there was catabolism (%15KD: 78 ± 2%, n=3) in the 2,000g pellet, but not the supernatant, of minced choroid plexus. In conclusion, the fetal choroid plexus may remove PGs from CSF by independent processes of catabolism and transport. Unlike lung, catabolism does not take place when the structural integrity is disrupted by homogenization, suggesting a special arrangement in the fetal choroid plexus. (NK supported by SIDS and Genesis).

308.14

INHIBITION OF CYTOKINE-INDUCED NF-κB BINDING ACITIVITY AND iNOS EXPRESSION IN CEREBRAL ENDOTHELIAL CELLS (CECs) BY AN HAIRPIN NF-κB OLIGONUCELOTIDE (HR-NF-κB) L.M.He, J. Xu, Y.Q. Yang, Y.J. Wu and C.Y. Hsu* Dept Neurology, Washington Univ Sch Med, St. Louis, MO 63110.

To explore novel mechanisms to modulate iNOS expression in CECs, we designed an HR-NF-κB in an attempt to block NF-κB binding activity and consequently iNOS expression in CECs. In murine CECs, LPS, TNFα, or IL-1β alone does not induce iNOS or increase nitrite/nitrate (NOx) content. TNFα+INFγ increased iNOS expression and NOx formation in a time-dependent manner peaking 3 and15 hr respectively after exposure. L- nitro-arginine completely blocked NOx production as did cycloheximide (CHX), an inhibitor of macromolecule synthesis. CHX, but not L-nitro-arginine, completely inhibited iNOS protein synthesis determined by Western blot analysis. These findings suggest that TNFα+INFγ induced increase in NOx formation was through iNOS induction. When CECs were pretreated with HR-NF-κB, NF-kB binding was blocked. This effect of HR-NF-κB was accompanied by a decrease in iNOS mRNA expression and NOx formation induced by TNFα+INFγ. Results showed that in CECs, TNFα +INFγ induction of iNOS expression and subsequent increase in NOx formation is subject to different mechanisms of regulation. HR-NF-kB offers an alternative and novel means to modulate cytokine-induced iNOS expression in CECs. (supported by an Office of Naval Research grant #4114503-01 and NIH NS28995).

308.15

CYTOKINES REGULATE PROSTAGLANDIN H SYNTHASE-2 IN CEREBRAL MICROVASCULAR ENDOTHELIUM. S.A. Moore*, E.J. Yoder, and G. Rich. Dept. of Pathology, University of Iowa, Iowa City, IA 52242-1181

Arachidonic acid metabolism is important in regulating the cerebral circulation and its response to CNS injury, a response that often involves vascular cell proliferation, inflammatory cell infiltrates, and glial cell reactions. Induction and regulation of prostaglandin H synthase-2 (PGHS-2) may be important in these processes. To study PGHS expression in cerebrovascular cells, murine-derived cerebral endothelial cultures are being incubated for up to 24h with a variety of inflammatory mediators. Over 24h, LPS and IL-1β cause PGE_2 and I_2 to accumulate in the medium to an 8- to 10-fold greater degree than control, but significant increases are not detected until 12h of incubation. IL-6 and IFN have no effect. Restimulation of LPS and IL-1β activated cells with 7.5 μM arachidonic acid reveal 3- to 10-fold increases in total PGHS activity, respectively. Levels of PGHS-2 mRNA and protein are rapidly induced by these same two proinflammatory mediators. Peak mRNA levels are seen by 3h and peak protein levels by 6h. Both times preceed the maximum effect of these compounds on PG accumulation in the medium. These data indicate that PGHS-2 expression and PGHS activity in cerebral endothelium are regulated by proinflammatory mediators. Differences in the time course of PGHS-2 induction and PG accumulation suggest that phospholipase A_2 may also be induced, but on a slower time course. Collectively the data suggest that cerebrovascular PG production may be modified by regulation of endothelial cell PGHS and/or PLA_2 in disease states such as trauma, stroke, infection, or neoplasia where proinflammatory mediators are produced.

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308.16

OXIDATIVE STRESS AND NF-κB IN LIPOPOLYSACCHARIDE (LPS) AND CYCLOHEXIMIDE (CHX) INDUCED APOPTOSIS IN BOVINE CEREBRAL ENDOTHELIAL CELLS (BCECs). J. Xu*, A. Wang, Y. Wu, L.M. Canzoniero, S. L. Sensi, C. Csernansky, L. Dugan, D. W. Choi, C.Y. Hsu Dept Neurology & Ctr Study Nervous System Injury, Washington Univ Sch Med, St. Louis, MO 63110

LPS priming (16 hr) followed by CHX exposure (6 hr) consistently induced BCEC apoptosis which was confirmed by characteristic DNA changes with Hoechst staining, immunoenzymatic demonstration of an increase in the cellular content of cytoplasmic histone-associated DNA fragments [DNA fragment enrichment factor (DNA FER): LPS (0.1 mg/ml) 0.93 ± 0.07; CHX (50 μg/ml) 1.16 ± 0.08; LPS+CHX 2.42 ± 0.01 , $p<0.01$] and the appearance of DNA laddering on agarose gel electrophoresis. N-acetyl-cysteine (NAC), which enhanced glutathione levels [NAC (-) 88 ± 10 nmol/ml; NAC (1 mM) 890 ± 30, $p<0.001$), prevented LPS/CHX-induced apoptosis in a dose-dependent manner [DNA fragment enrichment factor[DNA FER: NAC (-) 2.53 ± 0.01; NAC (0.01 mM) 1.92 ± 0.14; (0.1 mM) 1.11 ± 0.06; (1 mM) 0.64 ± 0.06, $p<0.001$]. In contrast, both maleic acid diethyl ester (MADE), a glutathione depletor, and DL-buthionine-(S,R)-sulfoximine (BSO), a glutathione synthase inhibitor, lowered glutathione levels [control 88 ± 10 nmol/ml; MADE (0.1 mM) not detectable; BSO (1 mM) not detectable] but enhanced apoptosis (DNA FER: control 2.39 ± 0.13; MADE (0.1mM) 4.38 ± 0.52; (BSO (1 mM) 5.54 ± 0.41, $p<0.01$). LPS/CHX substantially enhanced NF-κB binding activity. LPS or CHX alone had no effect. These results raise the possibility that LPS/CHX-induced apoptosis may involve a nuclear regulatory mechanism mediated by NF-κB which is subject to modulation by oxidative stress. (supported by an Office of Naval Research grant # 4114503-01 and an NIH grant NS 28995).

308.17

RMP-7, A BRADYKININ ANALOG, PERMEABILIZES BLOOD VESSELS IN A RAT BRAIN TUMOR. E. Sanovich*, H. Le, R. Bartus, R. Dean, P. Elliott, M. Brightman. N.I.H. Bethesda, MD 20892 & Alkermes, Inc., Cambridge, MA 02139.

The permeability of blood vessels supplying RG-2 gliomas, implanted in rat striatum, has been assessed with a newly applied probe: biocytin-lucifer yellow-lysine, visualized by fluorescence and electron microscopy. The probe is small (850 Da), to the extent that it does not bind to serum albumin. This probe or another one, horseradish peroxidase (40 kDa), were infused into the jugular vein and circulated for 2 minutes. Both probes are excluded by the barrier vessels of brain but they escaped from a variable number of vessels within the tumors and at the border with normal brain. In both saline infused rats and those given RMP-7 via the internal carotid artery, there were hemorrhages of variable size and number in the tumors and at their border. The hemorrhages were usually not associated with exudates which, therefore, escaped primarily across intact tumor vessels. More parenchymal exudates in the RMP-7 group coalesced into extensive extravascular pools. RMP-7 thus appears to increase the permeability of some vessels within the tumor and at its border with normal brain.

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308.18

PLASMA IN THE VENTRICULAR SYSTEM OF THE BRAIN INCREASES THE PRODUCTION OF CEREBROSPINAL FLUID. M.A. Maktabi* and G.C. Stachovic.
University of Iowa College of Medicine, Department of Anesthesia, Iowa City, IA 52242.

Blood in the ventricular system of the brain is a common sequela of head trauma and ruptured intracranial aneurysms. Hydrocephalus and increased intracranial pressure usually follows that complication. The rate of production of cerebrospinal fluid (CSF) is a major factor that influences intracranial pressure. Thus, we tested the hypothesis that presence of plasma in the ventricles of the brain increases the production of CSF. Experiments were performed in New Zealand White rabbits that were anesthetized, normothermic, and mechanically ventilated. The production of CSF was measured with the open ventriculo-cisternal perfusion method using C^{14} dextran as a nondiffusable indicator. Under control conditions, the production of CSF averaged 7.10 ± 0.99 µl/min (S.D., n=8). Infusion of plasma into the lateral ventricle of the brain at a rate of 6 µl/min, increased the production of CSF to 8.6 ± 1.6 µl/min (n=8). In a third group of animals administration of indomethacin (5 mg/kg, iv) inhibited increases in CSF production that were produced by plasma. In a fourth group of rabbits, administration of PGE_2 did not result in an increase in CSF production (n=5). These results suggest that presence of plasma in the ventricles of the brain increases CSF production via stimulation of prostaglandin synthesis. Since PGE_2, alone, did not increase CSF production, our results also suggest that this increase in CSF production may be due to an increase in prostacyclin or thromboxane synthesis in the ventricular system of the brain.

Dept. of Anesthesia, Univ. of Iowa

308.19

IMMUNOULTRASTRUCTURAL AND HIGH-VOLTAGE ELECTRON MICROSCOPIC STUDIES OF ENDOTHELIAL CELL TUBULES AND VESICULAR-VACUOLAR ORGANELLES IN HUMAN BRAIN TUMORS. A.S. Lossinsky*[1], K. Buttle[2], M. Marko[2], R. Pluta[3], M.J. Mossakowski[3], and H.M. Wisniewski[1]. [1]Dept. of Pathological Neurobiology, NYS Institute for Basic Research in Developmental Disabilities, Staten Island, NY 10314; [2]NYS Dept. of Health, Wadsworth Center for Labs. and Research, Albany, NY 12201; [3]Dept. of Neuropathology, Medical Research Center, Polish Academy of Sciences, Warsaw, Poland 00-784.

The vesicular-vacuolar organelle - VVO was recently described in murine ovarian carcinoma endothelial cells (ECs) (Dvorak et al., J. Leukoc. Biol. 59:100-115, 1996), who suggested their important role for macromolecular transport. The EC tubular system presented by us and others in the injured blood-brain barrier (BBB) described structural profiles that were purported to reflect passageways and conduits for macromolecules and inflammatory/tumor cells respectively. In the present study, we asked the question whether or not the VVO functions similar to the EC tubular profiles. Angioma and glioblastoma multiforme specimens were incubated with anti-human ICAM-1 antibodies using a pre-embedding technique and labeled with immunogold or immunoHRP probes. Electron microscopy demonstrated that both probes labeled the inner delimiting membrane surfaces of EC tubules and VVOs. A three-dimensional distribution of immunogold labeling could be visualized by stereo-pair images of thick-sections by high-voltage electron microscopy. Our data suggest that both ICAM-1-positive VVOs and EC tubular profiles may represent an important mechanism for transendothelial transport of both macromolecules and tumor cells in human brain tumors. Supported by the Office of Mental Retardation and Developmental Disabilities, the BMIRR of the Wadsworth Center (RR 01219), and the Medical Research Center of the Polish Academy of Sciences.

308.20

ANALYSIS OF TARGET ANTIGENS ON BLOOD-BRAIN BARRIER RECOGNIZED BY ANTI-ENDOTHELIAL CELL ANTIBODIES(AECA)

M.Yamawaki*, T.Iwasaki, T.Kanda, T.Ariga#, R.K.Yu#
Dept.Neurology Tokyo Medical & Dental Univ.,Tokyo, Japan.
#Dept.Biochemsitry, Medical College of VA, Richmond, VA.

Serum anti-endothelial cell antibody (AECA) is known to be associated with several inflammatory disease such as systemic vasculitis and systemic lupus erythematosis (SLE). In the present study we investigated the serum AECA activities in multiple sclerosis (MS) and further analyzed the target antigens of AECA on brain microvascular endothelial cells (BMECs). Sera from MS patients were collected according to clinical course (4 to 9 sera from each patients). AECA was detected by cellular enzyme-linked immunosorbent assay (cELISA). Target antigens of AECA were determined by ELISA, Western-blotting, and TLC-immunoblotting methods using cultured bovine BMECs and authentic standards of glycolipids as antigens. BMECs stimulated with interleukin-1(IL-1) beta were also used as antigens. We found that patients showed serum AECA activity of the IgG class against stimulated BMECs, but not against unstimulated cells. AECA reacted with sulfoglucuronosyl glycosphingolipids (SGGLs: a ligand for L-selectin) and a 65kD protein from the stimulated BMECs. Both of the antigens were upregulated with IL-1 beta. We conclude that the target antigens of AECA may play important roles in the course of inflammation on blood-brain barrier (BBB).

PRESYNAPTIC MECHANISMS: NEUROMUSCULAR JUNCTION

309.1

DETERMINANTS OF SYNAPTIC STRENGTH: PHYSIOLOGY MATCHED TO ULTRASTRUCTURE IN IDENTIFIED PHASIC AND TONIC CRAYFISH NEUROMUSCULAR JUNCTIONS. H.L. Atwood[1]*, M. Msghina[1], L. Marin[1], J. Pearce[2], and C.K. Govind[2]. [1]Dept. of Physiology and [2]Scarborough College, University of Toronto, Toronto, Ontario, Canada M5S 1A8.

In the crayfish leg extensor muscle, two excitatory motor neurons, phasic and tonic, innervate all muscle fibers in parallel. Neuromuscular junctions are readily observed and morphologically distinct. We recorded quantal currents with focal 'macro-patch' electrodes of 10-20μm diameter, marking the recording sites with fluorescent polystyrene microspheres (5 μm diameter) which are identifiable in both light and electron microscopy (Wojtowicz *et al.*, J. Neurosci. 14:3588, 1994). Preparations were then fixed for electron microscopy and the recorded regions of the neuromuscular junction reconstructed from thin serial sections. Synapses were measured and counted in the reconstructions.

Quantal content of synaptic currents was often 50 to 150-fold greater at low frequencies in recordings at phasic junctions, yet in the recorded area the number of individual synapses per μm of terminal was in some cases less for phasic than for tonic junctions. As well, contact areas of individual synapses were two-fold smaller on average in phasic junctions. On the other hand, there was a substantially greater percentage of synapses with more than one active zone ('complex' synapses) in phasic (30-50%) than in tonic (5-10%) junctions. Additionally, at these 'complex' synapses, paired active zones were more closely spaced in phasic junctions. While these latter two factors may account for some of the overall difference in synaptic strength (Cooper *et al.*, J. Neurophysiol. 75: 1996), they are not in themselves likely to account for all of the difference. Supported by grants from MRC and NSERC, Canada.

309.2

EFFECT OF MELATONIN ON SYNAPTIC TRANSMISSION AT CRAYFISH NEUROMUSCULAR JUNCTIONS. H.Brignull, W.North* & S.J. Velez
Dept. Biol. Sciences, Dartmouth College, Hanover, NH 03755.

Melatonin has been shown to decrease EPSPs when applied to neuronal populations in the hippocampus and the gastro-intestinal wall in vertebrates. The few melatonin studies in invertebrates have not looked at possible effects in synaptic transmission. We use a simple crayfish neuromuscular system to study how synaptic performance is affected by the application of drugs like caffeine (Soc. Neurosci. Abstr. 21:1089) and hormones like serotonin (Soc. Neurosci. Abstr. 20:1338), so we decided to investigate whether our system could be affected by the application of melatonin. We recorded junction potentials (jp's) produced by stimulation of the axons innervating superficial flexor muscle of the crayfish Procambarus clarkii. Recordings were made in normal Ringers before exposing preparations to different melatonin concentrations. JP's produced by axon 6, the largest excitor,did not change in the presence of melatonin but those produced by the other axons increased in size within minutes of exposure. The response was dosage dependent; no responses were seen with concentrations lower than 0.1mM, were first observed at 0.5mM, and increased with stronger dosages. Applying 2.3mM melatonin in Ringers of different calcium concentrations gave mixed results: in 30% calcium Ringer melatonin had no effect, but in 200% calcium Ringers the response was larger than controls, suggesting a presynaptic site of action for the hormone.

(Supported by Dartmouth College Research Funds)

309.3

EFFECT OF CAFFEINE ON CALCIUM RESPONSES AT CRAYFISH NEUROMUSCULAR SYNAPSES. E. Shugert and S. J. Velez*. Dept. of Biological Sciences, Dartmouth College, Hanover, NH 03755.

We had previously reported that caffeine decreased the junction potentials (jp's) recorded from crayfish neuromuscular synapses in Ringers varying in calcium concentrations (Soc. Neurosci. Abstr. 21:1089), and suggested that caffeine was reducing the amount of presynaptic calcium. To continue this study, we analyzed the responses of crayfish muscle fibers to (1) changes in calcium and to (2) the calcium ionophore A23187, before and after the application of caffeine. JP's elicited by stimulation of excitor axons were recorded in normal Ringers and in 11% calcium Ringers before exposing the fibers to a 5mM, 10mM or 20mM caffeine solution. After caffeine exposure, the jp's were recorded again in normal and 11% calcium Ringers; these jp's were smaller in size than controls and the facilitation observed previously (jp at 10 Hz/jp at 1 Hz) was reduced or absent, suggesting that caffeine's effects persist after drug removal. A23187 application created an initial enhancement of jp sizes at 1 and 10 Hz, followed within 4 min by a decline until sizes at 10 Hz were smaller than sizes at 1 Hz (i.e. terminals that facilitated now show antifacilitation). 10mM caffeine in A23187 does not eliminate the decline in jp size with time nor the antifacilitation, but slows down the appearance of the initial rise in jp and lowers its final size, suggesting that caffeine's effects do not involve calcium sequestration into internal stores.

(Supported by Dartmouth College Research Funds)

309.4

NO SYNTHASE LOCALIZATION AT THE FROG NEUROMUSCULAR JUNCTION. Laurence Descarries*, Chantale Marier and Richard Robitaille. Département de physiologie et Centre de Recherche en Sciences Neurologiques, Université de Montréal, Montréal (Qc) Canada H3C 3J7.

Nitric oxide (NO), a modulator of synaptic function, is produced by many cells of the nervous system including glial cells. This study aimed at determining whether the biosynthetic enzyme of nitric oxide, NO synthase, is present at the frog neuromuscular junction (NMJ), a synapse known to be sensitive to NO (Lindgren and Laird, 1994, Neuroreport 5, 2205). The histochemical technique involving NADPH-diaphorase staining revealed activity in cell body and processes of perisynaptic Schwann cells, glial cells at the NMJ. Muscle fibers were also stained, particularly near end plates, which presence was confirmed by revealing acetylcholinesterase activity. Incubation with NO synthase inhibitor Nω-Nitro-L-Arginine methyl ester greatly reduced the staining of NADPH-diaphorase, confirming that it was due to NO synthase activity. Omission of calcium did not affect this reaction, suggesting that the activity of the enzyme was not calcium-dependent. Staining was absent after denervation (seven days) and in younger frogs (< 20g).

Polyclonal antibodies against different types of NO synthases were used in an attempt to identify the type of enzyme present. Observation with fluorescent confocal microscopy revealed staining with neuronal and epithelial NO synthases antibodies in perisynaptic Schwann cells. Muscle fibers were labeled with epithelial NO synthase antibody only. No staining was observed with anti-induced NO synthase. The presence and functionality of NO synthase at the frog NMJ suggest that endogenous NO may modulate synaptic activity at this synapse. (Supported by MRC of Canada and the Sloan Foundation).

309.5

OSMOTIC ENHANCEMENT OF NEUROTRANSMITTER RELEASE AT NEUROMUSCULAR JUNCTIONS: COMPARISONS WITH STRETCH ENHANCEMENT. A.H. Kashani, B.M. Chen and A.D. Grinnell*, Jerry Lewis Neuromuscular Research Center, Department of Physiology, UCLA School of Medicine, LA, CA 90024

Hyperosmotic enhancement (HE) of spontaneous neurotransmitter release is widespread and poorly understood. We have shown that the maintained tonic component of HE at frog(*Rana pipiens*) neuromuscular junctions is mediated, at least in part, by integrins (Chen & Grinnell, Science 269, 1578-1580, 1995). Further experiments directed at elucidating the mechanism of osmotic modulation of release and comparing it with stretch enhancement (SE) show that: (a) both forms of enhancement are reduced 40-60% by the hexapeptide GRGDSP, which blocks integrin binding to natural ligands. Mammalian junctions also show GRGDSP sensitive HE; (b) Although both effects persist in 0 Ca^{++} Ringer, SE is much more strongly suppressed when internal Ca^{++} is buffered by BAPTA; (c) Like SE, HE is inversely proportional to resting mEPP frequency; (d) as mEPP frequency is elevated by stretch, HE decreases and, at high frequencies (>5Hz), reverses and becomes inhibitory to SE; (e) elevation of mEPP frequency by K^+-depolarization severely inhibits HE, while having no effect on the SE-mEPP frequency relationship. Although there are some striking differences between SE and HE in terms of dynamics and potency, there are sufficient similarities to suggest that at least part of HE may involve the same integrin-dependent mechanism(s) as SE. Supported by NS 30673

309.6

ADENOSINE FEEDBACK IS NOT THE PRIMARY BASIS FOR TETANIC FADE AT THE MAMMALIAN NEUROMUSCULAR JUNCTION. M. Malinowski, P.M. Barr, S. Cannady, Schmit, K.V., and D.F. Wilson*. Zoo Dept., Miami Univ., Oxford, OH 45056.

It has been suggested that tetanic fade (decline in transmitter release during repetitive stimulation) at the mammalian neuromuscular junction is due to increased negative feedback action of adenosine during repetitive neural stimulation. In the present study, the presynaptic effects of blocking adenosine A_1-receptors in the rat diaphragm with 1,10 or 100 μM 8-cyclopenty-ltheophylline (CPT) were examined. Intracellular recording techniques were used to monitor end-plate potentials and miniature end-plate potentials in the isolated cut-muscle and end-plate potentials in the curarized rat diaphragm-phrenic nerve preparation (40 stimuli of 1, 25, and 50 Hz were used). Quantal release and tetanic fade in the presence and absence of the antagonist were examined. CPT enhanced quantal release and end-plate potential amplitudes at the onset of tetanic stimulation in both the curarized and cut muscle preparations, but did not reverse tetanic fade at stimulus frequencies of 25 and 50 Hz. While adenosine may play a role in depressing transmitter release our results suggest that adenosine is not the major mechanism for causing tetanic fade. It is suggested that tetanic fade is due to calcium inactivation of calcium influx.

309.7

AN ACTIVITY-DEPENDENT ANTIBODY PROBE FOR USE AT THE SNAKE NEUROMUSCULAR JUNCTION. Y.-J. Son, J. C. Cole and R. S. Wilkinson*. Department of Cell Biology & Physiology, Washington University School of Medicine, St. Louis, MO 63110.

NMJs in the thin transversus abdominis muscle of the garter snake contain large (2-3 μm) terminal boutons which may be studied physiologically and then identified for light microscopic visualization. We have developed an antibody probe for visualization of one bouton component, recently active vesicular membranes, using this method. Our probe putatively recognizes the luminal domain of a vesicular membrane protein, synaptotagmin II, and is thereby taken up from the bath by living boutons undergoing exo- or endocytosis associated with neural activity (Malgaroli et al., Science 268: 1624-28, 1995).

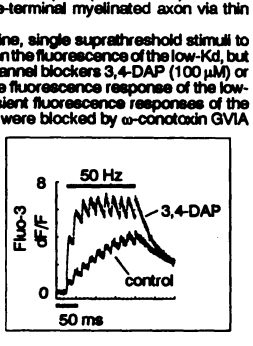
A snake spinal cord cDNA library was constructed and probed with marine ray synaptotagmin II (gift of R. Scheller, Stanford Univ.). A snake homologue was cloned, and the entire luminal domain of the gene utilized to construct a fusion protein for antibody generation. The resulting rabbit polyclonal antiserum recognized a ~50 kD spinal cord membrane fraction in Western blots, but neither brain membrane nor spinal cord cytosolic proteins. Bath-applied antiserum (but not pre-immune serum) stained living KCl-depolarized snake nerve terminal boutons brightly, but did not stain similar boutons bathed in normal reptilian saline (visualized with a fluorescent secondary antibody after fixation). High resolution 3-D confocal imaging revealed that stained regions of the bouton were punctate (<300 nm) and often opposed postsynaptic secondary folds (visualized with the anti-AChR monoclonal mAb 22, gift of J. Lindstrom, Univ. Pennsylvania). Supported by NIH grant NS24752, the McDonnell Center and the Keck Foundation.

309.8

FLUORESCENCE IMAGING OF STIMULATION-INDUCED [Ca^{++}] CHANGES IN LIZARD MOTOR NERVE TERMINALS. G.David*, J.N. Barrett and E.F. Barrett. Dept. of Physiology & Biophysics, Univ. of Miami Med. Sch., P.O. Box 016430, Miami, FL 33101.

Ca-sensitive dyes were ionophoresed into motor axons innervating the ceratomandibularis muscle of the lizard (Anolis sagrei). Fluorescence changes following stimulation were measured for both low-Kd (Fluo-3 and Ca Green-2N) and high-Kd (Ca Green-5N, Fluo-FF) dyes, using a confocal microscope. Most nerve terminals are composed of clusters of small (~1-3 μm diameter) interconnected boutons, which connect to the pre-terminal myelinated axon via thin (<0.25 μm) heminodal processes.

In boutons bathed in physiological saline, single suprathreshold stimuli to the nerve trunk evoked transient increases in the fluorescence of the low-Kd, but not the high-Kd, dyes. Addition of the K-channel blockers 3,4-DAP (100 μM) or TEA (5 mM) increased the amplitude of the fluorescence response of the low-Kd dyes (see Figure) and produced transient fluorescence responses of the high-Kd dyes. These fluorescence changes were blocked by ω-conotoxin GVIA (0.3 μM). The fluorescence response to a single stimulus started with a delay of ~1.5 ms after nerve stimulation and rose to 1/2 peak amplitude within ~ 1.5 ms.



Little or no fluorescence increase was observed in the myelinated region and terminal heminode of axons stimulated with single stimuli or short trains (5 @ 50 Hz). Longer trains (50-100 Hz, >500 ms), resulted in some increase in these regions, but this increase was always smaller in amplitude than that in boutons.

Supported by NS 12404

309.9

RECRUITMENT OF ACTIVE SYNAPSES AT THE CRAYFISH NEUROMUSCULAR JUNCTION VISUALIZED WITH THE FLUORESCENT DYE FM1-43. P.A. Quigley[1], R.L. Cooper[2], C.K. Govind*[1] and H.L. Atwood[2]. [1]Scarborough College and [2]Dept. of Physiology, University of Toronto, Toronto, Ontario, Canada M5S 1A8.

The crayfish neuromuscular junction consists of a series of varicosities, each of which contains 20-40 individual synapses with variable number of active zones (Cooper *et al.*, 1995, J. Neurosci. 15, 4209-4222). Frequency facilitation is very pronounced at neuromuscular junctions of 'tonic' motor neurons: quantal content of transmission at a varicosity is very low (often less than 1) at low frequencies, and increases dramatically at higher frequencies. We hypothesize that one of the mechanisms underlying the dramatic increase in quantal content with frequency is the recruitment of additional synapses.

An optical method to test this hypothesis is afforded by the fluorescent dye FM1-43, which is sequestered by synaptic vesicles at active synapses (Betz *et al.*, 1992, J. Neurosci. 12, 363-375). We have found that this dye is taken up at the crayfish neuromuscular junction, and that neurons releasing more transmitter take up more of the dye (Msghina *et al.*, 1995, Soc. Neurosci. Abstr. 21, 333). Individual varicosities show a few well-defined bright spots after stimulation in FM1-43. At intermediate frequencies, the number of bright spots is less than the number of individual synapses found on these varicosities by electron microscopy, suggesting that not all synapses are equally active. The staining pattern depends on frequency and duration of stimulation, indicating unequal and progressive synaptic participation linked to frequency facilitation.

Supported by grants from MRC and NSERC, Canada.

309.10

NERVE ACTIVITY DETERMINES THE TIME COURSE OF ENDOCYTOSIS AND SYNAPTIC DEPRESSION AT FROG NEUROMUSCULAR JUNCTIONS
L.G. Wu* and W.J. Betz. Department of Physiology, UCHSC, Denver, CO 80262

To measure the time course of endocytosis at frog cutaneous pectoris motor nerve terminals, we stimulated nerves at 30 Hz, then added FM1-43 at various times after the end of the stimulus train, and then washed and imaged terminals to measure the amount of dye taken up by the terminals. The endocytic rate declined exponentially after the end of the stimulus train, and the time constant was approximately equal to the duration of the train (we used trains lasting 10, 60, and 300 s, and the endocytic time constants were 23, 62, and 385 s respectively).

We also recorded end plate potentials (EPPs) and observed that the recovery from synaptic depression was also slowed after prolonged stimulation. To quantify these observations, we developed a three compartment model consisting of a reserve pool of vesicles (measured by nerve terminal brightness after maximum FM1-43 staining), a docked pool of vesicles (measured by EPP amplitude), and an externalized membrane pool (unretrieved vesicle membrane, measured by the post-stimulus dye uptake as described above). Results suggest that the rate constants of both endocytosis and vesicle moblization determine the depression time course.

We tested the hypothesis that the endocytic time constant increased after long stimulus trains due to a rise in $[Ca^{++}]_i$. Terminals were loaded with the salt form of Fura-2, and calcium concentrations were determined by conventional ratio imaging. Consistent with the hypothesis, we found that the post-stimulus rise in calcium concentration was more prolonged after long stimulus trains. However, calcium concentration during a stimulus train (up to 1 μM) was much higher than after the train, yet endocytosis (FM1-43 uptake) was not inhibited during a stimulus train. Thus, it appears that an increase in calcium concentration is not responsible for the dependence of the endocytosis rate on stimulation duration.

This work was supported by research grants from MDA and NIH(NS23466).

309.11

TETANUS-INDUCED-INTRACELLULAR Ca^{2+} DYNAMICS IN THE FROG MOTOR NERVE TERMINAL. KUBA, K[1,*], MITSUMOTO, T[2], OSANAI, M[3], NARITA, K[4], SHIRASAKI, T[2], SUZUKI, S.[3], SUZUKI, N[3], & KIJIMA, H[3]. Dept. of Physiology. Nagoya Univ. Sch. of Med.[1] Showa-ku. Nagoya 466, Dept. of Physiology. Saga Med. Sch.[2], Nabeshima, Saga; Dept. of Physics., Sch. of Science. Nagoya Univ.[3], Chikusa-ku, Nagoya; Dept. of Physiol., Kawasaki Med. Sch.[4], Kurashiki. Okayama. Japan

Fluorescence of indo-1 loaded in the motor nerve terminals of frog cutaneous pectoris muscles was recorded by line-scanning with a confocal laser-scanning microscope and the ratio of fluorescences in two wavelength ranges (F_{412}/F_{476}) was taken. Tetanic nerve stimulation (100 Hz, 0.5-5 sec) produced a rise in intracellular Ca^{2+} (tetanus-induced Ca^{2+} transient) which decayed in fast and slow phases (time constants: several 100 msec and more than several seconds). The fast phase was increased in amplitude, but not in time constant, with the number of pulses. Replacement of extracellular Na^+ with Li^+ increased both the amplitude and time constant of the fast component. Raising pH to 9 (to block the plasma membrane Ca^{2+} pump) or applying thapsigargin (to block the Ca^{2+} pump at the organelle membrane) did not affect Ca^{2+} transients. High pH, however, prolonged the both phases of the decay under the effect of Li^+ and increased the amplitude of the slow phase. The decay phase of tetanus-induced Ca^{2+} transient in the motor nerve terminals is thus determined predominantly by the Na/Ca exchange and partially by the Ca^{2+} pump at the cell membrane.

309.12

CHANGES IN THE KINETICS OF TRANSMITTER RELEASE DURING FACILITATION AT THE INHIBITORY OF THE CRAYFISH NEUROMUSCULAR JUNCTION. A. Vyshedskiy, Jen-Wei Lin*. Dept. Biology, Boston Univ. Boston, MA 02215.

The inhibitor of the crayfish opener muscle is used to investigate synaptic facilitation. Two electrode voltage clamp is used to control the amplitude and duration of presynaptic depolarizations. Inhibitory postsynaptic potentials (IPSP) are measured from a muscle fiber near the presynaptic voltage electrode. We investigate the F2 component of facilitation with controlled presynaptic pulses. Paired-pulse facilitation, tested by 5 ms presynaptic pulses, reveals an acceleration of neurotransmitter release kinetics. The acceleration is best shown as a reduction of delay measured between the onset of a presynaptic pulse and an IPSP at its 50% peak amplitude ($delay_{50}$). The reduced $delay_{50}$ decays with the same time constant as that measured from IPSP amplitudes. As we change the strength of facilitation, by varying the amplitudes of conditioning pulses, the reduction of $delay_{50}$ quantitatively correlates with the magnitude of facilitation measured by amplitudes. Similar to facilitations tested by action potentials, short presynaptic pulses, 2 ms pulses depolarized to 0 mV, detect facilitation with no reduction of $delay_{50}$. In contrast, longer presynaptic pulses, 5, 10 or 20 ms pulses depolarized to 0 mV, reveal a clear decrease in $delay_{50}$ during facilitation. The change in transmitter release time course has never been reported before and its mechanism is currently under investigation. (Supported by NIHNS31707)

309.13

MODULATION OF TRANSMITTER RELEASE BY 5-HT AT PHASIC AND TONIC NEUROMUSCULAR JUNCTIONS IN CRAYFISH: QUANTAL PARAMETERS ASSESSED.
M.E. Crider and R.L. Cooper* School of Biological Sciences, Section of Organismal & Integrative Biology, University of Kentucky, Lextington, KY 40506-0225

It is well established that serotonin (5-HT) enhances neurotransmitter release at crustacean neuromuscular junctions (NMJs) and that circulating 5-HT in the blood has a physiological relevance in regulating the animal's behaviour. Since there is synaptic differentiation, both morphologically and physiologically, among high- and low-output motor nerve terminals, we investigated if the neuromodulator, 5-HT, displayed differential effects on these terminals. To better understand the enhanced effects of 5-HT on synaptic release, quantal analysis was performed to determine *m* (mean quantal content), *n* (number of release sites), and *p* (probability of a vesicle releasing at a site). Recordings of synaptic currents were obtained with a focal macropatch electrode placed over terminals made visible with a fluorescent dye (4-Di-2-Asp). We will present the effects 5-HT has on release in high- and low-out tonic terminals as well as high-output phasic motor terminals in the crayfish limbs. (Supported by Univ. of Kentucky start-up funds to RLC).

309.14

QUANTAL ANALYSIS BASED ON SPECTRAL METHODS OF HIGH-OUTPUT, PHASIC & LOW-OUTPUT, TONIC NEUROMUSCULAR JUNCTIONS IN CRAYFISH.
A.E. Ditvatev* and R.L. Cooper[1] Dept. of Physiology, Univ. of Bern, CH-3012, Bern, Switzerland; [1]Sch. of Biol. Sci., Sect. of Organismal & Integrative Biol., Univ. of Kentucky, Lextington, KY 40506-0225.

Single of the muscle fibers in the leg extensor muscle receive phasic and tonic innervation. As observed by focal macropatch current recordings placed directly over visualized terminals, the phasic endings have a larger (>50 fold) quantal content then the tonic terminals at a 1 Hz stimulation frequency. Since the phasic output is so high at physiological $[Ca^{2+}]_o$ the gold standard of counting evoked quanta is not possible as with the low-output, tonic terminals. So, we applied spectral analysis to the distributions of amplitude and charge measures of observed evoked & spontaneous currents to determine if this approach may allow us to evaluate the quantal parameters of the synaptic transmission in the phasic and tonic terminals. This method was chosen because (1) it is effective when quantal content is high; (2) it does not require any parametrisation of quantal size distribution and; (3) it provides an estimate of reliability of peaks in the analyzed distributions. The spectral analysis allowed us to compare the values of quantal size of evoked and spontaneous events and to assess quantal variability among these diverse terminals in order to better correlate quantal parameters to the observed synaptic differentiation. (Funds: Swiss National Funds to AED; Univ. of Kentucky start-up funds to RLC).

309.15

INTRACELLULAR ACIDIFICATION ARRESTS ENDOCYTOSIS AT THE NEUROMUSCULAR JUNCTION. Clark A. Lindgren*, Dennis G. Emery, & Philip G. Haydon. Department of Zoology and Genetics, Iowa State University, Ames, Iowa 50011.

We investigated the pH dependence of synaptic vesicle recycling at neuromuscular junctions of the lizard (*Anolis carolinensis*). Endocytosis was assayed by measuring accumulation of the fluorescent dye FM1-43, which becomes trapped in nerve terminals during the retrieval of membrane into synaptic vesicles. Intracellular pH was monitored by loading terminals with the fluorescent pH indicator BCECF. Nerve terminals were acidified by replacing chloride in the external bathing solution with equimolar concentrations of propionate. (Intracellular pH is reduced as some of the propionic acid diffuses across the cell membrane and dissociates inside the nerve terminal, releasing free hydrogen ions.)

Whereas the exocytosis of synaptic vesicles (detected as the loss of fluorescence from terminals preloaded with FM1-43) was unaffected by internal acidification, we found that membrane retrieval stopped if intracellular pH was reduced by at least 0.6 pH units. This degree of acidification was generated by 100 mM propionate. Lower concentrations of propionate did not arrest endocytosis. Furthermore, endocytosis was restored if the change in intracellular pH created by 100 mM propionate was made less than 0.6 units by either raising extracellular pH or by adding ammonium chloride.

The results with FM1-43 were confirmed using electron microscopy (EM). Neuromuscular junctions were depolarized in 60 mM KCl for 15 minutes to induce extensive exocytosis of synaptic vesicles. Junctions were allowed to recover for 15 minutes, either in the presence or absence of propionate, and then prepared immediately for EM. Those junctions which recovered in the absence of propionate contained normal numbers of synaptic vesicles whereas those exposed to 100 mM propionate during the recovery period were nearly depleted of vesicles. (Supported by NIH Grant NS26650.)

309.16

Increased neuronal excitability conferred by a mutation in the *bemused* gene. K. Walters*, T. Yoder and M. Stern, Department of Biochemistry and Cell Biology, Rice University, Houston, TX 77251.

We are interested in identifying genes that control neuronal excitability and synaptic transmission at the larval neuromuscular junction. As part of this long term interest, we have isolated a mutation in a novel gene. This mutation, *bemused(bem)*, is the result of a P element insertion at cytological position 85D. Flies defective in *bem* display uncoordination, inability to fly and female sterility. The phenotype of female sterility is currently undergoing further characterization, though preliminary analysis suggests it may be due to incomplete oogenesis. Furthermore, *bem* mutant larvae display aberrant synaptic transmission at the larval neuromuscular junction, specifically a more rapid onset of augmentation than do wildtype larvae. This increased rate of augmentation is potentiated by application of the potassium channel blocking drug quinidine. This electrophysiological phenotype is conferred by several other mutations that increase neuronal excitability; therefore, we propose that the *bem* mutation causes increased neuronal excitability as well. This hypothesis is supported by the observation that the facilitated transmitter release that occurs in *bem* mutants is the result of prolonged nerve depolarization.

In order to clone *bem*, DNA flanking the P element was obtained by the plasmid rescue technique. The 6 kb fragment was used to isolate additional genomic DNA spanning the P element insertion region. This DNA is currently being used to screen head and ovarian cDNA libraries. *This work is supported by NSF predoctoral fellowship to K. Walters and grant and aid from the American Heart Association to M. Stern.*

MECHANISMS OF NEUROTRANSMITTER RELEASE I

310.1

CHARACTERIZATION OF N-SEC1 BINDING TO SYNTAXIN SUGGESTS A POSITIVE ROLE IN SECRETION. L. Frelin and J. Pevsner*, Dept. of Neuroscience, Kennedy Krieger Institute and Johns Hopkins School of Medicine, 707 N. Broadway, Baltimore, MD 21205

N-sec1 (also called Munc-18 or RbSec1) is a soluble 67 kDa protein implicated in synaptic vesicle trafficking in mammalian nerve terminals. N-sec1 is a high affinity ligand for syntaxin 1a, one of a family of 35 kDa plasma membrane proteins that may regulate synaptic vesicle docking and/or fusion. In *Drosophila*, the n-sec1 homolog rop is required for secretion in neuronal and non-neuronal pathways. Four lethal alleles of rop involve single amino acid substitutions (Harrison et al., Neuron 13:555-566, 1994). We generated corresponding mutant proteins in rat n-sec1 by site-directed mutagenesis. We in vitro translated these proteins and quantitated their binding to recombinant syntaxin 1a immobilized on agarose beads. In three of the n-sec1 mutants, binding to syntaxin was reduced >99% relative to wildtype n-sec1. In a fourth mutant, binding to syntaxin was reduced 24%. These results are consistent with the hypothesis that n-sec1 binding to syntaxin is required for secretion. *Drosophila* rop mutants are presumably lethal because of their failure to bind syntaxin.

310.2

MORPHOLOGICAL AND BIOCHEMICAL CHARACTERIZATION OF SYNVESICLIN IN RAT BRAIN. C.-J. Jeng*, E. Floor, and E. S. Schweitzer. Dept. of Neurobiol., UCLA Med. Sch., LA, CA 90095 and Dept. of Physiol. and Cell Biol., Univ. Kansas, Lawrence, KS 66045.

We have recently identified a novel protein, synvesiclin, which is a 27 kD vesicle-specific integral membrane protein component of both small clear vesicles and large dense-core vesicles in PC12 cells. We have now examined the subcellular and regional distribution of synvesiclin in rat CNS.

Immunohistochemical analysis of CNS reveals a fine punctate staining, similar to that seen for two other synaptic vesicle markers, SV2 and synaptophysin, consistent with synvesiclin's association with synaptic vesicles. It is present throughout the gray matter in the brain and spinal cord, but is absent from white matter. Synvesiclin staining is found in all layers and regions of the cerebral cortex, in the molecular and granule cell layers of cerebellar cortex, in the synaptic layers of the hippocampus, as well and in the inner plexiform and ganglion cell layers of the retina.

We have also demonstrated biochemically that synvesiclin is a component of two distinct populations of synaptic vesicles in brain. Using dot blot analysis, we find that synvesiclin copurifies with rat brain synaptic vesicles through centrifugation, density gradient fractionation, and Sephacryl S-1000 and controlled-pore glass sizing columns. These results confirm the conclusion that synvesiclin is a common component of both small synaptic vesicles and large, dense-core vesicles in brain as well as in PC12 cells, and is likely to play a fundamental role in regulated secretion.

Supported by NIH grants R01 NS23084 and AG12993.

310.3

DISTINCT FEATURES OF SYNAPTIC ENDOCYTOSIS LINKED TO DYNAMIN AND ATP LEVELS. O. Shupliakov*, E. Zotova, L. Bakeeva[], P. Löw, H. Gad, L. Brodin. Dept. of Neuroscience, Karolinska Institute, S-17177 Stockholm, Sweden; []Belozersky Inst., Moscow State Univ., Moscow, Russia.

It is still debated which mechanisms underlie synaptic vesicle (SV) recycling. One reason for this uncertainty is the diverse time courses of membrane retrieval reported in different studies, and another is the poor correlation between the level of synaptic activity and the incidence of morphological correlates of membrane retrieval including clathrin-coated (CC) pits (reviewed in Neuron 14:689-96, 1995). Here we test whether the intrinsic properties of a given type of synapse may influence these parameters. Sustained depolarization (30 mM KCl, 30 min) of lamprey axons adapted to low-level activity ("phasic" reticulospinal axons), resulted in a marked depletion of SVs and a massive increase in the number of CCs. In axons adapted to high-level activity ("tonic" dorsal column axons), however, this treatment did not alter the number of SVs or CCs. As the release sites in tonic axons contained a much larger number of mitochondria than phasic synapses, we repeated the depolarization in the presence of mitochondrial inhibitors (3.2 mM dinitrophenol or 40 mM NaN_3). Now a depletion of SVs also occurred in the tonic axons, and the number of CCs increased significantly. However, the number of CCs did still not approach that in phasic synapses, suggesting additional differences. Immunogold analysis with dynamin antiserum showed that the levels of dynamin were significantly higher in tonic synapses, both over SVs and the plasma membrane around active zones. The results indicate that synapses adapted to tonic activity have a far more efficient recycling machinery (with a shorter life-time of CCs), than those adapted to phasic activity. This differentiation appears to involve the ATP supply as well as the availability of "endocytic" proteins, such as dynamin. (Supported by the Swedish MRC).

310.4

BIOCHEMICAL AND FUNCTIONAL STUDIES OF SYNAPTOBREVIN-MEDIATED PROTEIN INTERACTIONS. L. Pellegrini, O. El Far, V. O'Connor, W. DeBello#, D. van Rossum*, G. Augustine# and H. Betz. Max-Planck-Institut für Hirnforschung, Frankfurt/M, Germany 60528, and #Duke University Medical Centre, Durham, 27710 USA.

Neurotransmitter release (NtR) occurs via the calcium-dependent fusion of a pool of synaptic vesicles (SV) that appear docked at the presynaptic plasmamembrane (PPM). A ternary complex containing the SV protein synaptobrevin (SyB) and the PPM proteins syntaxin and SNAP-25 has been assigned a critical role in both the docking and fusion processes. The clostridial neurotoxin tetanus toxin (TeTx) inhibits NtR by cleaving SyB into two fragments: a soluble N-terminal domain and a smaller C-terminal fragment that remains anchored to the SV. Despite SyB cleavage, SV docking appears unaffected in nerve terminals poisoned with TeTx. Biochemical evidence indicates that both fragments associate to form a ternary complex with the PPM proteins, thus the cleaved protein could still participate in a SyB-dependent mechanism of SV docking. This suggests that TeTx cleavage might define SyB domains with distinct binding sites in the ternary complex and/or distinct roles in exocytosis. Indeed, the small C-terminal domain consistently causes an irreversible inhibition of NtR when introduced into the squid presynaptic terminal. The effects of this peptide on the *in vitro* protein interaction that reflect SyB function and morphological analysis of the terminals inhibited by this peptide should provide further insight into the function of SyB. *Supported by the Deutsche Forschungsgemeinschaft (Leibniz-Programm and Be718/11).*

310.5

CHARACTERIZATION OF SPECIFIC SITES OF INTERACTION OF THE SYNAPSINS BY USING PHAGE DISPLAY LIBRARIES. P. Vaccaro, L. Dente, F. Onofri, A. Zucconi, F. Valtorta[1], P. Greengard[2], G. Cesareni, F. Benfenati*
Dept. of Biology and Exp. Medicine, II Roma University and [1]Dibit, Dept. of Pharmacology, Milano University, Italy; [2]The Rockefeller University, New York.

The synapsins are a family of phosphoproteins that are involved in the short-term regulation of neurotransmitter release and in the formation of synaptic contacts. The synapsins interact with the cytoplasmic surface of synaptic vesicles and with cytoskeletal proteins, namely actin. To identify and localize specific interaction sites of the synapsins, we have analyzed libraries of short peptides of random sequence (about 10^9 peptides), displayed on the surface of a filamentous bacteriophage. Oligonucleotides encoding for nonapeptides of random sequence were inserted by means of a phagemid vector into the gene of the capsid protein pVIII so that the peptides were expressed in multiple copies at the surface of the phage. Affinity selection of specific ligands of synapsin I (Syn I) was performed by screening the phage library on Syn I-coated polystyrene beads (biopanning). After a few cycles of biopanning and phage amplification, phage clones that bound Syn I with high affinity were obtained and sequenced. The amino acid sequences of the exposed peptides were determined and aligned according to their sequence similarity. Families of reactive clones exhibiting common motifs (QNYY, YxxWE, YYxxW; single letter code) could be identified. The most reactive phage clones were used to produce anti-peptide antibodies in mice and to synthetize the corresponding peptides. The resulting peptides and antibodies were then characterized for the ability to affect the binding of Syn I to synaptic vesicles and actin. The identification of the specific sites and sequences of interaction between Syn I and its partners in the nerve terminal may provide peptide tools capable of selectively interfering with the functional activity of Syn I in the regulation of neurotransmitter release and synapse formation. Supported by Telethon, Italy (grant # 753).

310.6

AXONAL TRANSPORT OF SYNAPTIC VESICLE PROTEINS IN THE RAT OPTIC NERVE A. Dahlström* and J.-Y. Li
Dept. of Anat. and Cell Biol., Göteborg University, S- 413 90 Göteborg, Sweden

The optic nerve, a part of the central nervous system (CNS), has been used to study axonal transport for decades. The present study has concentrated on the fast axonal transport of synaptic vesicle proteins in the optic nerve, as a model of the CNS, using the "stop-flow" (nerve crush) method. After blocking fast axonal transport, distinct accumulations of synaptic vesicle proteins developed during the first hour after crush-operation and marked increases were observed up to 8 hrs post-operation. Semi-quantitative analysis, using cytofluorimetric scanning (CFS) of immuno-incubated sections, revealed that the ratio between distal accumulations (organelles in retrograde transport) and proximal accumulations (organelles in anterograde transport) was much higher (up to 80-90%) for the transmembrane proteins than that for surface adsorbed proteins (only 10-20%). The pattern of axonal transport in the optic nerve was comparable to that in the sciatic nerve. However, immunoreactive clathrin and Rab3a were found to accumulate in much lower amounts than in the sciatic nerve. Most synaptic vesicle proteins were colocalized in the axons proximal to the crush. However, a differential distribution of synaptobrevin I and II was observed in optic nerve axons; synaptobrevin I was present only in large sized axons, while synaptobrevin II immunoreactivity was present in most axons, including the large ones. The two isoforms were, thus, partially colocalized, in the large axons.

The results demonstrate 1) that CFS techniques could be successfully used to study axonal transport not only in peripheral nerves, but also in the CNS, 2) that synaptic vesicles are transported with fast axonal transport in this nerve, and that 3) some quantitative differences were noted compared with the sciatic nerve, especially for Rab3a and clathrin.

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310.7

INTRANEURONAL TRAFFICKING AND DIFFERENTIAL DISTRIBUTION OF AMPHIPHYSIN AND SYNAPTOJANIN IN THE RAT PNS AND SPINAL CORD J.-Y. Li* and A. Dahlström
Dept. of Anat. & Cell Biol., Göteborg University, S-413 90 Göteborg, Sweden

Transmitter release takes place in a very short delay after nerve terminal depolarization, followed by a rapid endocytic reuptake of synaptic vesicle membranes. We investigated intraneuronal dynamics and distribution of two neuronal components, amphiphysin and synaptojanin, considered to be involved in the endocytosis process. The nerve crush ("stop flow") technique was used in combination with immunofluorescence, cytofluorimetric scanning (CFS), confocal laser scanning microscopy and immuno EM. After crushing, distinct accumulations of immunoreactive amphiphysin and synaptojanin were detected as early as 1 h post-operation, demonstrating fast axonal transport of these proteins. The proximal accumulations increased linearly between 1 and 8 h. CFS analysis showed that about 30% of anterogradely transported amphiphysin and synaptojanin was retrogradely transported (recycled), in contrast to the transmembrane vesicle protein, synaptophysin, 80% of which was recycled. The results suggest that amphiphysin and synaptojanin are predominantly involved in anterograde axonal transport and to a large extent degraded or metabolized in nerve terminals. Immuno-gold EM showed that anti-amphiphysin and anti-synaptojanin had primarily associated with heterogeneous membrane profiles in crushed sciatic nerves, and with small synaptic vesicles in nerve terminals of the spinal cord. In addition, peroxidase immuno-EM demonstrated that the two proteins were differentially distributed in subsets of nerve terminals, the intensity of immunolabelling of nerve terminals for amphiphysin and synaptojanin differing from negative to very intense. The study indicates 1) that amphiphysin and synaptojanin are transported with fast anterograde axonal transport in the sciatic nerve, 2) that only little (30%) is recycling, 3) that the two proteins are associated with heterogeneous membrane structures and 4) they are selectively present in subsets of spinal cord nerve terminals.

Supported by the Swedish MRC (2207) and the Royal Acad. of Sci and Arts in Göteborg. The generous supply of antibodies from Dr. P. De Camilli and Dr. R. Jahn is acknowledged.

310.8

Secretory Proteins SNAP-25 and Syntaxin Form Complexes at the Active Zone *in situ*: Evidence from Co-application of Botulinum A and C1. [1]D.A.Raciborska, [2]W.S.Trimble*, and [1]M.P.Charlton. *[1]Dept. Physiology, Univ. Toronto and [2]Center for Research in Neurodegenerative Diseases, Toronto, Canada, M5S 1A8.*

SNAP-25 and syntaxin have been identified as critical participants in exocytosis, associated with membrane and other compartments of the presynaptic cell. In *in vitro* studies they form complexes with each other and with other proteins. Cleavage of SNAP-25 and syntaxin by Botulinum A (BotA) and C1 (BotC) results in blockade of neurotransmitter release. We used immunocytochemistry and exploited the regularity of active zones at the frog neuromuscular junction to investigate protein distribution before and after poisoning. SNAP-25 and syntaxin were distributed in incomplete bands at 1μm intervals in register with post-synaptic acetylcholine receptors. BotA blocked transmitter release and caused a decrease in SNAP-25 C-terminus, but not in SNAP-25 N-terminus or syntaxin immunoreactivity. BotC blocked transmitter release and reduced syntaxin but not SNAP-25 immunoreactivity. This effect was strongest between active zones, presumably due to sensitivity of the cytoplasmic/vesicular pool of proteins to toxins. When BotA and BotC were applied together, both SNAP-25 N- and C-terminal, and syntaxin immunoreactivity were reduced, indicating that retention of cleaved SNAP-25 depended on intact syntaxin. Hence, these molecules form a complex at the active zone *in vivo* in frog nerve terminals, with syntaxin anchoring SNAP-25 and influencing its conformation. Because not all immunoreactivity disappeared upon cleavage of SNAP-25 and syntaxin, distinct pools of these proteins in the nerve terminals may be differentially cleaved by the toxins.

Supported by a grant to M.P.C. from MRC of Canada.

310.9

SYNAPSINS, SYNAPTOTAGMIN AND OTHER VESICLE MARKERS IN AXONAL NEUROTRANSMITTER VESICLES Gabriel Fried*[1,2], Andrew Czernik[3], Andrew Bean[4]
Dept. of [1]Physiology and Pharmacology, [2]Dept. of Woman and Child Health, Karolinska Institute, Stockholm, [3]Molecular and Cellular Neuroscience, Rockefeller University, New York and Dept. of [4]Neuroscience, Stanford University.

We have examined the subcellular distribution of several synaptic vesicle proteins (synapsins, synaptophysin, synaptotagmin, synaptobrevin (VAMP-1/2), rab3a, n-sec1 and dopamine-ß-hydroxylase (DBH)). in density gradients from bovine splenic nerve axons. Nerves were homogenized and centrifuged on linear density gradients (0.25-1.5 M sucrose) at 280,000xg for 90 min in a Beckman L70 using a SW40 rotor. Subcellular fractions were analyzed by immunoblotting. We found that synapsin I and synaptophysin, markers for small synaptic vesicles, peaked in low density fractions. DBH, a marker for large noradrenergic vesicles, showed a distinctly different pattern with a single peak at much higher density. Synaptotagmin was present throughout the gradient, with maximal levels below the DBH peak. VAMP-1/2, rab-3 and n-sec1 were only seen in low density fractions. The results show that the bulk of synapsin and synaptophysin transported in the bovine splenic nerve is present in fractions containing organelles with different size and density than organelles transporting DBH. Synaptotagmin was localized in both small and large vesicle fractions, which is compatible with its suggested role as a general calcium sensor in exocytosis.

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310.10

MgATP-DEPENDENT DISSOCIATION OF A 68-kDa SYNAPTIC PROTEIN FROM SYNTAXIN. T. Nishiki[1, 2], M. Sekiguchi[1], S. Kozaki[3], K. Kumakura[2] and M. Takahashi[1]*. [1]Mitsubishi Kasei Inst. of Life Sci., Tokyo, Japan, [2]Sophia Univ., Tokyo, Japan, [3]Osaka Pref. Univ., Osaka, Japan.

Neurons release neurotransmitters at synapses by exocytosis of synaptic vesicles. Syntaxin, a synaptic membrane protein, interacts with various synaptic proteins *in vitro* and is believed to form a core complex required for docking/fusion of synaptic vesicles. However it is still unclear how the protein-protein interactions are regulated. In this study, we have found a 68-kDa protein binding to syntaxin *in situ* and characterized the interaction between syntaxin and the syntaxin-binding protein. Digitonin-permeabilized rat pheochromocytoma cells were incubated with various chemical cross-linkers and the cell proteins were analyzed by immunoblotting with anti-syntaxin antibody. In the presence of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) a new immunoreactive band with 105 kDa was formed. The molecular mass of the syntaxin-binding protein was 68 kDa, deduced from the difference between the migration in SDS-PAGE of the conjugate and that of syntaxin. Treatment of the permeabilized cells with MgATP prior to cross-linking with EDC largely decrease the amount of cross-linked product. The cross-linked product was also formed when cell proteins were solubilized with Triton X-100 and incubated with EDC, indicating that the interaction between syntaxin and the 68-kDa protein is not influenced by the solubilization. The 105 kDa cross-linked band containing syntaxin was also detected in the detergent extract obtained from rat brain synaptosomes. These results suggest that the 68 kDa protein binds to syntaxin on plasma membranes and the interaction between them is dependent on MgATP. The 68-kDa syntaxin-binding protein may regulate the function of syntaxin in synaptic vesicle exocytosis.

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310.11

ISOLATION AND FEATURES OF A CA^{2+}-INDEPENDENT α-LATROTOXIN RECEPTOR. Yu. Ushkaryov*, B. Davletov, and O. Shamotienko. Biochemistry Department, Imperial College, London SW7 2AY, UK.

α-Latrotoxin (LTX), a neurotoxin from the Black Widow Spider venom, binds to the presynaptic membrane and causes massive neurotransmitter release in all vertebrate synapses both in the presence and in the absence of Ca^{2+}. Neurexins (Ushkaryov, Yu. A., et al. (1992) *Science* **257**, 50-56), isolated previously as LTX receptors interact with the toxin only in Ca^{2+} and, thus, may not represent the Ca^{2+}-independent receptor. We now report the isolation of a novel neuronal protein that binds LTX in EDTA. This high molecular weight protein, termed here latrophilin, has been purified from detergent-solubilised bovine brain membranes by affinity chromatography on immobilised LTX and wheat germ lectin. Latrophilin binds to LTX, immobilised on a column or immunobeads, with high affinity (Kd=0.5-0.7 nM). Sucrose density gradient centrifugations demonstrated that latrophilin forms with LTX stable complexes composed of one molecule of each protein. Latrophilin consists of a single polypeptide chain that is heavily N-glycosylated. When analyzed by the two-phase partitioning method, latrophilin behaved as a hydrophobic protein, indicating that it is normally membrane-bound. Anti-latrophilin antibodies detected this protein in a glycoprotein-enriched membrane fraction from bovine cerebral cortex and cerebellum but not from non-neuronal tissues. Four times more latrophilin was present in the cortex than in the cerebellum, and the level of expression did not exceed 0.8 pmol latrophilin/mg total membrane protein. Sequences of peptides, purified from protease-digested latrophilin, showed no homology to proteins in the current data bases. All features of latrophilin are in good agreement with known characteristics of the Ca^{2+}-independent LTX receptor. Our data suggest that latrophilin may represent the presynaptic receptor and/or molecular target for α-latrotoxin. - This work was supported by a Wellcome Senior European Research Fellowship (to Y.A.U.).

310.12

CA-DEPENDENT MODULATION OF ASSOCIATION AND DISSOCIATION OF SYNAPTOTAGMIN TO SYNTAXIN. A. Yoshida*, T. Yoshioka. Dept. of Mol. Neurobiol., Waseda Univ., Tokorozawa, 359, Japan.

Synaptotagmin, that has been postulated to act as a Ca^{2+} sensor for neurotransmitter release, Ca^{2+}-dependently forms a complex containing syntaxin. In this study, we examined the dynamical interaction of synaptotagmin to syntaxin by BIAcore system based on surface plasmon resonance. A glutathione-S-transferase (GST)-fusion protein of syntaxin amino- or carboxyl-terminal region (GST/Stx-Nt or -Ct) were immobilized by anti-GST antibody crosslinked on the sensor chip surface. The cytoplasmic region of rat synaptotagmin 1 with a maltose binding protein (MBP/Syt) slightly bound to the immobilized GST/Stx-Ct in the absence of Ca^{2+}. It showed two component of the association and dissociation. In the presence of Ca^{2+} (0.5 mM), the amount of the fast associated MBP/Syt and the rate of the slow association component were increased. The amount of slowly dissociated MBP/Syt from the complex were increased in the presence of Ca^{2+}. No binding activity of MBP/Syt to the GST/Stx-Nt nor GST was found. These results suggested that the Ca^{2+} might modulated the state of the complex of synaptotagmin and syntaxin.

This work was supported by the Ministry of Education, Science and Culture of Japan.

310.13

CHARACTERIZATION OF RAB3A, RAB3B AND RAB3C -DIFFERENT BIOCHEMICAL PROPERTIES AND INTRACELLULAR LOCALIZATION IN BOVINE CHROMAFFIN CELLS [2]Chong-Gee Lin , [3,4]Yu-Chi Lin, [3,4]Hwan-Wun Liu and [1,2]Lung-Sen Kao.* [1]Institute of Biomedical Sciences, Academia Sinica; [2]Department of Biochemistry, National Yang-Ming University; [3]Department of Anatomy, National Defense Medical Center; [4]Institute of Life Science, National Defense Medical Center; Taipei, Taiwan, R. O. C.

Rab3 proteins are the only group of small G proteins that is thought to be involved in regulated exocytosis. Although amino acid sequences of Rab3 proteins are highly homologous, their functions are distinct. Compartmentation and biochemical characteristics are main determinants for specific functions of small G proteins. In this study, we examined the biochemical properties and subcellular localization of Rab3A, Rab3B and Rab3C in bovine adrenal chromaffin cells. The Kd for GTPγS of the three Rab3 proteins was 15 nM, 2700 nM and 204 nM for Rab3A, Rab3B and Rab3C, respectively. The intrinsic GTPase activity of the three Rab3 proteins was similar. It appears there are different GAPs for different Rab3 proteins in bovine chromaffin cells. Truncation of C-terminal 31 amino acids decreases the binding affinity for GTPγS of the three Rab3 proteins. When the C-terminal of Rab3C is replaced with that of Rab3A, the binding affinity of Rab3C for GTPγS is decreased, but the replacement does not affect the affinity of Rab3B for GTPγS. Immunostaining and subcellular fractionation experiments show that Rab3A, Rab3B and Rab3C were separately localized within chromaffin cells. Rab3A is predominantly localized in fractions of chromaffin granule, plasma membrane and cytosol. Anti-Rab3B antibody distinctly stained the plasma membrane. Rab3C was rich in fractions of chromaffin granules and mitochondria. In summary, bovine chromaffin cells express the three Rab3 proteins but the subcellular localization and biochemical properties of the three Rab3 proteins are distinct. The work is supported by grants from National Science Council (NSC84-2331-B001-053) and Academia Sinica, Taipei, Taiwan, R. O. C..

310.14

SEC-1/UNC-18 REGULATES NEUROTRANSMITTER RELEASE AT A STEP THAT FOLLOWS VESICLE DOCKING M.E. Burns*, T. Dresbach, V. O'Connor, W.M. DeBello, H. Betz, and G.J. Augustine. Dept. Neurobiology, Duke University Medical Center, Durham, NC USA and Max-Plank Institute for Brain Research, Frankfurt Germany.

Sec-1/unc-18 is a presynaptic protein that regulates SNARE complex assembly *in vitro*. We have cloned the gene for the squid homolog of sec-1/unc-18 and tested the function of this protein in neurotransmitter release. Microinjection of sec-1/unc-18 into the presynaptic terminal of the squid giant synapse produced a reversible inhibition of transmitter release evoked by presynaptic action potentials. This inhibition did not occur when sec-1/unc-18 was co-injected with syntaxin, suggesting that sec-1/unc-18 inhibits release via an association with endogenous syntaxin. A synthetic peptide from a conserved region of sec-1/unc-18 also inhibited release reversibly, while a scrambled version of this peptide, as well as other peptides from other regions of the protein, had no effect. Electron microscopy of terminals injected with the active peptide revealed an accumulation of vesicles located at the plasma membrane of active zones. These results are consistent with the hypothesis that sec-1/unc-18 is a negative regulator of transmitter release and that this regulation is conferred by binding to syntaxin. Because sec-1/unc-18 regulates SNARE complex assembly *in vitro*, our results further suggest that SNARE complex assembly is important for release at a step that follows the docking of vesicles at the plasma membrane. Supported by NIH NS-21624 and Sigma Xi.

310.15

NSF IS INVOLVED IN A KINETICALLY IMPORTANT STEP IN NEUROTRANSMITTER RELEASE. F.E. Schweizer*, W.M. DeBello, T. Dresbach, V. O'Connor, H. Betz, and G.J. Augustine. Dept. Neurobiology, Duke Univ. Med. Cntr, Durham, NC 27710, MBL, Woods Hole, MA 02543, and Max-Planck Inst., Frankfurt, Germany.

A peptide derived from the N-terminal domain of NSF (NSF-1) alters synaptic transmission when microinjected into the giant presynaptic terminal of squid. This peptide both reduces the amplitude and slows the time course of the postsynaptic potentials evoked by presynaptic action potentials (Soc. Neurosci. Abs. **21**, 325). To understand the mechanism of NSF-1 action, we monitored pre- and postsynaptic currents while injecting NSF-1. Control postsynaptic currents (PSCs) evoked by presynaptic action potentials rose with a mean rise time (20% to 80%) of 0.40±0.06 ms. PSC decay could usually be described as the sum of two exponentials with time constants of 0.58±0.09 ms and 4.09±1.27 ms. Presynaptic injection of NSF-1 slowed in a dose dependent manner both the onset and the decay of the PSC. The rapid component of the decay was slowed ~2-fold more than the rise time, whereas the slow component of the decay was not affected by NSF-1. To determine whether the slowing is due to changes in the rate of presynaptic Ca^{2+} entry we measured voltage-dependent presynaptic Ca^{2+} currents and monitored PSCs triggered by these currents. The Ca^{2+} currents were not affected by NSF-1 injections even though the time course of the PSCs was slowed. We conclude that NSF participates in transmitter release by catalyzing a step that influences the kinetics of exocytosis. NSF-1 is thus a valuable tool for understanding both the presynaptic function of NSF and the reactions that determine the speed of transmitter release. Supported by NIH NS-21624.

310.16

REGULATION OF SNARE COMPLEX ASSEMBLY *IN VIVO* BY THE SNAP-25-BINDING DOMAIN OF SYNTAXIN 1A C. Heuss, W.M. DeBello, T. Dresbach, V. O'Connor, T. Schäfer*, M.M. Burger, H. Betz, and G.J. Augustine. Friedrich Miescher Institute, Basel, Switzerland; Dept. Neurobiology, Duke University Medical Center, Durham, NC, U.S.A.; and Max-Planck Institute for Brain Research, Frankfurt , Germany.

Syntaxin, an integral membrane protein found in nerve terminals, interacts *in vitro* with the plasma membrane-associated protein SNAP-25. It has been suggested that both proteins mediate Ca-dependent neurotransmitter release *in vivo*. In order to assess the role of the SNAP-25-syntaxin interaction in transmitter release, we microinjected syntaxin fragments into giant presynaptic terminals of squid. A squid homolog of syntaxin 1A was cloned and two recombinant peptides were prepared: TAX86, corresponding to the 86 C-terminal residues of mammalian syntaxin 1A, a region known to bind SNAP-25, and TAX50, corresponding to the 50 N-terminal residues of TAX86. Gel overlays showed that TAX86, but not TAX50, bound squid SNAP-25. TAX86 also competitively inhibited the binding of syntaxin to SNAP-25. Further, *in vitro* binding measurements showed that TAX86 forms SDS-sensitive trimeric SNARE complexes with recombinant SNAP-25 and synaptobrevin. Microinjection of TAX86 caused a rapid and irreversible inhibition of release evoked by single action potentials. In contrast, comparable injections of TAX50 had no effect on release. Our interpretation is that TAX86 inhibits release by binding to endogenous SNAP-25, and thereby prevents the binding of endogenous syntaxin. The irreversible action of TAX86 on release is consistent with this interpretation, because SNARE complexes formed with TAX86 should be insensitive to ATP hydrolysis by NSF (Neuron 14:991-8). We conclude that the interaction between syntaxin and SNAP-25, and the subsequent NSF-dependent disassembly of the SNARE complex, are critical for neurotransmitter release. Supported by NIH grant NS-21624.

310.17

DEVELOPMENTAL EXPRESSION OF BRAIN/KIDNEY (B/K) GENE ENCODING A PROTEIN WITH TWO C2 DOMAINS THAT IS SELECTIVELY EXPRESSED IN THE BRAIN AND KIDNEY. O.-J. Kwon[1]*, H. Kim[2], and H. Chin[3]. [1]Dept. of Biochemistry, Catholic University Medical College, [2]Dept. of Anatomy, Korea University Medical College. Seoul, Korea, and [3]Lab of Neurochemistry, NINDS, NIH, Bethesda, MD 20892.

We have previously isolated and characterized a rat brain cDNA clone that encodes a protein with structural similarity to the synaptotagmins, and called it B/K protein based on its selective expression in the brain and kidney (Kwon, et. al., FEBS Letters, 378:135, 1996). We now isolated a human brain cDNA encoding the B/K protein. The 1.5 kb hB/K12 cDNA clone, which exhibited ~90% nucleic acid sequence identity with the rat B/K, contained the entire coding sequence of 474 amino acid B/K protein. On Northern blot analysis, a hB/K cDNA probe hybridized to a 3.0 kb B/K transcript present only in human brain and kidney.

In situ hybridization histochemistry analysis of developing and adult rat brains showed that B/K expression gradually increases during development. In the prenatal stage, low levels of B/K transcript was found in the brain and spinal cord. After birth, the B/K expression continually increased, but became restricted to the frontal brain. In the adult rat brain B/K mRNA was highly expressed in the superficial layer of cerebral cortex, cingulate gyrus, dentate gyrus and CA1 area of Ammon's horn. This spatiotemporal pattern of expression suggests that B/K protein may function in membrane trafficking process in this region of the brain. This work is funded by the grants from Korean Science Foundation and by the NINDS, NIH, Intramural Program.

310.18

METAL SELECTIVITY OF THE INTERACTION BETWEEN SYNAPTOTAGMIN AND PHOSPHOLIPID BILAYERS. J.L. Tomsig*, X. Sun and J.B. Suszkiw. Dept. of Molecular and Cellular Physiology. University of Cincinnati. Cincinnati, OH 45267-0576.

We have previously reported (*J. Neurochem.* **66**, 644-650, 1996) the metal selectivity of exocytosis in α-toxin permeabilized chromaffin cells. Metal cations that activate exocytosis exhibit the following potency sequence: $Pb^{2+} >> Cd^{2+} > Ca^{2+} \approx La^{3+} >> Ba^{2+} \approx Sr^{2+}$. We report here the effect of these metals on the interaction between phospholipid bilayers and synaptotagmin, a molecule that has been proposed as the calcium sensor that triggers the mechanism of exocytosis. We studied the association of histidine-tagged synaptotagmin and liposomes labeled with dansyl-phosphatidylethanolamine using a fluorescence resonance energy transfer assay. We found that the interaction between synaptotagmin and phospholipid bilayers is promoted by a metal series that resembles the potency sequence described for activation of exocytosis. The concentrations of metal cations required for half-maximal activation were: Pb^{2+}, 7.2 X 10^{-9} M; Cd^{2+}, 2.1 X 10^{-7} M; Ca^{2+} 5.2 X 10^{-7} M; La^{3+}, 5.8 X 10^{-6} M; Tb^{3+} 3.0 X 10^{-5} M; Sr^{2+} 6.5 X 10^{-4} M; Ba^{2+}, 1.1 X 10^{-3} M. The similarity between these two sequences supports the hypothesis that synaptotagmin or a protein structurally related to synaptotagmin can function in chromaffin cells as the intracellular receptor that promotes exocytosis. Supported by NIEHS grant ES-04090.

310.19

INHIBITION OF NEURONAL NITRIC OXIDE SYNTHASE BY A NOVEL ASSOCIATED PROTEIN, S. R. Jaffrey* and S. H. Snyder. Departments of Neuroscience, Pharmacology and Experimental Therapeutics, and Psychiatry, Johns Hopkins University, School of Medicine, Baltimore, MD, 21205.

Neurotransmitter functions of nitric oxide are dependent upon dynamic regulation of its biosynthetic enzyme, neuronal nitric oxide synthase (nNOS). A yeast two-hybrid screen was employed to identify a regulatory protein that binds nNOS. A small, ~10 kD protein was identified which physically interacts with and inhibits the activity of nNOS. This protein inhibitor of nNOS (PIN-1) appears to be one of the most conserved proteins in biology with 92% amino acid identity between *C. elegans* and man. Binding destabilizes the nNOS dimer, a conformation necessary for activity. PIN-1 provides a novel mode of regulation of NOS activity and may regulate numerous biological processes.

This Research was supported by grants from the National Institutes of Drug Abuse.

MECHANISMS OF NEUROTRANSMITTER RELEASE II

311.1

INSULIN TRIGGERS PEPTIDE SECRETION FROM THE BAG CELL NEURONS OF *APLYSIA*. C.M. Smith[1], E.A. Jonas[1], N.L. Wayne*[2], and L.K. Kaczmarek[1]. Dept. of Pharmacology[1], Yale Univ. Sch. of Med., New Haven, CT 06520. Dept. of Physiology[2], Univ. of California Sch. of Med., Los Angeles, CA 90095.

Localized increases in cytoplasmic free Ca^{2+} levels are critical in regulated exocytosis. Insulin elevates intracellular Ca^{2+} in both the somata and neurite tips of the peptidergic bag cell neurons of Aplysia (Jonas et al., *Neurosci. Abstr.*, 1995). We studied the effect of insulin on the secretion of egg-laying hormone (ELH) from the bag cells. Bag cell clusters were treated with insulin (5 μM) dissolved in artificial sea water. In some experiments, the afferent input to the clusters was electrically stimulated to fire a repetitive series of action potentials (afterdischarge; AD). Perfusate from clusters was collected by regular bath exchange, and the amount of ELH in each sample was quantified by radioimmunoassay. Clusters that were not stimulated to AD nor treated with insulin did not secrete ELH. However, control clusters secreted ELH in response to an AD. In the absence of action potential firing, insulin-treated clusters secreted an amount of ELH comparable to the amount secreted by control cells that afterdischarged. The ability of insulin to trigger ELH release contrasts with that of thapsigargin (5 μM), which failed to elicit secretion. Calcium imaging of isolated, fura-2-loaded bag cell neurons showed that thapsigargin elevates cytoplasmic free Ca^{2+} primarily in the somata (Knox et al., *J. Physiol.*, 1996), and less in the neurites of bag cells, where secretion is believed to occur. The results suggest that ELH secretion from the bag cells can be supported at least in part by Ca^{2+} released from an insulin-sensitive, intracellular Ca^{2+} store that is localized to the site of exocytosis. Work supported by NIH grants NS18492 (L.K.K.) and NS33548 (N.L.W.).

311.2

CHARACTERIZING A NOVEL INTRACELLULAR CALCIUM POOL THAT MAY REGULATE NEURONAL PEPTIDE SECRETION. E.A. Jonas*, R.I. Knox, J.A. Connor, L.K. Kaczmarek. Dept. Pharmacology, Yale Univ. Sch. of Med., New Haven, CT 06520. Lovelace Institute, Albuquerque, NM 87108.

The bag cell neurons of *Aplysia* are a model system in which to study the regulation of neuronal excitability and secretion. In fura-2 loaded bag cell neurons, activation of the plasma membrane insulin receptor stimulates Ca^{2+} release from a novel intracellular Ca^{2+} source (Jonas *et al., Neurosci. Abstr.*, 1995) which is particularly prominent in neurites, where peptides are secreted from these cells. Insulin also stimulates secretion of neuropeptide from bag cell neurons (Smith, *et al., Neurosci. Abstr.*, 1996). We developed a technique to form *giga*-ohm seals on intracellular membranes, so that we could attempt to measure channel activity associated with the insulin-stimulated Ca^{2+} release. Following seals on intracellular organelle membranes, we identified three different intracellular membrane conductance levels of 7, 13, and 78 pS with reversal potentials of +70, 0, and -20 mV respectively. The 7 pS conductance may be activated by extracellular insulin application. Further studies of the insulin-sensitive store will attempt to identify the type of organelle responsible for the intracellular Ca^{2+} release. Peptide-containing vesicles are present in bag cell neurite tips, therefore the novel insulin-sensitive store may be one such vesicle located near the sites of neuropeptide release. Work supported by NIH Grant # NS18492 (L.K.K.).

311.3

LOCAL CALCIUM GRADIENTS GENERATED BY RELEASE FROM INTRACELLULAR CALCIUM STORES REGULATE EXOCYTOSIS IN PITUITARY GONADOTROPES. F.W. Tse*[1], A. Tse[1], B. Hille[2] & W. Almers[3] [1]Dept. Pharmacol., U. Alberta, Edmonton, Alberta, Canada. [2]Dept. Physiol. & Biophys., U. Washington, Seattle, Washington, USA. [3]Max-Planck-Inst. Med. Forschung, Heidelberg, Germany.

In single identified gonadotropes of male rat, the hormone GnRH induces release of intracellular Ca^{2+} from IP_3-sensitive stores which in turn stimulates exocytosis (Tse et al., *Science* 260:82). The rate of GnRH-induced exocytosis (increase in membrane capacitance) was >50 vesicles/s when spatially averaged $[Ca^{2+}]_i$ was rising from 0.5 μM to 1.5 μM. When Ca^{2+} was released from intracellular stores by flash photolysis of caged IP_3 (either in the presence or absence of extracellular Ca^{2+}), the exocytic rate at $[Ca^{2+}]$ in between 0.5 μM to 1.5 μM could be as fast as that induced by GnRH; however, the rate of exocytosis was more closely correlated with the *rate of rise of* $[Ca^{2+}]_i$ than with $[Ca^{2+}]_i$ itself. In addition, when $[Ca^{2+}]_i$ was raised uniformly to comparable levels by flash photolysis of caged-Ca, or by intracellular dialysis of Ca-buffered solutions, the exocytic rate was <1 vesicle/s. These observations suggest that the exocytic sites are selectively localized near IP_3 receptors and are stimulated by local high elevations of $[Ca^{2+}]$ around active IP_3 receptors rather than by the lower average $[Ca^{2+}]_i$ measured by photometry. (Supported by Canadian MRC, AHFMR, US NIH HD-12629, AR-17803.)

311.4

STIMULUS-SECRETION COUPLING IN PITUITARY CORTICOTROPES. A. Tse*, A.K. Lee & F.W. Tse Dept. Pharmacology, U. Alberta, Edmonton, Alberta, Canada.

Corticotropes in the anterior pituitary secrete the stress hormone, adrenocorticotropic hormone (ACTH) in response to arginine vasopressin (AVP). Single, rat corticotropes were identified with the reverse hemolytic plaque assay and voltage-clamped at -50 or -70 mV. AVP (100 nM) triggered a "spike and plateau" elevation in $[Ca^{2+}]_i$ (monitored by indo-1 fluorescence). Peak $[Ca^{2+}]_i$ elevation ranged 1- 2 μM. Several lines of evidence suggest that AVP acts via a G-protein coupled phosphoinositide pathway to release Ca^{2+} from the IP_3-sensitive stores and triggers exocytosis. (1) AVP response was blocked by intracellular dialysis of 2 mM GDP-β-S. (2) Dialysis of IP_3 (10 μM) or flash photolysis of caged IP_3 (10 μM) mimicked the response. (3) Intracellular dialysis of the IP_3 blocker, heparin (300 μM) abolished the response. (4) AVP could trigger $[Ca^{2+}]_i$ elevation in the absence of extracellular Ca^{2+}. (5) Simultaneous measurement of $[Ca^{2+}]_i$ and changes in membrane capacitance showed that exocytosis (20-80 fF) was triggered when AVP-induced $[Ca^{2+}]_i$ elevation was >1 μM. (6) Flash photolysis of caged IP_3 raised $[Ca^{2+}]_i$ (2-3 μM) and triggered exocytosis (80 - 120 fF). (Supported by Canadian MRC and AHFMR).

311.5

THE RELATION BETWEEN CALCIUM INFLOW AND TRANSMITTER RELEASE AT A FAST GLUTAMATERGIC SYNAPSE. J.G.G. Borst, A. Villarroel* and B. Sakmann. Max-Planck-Institut für medizinische Forschung, Jahnstraße 29, 69120 Heidelberg, Germany.

Each principal cell in the medial nucleus of the trapezoid body (MNTB) is excited by a single, large synaptic terminal, called the calyx of Held. We measured synaptic currents and presynaptic calcium currents in transversal rat brainstem slices containing the MNTB, to obtain an estimate of the number of calcium ions needed to release a single vesicle. The calcium current flowing into the calyx of Held during a presynaptic action potential was measured by voltage clamping it with an action potential waveform, after blocking the sodium and potassium currents. The calcium current activated shortly after the peak of the action potential and ended before repolarization was complete. It had a peak amplitude of 2.6 ± 0.2 nA and its integral was 0.89 ± 0.04 pC (n=12), at 2 mM external calcium.

An estimate for the quantal content of the excitatory postsynaptic currents (EPSCs) was obtained in separate experiments by voltage clamping principal cells during afferent stimulation and taking the ratio of the peak amplitude of the EPSCs, at 2 mM external calcium and the quantal currents (43 ± 5 pA), measured at low (0.25 mM) calcium concentration. Quantal content was 151 ± 14 (n=10), indicating that around 18000 calcium ions enter for each vesicle that is released. Given the low conductance of calcium channels this may mean that the simultaneous opening of many calcium channels is needed to release a single vesicle at this fast excitatory synapse.

J.G.G.B was supported by EMBO and Max-Planck Gesellschaft.

311.6

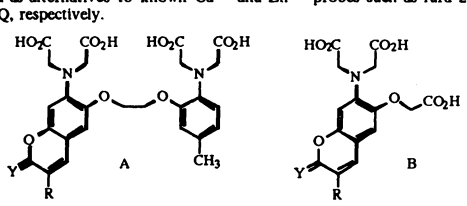
EXOCYTOSIS AND RE-SUPPLY OF SUBPLASMALEMMAL SECRETORY GRANULES OBSERVED WITH EVANESCENT WAVE FLUORESCENCE MICROSCOPY. Jürgen Steyer and W. Almers*. Max-Planck-Institut. f. Medizin. Forschung, Heidelberg, Germany.

Secretory granules of bovine chromaffin cells (average diameter 340 nm) were stained with the fluorescent dye acridine orange. Cells adhering tightly to a coverslip were viewed. The granules immediately adjacent to the coverslip were selectively illuminated with the evanescent wave (EW) caused by total reflection of light at the glass/ aqueous interface. Photometry with 280 nm fluorescent beads showed that we collect EW fluorescence from a distance of up to 300 nm from the coverslip. Single fluorescent spots (FS) representing subplasmalemmal granules were readily seen. Most dithered randomly around a fixed position, while rare FS's migrated several μm's parallel to the plasmalemma within 10 s. When high $[K^+]$ triggered Ca entry and exocytosis, single FS's disappeared abruptly as granules exocytosed and lost their dye. Indeed, the loss of FS's was accompanied by exocytic release of epinephrine from single granules, as recorded simultaneously with a carbon fiber. A 2 min exposure to high $[K^+]$ caused depletion of FS's. New FS's replaced the old within 20 min, though not at the same position. Since no dye was present externally, the new FS's represent chromaffin granules that entered the EW from regions deeper within the cell. The method allows us to watch single granules as they migrate and exocytose, and to study how they travel the last 300 nm of their way to the plasmalemma. Supported by the Max Planck Society.

311.7

COUMARINE - BASED FLUORESCENT ION INDICATORS. H. E. Katerinopoulos, E. Foukaraki, A. K. Moschovakis*, M.A. Kuhn[2]. Dept. of Chem. and [1]Div. of Basic Sciences, Sch. of Med., Univ. of Crete, 71409 Greece, and [2]Molecular Probes Inc. Eugene, OR., 97402, USA.

A number of cell permeable tetracarboxylate (A) and tricarboxylate (B) fluorescent indicators have been synthesized and tested as probes of intra as well as extra cellular ion concentrations. Coumarins with substituents, causing bathochromic shift, were used as chromophores. Tetracarboxylate dyes (A) were found to be selective for Ca^{2+} ions. Tricarboxylate dyes (B) appear to be selective for Zn^{2+} ions. In both cases the indicators with the benzothiazolyl substituent gave the longest excitation wavelength and the largest shift upon ion binding. These properties enable the compounds to be used as alternatives to known Ca^{2+} and Zn^{2+} probes such as fura 2 and TS-Q, respectively.



Y = O, S R = -COMe, -CO_2Me, -Benzoxazolyl, - Benzothiazolyl

This research was supported by a grant from the Greek Ministry of Industry and Research.

311.8

NH_4^+ DECREASES TRANSMITTER RELEASE INDEPENDENT OF pH_i AND $[Ca^{2+}]_i$. W. Raabe* and R. Nitzan, Departments of Neurology and Physiology, University of Minnesota and VA Medical Ctr., Minneapolis, MN, 55417.

NH_4^+ is involved in the pathogenesis of hyperammonemic encephalopathies such as hepatic encephalopathy. To investigate how NH_4^+ causes encephalopathy, we studied the effects of NH_4^+ on transmitter release from cerebellar neurons grown in tissue culture. MiniEPSCs were recorded by whole cell patch clamp in cultures treated with bicuculline and TTX to suppress IPSCs and action potential dependent transmitter release.

NH_4^+ (2.5-5 mM) reversibly decreased the frequency of miniEPSCs without effects on amplitude. The effect of NH_4^+ did not depend on an increase of pH_i since intracellular alkalinization with trimethylamine did not affect miniEPSC frequency. pH_i measurement with the fluorescent dye BCECF showed that NH_4^+ (5 mM) and trimethylamine (5 mM) increased pH_i to the same extent. pH_i changes by NH_4^+ were transient and followed by an "undershoot" of pH_i upon NH_4^+ washout. In contrast, trimethylamine increased pH_i throughout application and without "undershoot" upon washout. The decrease of miniEPSC frequency by NH_4^+ was not altered by pretreatment of cultures with the $[Ca^{2+}]_i$-buffer BAPTA-AM.

These observations indicate that NH_4^+ decreases transmitter release by effects independent of changes in pH_i and $[Ca^{2+}]_i$, suggesting an effect of NH_4^+ on synaptic vesicle proteins involved in vesicle fusion and exocytosis of transmitter. Such an effect of NH_4^+ causes or contributes to the encephalopathy associated with hyperammonemia, e.g. hepatic encephalopathy.

Supported by the Department of Veterans Affairs and the Minnesota Medical Foundation.

311.9

THE NUMBER OF DOCKED VESICLES DETERMINES THE RELEASE PROBABILITY OF HIPPOCAMPAL NEURONS, T. Schikorski, and C.F. Stevens* Howard Hughes Medical Institute at the Salk Institute, 10010 N. Torrey Pines Rd., La Jolla, CA 92037

Physiological experiments have indicated that release probability at central synapses are very variable from synapse to synapse, and that the number of docked vesicles is an important determinant of this release probability. For this reason we reconstructed synapses in the CA1 region of adult mice and in rat hippocampal neurons in cell culture from EM serial sections. An average of 7.7 docked vesicles/presynapse was found in mice (std 4.19) and 4.6 docked vesicles in cell cultured neurons (std 3.0). The number of docked vesicles is strongly correlated with the size of the active zone (r=0.745 for mice; r=0.934 for cell cultured neurons), which is in turn strongly correlated with the size of the postsynaptic densities. The distribution of docked vesicles over the active zone appears random.

To determine if the release probability measured for synapses of hippocampal CA1 neurons could be explained by a varying number of release sites , we compared the distribution of release probability in hippocampus with the distribution of docked vesicles/synapse. If every docked vesicle is assigned a constant release probability of .04, both distributions match (Kolmogorov-Smirnov test $p > 0.1$). Therefore, varying the number of docked vesicles at these synapses, seems to correlate with release probability.

311.10

RUTHENIUM RED POTENTIATES EXOCYTOSIS AT SINGLE NERVE ENDINGS. D.R. Giovannucci* and E.L. Stuenkel. Department of Physiology, University of Michigan Medical School, Ann Arbor, MI 48109.

Mitochondria provide low affinity, high capacity Ca^{2+} buffering in neurons, and may play a role in Ca^{2+} homeostasis under physiological conditions. For example, strong stimulus demand or the generation of local Ca^{2+} domains may raise $[Ca^{2+}]_i$ to ≥ 1 µM, a level high enough to fully activate mitochondrial Ca^{2+} uptake. In the present study, the role that mitochondrial Ca^{2+} buffering may play in modulating exocytosis was investigated at the rat single neurohypophysial nerve ending level using time-resolved membrane capacitance (C_m) measurements in combination with fura-2 microfluorometry. Treatment with 10 µM Ruthenium Red (RuR), a blocker of the mitochondrial Ca^{2+} uniporter, introduced via patch pipette whole cell dialysis, was shown to have no effect on resting $[Ca^{2+}]_i$, C_m, or Ca^{2+} currents (I_{Ca}). Most nerve endings tested contained mitochondria as shown by EM and fluorescence microscopy, and exhibited a RuR-sensitive Ca^{2+} buffering component that limited depolarizing stimulus-induced spatially averaged $\Delta[Ca^{2+}]_i$ to ~ 500 nM. RuR treatment enhanced the magnitude of exocytotic response to repetitive depolarizing pulses from a release-ready granule pool (170 granule fusions/pulse train; n=7) by ~ 5-fold over control nerve endings. In addition, the rise in C_m continued after cessation of the stimulus for a longer period, and the rate of recovery of C_m to pre-stimulus levels was slowed 4-fold in RuR-treated nerve endings ($\tau_{1/2}$ = 44 s) compared to control. These effects were abolished by co-treatment with 5 mM EGTA (n=7) and indicated that the potentiation of exocytosis by RuR was dependent on the reduced rate of Ca^{2+} buffering and enhanced rises in $[Ca^{2+}]_i$. These studies suggest a role for mitochondria in modulating secretion in peptidergic nerve endings. (Supported by NIH and NSF grants to ELS and AHA/MI fellowship to DRG.)

311.11

SNAP-25 ANTIBODIES ARE POTENT BLOCKERS OF SYNAPTIC RELEASE IN VIVO. P. Löw, O. Shupliakov, T. Norlin, C. Risinger[1], D. Larhammar[2], V.A. Pieribone[3] and L. Brodin*, Dept. of Neuroscience, Karolinska Institutet, S-171 77 Stockholm, Sweden, [1]Dept. of Molecular & Cell Biology, Berkeley, CA 94720, [2]Dept. of Medical Pharmacology, Uppsala University, S-751 24 Uppsala, Sweden, [3]Dept. of Molecular & Cellular Neuroscience, The Rockefeller University, NY 10021.

SNAP-25 is a plasma membrane-associated protein which can engage in a protein complex (SNAP-25+syntaxin+VAMP) thought to mediate the targeting and/or fusion of synaptic vesicles (Calakos & Scheller, Physiol. Rev. 76:1-29, 1996). The C-terminal fragment of SNAP-25 can be selectively cleaved by Botulinum toxin A, which results in a partial inhibition of transmitter release (Dreyer and Schmitt, Pflügers Arch. 399:228-234, 1983; Niemann et al. TIBS 4:179-185, 1994; Owe-Larsson et al. Soc. Neurosci. Abstr 1996). Here we test the effect of microinjection of a polyclonal antibody raised against the whole SNAP-25 protein into the presynaptic element of the lamprey giant reticulospinal synapse. The reticulospinal neurons were shown by *in situ* hybridization to express high levels of SNAP-25. The antibody was affinity-purified, labeled with CY5, and microinjected presynaptically by pressure. Following injection the antibody accumulated in spots within the axon, with a location corresponding to that of release sites (Pieribone et al., Nature 375:493-497, 1995). The synaptic response, monitored by intracellular recording from target cells, showed a rapid decay, which coincided with the occurrence of antibody fluorescence in the synaptic area. Within minutes, no synaptic release could be detected. Neither trains of high-frequency stimulation, nor broadening of the presynaptic spike (by current pulses) were effective in restoring synaptic responses. Our results are consistent with an essential role of SNAP-25 in synaptic exocytosis. (Supported by the Swedish MRC).

311.12

ACTION OF α-LATROTOXIN (α-LT) ON QUANTAL RELEASE FROM EXCITABLE ENDOCRINE CELLS. J.Liu *, D.W. Barnett, and S. Misler. Depts. of Medicine and Cell Biology and Program in Neurosciences, Washington Univ., St. Louis MO 63110

α-LT, from the black widow spider, produces rapid and massive "spontaneous" quantal release of neurotransmitters from a variety of vertebrate nerve terminals with small synaptic vesicles. It also has variable effects on evoked transmission and vesicle recycling. Its action at excitable endocrine cells containing dense core granules is controversial. Using capacitance measurements to track granule fusion and/or carbon fiber amperometry to detect epinephrine release, we now report that in rat adrenal chromaffin cells, α-LT, in the nM range, produces exocytosis, over tens of seconds, of many hundreds to several thousand quanta stored in dense core secretory granules. Isolated cells were maintained in culture for 2-5 days and then exposed to a physiological saline for recording. The onset of release is preceded by the development of a large inward cation current. The time course of the subsequent capacitance increase parallels the cumulative amperometric spike release, where each spike corresponds to the release of at least a million molecules of catecholamine. Termination of the discharge can be followed by delayed endocytosis. After the discharge, the voltage-dependent Na current is intact and, in some cells, the Ca current still supports depolarization-evoked release. In pilot studies with dog pancreatic islet β-cells, α-LT produced comparable effects on the time course of membrane current and capacitance, and quantal release of serotonin. Since neither endocrine cell is reported to contain neurexin, the putative α-LT receptor at the nerve terminal surface, these results raise new questions about the mechanism(s) of α-LT action. Support: NIH (DK37380) and Jewish Hospital Research Fund to S.M.

311.13

CHARACTERIZATION OF SMALL SYNAPTIC AND LARGE DENSE CORE VESICLE EXOCYTOSIS USING CARBON FIBERS AS ELECTROCHEMICAL DETECTORS. D. Bruns, S. Engers, A. Jeromin and R. Jahn*, Howard Hughes Medical Institute, Yale Univ. Sch. of Med., New Haven, CT, 06510

Carbon fiber electrodes were used to measure transmitter discharge from small synaptic (SSV) and large dense core vesicles (LDCV) in cultured serotonergic neurons of the leech (Hirudo medicinalis). Transmitter release from individual vesicles was monitored as discrete spike-like oxidation currents. The frequency of oxidation signals was greatly reduced in neurons injected with the neurotoxin Tetanus toxin (TeNT-LC), which blocks transmitter release in other preparations by cleaving the synaptic vesicle protein synaptobrevin. The leech synaptobrevin homologue was cloned and found to be also sensitive to TeNT-LC. Furthermore, western blot analysis of single cell protein demonstrates the successful cleavage of leech synaptobrevin in toxin injected neurons compared with control cells. These results support our hypothesis that the identified signals reflect vesicle mediated exocytosis. At axonal recording sites two types of oxidation signals, small and large events, were observed that likely correspond to exocytosis of SSV and LDCV, respectively. At somatic sites only large events were measured. Electron microscopy demonstrates that both vesicle types are present in the axon stump, whereas solely LDCV were found in the soma of these neurons. SSV and LDCV exocytosis differ with respect to the number of released molecules, the kinetics of transmitter discharge and the time dependent coupling to action potentials. Funding sources: HHMI, NIH-RO1-NS33709-0141.

311.14

EVIDENCE FOR A ROLE OF CALCINEURIN IN Ca^{2+}-INDUCED NEUROTRANSMITTER RELEASE: EFFECTS OF ANTI-CALCINEURIN ANTIBODIES AND PHOSPHATASE INHIBITORS IN PERMEATED NERVE TERMINALS. J.J.H. Hens[1], M. De Wit[1], W.E.J.M. Ghijsen[2], A.G.M. Leenders[2], H.W.G.M. Boddeke[3], R. Kissmehl[4], U. Weller[5], W.H. Gispen[1] and P.N.E. De Graan[1].
[1]Rudolf Magnus Inst. for Neurosciences, Utrecht Univ. and [2]Graduate School for the Neurosciences, Univ. A'dam, Nl, [3]Sandoz Pharma, Basle, Switzerland, [4]Biology Dept., Univ. Konstanz and [5]Inst. Med. Microbiology, Univ. Mainz, Germany. (SPON: European Neuroscience Association).

Several proteins that have been implicated in Ca^{2+}-induced neurotransmission have been identified as protein kinase substrates, e.g.: the synapsins, synaptotagmin, rabphylin-3A, synaptobrevin, MARCKS, dynamin I and B-50/GAP-43. Recent evidence, however, shows that protein phosphorylation is not a crucial step in Ca^{2+}-induced release. Therefore, we investigated the role of Ca^{2+}/calmodulin-dependent phosphatase calcineurin (CaN) and protein dephosphorylation in Ca^{2+}-induced exocytosis and subsequent vesicle recycling. We introduced CaN-neutralizing antibodies (IgGs) into streptolysin-O-permeated synaptosomes from rat cerebral cortex and measured the release of glutamate (glu), noradrenaline (NA) and neuropeptide cholecystokinin-8 (CCK). The vesicular nature of Ca^{2+}-induced glu release (obtained by an elevation of free $[Ca^{2+}]_i$ from 10^{-8} to 10^{-4} M) was demonstrated by its complete block by the light chain fragment of tetanus toxin (300 nM). Specific poly- and monoclonal anti-CaN IgGs, that inhibited CaN-mediated dephosphorylation of protein kinase C-substrates B-50 and MARCKS, inhibited Ca^{2+}-induced release of glu, NA and CCK. Control IgGs were without effect. Moreover, CaN-inhibitor cyclosporin A (10 µM) decreased Ca^{2+}-induced glu release from permeated synaptosomes. Okadaic acid (≤10 µM) did not significantly inhibit Ca^{2+}-induced release of glu, NA or CCK, indicating that phosphatase 1 and 2A are not involved in this release process. Altogether, these data identify CaN as a key protein phosphatase involved in the regulation of Ca^{2+}-induced exocytosis and/or vesicle recycling of all major neurotransmitter types. J.J.H.H. is supported by grant 903-53-091 of the Netherlands Organization for Scientific Research (MW-NWO).

311.15

SYNAPTIC PROPERTIES OF BOTULINUM TOXIN A-TREATED RAT HIPPOCAMPAL NEURONS. B. Owe-Larsson*, K. Kristensson and L. Brodin. Dept. of Neuroscience, Karolinska Institutet, S-171 77 Stockholm, Sweden.

Botulinum toxin A (BoNT/A) cleaves off a C-terminal fragment of SNAP-25, which results in a decreased stability of the "SNARE-complex" (SNAP-25+VAMP+syntaxin). In neuromuscular synapses BoNT/A reduces, but it does not abolish, synaptic transmission . To test the effect of BoNT/A on the properties of a central synapse, we recorded autaptic responses in rat hippocampal neurons, grown at low density in culture, using voltage clamp, whole cell recordings. Control cells showed an autaptic response in 70 % of the cells. The response remained stable during 0.2 Hz stimulation, and showed a gradual depression during 5 Hz stimulation. Cells exposed to high BoNT/A concentrations (40 nM for 4 h) showed virtually no response during 0.2 Hz stimulation. However, when cells were stimulated at 5 Hz, a synchronous response occurred in 30 % of the cells. In contrast to the untreated cells, this autaptic response facilitated markedly during the course of the pulse train. At intermediate concentrations of BoNT/A (10 nM), a good half of the responding cells showed facilitation, while at low concentrations (1 nM) most of the responding cells showed depression. For comparison, in cells treated with high concentrations of Tetanus toxin (6.7 nM), the autaptic response was almost completely abolished. Our results shows that the blocking effect of BoNT/A can be overcome by raising the stimulation frequency, indicating that the C-terminal portion of SNAP-25 is not essential for action potential-induced fusion of synaptic vesicles in central synapses. This may suggest that the threshold for activation of synaptic vesicle fusion is linked to the stability of the "SNARE-complex".

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311.16

BLOCKADE OF ACH RELEASE AT A SYNAPSE IN *APLYSIA* BY A PEPTIDE THAT MIMICS THE CARBOXY-TERMINAL DOMAIN OF SNAP-25. J.P. Aplandi[1], M. Adler[1], A.Y. Ferrer-Montiel[2], M. Montal[2], and M.G. Filbert[1]. [1]Neurotoxicology Branch, USAMRICD, Aberdeen Proving Ground, MD 21010-5425, and [2]Dept. of Biology, UCSD, La Jolla, CA 92083-0366.

Neurotransmitter exocytosis is preceded by docking of synaptic vesicles at active sites on the presynaptic membrane. SNAP-25 (synaptosomal-associated membrane protein of 25 kDa) is one of several proteins forming the fusion complex at the active sites. Botulinum neurotoxins serotypes A and E cleave SNAP-25. This is presumed to be the mechanism by which they block neurotransmitter release. The SNAP-25 C-terminal 20-amino acid peptide (named ESUP, for excitation-secretion uncoupling peptide) was recently shown by Gutierrez *et al.* (FEBS Lett. 372:39,1995) to inhibit transmitter release from permeabilized bovine chromaffin cells. The effect of this peptide on acetylcholine (ACh) release at an identified cholinergic synapse of *Aplysia* neurons was investigated.

Recordings were obtained from isolated buccal ganglia of *Aplysia*. The presynaptic neuron was current-clamped and stimulated electrically at 0.1 Hz to elicit action potentials. The postsynaptic neuron was voltage-clamped, and evoked inhibitory postsynaptic currents (IPSCs) were recorded. ESUP was pressure-injected into the presynaptic neuron, and its effect on the amplitude of the IPSCs was studied. ACh release from presynaptic cells, as measured by the amplitudes of IPSCs, was consistently inhibited by the peptide. The inhibition was gradual, requiring 2-3 hr to produce a 50-60% reduction of IPSC amplitude. A random-sequence peptide of the same amino acid composition had no effect. These results suggest that ESUP competes with the intact SNAP-25 for binding with other fusion proteins, thus inhibiting exocytosis of neurotransmitter. This effect may account, in part, for botulinum toxin-induced inhibition of transmitter release. *Supported by DoD.*

311.17

SYNAPTIC ACTIVITY IN PRIMARY POSTNATAL RAT NEOSTRIATAL MICROCULTURES. P.B. Senatus and D.D. Potter. Department of Neurobiology, Harvard Medical School, Boston, MA 02115.

GABA/ peptidergic medium spiny projection neurons comprise the predominant neuronal cell type in the adult rat striatum (e.g., Wilson and Groves [1980] J. Comp. Neurol. 194: 599-615). There is extensive collateral synapse formation among these neurons (e.g., Somogyi et al. [1981] J. Comp. Neurol. 195: 567-584; Kitai et al. [1979] Adv. Neurol. 24: 45-51). To study these synapses *in vitro*, neostriatal microcultures were prepared from postnatal rats and cultured several weeks using methods previously reported (Senatus [1995] Soc. Neurosci. Abstr. 21: 1566); the microcultured neurons contain GABA and leu-Enkephalin immuno-reactivity, and NADPH diaphorase activity in proportions similar to those in adult neurons.

In single, dual and multi-neuron striatal microcultures one to four weeks old, stable perforated patch recordings up to three hours in duration in current clamp were obtained using electrodes containing Gramicidin D. In Tyrode's solution containing 1mM Mg^{++} and 3mM Ca^{++}, conventional spontaneous depolarizing synaptic potentials as well as robust evoked autaptic and synaptic potentials were present; they were sensitive to 10-20μM bicuculline and 10-100 μM baclofen. Spontaneous depolarizing shifts (DS's) of varying amplitude and duration occurred occasionally and sometimes rhythmically; action potentials were usually present on their crests. Transitions in striatal neurons between metastable membrane potential states have been reported *in vivo* and in slices (e.g., Wilson and Kawaguchi [1996] J. Neurosci. 16(7): 2397-2410).

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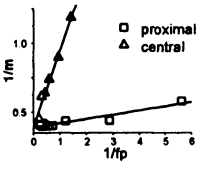
311.18

QUANTAL DEMOBILIZATION DETERMINES FREQUENCY FACILITATION M.K.Worden[1], M.Bykhovskaia [1,2] and J.T.Hackett*[1] [1]Dept Physiology, UVa Health Sci. Ctr., Charlottesville, VA 22908, [2]Sechenov IEPhB, S-Petersburg 194223, Russia

In the lobster dactyl opener muscle, the proximal synapses of the excitory motoneuron have a larger quantal content *(m)* but less frequency facilitation than central synapses. We compared proximal and central synapses using focal extracellular recordings of postsynaptic quantal currents at frequencies of stimulation in the range from 1 to 10 Hz. The number of quanta released in response to each stimulus was counted, and from a statistical analysis of the distribution of quantal content the number of releasable quanta *(n)* and the probability of quantal release *(p)* were estimated. At low frequencies both n and p were found to be higher at proximal synapses. At high frequencies (f>5 Hz) the value of p saturated at the same level for both central and proximal synapses. All central synapses had a statistically significant increase in n as f increased, whereas at proximal synapses n did not increase. We interpreted this result in terms of stimulus-dependent mobilization model, which predicts that the relationship between *(1/m)* and *(1/fp)* should be linear, with a slope and intercept equal to $(k_D/\langle n_s \rangle)$ and $(1/\langle n_s \rangle)$ respectively, where $\langle n_s \rangle$ is the average number of quanta newly mobilized with each stimulus, and k_D is a rate constant of demobilization of docked quanta back to cytoplasm. The relationship between *(1/m)* and *(1/fp)* was linear both for proximal and central synapses with similar intercepts (see plot). However the slopes of this relation for central synapses are steep, whereas for proximal synapses the slopes were nearly zero. These results indicate that central synapses differ from proximal synapses by higher rates of demobilization of docked quanta. At central synapses quantal demobilization between sequential stimuli decreases as f increases, therefore more quanta are released. In contrast, quantal demobilization for proximal synapses is always low resulting in more releasable quanta independent of f.



Supported by NIH grant RR10481 to M.K.W.

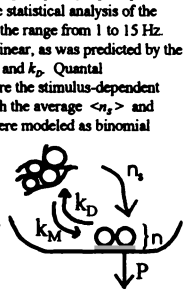
311.19

STIMULUS-DEPENDENT MOBILIZATION MODEL OF QUANTAL NEUROSECRETION. M.Bykhovskaia [1,2] J.T.Hackett[1] and M.K.Worden*[1] [1]Dept of Physiology, UVa Health Sci. Ctr., Charlottesville, VA 22908, [2]Sechenov IEPhB, S-Petersburg 194223, Russia

We have developed a new model of neurosecretion which explains the phenomenon of frequency facilitation in terms of quantal mobilization and demobilization. We propose that each stimulus causes mobilization and docking of a number of quanta (n_s, see the schematic) independent of frequency of stimulation *(f)*. Based on this model we derived and solved the kinetic equations describing quantal mobilization, demobilization and release. This enabled us to predict a linear dependance between *(1/m)* and *(1/fp)*:

$$1/m=(k_D/\langle n_s \rangle)\,(1/fp)+1/\langle n_s \rangle ,$$

where m is the average quantal content, p is the probability of release and k_D is the rate constant of quantal demobilization back to cytoplasm. This prediction was tested on lobster dactyl opener muscle with focal extracellular recordings of postsynaptic quantal currents. In each experiment m and p were estimated by the statistical analysis of the distribution of the quantal content for several values of f in the range from 1 to 15 Hz. The relationship between *(1/m)* and *(1/fp)* was found to be linear, as was predicted by the model, and from its slope and intercept we estimated $\langle n_s \rangle$ and k_D. Quantal neurosecretion was simulated by Monte-Carlo method, where the stimulus-dependent quantal mobilization was modeled as a random process with the average $\langle n_s \rangle$ and variance $var(n_s)$, and quantal demobilization and release were modeled as binomial processes with the probabilities $(1-exp(-k_D/f))$ and p, respectively. The parameters of the model were obtained by fit of experimental and predicted distributions of quantal content by χ^2-minimization. The values of the parameters of the model $\langle n_s \rangle$, k_D and p obtained by these two methods, were found to be in a good agreement, as were the simulated and predicted distributions of quantal content.



Supported by NIH grant RR10481 to M.K.W.

311.20

A MODEL OF TRANSMITTER RELEASE THAT ACCOUNTS FOR FAST FACILITATION AND AUGMENTATION. R. Bertram[1]* and A. Sherman[2]. [1]Division of Science, Penn State Erie, Erie, PA 16563 and [2]Mathematical Research Branch, NIDDK, NIH, Bethesda, MD 20892.

We describe a simple mathematical model of transmitter release that can account for F1 and F2 fast facilitation as well as longer-lasting augmentation. This model reconciles two recent experimental findings, one which showed that exogenous buffers reduce fast facilitation (Kamiya and Zucker, Nature, 371:603), and one which showed that they did not (Winslow et al., J. Neurophysiol., 72:1769). We show that these apparently contradictory findings are due to the different experimental protocols, and are both consistent with our model. The model we present is a modification of an earlier one based on the colocalization of Ca^{2+} channels and transmitter release sites, each release site having four Ca^{2+} binding sites with different affinities and kinetic rates (Stanley et al., Soc. Neurosci. Abstr., 21:24). The modification consists of placing one of the binding sites away from the Ca^{2+} channel, so that it senses the bulk cytosolic Ca^{2+}, but not the domain Ca^{2+} surrounding an open channel. Augmentation is due to the accumulation of Ca^{2+} bound to this distant site, while F1 and F2 facilitation are due to the accumulation of Ca^{2+} bound to two of the three sites adjacent to the Ca^{2+} channel. In addition to these features, the model preserves features of the earlier version including fourth power cooperativity with respect to external Ca^{2+}, a release time course that is independent of an increase in quantal content, an inverse relation between external Ca^{2+} and the degree of facilitation, and frequency-dependent steps in synaptic enhancement.

312.1

MORPHOLOGIOCAL PLASTICITY OF DENDRITIC SPINES: CHANGES IN SPINE NUMBER AND SHAPE AFTER DEAFFERENTATION OR APPLICATION OF GLUTAMATE RECEPTOR ANTAGONISTS IN HIPPOCAMPAL SLICE CULTURES.

R.A. McKinney, R. Dürr, M. Müller*, B.H. Gähwiler and S.M. Thompson.
Brain Research Institute, University of Zurich, CH-8029 Zurich, Switzerland

Changes in synaptic structure may represent a mechanism underlying lasting changes in synaptic strength. The determinants of the number and shape of dendritic spines on CA1 pyramidal cells in hippocampal slice cultures were investigated with confocal microscopy of cells that had been filled with Lucifer Yellow. In control cultures, the average spine density was ~1/μm and spine length was up to 1.5μm. Transection of Schaffer collateral afferents in mature cultures resulted in a transient increase in spine length (up to ~2.5μm) at 3 days post-lesion, followed at 7 days by a marked decrease in spine length to <0.5 μm and massive decreases in density. By times >14 days post-lesion, spine density and length had returned to almost control values, due to reinnervation from area CA3 (as seen immunohistochemically and electrophysiologically). Do these morphological changes result from the absence of glutamatergic excitation after axonal degeneration? In contrast to axonal transection, 'chemical deafferentation', by incubation of mature cultures with the AMPA receptor antagonist NBQX for 3 days, produced only modest decreases in spine length and number. Blocking NMDA receptors with MK-801 for 3 days, in contrast, led to striking decreases in spine density and a dramatic increase in the proportion of mushroom-shaped or calciform spines having long spine necks (~2.5μm) and enlarged heads. NBQX or MK-801 applied for 14 days after axonal transection reduced, but did not prevent, the re-establishment of spines upon reinnervation.

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312.2

REGIONAL DIFFERENCES IN THE SUBCELLULAR LOCALIZATION OF GLYCINE RECEPTOR α1 AND α2 mRNAs IN RAT CNS. A. Triller*, A. Gardiol and C. Racca. Laboratoire de Biologie Cellulaire de la Synapse, INSERM CJF 94-10, Ecole Normale Supérieure, Paris, France.

We have shown previously that neurones in the ventral horn of adult rat spinal cord express α1 and α2 glycine receptor (GlyR) subunit mRNAs in somata and dendrites, whereas those for β and gephyrin are in the somata only. We have studied here the subcellular distribution of GlyR complex mRNAs with respect to the regional localization of neurones in the adult rat CNS. Nonradioactive *in situ* hybridization with digoxigenin-labeled oligonucleotide and cRNA probes specific for the GlyR subunits and gephyrin was used to detect these mRNAs.

As seen by others (refs in Kirsch et al., 1993, EJN *5*: 1109), we observed that β subunit and gephyrin mRNAs had similar regional and subcellular distributions in the whole CNS. Although, α1 and α2 subunit mRNAs predominated in the diencephalon, mesencephalon, medulla, and spinal cord, their regional distributions were comparable to that of β and gephyrin transcripts. However, in the neocortex and thalamus, α1 and α2 subunit mRNA hybridization signals were restricted to cortical layers II-III, V, and VI, and certain thalamic nuclei, whereas β subunit and gephyrin transcripts were widely present all over these regions. The transcripts for α1 and α2 subunits were found in dendrites only in the globus pallidum, amygdaloid complex, substantia innominata, substantia nigra, deep cerebellar nuclei, medulla, and spinal cord.

These data provide further support for a role of GlyR inhibition in higher structures of the brain. The main result of this study was that the dendritic localization of α1 and α2 GlyR subunit mRNAs depended on the brain region. This finding suggests that different regulatory mechanisms for the localization of α1 and α2 subunit transcripts are used by distinct populations of neurones.

This work was supported by Institut de Recherche sur la Moelle Epinière, France.

312.3

GLYCINE RECEPTOR α SUBUNIT mRNAs IN DENDRITES OF RAT SPINAL CORD NEURONES. C. Racca*, A. Gardiol and A. Triller. Lab. Biologie Cellulaire de la Synapse, INSERM CJF 94-10, ENS, Paris, France.

The presence of neurotransmitter receptor mRNAs in dendrites, close to synapses, may be relevant for local modifications of the responsiveness of the postsynaptic membrane. The glycine receptor (GlyR) is the only ligand-gated channel to be present exclusively at the postsynaptic differentiations on both neuronal somata and dendrites (Triller et al., 1985, JCB *101*: 683).We have therefore investigated the subcellular distribution of the mRNAs encoding for the various GlyR subunits in adult rat spinal cord neurones.

The transcripts were detected using light and electron microscopic nonisotopic *in situ* hybridization (ISH) with specific complementary oligonucleotide and cRNA probes for α1, α2, and β GlyR subunits and gephyrin. With digoxigenin-labeled probes and the alkaline phosphatase reaction we found that α1 and α2 subunit transcripts were present in somata and neurites of most neurones. In contrast, those for β and gephyrin were detected in somata only. Fluorescent double-labeling experiments (ISH for all GlyR complex mRNAs and immunodetection for α1, gephyrin, or GlyR) showed that α1 and α2 transcripts aggregate in particles and tend to predominate at the periphery of the dendrites. Nanogold silver-enhanced labeling confirmed that α1 and α2 transcripts were within the dendroplasm and revealed their frequent localization near synaptic sites in association with submembranous cisternae.

GlyR α subunit transcripts are the first mRNAs for neurotransmitter receptors which are shown to be localized in dendrites *in situ*. The presence in dendrites of organelles involved in protein synthesis (the RER: Peters et al., 1991, 3rd ed.; polyribosomes: Steward & Reeves, 1988, J. Neurosci. *8:* 176; components of the Golgi apparatus: Triller, unpublished results), and of mRNA suggests a local synthesis of GlyRs.

In conclusion, mRNA targeting and localized protein synthesis might contribute to a fine regulation at postsynaptic sites of the expression of GlyR subunits.

This work was supported by grants from AFM and AC-SV 11, France.

312.4

DEVELOPMENTAL EXPRESSION OF THE BIDIRECTIONAL CONTROL OF MAP2 PHOSPHORYLATION AT GLUTAMATE SYNAPSES

E.M. Quinlan and S. Halpain* Department of Neuroscience and The Center for Cell Signalling, University of Virginia, Charlottesville, VA 22908

Although profound changes in neuronal morphology occur as synapses mature, little is known about how synaptic transmission regulates the developing neuronal cytoskeleton. Previously we showed that the postsynaptic microtubule-associated protein MAP2 is a target of bidirectional, calcium-dependent pathways stimulated by glutamate receptor activation in the hippocampus. To examine the regulation of MAP2 phosphorylation by synaptic transmission over development, slices of adult and neonatal hippocampus were depolarized with 40mM KCl. Depolarization produced a time-dependent bidirectional change in MAP2 phosphorylation in adult (postnatal day 42-44) and P21-23 hippocampus: a large transient increase was observed within 30 sec, followed by a pronounced decrease that persisted for ≥10 min. In contrast, in P17-18 and P7-8 hippocampus, depolarization induced only a net increase in MAP2 phosphorylation, which remained elevated relative to baseline for ≥10 min of depolarization. The depolarization-induced increase in MAP2 phosphorylation was mimicked by the glutamate receptor agonist 1S,3R-ACPD; however, the magnitude of the MAP2 phosphorylation observed after 5 min of ACPD was 2-fold higher in P7-8 versus P42-44. The depolarization-induced decrease in MAP2 phosphorylation was mimicked by NMDA; however, the magnitude of the dephosphorylation was significantly less at P7-8 than P42-44. Thus, early in neonatal development, signalling pathways that stimulate a net increase in MAP2 phosphorylation are favored. Because phosphorylation reduces the affinity of MAP2 for F-actin and microtubules, glutamate-induced increases in MAP2 phosphorylation may reduce cytoskeletal stability and promote morphological plasticity early in postnatal development. Supported by NIMH.

312.5

GLUTAMATE-INDUCED ACTIVATION OF PRE- AND POSTSYNAPTIC PKC INVOLVES DISTINCT METABOTROPIC GLUTAMATE RECEPTOR SUBTYPES G.M.J. Ramakers, P. Pasinelli, W.H. Gispen and P.N.E. De Graan Rudolf Magnus Institute for Neurosciences, P.O. Box 80040, 3508 TA Utrecht, The Netherlands (SPON: European Neuroscience Association).

Activation of protein kinase C (PKC) is thought to be an important step in long-term potentiation (LTP) of glutamatergic transmission in the hippocampus of the rat. Both pre- and postsynaptic PKC is implicated, because LTP induction in the CA1 field of the hippocampus increased the phosphorylation state of presynaptic protein B-50 (a.k.a. GAP-43, F1) as well as that of postsynaptic protein RC3 (a.k.a. neurogranin, BICKS), effects that could be prevented by the NMDA receptor antagonist AP5. In this study we investigated the effect of glutamate receptor (GluR) activation on pre- and postsynaptic PKC activity, as monitored by B-50 and RC3 phosphorylation. Hippocampal slices (450 μm) were labelled with $^{32}P_i$ (100 μCi/ml) for 90 min at 30°C, treated with L-Glutamate (Glu), and in situ B-50 and RC3 phosphorylation was determined using quantitative immunoprecipitation and phosphorimaging. Glu treatment induced a time- and concentration dependent increase in the phosphorylation state of both RC3 and B-50, which could be blocked by PKC inhibitors. Antagonists for the ionotropic GluRs, CNQX (100 μM) and AP5 (100 μM) did not affect the Glu induced increase in RC3 and B-50 phosphorylation. Pretreatment with MCPG (500 μM), an antagonist for the metabotropic glutamate receptor (mGluR) prevented the increase in RC3 and B-50 phosphorylation. In addition, ACPD (100 μM), a broad spectrum mGluR agonist mimicked the Glu effect. mGluRs have been classified into three major classes (group I, II and III) based on their second messenger coupling and pharmacological profile. DHPG (500 μM), a specific group I agonist, only affected RC3 phosphorylation, whereas L-AP4, a group III and presynaptic mGluR agonist (50 μM) specifically increased B-50 phosphorylation. In addition, the Glu-induced increase in RC3 phosphorylation could be prevented by pretreatment of slices with thapsigargin (10 μM), showing that IP_3 sensitive Ca-stores were essential in this proces. Our results show that Glu increased the *in situ* phosphorylation of PKC substrates B-50 and RC3 in a concentration- and time-dependent manner. This activation of pre- and postsynaptic PKC involves distinct mGluR subtypes, coupled to different second messenger pathways. (This work was supported by an ENP grant of the ESF)

312.6

AFFERENT STIMULATION CAUSES DEPOSITION O F SOMATICALLY SYNTHESIZED PROTEIN AT DENDRITIC SPINES IN THE GUINEA-PIG HIPPOCAMPUS SLICE S. Feig and P. Lipton* Depts. Anatomy and Physiology and Center For Neuroscience, University of Wisconsin Medical School, Madison, WI, 53706.

Local synthesis of protein is activated in CA1 pyramidal cell dendrites and spines by pairing Schaffer collateral stimulation with carbachol (J. Neuroscience 13: 1010). The possibility that the newly synthesized protein might enhance deposition of protein arriving via dendritic transport (dendritic flow) from the cell somata, was tested. This would allow appropriate stimulation to cause long-term synaptic change.

Rapid dendritic transport was characterized in control slices by L.M.autoradiography. Nocodazole-inhibitible protein accumulation in the dendrites peaked at 30' and reached a plateau after 2 hours.

Effects of paired Schaffer collateral stimulation and carbachol on dendritic transport was tested using E.M.autoradiography to *localize* transported protein. 90' after stimulation, when local synthesis,but not the newly synthesized protein, had disappeared, slices were pulse-labeled with 3H-leucine; flow was allowed to occur for 2 hours.

Accumulation of 3H-leucine at dendritic spines of the CA1 pyramidal cells was 2-fold greater ($p<.01$) in slices which had been stimulated than in control (exposed to carbachol but not stimulated) slices. There was no enhancement of accumulation over other portions of the dendrite. Thus the same stimulation that induces local synthesis of protein, causes deposition of somatically synthesized protein at dendritic spines.

Studies are in progress to determine whether the deposition is dependent on increased local protein synthesis or on other aspects of the stimulation. Support: American Heart Association, Wisconsin.

312.7

PEPTIDE SUBSTRATES FOR IN VITRO ASSAY OF ERK (MAP KINASE) ACTIVITY. John W. Haycock*. Dept. Biochemistry and Molecular Biology, Louisiana State University Medical Center, New Orleans, LA 70119.

Extracellular signal-Regulated protein Kinases (ERKs) are a family of proline-directed Ser/Thr kinases. ERK1 (44 kDa) and ERK2 (42 kDa) phosphorylate both microtubule-associated protein 2 and myelin basic protein (MBP) avidly in vitro (high V/Km). Pelech and coworkers developed a synthetic peptide substrate (APRTPGGRR) for the ERKs, modeled after the phosphorylation site in MBP. Although this peptide contains the --P-X-S/T-P-- motif thought to represent the consensus substrate recognition sequence for the ERKs, several other ERK substrates do not contain the Pro residue in the -2 position. For example, this is absent from the ERK phosphorylation site (rat: -EAVTS^{31}PRF-; human, -EAIMS^{31}PRF-) in tyrosine hydroxylase (TH), one of the first physiological substrates of ERK1/ERK2 described. In the present studies, several synthetic peptides modeled after S^{31} in TH were evaluated as in vitro substrates for the ERKs. The phosphorylation of a S^{31}-containing peptide from type 1 human TH by activated, recombinant ERK2 was found to exhibit a V/Km approximately 50% higher than that for the MBP peptide. In extracts from untreated PC12 cells, the phosphorylation of MBP peptide was higher than that of the Ser31-TH peptide. However, the relative increase in extracts of NGF-treated PC12 cells was much greater for the Ser31 peptide, suggesting that the latter peptide may be a more specific substrate for the ERKs. [Supported by USPHS grants NS25134 and MH00967.]

312.8

CO-LOCALIZATION OF PROTEINS FROM THE POSTSYNAPTIC DENSITY FRACTION AT GLUTAMATERGIC SYNAPSES IN HIPPOCAMPAL NEURONS IN CULTURE. M.L. Apperson*, L. Jia, J.C. Lee, M. J. Kiely, L.T. Schenker, and M.B. Kennedy. Division of Biology 216-76, Caltifornia Institute of Technology, Pasadena, CA 91125.

We have recently identified several abundant proteins associated with the postsynaptic density fraction prepared from homogenates of rat forebrain. One of these proteins is the 2B subunit of the NMDA-type glutamate receptor (NR2B) [1]. A second is PSD-95, a homologue of discs-large which is a component of *D. melanogaster* septate junctions and ZO-1 which is a component of mammalian tight junctions [2]. PSD-95 binds tightly to the terminal seven amino acids of the type 2 subunits of NMDA receptors, including NR2B, via the second of three PDZ motifs located in its amino terminal half. [3]. A third PSD component is densin-180, a brain specific transmembrane protein that appears to belong to the family of O-sialoglycoprotein adhesion molecules [4]. To determine whether these proteins are all present in most excitatory synapses, or are present in distinct subsets of synapses, we have carried out pairwise double-label immunohistochemical labeling of dissociated, 3-4 week cultured hippocampal neurons with antibodies specific for each of these proteins and for the α subunit of CaM kinase II, which is also associated with the PSD fraction. We find that all four molecules are present in the vast majority of excitatory synapses in the cultures. Thus, we conclude that several of the most abundant proteins purified from the PSD fraction are present together in vivo, perhaps in an organized complex, in glutamatergic synapses in the forebrain.

1. Moon et al. 1994. PNAS **91**: 3954-3958.
2. Cho et al. 1992. Neuron **9**: 929-942.
3. Kornau et al. 1995. Science **269**: 1737-1740.
4. Apperson and Kennedy 1995. Abstr. Soc. Neurosci. **21**: 593.
Support:NIH-NS28710, NSF GER-9023446

312.9

CALCIUM OSCILLATION FREQUENCY DEPENDENCE OF MULTIFUNCTIONAL CaM KINASE II ACTIVATION. P. De Koninck* and H. Schulman. Dept. of Neurobiology, Stanford Univ. School of Medicine, Stanford, CA 94305-5401.

Most external stimuli produce intracellular Ca^{2+} oscillations of various durations and frequencies; these oscillations may encode specific signals within cell compartments, such as the nucleus or dendrites. Specialized molecules in these compartments could serve as decoders and translators of Ca^{2+} signals. The cellular localization and biochemical properties of multifunctional CaM kinase II (CaMKII) make this enzyme a good candidate for such role. To determine the dependence of CaMKII activation on the duration and frequency of Ca^{2+} oscillations (or spikes), we developed a rapid perfusion technique: Recombinant α-CaMKII is immobilized via antibody onto plastic tubing connected to pressurized chambers and manifold valves. This setup is used to exchange the solution to which the enzyme is exposed (high/low Ca^{2+}, EGTA, calmodulin or ATP) within ≤ 20 ms. Preliminary results indicate that application of 20-30 Ca^{2+}/CaM spikes (≤ 200 ms each) at frequencies ≥ 3 Hz induces high levels of CaMKII autonomy (Ca^{2+}/CaM-independent activity). In contrast, the same number of spikes given at frequencies ≤ 1 Hz produce little CaMKII autonomy. These results support the notion that CaMKII serves as a Ca^{2+} spike frequency decoder, a role that may underlie the involvement of CaMKII in synaptic plasticity.

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312.10

CHANGES IN PSD-ASSOCIATED CAMKII ACTIVITY IN ANIMALS CHARACTERIZED BY ALTERED SYNAPTIC PLASTICITY. A. Caputi, F. Gardoni, L. Pastorino, M. Cimino, F. Cattabeni* and M. Di Luca. Inst. of Pharmacol. Sci., Univ. of Milano, V. Balzaretti 9, Milano 20133 Italy

To investigate the function of the autophosphorylated form of CaM Kinase II in synaptic plasticity we generated a rat characterized by lack of LTP in the CA1 region of the hippocampus (Ramakers et al, Neurosci., 1993). This animal model has been achieved by administering an antiproliferative agent selective for neuroepithelial cells (Methylazoxymethanol, MAM-rats; Cattabeni and Di Luca, Physiol. Rev., in press) during development, inducing a targeted neuronal ablation in hippocampus. At presynaptic level, these animals show molecular alterations, i. e. increased traslocation of PKC, leading to facilitation of glutamate release in hippocampal synapses (Di Luca et al., Eur. J. Neurosci., 1995). We now show that at postsynaptic site the Ca^{++} dependent component of Post Synaptic Densities (PSD)-associated CaMKII autophosphorylation is decreased in MAM-rats to 35% of control levels, although concentration of the enzyme is not altered. In addition the Ca^{++}/Calmodulin dependent phosphorylation of NR2A/B NMDA receptor subunits is decreased to a similar extent in MAM rats when compared to controls, as revealed in back-phosphorylation experiments. The alteration in CaMKII activity appears to be confined to PSD-associated CaMKII, since cytosolic enzyme activity is unchanged in MAM-rats. These data design a framework in which "in vivo" an enhanced presynaptic release of glutamate coexists with down-regulation of PSD associated CaM-Kinase activity. Endogenous autophosphorylation of the kinase might thus regulate the synaptic events that couple presynaptic activity and production of postsynaptic responses, such as LTP.

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312.11

IDENTIFICATION OF FYN SUBSTRATES IN THE POSTSYNAPTIC DENSITY FRACTION PREPARED FROM THE RAT FOREBRAIN. T. Suzuki*[1], K. Okumura-Noji[2] [1]Department of Neuroplasticity, Reaseach Center for Aging and Adaptation, Shinshu University School of Medicine, 3-1-1 Asahi, Matsumoto 390, Japan, [2]Department of Biochemistry, Nagoya City University Medical School, Mizuho-ku, Nagoya 467, Japan

The efficacy of neuronal signal transmission can be augmented or diminished according to the synaptic input into neurons, and the experimental models for these synaptic plasticities are long-term potentiation (LTP) and long-term depression (LTD), respectively. Various kinases control synaptic strength. Besides CaMKII and protein kinase C, some tyrosine kinases can modulate synaptic strength. The involvement of protein tyrosine kinase(s) has been suggested by experiments using specific inhibitors for tyrosine kinases. Furthermore, *fyn*-deficient mice have an impairment in LTP and in spatial learning. In this report, we investigated the synapse-specific substrates for Fyn. Fyn, protein tyrosine kinase, and its substrates were highly concentrated in the postsynaptic density (PSD) fraction prepared from the rat forebrain (Wistar, male). There were a number of Fyn substrates unique to the PSD fraction. One of the major substrates in the PSD fraction was found to be a concanavalin A-binding glycoprotein, PSD-gp180, which is the N-methyl-D-aspartate (NMDA) receptor subunit ε2 (NR2B). Western blotting and immunoprecipitation supported the phosphorylation of ε2 by Fyn. NMDA receptor subunit ε1 (NR2A) was also a substrate for Fyn. These results suggest that Fyn is involved in the modulation of synaptic efficacy through the phosphorylation of synapse-specific substrates such as the NMDA receptor/channel. Suported by a Grant-in-Aid for Scientific Research from the Japanese Ministry of Education, Science and Culture.

312.12

ALTERNATION OF GTP BINDING PROTEINS IN THE POSTMORTEM BRAINS OF HEROIN ADDICTS. S. Shichinohe[1], E Hashimoto[1], H. Ozawa[1], S. Toki[1], M. Yamamoto[1*], T. Saito[1], L. Frölich[2], P. Riederer[3] and N. Takahata[1]
[1] Dept. of Neuropsychiatry, School of Medicine, Sapporo Medical University Sapporo, Japan. [2] Dept. of Psychiatry, University of Frankfurt am Main, Frankfurt, Germany. [3] Dept. of Psychiatry, University of Würzburg, Würzburg, Germany.

Opioid receptors couple to GTP binding (G) proteins which play an obligatory role in the transduction of extracellular, receptor-detected signals across cell membranes to intracellular effectors. It is found that G proteins are modulated by various psychotropic drugs such as antidepressants and opiates. However, there are few direct information on the status of G proteins in the human brain during chronic opiate addiction. In the present study we examined the alternations of G proteins in membrane preparations in postmortem human brains obtained from heroin addicts and control subjects without a history of drug abuse. The quantity of G proteins (G αs, G αi-1,2, G αi-3, G αo, G αq, G β) were investigated by immunoblotting with poly clonal antibodies (RM/1, AS/7, EC/2, GC/2, QL, SW/1, respectively). The immunoreactivities of G β subunit in temporal cortex were significantly increased in heroin addicts compared with controls. Recently it has been demonstrated that β subunit of G proteins play an important role in the regulation of post-receptor signal transduction as well as α subunit.

The present findings suggest that alterations in the density of G proteins may be involved in pathophysiology of opiate tolerance and dependence.

312.13

MECHANISMS INVOLVED IN THE ANTIDOPAMINERGIC ACTION OF CLONAZEPAM AND MELATONIN IN RAT STRIATUM, L.P. Niles* and C.C. Tenn. Biomedical Sciences, McMaster Univ., Hamilton, ON, Canada L8N 3Z5.

The $GABA_A$ receptor antagonist, bicuculline (2.5 mg/kg, i.p.), causes a significant inhibition (~ 65%) of the antidopaminergic effect of clonazepam or melatonin in 6-hydroxydopamine-lesioned rats, indicating that this effect is mediated primarily by a GABAergic mechanism (1). To examine the extent of GABAergic involvement, direct intrastriatal injection of bicuculline was utilized in order to circumvent the convulsant effect of high doses of this antagonist. In addition, the intrastriatal effect of the peripheral-type BZ antagonist, PK11195, was examined since this agent caused a modest but significant attenuation of the above effect, when administered peripherally (1).

The intrastriatal injection of bicuculline (1-10 nmoles), was more effective than peripheral treatment, but did not completely block the antidopaminergic effect of clonazepam or melatonin. However, the intrastriatal infusion of bicuculline (5 nmoles) together with PK11195 (20 nmoles) completely abolished this effect. These findings confirm the predominant role of a GABAergic mechanism in the antidopaminergic action of clonazepam and melatonin. Moreover, since PK11195 can block the inhibitory effect of BZs and melatonin on cyclic AMP production in the striatum (2), it appears that suppression of this pathway is also involved in the antidopaminergic action of these drugs. 1. Tenn, C.C. and Niles, L.P.: J. Pharmacol. Exp. Ther. 274, 84-89 (1995). 2. Tenn, C.C. Neu, J.M. and Niles, L.P.: Soc. Neurosci, Abstr. Vol 21, Part 3, p. 2069 (1995). *(Supported by NSERC, Canada).*

312.14

SENSITIZATION OF DIAZEPAM INHIBITORY EFFECT ON ADENYLATE CYCLASE ACTIVITY IN 6-HYDROXYDOPAMINE LESIONED ANIMALS. CC. Tenn*, LP. Niles and L.J. Smith. Department of Biomedical Sciences, McMaster University, Hamilton, Ontario, Canada, L8N 3Z5.

Recent studies indicate that benzodiazepines, such as diazepam and Ro5-4864, inhibit adenylate cyclase activity (AC) via a pertussis toxin-sensitive G protein-coupled benzodiazepine (BZ) receptor. Since the inhibition of cAMP production is involved in the antidopaminergic effect of BZs in lesioned animals, the effects of diazepam on AC activity was investigated in these animals. Unilateral lesioning of the nigrostriatal pathway with 6-hydroxydopamine was carried out in male Sprague Dawley rats (200-250g). Functional studies revealed a biphasic inhibition of forskolin-stimulated AC activity by diazepam for both the denervated and intact striatum. While there was no difference in the enzyme activity between striata from sham-operated animals, there was a significant difference in AC activity between the intact and denervated striata of lesioned rats. For the receptor-mediated (first) phase of diazepam-induced inhibition, the EC_{50}'s were 10.2 nm and 4.2 nm, for intact and lesioned striata respectively. The second phase of inhibition, which involves a direct action on AC, showed no significant differences. The EC_{50}'s were 70.3 μM and 68.2 μM for intact and lesioned striata, respectively. These results indicate a sensitization of the receptor-mediated inhibitory effect of diazepam on AC activity in lesioned animals.

In addition, we have observed a significant increase in [^{3}H] diazepam binding in denervated striatal tissue as compared to the controls, with no change in the dissociation constant. The possibility that enhanced receptor G-protein coupling is involved in this sensitization, is currently being investigated (Supported by a NSERC (Canada) Strategic grant).

POSTSYNAPTIC MECHANISMS Ach, ATP AND PEPTIDE SIGNAL

313.1

POSTSYNAPTIC ACTIONS OF CHOLINERGIC RECEPTOR ACTIVATION ON MULTIPLE TYPES OF INTERNEURONS IN CA1 REGION OF THE RAT HIPPOCAMPUS. A.R. McQuiston* and D.V. Madison. Department of Molecular and Cellular Physiology, Stanford University School of Medicine, Stanford, CA 94306.

Previous studies have shown cholinergic receptor activation have several diverse modulatory actions on the electrophysiological properties of the mammalian hippocampus. Investigations have demonstrated that the mixed muscarinic and nicotinic agonist carbachol has excitatory actions on interneurons in area CA1 of the mammalian hippocampus. Indirect studies have shown an increase in the spontaneous inhibitory postsynaptic currents in pyramidal cells whereas direct studies have demonstrated that carbachol can depolarize some interneurons in stratum oriens (SO) of area CA1. We have further investigated the postsynaptic actions of cholinergic receptor activation in 109 interneurons of strata oriens, radiatum and lacanosum moleculare in area CA1 of the rat hippocampus. Consistent with previous findings, 38 of these cells depolarized (13.7 ± 0.9 mV) in response to the activation of muscarinic receptors with no significant effect on the input resistance of the cell (percent change 0.24 ± 3.32 %). A further four cells depolarized (14.0 ± 2.9 mV) as a result of the activation of nicotinic receptors which was accompanied by a decrease in input resistance (-23.7 ± 9.3 %). These depolarizations were often enough to bring a quiescent cell to the threshold for action potential firing. In contrast to previous studies, a subpopulation of interneurons hyperpolarize in response to muscarinic receptor agonists. Seventeen cells from the 3 regions investigated hyperpolarized (-12.8 ± 1.0 mV) with a concomitant decrease in input resistance (-33.9 ± 7.4 %). Furthermore, the outward current generated by carbachol or muscarine on these hyperpolarizing cells reverses at the expected reversal potential for the activation of a selective potassium conductance. Perhaps more interesting, some interneurons produced an afterdepolarization (ADP) in response to muscarinic agonist application following the injection of a depolarizing current pulse. This ADP was often sufficient to evoke bursts of action potentials following the depolarizing pulse. The ADP was insensitive to tetrodotoxin and inhibition of calcium influx.

Supported by NIH grant NS1451.

313.2

CARBACHOL-INDUCED OSCILLATIONS IN INTERNEURONS IN AREA CA1 OF THE RAT HIPPOCAMPAL SLICE. L.L. McMahon*, J.H. Williams, and J.A. Kauer. Department of Neurobiology, Duke Univ. Med. Cntr., Durham, NC 27710.

The septal cholinergic system plays an important role in the generation of hippocampal theta activity. *In vitro*, bath application of the muscarinic agonist, carbachol, produces oscillatory behavior in extracellular and intracellular recordings of CA1 pyramidal cells (Bland et al., 1988). We are testing the idea that interneurons in the CA1 region of the hippocampal slice undergo carbachol-induced oscillatory behavior as well. Whole-cell current clamp recordings were obtained from visually identified s. oriens and s. radiatum interneurons in 400 μm thick slices from 24-28 day old rats. Slices were submerged in a recording chamber and perfused with ACSF containing 5 mM KCl at 32°C. Upon carbachol application (50 μM), s. oriens interneurons were depolarized 5-15 mV from RMP (-60±3 mV; n=3). After 1-2 min in the continued presence of carbachol, rhythmic oscillations, 10-20s in duration, occurred at 5-10s intervals and continued for the duration of drug application. The oscillations consisted of a 6-8 mV depolarization to threshold, with a spike frequency of 10-12 Hz at the peak. Stratum radiatum interneurons were also depolarized by carbachol (3-40mV, n=5), however, consistent oscillatory behavior was not observed. In some cases (n=2), the carbachol-induced depolarization elicited tonic firing (3-6 Hz) which was interrupted by random 5-20s quiet periods. These data suggest that oscillatory behavior in interneurons may contribute to the rhythmic behavior observed in pyramidal cells. NIH NINDS NS30500 to J.A.K. and NIH NRSA NS09734 to L.L.M.

313.3

CARBACHOL-INDUCED OSCILLATIONS IN AREA CA1 OF THE HIPPOCAMPAL SLICE. J.H. Williams* and J.A. Kauer. Dept. of Neurobiology, Duke University Medical Center, Durham NC27710.

Brief applications of carbachol (CCH), a cholinergic receptor agonist, have been reported to generate oscillatory behavior in all regions of the hippocampus *in vitro* (Bland et al, 1988). CCH-induced oscillations may provide a convenient *in vitro* model for studying the synaptic processes that contribute to the behaviorally important type 2 theta observed *in vivo*.

We have examined the pharmacology of CCH-induced oscillations in CA1. Slices were maintained at 33° C in a submersion bath perfused with ACSF containing 2.5mM KCl. Extracellular EPSP's were recorded in s.radiatum; in some experiments an intracellular microelectrode was also used to record from s.pyramidale. CCH (50μM, 7-10 mins) induced oscillations in both the field (34-41 slices) and intracellular records (5-5 slices). The oscillations were sustained for the duration of the CCH application and were comprised of discrete bursts that were remarkably regular in terms of burst length (1.5±0.1secs n=20) and inter-burst interval (14±0.8 secs n=16) both within and across slices. The peak frequency of the oscillations was 7.2± 0.4Hz, well within the range of frequencies reported for theta *in vivo*.

The CCH-induced oscillations were blocked by atropine (5 μM, n=2) or DNQX (10 μM, n=2) but were largely unaffected by DL-AP5 (100 μM, n=4). Simultaneous recording in CA3 and CA1 suggested that the CCH-induced oscillation was initiated in CA3. Intriguingly while removal of CA3 (n=6) markedly attenuated the CCH-induced oscillations recorded in CA1 it failed to abolish it completely. Surprisingly bicuculline (10 μM, n=4) reversibly desynchronized the CCH-induced oscillations. These data are consistent with interneurons playing a role in determining the temporal properties of the CCH-induced oscillations. Bland et al, 1988. Brain Res. 447: 364-368. Supported by: NINDS NS30500.

313.4

CHOLINERGIC-DEPENDENT PLATEAU POTENTIALS SYNAPTICALLY EVOKED IN HIPPOCAMPAL PYRAMIDAL NEURONS. D. Doll, D.D. Fraser and B.A. MacVicar*, Neuroscience Research Group, University of Calgary, Calgary, AB, Canada T2N 4N1

The intrinsic activation of cholinergic-dependent plateau potentials (PP) involves the interplay between calcium entry and the Ca^{2+}-activated non-selective cation conductance (Fraser and MacVicar, *J. Neurosci.* In press). We have examined the response of pyramidal neurons to synaptic activation following muscarinic stimulation to determine if similar PPs could be synaptically evoked. Whole cell current clamp recordings were obtained from CA1 pyramidal neurons in 450 μm slices and synaptic responses were elicited from Schaeffer collateral stimulation. In oxotremorine (OXO; 15 μM; muscarinic agonist) PPs could be evoked intrinsically with intracellular current injection but not by synaptic stimulation even at 100 Hz for 800 ms. However when GABAergic receptors were inhibited with bicuculline ($GABA_A$ antagonist; 30 μM) and CGP35348 ($GABA_B$ antagonist; 50 μM) synaptically evoked PPs were recorded in OXO that were identical in duration, amplitude and waveform to intrinsically evoked PPs. These results indicate that synaptic inputs can trigger the generation of PPs within pyramidal neurons following muscarinic receptor activation when GABA receptors are also inhibited. Supported by MRC Canada.

313.5

PURINERGIC RECEPTORS INVOLVED IN VOLUME RECOVERY IN SPREADING DEPRESSION. B. Hampel*, S. Duffy and B.A. MacVicar, Neuroscience Research Group, University of Calgary, Calgary, Alberta, Canada T2N 4N1

The role of ATP-activated purinergic receptors in the various phases of spreading depression was investigated using receptor antagonists and imaging of intrinsic optical signals. Spreading depression was induced in rat hippocampal slices (400 μm) by superfusion of ouabain (100 μM) and was monitored by imaging changes in light transmittance at 700±10 nm. As we have previously shown spreading depression is associated with a propagating wave-like increase in light transmittance that is followed in dendritic regions by a rapid decrease below resting levels. The decrease in light transmittance following spreading depression was attenuated reversibly by the purinergic antagonist suramin (100 μM to 1 mM). These results suggest that activation of purinergic receptors plays an important role in regulatory volume decrease following spreading depression. Furthermore the propagation of spreading depression and the inhibition of the dendritic transmittance decrease by suramin was still observed in 0 external Ca^{2+} indicating that these effects are independent of Ca^{2+} influx.

Supported by MRC Canada.

313.6

REGULATION OF VOLUME RESPONSES DURING SPREADING DEPRESSION IN HIPPOCAMPAL SLICES. T.A. Basarsky*, B.A. MacVicar. Neuroscience Research Group, University of Calgary, Calgary, AB, Canada, T2N 4N1.

Previously our lab has examined the dynamics of intrinsic optical signals in hippocampal slices during ouabain induced spreading depression. Intrinsic optical signals (light transmittance) at 700 ± 10 nm were imaged from 15-25 day old (400 μm) hippocampal slices. At these wavelengths changes in the intrinsic signals are largely dominated by changes in cell volume. During spreading depression there is a transient increase in light transmittance that undergoes a wave-like propagation across the entire hippocampal slice, which is followed by a rapid decrease in dendritic regions to below control levels, indicating extensive cellular volume changes during spreading depression. Since volume-sensitive organic osmolyte-anion channels are present in a number of cells we have examined the involvement of these channels in spreading depression. The anion channel inhibitors NPPB (5-nitro-2-(3-phenylpropylamino) benzoic acid) (100 μM) or niflumic acid (100 μM) did not significantly affect the onset of spreading depression, whereas the subsequent decrease in light transmittance was attenuated. This raises the possibility that these channels mediate flux of ions or other compounds, such as amino acids, that play an active role in spreading depression.

Supported by the MRC of Canada (BAM) and an AHFMR Post-doctoral Fellowship (TAB).

313.7

EXPRESSION OF CHOLINERGIC-DEPENDENT PLATEAU POTENTIALS REQUIRES PHOSPHATASE-INDUCED DEPHOSPHORYLATION. D.D. Fraser* and B.A. MacVicar, Neuroscience Research Group, University of Calgary, Alberta, Canada. T2N 4N1.

We have previously identified a novel cholinergic-dependent plateau potential (PP) in CA1 pyramidal neurons that relies on the interplay between HVA Ca^{2+} channels and the Ca^{2+}-activated non-selective cation conductance (Fraser and MacVicar, *J. Neurosci.* in press). In the presence of carbachol, the PP was elicited by action potential firing and is reminiscent of ictal depolarizations. The PP was depressed by the non-selective muscarinic antagonist atropine (1 μM; n=7) or individual M_1/M_3 receptor antagonists (4-DAMP > pirenzipine > > *p*-F-HHSiD; n=3 at 1 μM for each), and completely inhibited by intracellular perfusion with 2 mM GDP-β-S (n=3). Surprisingly, the PP was still evoked in the presence of a PLC inhibitor [either 3 μM U-73122 (n=6) or 10 μM manoalide (n=3)] or following disruption of $[Ca^{2+}]_i$ stores [either 1 μM thapsigargin (n=4) or 400 μM IP_3 (n=4)]. PPs were also observed following intracellular perfusion with a non-selective protein kinase inhibitor (300 μM H-7; n=4) or a phosphorylation-inhibiting cocktail (0 Mg^{2+}, 5 mM ADP-β-S, 50 μM dinitrophenol, 0.1 mg/ml alkaline phosphatase; n=4). In contrast, the PP could not be generated following incubation of the slices in 1-2 μM calyculin-A, an inhibitor of protein phosphatases (PP-1, PP-2A; n=3), or following a 20 min intracellular perfusion with 5 mM ATP-γ-S (n=6). These data indicate that M_1/M_3 receptors are coupled to protein phosphatases, either directly or indirectly, via G-proteins. Furthermore, the expression of cholinergic-dependent PPs requires phosphatase-induced dephosphorylation.

313.8

INHIBITORY SYNAPTIC CURRENTS AND AGONIST-INDUCED RESPONSES IN GANGLION CELLS FROM RAT RETINAL SLICES. D.A. Protti*[1], H. M. Gerschenfeld[2] and I. Llano[1]. [1]Arbeitsgruppe Zelluläre Neurobiologie, Max-Planck-Institut für Biophysikalische Chemie, Göttingen, 37077, FRG and [2]Ecole Normale Supérieure, Paris, France.

Ganglion cells in 200 μm thick retinal slices from rats aged 4 to 7 weeks were studied using tight-seal whole cell recordings (wcr). Ganglion cells, selected by their position in the ganglion cell layer, were characterized by the presence of extracellularly recorded action potentials in the cell-attached configuration and of TTX-sensitive, voltage dependent sodium currents in wcr. In all cells studied, the prevalent spontaneous postsynaptic currents were inhibitory (IPSCs). Two different types of IPSCs were found. The first was sensitive to bicuculline (2-10 μM) and SR95531 (1-3 μM) while the second was sensitive to strychnine (0.3-2 μM) and insensitive to $GABA_A$ receptor antagonists. They were respectively identified as GABAergic and glycinergic. GABAergic IPSCs occurred in a higher proportion of cells and their frequency was usually diminished in the presence of TTX. Glycinergic IPSCs often had a slow decay phase with discrete single channel closings and reopenings having a prevalent conductance level of 45-50 pS. Bath applications and puffs of GABA and glycine elicited responses in all ganglion cells tested. In symmetrical Cl^- solutions, GABA-activated currents had outwardly rectifying I-V relations, while currents elicited by glycine had linear I-V relations. In cells dialyzed with low internal Cl^-, IPSCs and agonist-induced currents reversed at membrane potentials close to E_{Cl^-}, indicating that the underlying channels were Cl^- selective. These data provide a first characterization of IPSCs in ganglion cells from mammalian retinal slices. D.P holds an A. von Humboldt fellowship.

313.9

CHARACTERISTICS OF SYNAPTIC TRANSMISSION IN RAT OTIC GANGLION CELLS. R. J. Callister* & P. Sah. The Neuroscience Group, Faculty of Medicine & Health Sciences, University of Newcastle, NSW 2308, AUSTRALIA

Neurons in the otic ganglion (OG) innervate several important structures in the head, including the parotid salivary gland and cerebral blood vessels. In this report we describe the physiological properties of OG neurons and their synaptic connections. Ganglia were removed from halothane-anesthetized adult rats and pinned out in a perfusion chamber. Recordings were made at room temperature (20-23° C) with KCl-filled microelectrodes (50-140 MΩ) using standard intracellular techniques. Neurons (n = 32) had resting potentials of -56 ± 6.6 mV (mean ± SD), input resistances of 119 ± 60 MΩ, and membrane time constants of 12.8 ± 5.7 ms. Action potentials had amplitudes of 83 ± 8.3 mV and were followed by an afterhyperpolarization lasting 331 ± 97 ms (time to 90% decay). Most neurons (70%, n = 52) were innervated by a single preganglionic axon. Stimulation of preganglionic afferents generated a suprathreshold synaptic input which was markedly reduced by the nicotinic antagonists mecamylamine (20-100 μM) and d-tubocurarine (100 μM). In the presence of eserine (10-20 μM), low frequency synaptic stimulation (1-2 Hz) resulted in a sustained depolarization (9.5 ± 4.5 mV, n=10) which persisted throughout the period of stimulation (up to 180 s). This depolarization was accompanied by an increase (58 ± 16%) in conductance, was insensitive to atropine (1 μM) and was blocked by d-tubocurarine (100 μM). Upon cessation of stimulation, membrane potential slowly (16.6 ± 6.7 s recovery time constant) recovered to a level below (4.5 ± 2.9 mV) that of control. This hyperpolarization was accompanied by a decrease (21 ± 11 %) in conductance. Together, these results show that fast synaptic transmission is mediated by nicotinic acetylcholine receptors in OG neurons. Furthermore, our data suggests that when acetylcholinesterase is blocked, diffusion of acetylcholine away from the synaptic cleft may proceed slowly. (Supported by the National Health & Medical Research Council of Australia)

313.10

THE MUSCARINIC POSTSYNAPTIC EFFECT OF DEXMEDETOMIDINE (DMT) ON SYMPATHETIC GANGLIONIC TRANSMISSION J. B. McCallum, Q. Hogan, and Z. J. Bosnjak.* Department of Anesthesiology, Medical College of Wisconsin, Milwaukee, WI 53226.

The present study provides direct evidence for a muscarinic rather than nicotinic site of action for the α_2 agonist DMT. Twelve stellate ganglia were removed from adult mongrel dogs after halothane anesthesia, desheathed and superfused with Krebs' solution equilibrated with a mixture of 97% O_2 and 3% CO_2 and maintained in a tissue chamber at pH of 7.4±0.05 and 37±0.5°C. Fast and slow excitatory postsynaptic potentials (EPSP) were generated by subthreshold stimulations of the preganglionic T_3-ramus at a frequency of 0.4 Hz to reduce the number of postsynaptic receptors occupied by acetylcholine. Nicotinic stimulation was elicited with 1,1-dimethyl-4-phenylpiperazinium (DMPP, 10 μM) in the presence of atropine sulfate (20 μM), and muscarinic stimulation was evoked by (+)-*cis*-dioxolane (CD, 10 μM) in the presence of hexamethonium (20 μM). Nicotinic and muscarinic stimulations were given before and after application of 70 μM DMT. DMT abolished the increase in fast (550%) and slow (275%) EPSP after muscarinic stimulation, while DMT did not reduce the increase (42%) in fast EPSP after DMPP application. These results indicate that the inhibitory action of DMT resides in the late portion of the action potential, possibly via cellular hyperpolarization due to potassium channel activation.

313.11

ELECTRICAL PROPERTIES AND SYNAPTIC CURRENTS OF RAT CHROMAFFIN CELLS IN SITU. R. Kajiwara[1], O. Sand[2], Y.Kidokoro*[3], M.Barish[4] and T. Iijima[1]1 Electrotechnical Lab., Japan, 2 Univ. Oslo, Norway, 3 Univ. Gunma, Japan, 4 Beckman Res. Inst., USA

Adrenal chromaffin cells originate from the neural crest and are innervated by terminals from the splanchnic nerve. The cells have neuron-like electrical properties and have been extensively used as a model system for studies of voltage-gated ion channels and non-muscle-type nicotinic acetylcholine receptors. Most of these studies have been performed on cultured cells, and only data obtained using traditional microelectrodes have previously been reported from chromaffin cells in situ. We have performed whole-cell "tight seal" voltage clamp recordings from chromaffin cells in 300 μm slices of rat adrenal medulla. The general electrophysiological properties of the cells in situ were in agreement with previous reports on cultured cells. In particular, the input resistance was usually larger than 1 Gohm, a result not consistent with previous suggestions of electrical coupling between chromaffin cells in situ. The peak inward current evoked by depolarizing voltage steps reached a maximum value of 4-6 nA at about 0 mV, and displayed an I/V-relation and inactivation properties characteristic of voltage-gated Na^+-current. The outward current showed the bell-shaped I/V-curve characteristic of Ca^{2+}-activated K^+-current. Although preparation of the slices is likely to disconnect superfical chromaffin cells from their nerve input, about 10% of the cells studied displayed excitatory postsynaptic currents (EPSC) of 200 pA-1 nA upon direct electrical stimulation of sympathetic nerve branches in the slice. An EPSC of this magnitude will provide a cell exhibiting Gohm input resistance with a large safety factor for spike initiation. The EPSCs recorded from a particular cell frequently showed several discrete amplitudes, suggesting the presence of multiple active synaptic sites. The EPSC was completely and reversably blocked by 100 μM hexamethonium, an indication that only nicotinic ACh receptors contributed to the observed EPSCs. This is the first reported description of the EPSCs of chromaffin cells.

313.12

EXPERIMENTAL AND MODELING ANALYSIS OF MINIATURE ENDPLATE CURRENT VARIABILITY: EFFECTS OF TEMPERATURE AND CHOLINESTERASE INHIBITION. J.R. Stiles*, I.V. Kovyazina, E.E. Salpeter and M.M. Salpeter. Section of Neurobiology & Behavior, Cornell University, Ithaca, NY 14853-2702.

We investigated synaptic current variability at endplates, where acetylcholine receptors (AChR's) are present at high density and in great excess over released ACh. Acetylcholinesterase (AChE) density is species dependent and varies widely, predicting that miniature endplate current (mEPC) fall time (t_f) is critically dependent over a very narrow range (Anglister et al., 1994, Neuron 12:783). The mEPC shape and short rise time (t_r, 20-80% ~80 μs) can be measured accurately using extracellular (EC) recording, and can be modeled using passive diffusion of ACh through a rapidly expanding vesicular fusion pore (Stiles et al., 1996, PNAS, in press). In the present study, we examined the dependence of t_r and t_f on temperature and AChE inhibition using EC recording and lizard intercostal muscle. We used a wide range of AChE inhibition conditions, all of which gave complete (>98%) block by biochemical and histochemical criteria. Increased mean t_f and t_f variability (up to 3-4 fold) nevertheless showed a marked dose response to inhibition conditions, while t_r increases were less sensitive. These findings are consistent with 3D Monte Carlo modeling predictions, and indicate that cleft geometry and sparsely packed transmitter removal sites can profoundly influence current decay times. Temperature changes from 40°C down to ~12°C increased t_f and its variability by only ~60%. However, at lower temperatures (down to 3°C), 2-3 fold increases were seen. Similar results were obtained for t_f. We hypothesize that a change in membrane fluidity underlies this temperature sensitivity, and from modeling studies suggest that the primary effect is on AChR gating rather than on ACh release. Additional studies utilizing partial AChR blockade and quantitative voltage clamp may help to further define pre- and postsynaptic factors contributing to mEPC variability.

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313.13

ATP-RECEPTOR MEDIATED SYNAPTIC TRANSMISSION IN THE DORSAL HORN OF THE RAT SPINAL CORD. P. A. Goldstein*, C. J. Lee, R. Bardoni, J. G. Gu & A. B. MacDermott. Depts. of Physiology and Cellular Biophysics and Anesthesiology and the Center for Neurobiology & Behavior, Columbia Univ New York, NY 10032.

ATP-receptor mediated synaptic transmission has been demonstrated in only one area of the CNS, the habenula (Edwards *et al.* 1992). While ATP excites a subpopulation of rat dorsal horn (DH) neurons (Jahr & Jessell 1983), direct evidence of ATP-receptor mediated synaptic transmission in the spinal cord has been elusive. Using Fura-2 Ca^{2+}-imaging we have demonstrated that exogenously applied ATP elevates intracellular Ca^{2+} ($[Ca^{2+}]_i$) by P_{2X} receptor activation and direct electrophysiological measurements of evoked EPSCs have demonstrated that these receptors are synaptically activated. Ca^{2+} measurements were made on acutely dissociated DH neurons prepared from young (P7-10) animals. ATP, but not α,β-Me-ATP, evoked large increases in $[Ca^{2+}]_i$ and this increase could be completely or partially blocked by the P_{2x} receptor antagonist, suramin (n=4). This pharmacological profile suggests that these neurons express the P_{2X2}, P_{2X4}, P_{2X5}, and/or P_{2X6} subunits, although the distribution of cRNA coding for those subunit excludes P_{2X5} (Collo *et al.* 1996). Synaptic recordings were made on lamina II DH neurons in acutely prepared transverse sections of cervical spinal cord. In the presence of CNQX, APV, bicuculline, and strychnine, a small (-10 to -30 pA at -70 mV) EPSC was detected in some cells. This synaptic current was completely blocked by suramin in 3 cells, partially blocked (63.9 ± 8.9%) in 12 cells, and minimally blocked in 3 cells (12.8 ± 4.2%). These data indicate that P_{2X} receptors contribute to synaptic transmission in the spinal cord dorsal horn.

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313.14

OXYTOCIN-EVOKED CURRENT IN VAGAL NEURONS: NEGATIVE MODULATION BY cAMP. S. Alberi* and M. Raggenbass, Department of Physiology, University Medical Center, CH-1211 Geneva 4, Switzerland

The nonapeptide oxytocin can increase the excitability of vagal neurons in brainstem slices of the rat by generating a sustained inward current which is sodium-dependent and TTX-insensitive. This action is mediated by G protein activation and is independent of an increase in the intracellular calcium concentration (Soc. Neurosci. Abstr., 21, 591, 1995). We have used whole-cell recordings to investigate a possible interaction between the cAMP-dependent pathway and the peptide-induced current. We found that whereas in control neurons the current generated by oxytocin at 0.2 μM was on average 55 ± 5 pA, in neurons loaded with 100 to 500 μM Br-cAMP, this current was reduced to 25 ± 5 pA (n = 36). A similar decrease in the amplitude of the peptide-specific current was also observed following inclusion into the patch pipette of forskolin, an activator of adenylyl cyclase, at 10 μM and 20 μM. By contrast, loading neurons with Br-cGMP, at 100 to 500 μM, had no sinificant effect on the oxytocin response. The action of Br-cAMP was probably direct, rather than mediated by protein kinase A (PKA). Indeed, Rp-cAMPS and Sp-cAMPS, an inhibitor and a activator of PKA respectively, when included into the patch pipette at 500 μM, did not modify the average amplitude of the oxytocin-induced current. These results suggest that part of this current could be mediated by cAMP. Alternatively, cAMP could negatively regulate the peptide-evoked current without being a second messenger by itself.

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POSTSYNAPTIC MECHANISMS: GABA SIGNALS

314.1

ROLE OF BICARBONATE IN THE GENERATION OF GABAERGIC SPONTANEOUS ACTIVITY IN THE INHIBITORY NETWORK OF THE RAT HIPPOCAMPAL SLICE. K. Lamsa* and K. Kaila, Department of Biosciences, Division of Animal Physiology, P.O. Box 17, 00014 University of Helsinki, Finland.

4-aminopyridine (4-AP) is an interesting convulsant agent in that it can evoke epileptiform activity in the hippocampus despite its potentiating effect on GABAergic transmission. In brain slices exposed to blockers of ionotropic glutamate antagonists, 4-AP has been shown to induce a bicuculline-sensitive wave of activity that spreads through the preparation (e.g.Perreault & Avoli 1992: J.Neurosci.12:104). This is associated with a $GABA_A$ receptor-mediated long- lasting depolarization (LLD) in pyramidal neurons and with an increase in the extracellular K^+ concentration ($[K^+]_o$). The present work was done on rat hippocampal slices in an interface chamber to examine the ionic basis of the generation of the LLDs. We employed conventional intra- and extracellular microelectrodes, as well as ion-selective electrodes to measure changes in $[K^+]_o$ and extracellular pH (pH_o). The LLDs were linked to an alkaline pH_o transient and to a transient rise in $[K^+]_o$ with a time course similar to the depolarization. All these responses were inhibited in the nominal absence of CO_2/HCO_3^- and following application of a membrane- permeant inhibitor of carbonic anhydrase (ethoxyzolamide, 50 μM). Inhibition of extracellular carbonic anhydrase with benzolamide (10 μM) blocked the alkaline pH_o transients but had no significant effect on the LLDs or on the $[K^+]_o$ shifts. Application of pentobarbiturate (100 μM), a positive allosteric modulator of $GABA_A$ receptors, enhanced the amplitude and frequency of the LLDs and of the $[K^+]_o$ and pH_o transients. These results suggest that depolarizing bicarbonate currents mediated by $GABA_A$ receptors play a critical role in the excitatory coupling of interneurons within the inhibitory network, and hence (see abstract by Voipio et al.) in the generation of the LLDs in principal neurons. *Supported by the Academy of Finland and by the Sigrid Juselius Foundation.*

314.2

RISE IN EXTRACELLULAR K^+ AND FALL IN Ca^{2+} INDUCED BY TETANIC STIMULATION OF INHIBITORY INTERNEURONS IN AREA CA1 OF RAT HIPPOCAMPAL SLICES. J. Voipio*, K. Lamsa, A.-M. Autere, S. Smirnov, T. Taira, K. Kaila, Department of Biosciences, Division of Animal Physiology, P. O. Box 17, 00014 University of Helsinki, Finland.

Brief tetanic stimulation (40 or 100 pulses applied at 100 or 200 Hz) induced fast rise in the interstitial K^+ concentration ($[K^+]_o$) from 3 to 8 mM, as measured with K^+-selective microelectrodes placed at a distance of 0.5 mm from the site of stimulation in the CA1 stratum radiatum of rat hippocampal slices in an interface recording chamber. Application of ionotropic glutamate antagonists (10 μM NBQX + 80 μM AP5) had a negligible effect on the K^+ shift, but it was fully blocked by the $GABA_A$ receptor antagonist, picrotoxin (100 μM). The rise in $[K^+]_o$ was associated with a fall in free extracellular Ca^{2+} from 1.6 mM to 1.4 mM, which indicates that synaptic activation of $GABA_A$ receptors can lead to neuronal uptake of calcium in the adult hippocampus. Both ionic shifts were inhibited in the presence of a membrane-permeant inhibitor of carbonic anhydrase (ethoxyzolamide, 50 μM), or in the nominal absence of CO_2/HCO_3^-. The $GABA_A$ receptor-mediated K^+ transient had a latency of 100 ms and a time-to-peak of 900-1200 ms, which closely paralleled the time course of the depolarizing phase of the biphasic hyperpolarizing/depolarizing postsynaptic potential that was simultaneously recorded in pyramidal neurons. Experiments with intracellular inhibitors of K^+-Cl^- cotransport (such as 100 μM DIOA), applied via whole-cell recording electrodes, suggested that the change from hyperpolarizing to depolarizing of the postsynaptic voltage response is due to a carrier-mediated influx of Cl^-, driven by the rise in $[K^+]_o$ that appeared to originate from the activity of inhibitory interneurons. Taken together, the present data suggest that ionic shifts evoked upon activation of the inhibitory network play an important role both in the generation and modulation of neuronal activity evoked by tetanic stimulation in area CA1. *Supported by the Academy of Finland and by the Sigrid Juselius Foundation.*

314.3

$GABA_A$ RECEPTORS MEDIATE A POST-TETANIC EXCITATION IN CA1 AREA IN RAT HIPPOCAMPAL SLICES. T. Taira*, K. Lamsa and K. Kaila, Department of Biosciences, Division of Animal Physiology, P.O. Box 17, 00014 University of Helsinki, Finland. Email Taira@katk.helsinki.fi.

The main excitatory drive in the adult hippocampus is thought to be provided by glutamate. However, in the absence of glutamatergic transmission $GABA_A$ receptor-mediated depolarizing postsynaptic potentials can be evoked in CA1 pyramidal neurons of rat hippocampal slices upon high-frequency stimulation of interneurons. To elucidate the role of GABA as an excitatory transmitter we studied the contributions of glutamatergic and GABAergic synaptic inputs to excitation of CA1 pyramidal neurons. The experiments were done with conventional intracellular microelectrodes in an interface chamber. Under control conditions, tetanic (100-200 Hz/0.5 s) stimulation applied to s. radiatum typically resulted in a triphasic depolarization/hyperpolarization/sustained depolarization sequence. The late depolarization gave rise to a prolonged train of spikes. Antagonists of ionotropic glutamate receptors (10 μM NBQX, 80 μM AP5, 50 μM ketamine) blocked the initial fast depolarization, but they had little effect on the late depolarization and the associated spiking. In contrast to this, the late depolarization and spike firing were selectively blocked (within a time window of 3-6 minutes) following exposure to the $GABA_A$ receptor antagonist, picrotoxin (PiTX, 100 μM). Paradoxically, at this early stage of PiTX application, overall neuronal excitability was attenuated to a higher degree than what was achieved by the glutamate antagonists. The present results suggest that intense activation of GABAergic interneurons may accentuate neuronal excitation under physiological and pathophysiological conditions. *Supported by the Academy of Finland and by the Sigrid Juselius Foundation.*

314.4

SPATIO-TEMPORAL MATURATION OF INHIBITORY SYNAPTIC TRANSMISSION IN GRANULE CELLS OF THE RAT DENTATE GYRUS. G.S. Hollrigel* and I. Soltesz, Department of Anatomy and Neurobiology, University of California Irvine, Irvine, CA 92717.

Granule cells in the adult dentate gyrus receive tonic inhibitory input from GABAergic synapses located near the soma (Soltesz et al., 1995). A higher concentration of GAD-containing cells can be found slightly above the outer border of the developing granule cell layer (Dupuy and Houser, 1995), and the first synapses onto granule cells appear on proximal dendrites (Lübbers and Frotscher, 1988). We determined whether these early synaptic contacts were functional, and to what extent their location influences the kinetics of the synaptic currents.

Miniature IPSCs (mIPSCs) were recorded from granule cells in the dentate gyrus in brain slices from neonatal and adult rats. Slices were perfused with 36°C artificial cerebral spinal fluid containing glutamate receptor antagonists and tetrodotoxin.

Miniature IPSCs were detected within the first 24 hours after birth. Between P0 and P4 the mean frequency of the mIPSCs was 0.59±0.21Hz, and it increased with age to the adult value of 6.04±1.62Hz. The rise time constants were slower for mIPSCs recorded from P0-P4 rats (0.32±0.04ms) and attained adult values by P21 (0.18±0.01ms). Similarly, the mean decay time constant for P0-P4 granule cells equaled 10.63±0.96ms, and by P21, it was similar to that of adult granule cells (4.91±0.09ms). In contrast, the peak amplitude of the averaged mIPSCs was not significantly different from the adult value (64.75±3.19pA).

These data indicate that immediately after birth, IPSCs are slower rising and slower decaying than in adult granule cells. The slower kinetics of IPSCs in young granule cells may be due to both electrotonic filtering and differential $GABA_A$ subunit expression patterns.

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314.5

NEURONAL PROPERTIES IN ORGANOTYPIC RAT HIPPOCAMPAL SLICE CULTURES. R.J.Holland, N.Best, J.Mitchell, J.E.Chad* & H.V.Wheal, Neuroscience Research Group, University of Southampton, SO16 7PX, UK.

The ability to maintain neuronal architecture and connectivity in a long-term culture permits detailed investigations into the effects of neuroprotective agents. We are using the method of Stoppini *et al* (1991) to produce cultures derived from 400μm hippocampal slices from P8 rats. Experiments were conducted after 10-15 days in culture. Neurones in control slices were shown to be viable due to their fluorescent dye exclusion, maintenance of Ca^{++} homeostasis and electrophysiological properties. We are investigating the role of synaptic inhibition in controlling inhibitory neurones. Within these cultures we routinely observe non-pyramidal neurones lying at the CA1/2 stratum pyramidale/radiatum boundary. We have compared the electrophysiological properties of these neurones to pyramidal cells were recorded from in current clamp and set to -60mV. Preliminary data indicates that the number of action potentials induced by a 300ms 0.2nA current pulse was greater in the pyramidal cells (7±0.82, n=4) compared to non-pyramidal cells (3.25±0.96, n=4). Accomodation was greater in the non-pyramidal cells and the latency of the first spike was larger (40ms±40) than the pyramidal neurones (15ms±4).

From EM analysis of morpologically identified interneurones filled with neurobiotin there is evidence for the presence of inhibitory synaptic inputs. Local (200μm) monopolar stimulation appears to induce IPSCs, as well as EPSCs. We are presently investigating the source of inputs to these neurones and tracing their outputs in order to determine their role in hippocampal function.

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314.6

DELTA-RHYTHM OSCILLATIONS IN THE ENTORHINAL CORTEX MEDIATED BY INHIBITORY INTERNEURON ACTIVITY. V. Lopantsev* and M. Avoli, Montreal Neurological Institute and Dept. of Neurology & Neurosurgery, McGill University, Montreal, QC, H3A 2B4, Canada

Spontaneous rhythmic, oscillatory potentials were recorded extra- and intracellularly in adult rat entorhinal cortex slices in the presence of 4-aminopyridine (4AP, 50 μM). Field potential oscillations occurred at a frequency of 1.2±0.3/s (mean±SD; n=30 slices) either continuously or following GABA-mediated synchronous events. Simultaneous intracellular recordings showed corresponding rhythmical membrane depolarizations that had amplitude of 11.4±4.3 mV (n=24) and lasted 319±124.9 ms (n=16). These depolarizations gave rise to occasional single action potentials and were associated with a decrease (up to 86 %) of the input membrane resistance. The rhythmic depolarization amplitude varied linearly during steady changes of the membrane potential and had reversal potentials of -66.7±5.7 mV (n=13). These depolarizations increased in amplitude (up to 31.0±6.5 mV, n=3) after intracellular Cl^- injection, were blocked by a non-NMDA receptor antagonist, but were not influenced by antagonists of the NMDA receptor. The amplitude of the rhythmic field potentials was larger at depths 700-900μm, corresponding to entorhinal cortex layers IV-V. Intracellular recordings from presumably inhibitory interneurons revealed rhythmic depolarizations which gave rise to regular action potential discharge; and did not increase in amplitude during intracellular Cl^- injection. Thus these depolarizations may represent large, synchronous EPSPs generated by inhibitory interneurons. Our findings indicate that entorhinal cortex neurons can generate in the presence of 4AP delta-rhythm oscillations representing Cl^--dependent, depolarizing IPSPs, that are driven by inhibitory interneurons via excitatory, non-NMDA-mediated circuits. Supported by the MRC of Canada.

314.7

ANALYSIS OF INHIBITORY SYNAPTIC CURRENTS IN PYRAMIDAL AND NONPYRAMIDAL NEURONS OF THE RAT VISUAL CORTEX. Mario Galarreta* and Shaul Hestrin, University of Tennessee, Dept. of Anatomy & Neurobiology, TN 38163.

To characterize the properties of the $GABA_A$ receptors that mediate the inhibitory synaptic currents in the visual cortex, we have analyzed the miniature $GABA_A$ receptor-mediated inhibitory synaptic currents (mIPSCs), and the currents elicited by rapid application of GABA on somatic outside-out membrane patches, from excitatory pyramidal and inhibitory nonpyramidal neurons. Whole-cell recordings (20-25 °C) were made from visually identified cells, in slices of the visual cortex of 14-18 day-old rats.

mIPSCs (in the presence of CNQX, 10μM and TTX, 0.5 μM) recorded from pyramidal and nonpyramidal cells were similar. Their decay time course was described by a biexponential function with the following parameters: τ fast= 6.2 ± 1 ms (68.6 ± 14 %) and τ slow= 22.0 ± 7 ms for pyramidal cells in layer V (n= 17, mean ± SD); and τ fast= 5.0 ± 2 ms (61.7 ± 12 %) and τ slow= 24.7 ± 12ms, for 10 nonpyramidal cells (4 in layer V and 6 in layer I).

Decay of currents elicited by a brief (1ms) pulse of GABA (1 or 10mM) in outside-out patches from both types of cells, also required a biexponential fitting. The responses obtained in patches excised from pyramidal neurons (n=29) were described with a τ fast= 4.8 ± 3 ms (49.0 ± 9 %) and a τ slow= 48.3 ± 15, and those from nonpyramidal cells (n=7) with a τ fast= 4.6 ± 2 ms (52.4 ± 12 %), and a τ slow= 34.1 ± 8 ms.

The patch responses under these conditions did not accurately mimic the mIPSCs. However, the general resemblance we observed suggests that the kinetics of synaptic currents in these cells, primarily reflects receptor properties.

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314.8

SPONTANEOUS IPSCs IN RAT NEOCORTICAL LAYER I NEURONS. F.-M. Zhou* and J. J. Hablitz, Neurobiology Research Center, University of Alabama at Birmingham, Birmingham, AL 35294

The properties of inhibitory postsynaptic currents (IPSCs) in neocortical interneurons are not well described. Since layer I neurons of the rat neocortex are fast-spiking GABAergic interneurons, we have used these cells as model interneurons to study spontaneous (s) and miniature (m) IPSCs. Whole-cell patch clamp techniques were used in brain slice preparations. All recordings were made at ~22 °C, in the presence of 20 μM D-APV and 10 μM CNQX to block EPSCs. At a holding potential of -60 mV, using symmetrical Cl^- concentrations, amplitudes of sIPSCs ranged from 5 to 500 pA. mIPSC amplitudes were less than 80 pA at -60 mV. Both s- and mIPSCs reversed polarity at +5 mV, near the Cl^- equilibrium potential. Bicuculline (10 μM) reversibly blocked sIPSCs and mIPSCs, indicating mediation by $GABA_A$ receptors. At -60 mV, the 10-90% rise time was ~1.3 ms for averaged sIPSCs (13 cells) and ~1.2 ms for averaged mIPSCs (10 cells). The majority of individual sIPSCs and mIPSCs had double exponential decays with time constants of ~1-10 ms and ~20-70 ms. The decay was slower at more positive potentials. The slow component contributed 30-70% to the total amplitude. Dendritic filtering was not solely responsible for the observed variability. Differences in subunit composition and/or gating schemes for the $GABA_A$ receptors at different synapses may also be responsible. sIPSCs and mIPSCs were also recorded from 6 pyramidal neurons and the kinetics of sIPSCs and mIPSCs from layer I and pyramidal neurons were similar, suggesting that $GABA_A$ receptors were similar between the two cell types. Supported by NIH grant NS18145.

314.9

GABA-INDUCED MEMBRANE CONDUCTANCE AND THE INTEGRATIVE PROPERTIES OF NEOCORTICAL PYRAMIDAL CELLS. Christian Schmidt*, Peter König, and Ronald B. Langdon. The Neurosciences Institute, San Diego, CA 92121.

Total somatic input conductance (G_n) and cable response properties must play a critical role in determining (1) the number of synchronous inputs needed to drive cortical neurons to fire and (2) the time interval over which asynchronous inputs are effectively summed. We have studied the ability of GABA to modify membrane potential excursions (ΔV_m) induced by current injection and synaptic input, since summation of excitatory inputs must occur against a background of inhibitory, GABA-induced shunting conductance during normal cortical function. Most GABA receptors are located proximally, so GABA-gated conductance could hypothetically alter ΔV_m amplitudes at the soma more than ΔV_m time-course.

Our data were obtained by whole-cell, current-clamp recording from pyramidal cells (n = 7) in layer 3 of slices of occipital cortex from adult (130 to 235 g) rat. Under control conditions, cells exhibited a mean resting G_n of 20.5 nS, a mean principal response time-constant (τ_0) of 14.2 msec, and resting V_m between -65 and -83 mV. Current step and synaptic input levels were adjusted to have $-10 < \Delta V_m < +5$ mV. GABA (applied by dropping into the bath, $0.10 <$ final bath [GABA] < 7.5 mM) increased G_n by up to 500 nS (the upper limit observable) in a concentration-dependent manner (apparent $EC_{50} = 0.2$ mM). G_n and EPSP amplitudes were somewhat more sensitive to GABA than was τ_0. At 0.25 mM, GABA increased the mean G_n 2.7-fold and reduced the mean peak EPSP amplitude by 58%, while reducing τ_0 by only 38%. These data imply that GABA action *in vivo* would strengthen the requirement for spatial summation at the soma while shortening, to a lesser extent, the interval of effective temporal integration. Supported by Neurosciences Research Foundation.

314.10

INFLUENCE OF CORTICAL HYPERSYNCHRONISATION ON POSTSYNAPTIC ACTIVITY OF THE RAT NEOSTRIATUM *IN VITRO*. B. Schlösser, F. Rucker and G. ten Bruggencate. Department of Physiology, University of Munich, D-80336 Munich, Germany. Spon: EBBS.

Neuronal activity within the neostriatum is mainly determined by cortical afferents. In the present study, we investigated the influence of cortical hypersynchronisation on synaptic activity in the striatum *in vitro* using intracellular recordings.

Experiments were performed on rat neostriatal slices with preserved inputs from the medial agranular cortex. In the recording chamber the slices were kept submerged in artifical cerebrospinal fluid. Synaptic activity was evoked by intracortical electrical stimulation.

Focal application of the $GABA_A$ receptor antagonist bicuculline into the neocortex induced transient paroxysmal depolarisations in cortical neurons associated with large epileptiform field potentials indicating hypersynchronous activity. During the state of hypersynchronisation, the cortically evoked synaptic activity in the striatum was strongly potentiated as indicated by the marked increase in amplitude and duration of synaptic responses. The potentiated synaptic potentials were composed of an EPSP and a depolarizing $GABA_A$ receptor-mediated IPSP. The contribution of the $GABA_A$-receptor-mediated IPSP to the synaptic response increased from 14% under control conditions to 41% during hypersynchronisation of the neocortex.

The results demonstrate that hypersynchronous activity in neocortical neurons potentiates both excitatory and inhibitory synaptic transmission in striatal neurons. The pronounced increase of striatal inhibition can be attributed to a more than proportional activation of inhibitory striatal interneurons and/or to the activation of recurrent inhibitory mechanisms within the striatum. *Supported by the German Research Ministry.*

314.11

IMPACT OF SPONTANEOUS NETWORK ACTIVITY AND MINIATURE SYNAPTIC POTENTIALS ON MEMBRANE PROPERTIES OF CORTICAL PYRAMIDAL NEURONS: AN IN VIVO INTRACELLULAR STUDY H. Gaudreau*, E. J. Lang, A. Destexhe and D. Paré. Dept. of Physiol., Sch. of Med., Laval Univ., Quebec, Canada G1K 7P4.

In vivo intracellular recordings were obtained from area 5-7 cortical pyramidal neurons with pipettes containing KCl. TTX was applied locally to the recording area to block all network activity as shown by the lack of evoked responses to local stimuli and by the loss of Na spikes. Under these conditions cells had R_{IN} of 40-60 MΩ, whereas in the absence of TTX the R_{IN} ranged between 20-30 MΩ. Cells recorded with pipettes containing KCl and QX314, had intermediate R_{IN} values of 30-40 MΩ (no TTX present). TTX increased the time constants ≈2-fold. Thus, spontaneous network activity significantly influences the membrane properties of pyramidal neurons.

After TTX application, we observed frequent (5-20/sec), low amplitude (0.5-2 mV) depolarizing events, which were interpreted as miniature synaptic potentials (minis) independent of Na spikes. The amplitude of the minis was correlated with the R_{IN} of the cell, suggesting the presence of additional unobserved events occurring at more distant sites. Larger potentials were also seen, and appeared to result from summating minis. Presumably, the minis were generated at sites electrotonically close to the soma where most synapses are GABAergic. Consistent with the idea that the minis were reversed $GABA_A$ IPSPs, membrane depolarization reduced their amplitude. Experiments are currently underway to identify the involved transmitter. The minis produced transient drops in R_{IN}, suggesting that ongoing action potential-independent transmitter release is a major determinant of the membrane properties of pyramidal neurons in intact networks. Support: MRC, NINDS and FRSQ.

314.12

SYNAPTIC AND SYNAPTICALLY-ACTIVATED HYPERPOLARIZING CONDUCTANCES REGULATE THE RESPONSIVENESS OF LATERAL AMYGDALOID (LAT) PROJECTION NEURONS. E. J. Lang* and D. Paré. Dept. of Physiol., Sch. of Med., Laval Univ., Quebec, Canada G1K 7P4.

The inhibitory processes controlling LAT projection neurons (n=174) were studied with *in vivo* intracellular recordings. In contrast to *in vitro* results, spontaneous and synaptic potentials evoked by stimulation of major input and output structures of the LAT nucleus always consisted of an early EPSP truncated by a large monophasic hyperpolarization, which reversed at -84.7±1.3 mV when recorded with K-acetate (K-ac). The monphasic IPSPs probably resulted from divergent activity through intact cortical and LAT networks that smeared the activation of interneurons in time. Supporting this, convergence from widespread perirhinal cortical regions onto LAT neurons was demonstrated by evoking responses whose amplitudes and durations varied with stimulation site. At each site, the responses were intensity-dependent: low intensities evoked depolarizing responses and higher intensities hyperpolarizing ones. Orthodromic spikes could only be evoked in a narrow range of stimulus intensities.

The underlying conductances were investigated using K-ac, KCl, Cs-ac, and BAPTA filled pipettes. The hyperpolarization was demonstrated to be mainly comprised of a short latency Cl IPSP (presumably GABAA mediated) and Ca-dependent K conductance. A slowly activating K conductance (presumably a GABAB IPSP) also made a small contribution.

These results suggest that information processing in the LAT nucleus may be based primarily on quantitative differences in activation of intra-LAT circuitry and intrinsic conductances. Therefore, inputs from relatively small cortical ensembles may be the most effective stimulus for activating LAT neurons. Support: NINDS: 1F32NS1000601, MRC: MT-11562.

314.13

MODULATION OF EVOKED HIPPOCAMPAL FIELD POTENTIALS *IN VIVO* BY STRESS AND NEUROSTEROIDS

H.J.A. Beldhuis*, I.M. Nijholt, E. Dijkstra, T. Suzuki, S.F. de Boer and B. Bohus. Center for Behavioral and Cognitive Neurosciences, Dep. Animal Physiology, University of Groningen, PO Box 14, NL-9750 AA Haren, The Netherlands.

Neurosteroids (NSs) can rapidly alter the excitability of neurons by nongenomic mechanisms. Because the physiological significance of neurosteroids in intact organisms is still poorly understood, the main goal of this study was to examine the effects of NSs on transmission in the Schaffer collateral pathway to pyramidal cells in CA1 hippocampus in freely moving rats. In addition, since the hippocampal formation is implied in stress responses and levels of certain NSs are increased during stress, we investigated the effect of stressful stimuli in the same neuronal network.

Animals were chronically implanted with electrodes in the dorsal hippocampus to stimulate the Schaffer collaterals (SC), and recording electrodes to record dendritic pEPSPs in the CA1 stratum radiatum. Pharmacological manipulation of NS levels was performed by *ip* application of synthetic neurosteroids, *i.e.* dehydroepiandrosterone (DHEAS), allopregnanolone (ALLO) and 5α-dihydroprogesterone (5α-DHP). Social interactions with dominant conspecifics were used to induce stressful situations. Single and double pulse responses were evoked before and after experimental treatment.

Application of 5α-DHP, the main progesterone metabolite, had no effect on evoked responses. DHEAS increased pEPSP amplitude and suppressed paired-pulse inhibition, whereas ALLO and a conflict situation had the opposite effects. Together, these data suggest a role for NSs in modulating neuronal excitability at the SC-CA1 synapse *in vivo*.

These investigations are supported by the Dutch National Epilepsy Foundation.

315.1

CONDUCTION OF POSTSYNAPTIC POTENTIALS IN THE DENDRITES OF CULTURED HIPPOCAMPAL NEURONS AS MEASURED BY HIGH-SPEED LASER-SCANNING MICROSCOPY. A. Bullen* and P. Saggau, Division of Neuroscience, Baylor College of Medicine, Houston, TX 77030.

The conduction and integration of postsynaptic potentials in the dendrites of cultured hippocampal neurons was investigated using a high-speed laser-scanning microscope. Postsynaptic potentials were elicited at one or more presynaptic sites by focal stimulation (Bekkers & Stevens, 1995). A random-access laser-scanning microscope developed in our lab was used to acquire signals from fluorescent voltage-sensitive membrane probes (Patel et al, 1995). Transients in membrane potential were derived from the fractional change in fluorescence ($\Delta F/F$). Typically, recordings were made at 2 kHz sampling rate (per scanning spot) and digitially filtered at 300 Hz. Spatial resolution was limited by spot size at the level of the preparation (2 μm). Hippocampal neurons were harvested from E19 rat pups and plated at a density of 10^4 cells/cm^2. These cells were stained by bath application of voltage-sensitive dye (VSD; Di-8-ANEPPS, 100 μM). A DIC image was used to interactively select multiple locations for optical recording. Focal stimulation was accomplished by the application of hyperosmotic solution (bath solution + 500 mM sucrose) applied through a patch pipette positioned at strategic locations in the dendrites. Both EPSPs and IPSPs could be elicited depending on the nature of synapses present. Optical transients recorded from the soma with VSDs were highly correlated with corresponding current-clamp records. After noise reduction procedures (oversampling, ratioing and digital filtering) a signal-to-noise ratio of 5-10 could be achieved (detector bandwidth = 15 kHz) for postsynaptic potentials in small dendritic structures. The conduction and summation of postsynaptic potentials were examined in small dendritic processes and at branch points, up to 150 μm from the soma. Dendritic recordings were compared to electrical recordings made concurrently from the soma via a patch pipette. The conduction and integration of postsynaptic potentials elicited at different points throughout the dendritic tree of hippocampal neurons will be described. *Supported by NSF BIR-9521685.*

315.2

EFFECTS OF DENDRITIC MEMBRANE CURRENTS ON THE SHAPE OF EPSP's IN PYRAMIDAL NEURONS Paul A. Rhodes* and Alex M. Thomson, Mathematical Research Branch, NIDDK, National Institutes of Health, Bethesda, MD and Dept. of Physiology, Royal Free Hospital School of Medicine, London

Measurements of the EPSP's produced in neocortical pyramidal neurons by the firing of a single presynaptic pyramid reveal a wide variety of EPSP shapes, as well as a pattern of shape change as postsynaptic membrane potential is increased. In an effort to understand the generation of these features, in vitro measurements from connected pairs of layer V pyramidal neurons were simulated using compartment model methods, with reconstruction of the presynaptic axon (Deuchars et al. 1994) guiding the placement of presumed synaptic contacts at appropriate locations on the postsynaptic dendritic tree. Simulations were constrained both to match EPSP shape parameters measured under control conditions, and to account for the changes in EPSP shape observed in vitro as membrane potential of the postsynaptic neuron was varied, or NMDA antagonist added. The results indicate that for the first spike in a train much of the EPSP broadening seen with postsynaptic depolarization could be reproduced in the model by inactivation of the transient potassium (A) current, along with increased activation of the low threshold calcium (T) current, suggesting that dendritic voltage-gated currents could play an unexpectedly large role in controlling the shape of subthreshold EPSP's. Further, in these simulations dendritic Ca(T) (but not Na) current enhanced the broadening effect of synaptic NMDA current. These predicted consequences of dendritic voltage-gated channels draw attention to the local depolarization in the dendritic branches receiving input, and call into question approaches to the analysis of EPSP's which neglect currents in the dendritic membrane. We conclude that the dendritic currents activated by subthreshold synaptic conductance may considerably transform the impact of synaptic input.

315.3

A DECREASE IN Na^+ CURRENT CONTRIBUTES TO LOSS OF ACTION POTENTIAL AMPLITUDE IN DENDRITIC SPIKE TRAINS C. M. Colbert* and D. Johnston, Division of Neuroscience, Baylor College of Medicine, Houston, TX 77030

Action potentials initiated in the axon of central pyramidal neurons propagate "backwards" through the soma and dendrites. When the neuron fires trains of action potentials, the amplitude of action potentials recorded in the apical dendrites decreases toward a steady-state value in a frequency dependent manner (Spruston et al., Science 268:297-300, 1995; Callaway and Ross., J. Neurophysiol. 74:1395-1403, 1995). The mechanisms of the decrease in dendritic action potential amplitude are unknown. To test the hypothesis that a decrease in available Na^+ current contributes to the loss of action potential amplitude in the dendrites, we measured Na^+ currents in cell-attached patches on apical dendrites (20–200 μm from the soma) of pyramidal neurons in layer V neocortex, CA1 of the hippocampus, and the subiculum. K^+ currents were blocked by 4AP and TEA in the patch pipette. Action potentials were simulated by a step to a command potential of −10 mV for 2 ms from a holding potential near rest. Trains of action potentials were simulated by 7 steps at an interval of 20 ms. Ensemble averages were computed from at least 25 trains. A ratio of the ensemble current elicited by the last step to that elicited by the first step in a train was computed for each patch. When holding the patches at rest (~ −65 mV) between simulated action potentials, the ratio was 0.49±0.04 (mean±SEM, n = 8) (i.e. Na^+ current decreased during the train). When holding at 20 mV hyperpolarized to rest (~ −85 mV), the ratio was 0.72±.06 (n = 6). Thus, the voltage-dependence of the loss of Na^+ current is consistent with mechanisms such as slow Na^+ channel inactivation and voltage-dependent return from inactivation, but inconsistent with Na^+ accumulation. These results suggest that a decrease in Na^+ current contributes to the loss of action potential amplitude seen in the apical dendrites of pyramidal neurons. (NS11535, MH44754, MH48432 and MH10896).

315.4

PHARMACOLOGICAL MODULATION OF SPIKE PROPAGATION IN THE APICAL DENDRITES OF HIPPOCAMPAL PYRAMIDAL CELLS. H. Tsubokawa and W.N. Ross*, Dept. of Physiology, New York Medical College, Valhalla, NY 10595.

Na^+ dependent action potentials backpropagate over the apical dendrites of CA1 pyramidal neurons in an activity dependent manner (Jaffe et al., 1992; Spruston et al., 1995; Callaway and Ross, 1995). When recorded in the middle dendrites, the amplitudes of each spike in a train gradually decrease, but propagation of all spikes appears to be regenerative. In the distal dendrites the fall in amplitude after the first spike is more dramatic, and only the first spikes propagate actively to the end of the cell. Using whole cell recording from the middle dendrites we studied the gradual reduction in amplitude in trains of action potentials antidromically activated at 10 ms to 2 s intervals. The bath contained 50 μM DL-APV, 10 μM CNQX, and 10 μM bicuculline methiodide to block fast synaptic responses. Their presence had no effect on spike propagation. The recording pipette contained (in mM): 130 K-glu, 10 Na-glu, 4 NaCl, 4 Mg-ATP, 0.3 GTP, 4 Na_2-phosphocreatine, 10 Hepes (pH=7.2). Under these conditions addition of 50 μM carbachol reduced the amplitude modulation, making the spike heights almost equal. 10 μM carbachol or 20 μM norepinephrine had little or no effect on the amplitude profile. 500 μM Cs^+, 200 μM Ni^{2+} or 200 μM Cd^{2+} also reduced the amplitude modulation, but were often irreversible. These results suggest that the M-current, Q-current, and possibly the current underlying the slow AHP regulate spike propagation into the dendrites. However, changes in the resting potential and the composition of the intracellular solution complicate this interpretation.

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315.5

ACTIVATION OF PERSISTENT SODIUM CURRENT IN NEOCORTICAL LAYER V NEURONS CORRELATES WITH INCREASED INTRADENDRITIC SODIUM CONCENTRATION. T. Mittmann*, S.M. Linton, P.C. Schwindt and W.E. Crill, Dept, Physiol. & Biophys., Univ. Washington, Sch. of Med., Seattle, WA 98195.

In neocortical layer V neurons fluorescence imaging technique and intracellular recordings were combined to study changes in the intracellular sodium concentration ($[Na^+]_i$) due to activation of persistent sodium current (I_{NaP}). Brain slices (200-250 μm) were prepared from ketamine anaesthetized 15-36 day old rats. Neurons were loaded through the intracellular electrode with the sodium sensitive dye SBFI (8-12 mM). Images were recorded with a cooled CCD-camera system at 380 nm illumination. I_{NaP} was activated by both current clamp and voltage clamp methods. In current clamp a short depolarizing current pulse (20 ms) in Ca^{2+}-free ACSF containing Mn^{2+} (2 mM) and TEA (20 mM) evoked a single action potential (AP) following by a plateau depolarization (PD) lasting >1s. The TTX-sensitive PD is caused by I_{NaP} activation (Stafstrom et al., 1985). The PDs are associated with SBFI-fluorescence changes in the soma and apical dendrite, indicating an increased $[Na^+]_i$. The SBFI changes (ΔF and $\Delta F/F$) reached into the dendrites as far as dye could be visualized (50-300 μm) (n=10). Single APs induced no detectable SBFI fluorescence changes (n=3). During voltage clamp in normal ACSF a depolarizing voltage ramp (40mV/s) evoked persistent inward current and associated increase in somatic and dendritic $[Na^+]_i$ that was blocked by TTX (1μM). We conclude that both somatic and dendritic membrane generate I_{NaP}. Supported by NIH Grant NS16792, the Keck Foundation & an Alexander v. Humboldt fellowship to T. Mittmann.

315.6

THE MONOSYNAPTIC PERFORANT PATH INPUT TO CA3 PYRAMIDAL NEURONS: II. THE ROLE OF VOLTAGE-DEPENDENT CONDUCTANCES IN PROPAGATION OF EPSPS. N.N. Urban* and G. Barrionuevo *Department of Neuroscience and Center for the Neural Basis of Cognition, University of Pittsburgh, Pittsburgh, PA 15260.*

Models of CA3 pyramidal cells based on passive cable theory predict that perforant path (PP) EPSPs reaching the soma will be reduced in amplitude by ≈95% and will have very slow rise times. In contrast with these predictions, we have found that in whole-cell recordings from CA3 pyramidal cells, PP stimulation results in large (>25 mV peak amplitude), and fast (<8 msec rise time) EPSPs. These observations suggest that voltage dependent (VD) conductances in CA3 neurons influence the propagation of perforant path synaptic responses to CA3 cell somas. To test this hypothesis we examined the effect of blockers of VD channels on the somatic depolarization observed following stimulation of distal dendrites.

Distal dendrites of CA3 cells were stimulated either by PP synaptic activation or by direct depolarization using an extracellular stimulating electrode placed in *s. lacunosum moleculare* of CA3. When direct depolarization was used, synaptic transmission was blocked using either 500 μM $CdCl_2$ or a combination of glutamatergic antagonists. Local application of 1 μM TTX to the CA3 cell dendrites in *s. radiatum* blocked action potentials evoked by distal stimulation (N=5). This same application of TTX did not affect the amplitude of action potentials induced by somatic depolarization. In other experiments, subthreshold responses to synaptic and direct stimulation of distal dendrites were significantly attenuated by TTX as well as by low concentrations (10-30 μM) of $NiCl_2$ (average reduction by $NiCl_2$ 32%; N=6). These data suggest that VD sodium channels and T-type calcium channels may play a role in the amplification of perforant path inputs to CA3 cells.

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315.7

THE MONOSYNAPTIC PERFORANT PATH INPUT TO CA3 PYRAMIDAL NEURONS: I. PHARMACOLOGICAL CHARACTERIZATION.

Julia Berzhanskaya*, N.N.Urban and G. Barrionuevo *Department of Neuroscience and Center for the Neural Basis of Cognition, University of Pittsburgh, Pittsburgh, PA 15260.*

Much of our current understanding of hippocampal function is based on the classical view of the hippocampus as a "trisynaptic circuit".This view ignored the direct projection from the entorhinal cortex to CA3 and CA1 via the direct perforant path (PP). However, *in vivo* experiments have demonstrated that PP stimulation can elicit monosynaptically population spikes in CA1 and CA3 cells. In our experiments on CA3 pyramidal cells we have observed isolated PP-evoked EPSPs as large as 25 mV. These observations suggest that perforant path may provide an important excitatory input to CA3 pyramidal cells.

PP-evoked EPSCs in CA3 pyramidal cells were studied using whole-cell recording techniques. EPSCs were evoked by stimulation of PP fibers as they travel in the subiculum. Selectivity of PP stimulation was confirmed by laminar profile analysis showing a single current sink in the apical dendrites of CA3 cells at about 450 μm from pyramidal cell layer. A series of cuts was made to exclude polysynaptic contamination produced by possible activation of the mossy fibers.

Monosynaptic EPSCs were studied following blockade of inhibition. Application of D-APV(25 μM) and CNQX (10 μM) revealed that PP-evoked EPSCs have both AMPA and NMDA receptor mediated components. The NMDA component accounted for 30%+ 7% of the peak EPSC (at -50mV, N=6).

When inhibition was intact, PP stimulation elicited both EPSCs and IPSCs. Both fast and slow IPSCs were observed. The fast component of the IPSC reversed at the chloride equilibrium potential and was blocked by bicuculline (10 μM). The slow component of the IPSC reversed at K^+ equilibrium potential and was partially blocked by CGP3548 (50-100 μM). Supported by NS24288 and Howard Hughes Medical Institute Predoctoral Fellowship (to NNU).

315.8

PLATEAU POTENTIALS IN AMBIGUUS NEURONS IN NEWBORN MOUSE BRAIN STEM *IN VITRO*.

J.C. Rekling* and J. L. Feldman, Departments of Physiological Science and Neurobiology, UCLA, Box 951527, Los Angeles, CA, 90095-1527.

Whole-cell patch recordings from neurons located in the rostral part of the nucleus ambiguus were performed in rhythmic brain stem slices of newborn (1-3 day) mice (Funk et. al, J. Neurophys. '94). Neurons were visualized using IR video microscopy. Some neurons were weakly respiratory-modulated, showing trains of IPSPs in synchrony with inspiratory bursts of the XII nerve. Following suprathreshold depolarizing pulses or strong hyperpolarizing pulses, neurons displayed long-lasting depolarizing plateau potentials. A two-pulse stimulation paradigm revealed that the plateau potential was voltage-dependent, increasing in amplitude and duration more positive potentials. At more positive potentials the plateau potential lead to sustained repetitive firing that could be terminated by ~5 s hyperpolarizing pulses. The plateau potential was linked to a rise in intracellular Ca^{2+} because it was blocked by high pipette BAPTA concentrations. When the plateau potential was blocked with BAPTA a number of active properties resembling that of adult ambiguus neurons were uncovered, such as delayed excitation and postinhibitory rebound responses.

We conclude that ambiguus neurons in newborn mice have bistable properties leading to long-lasting firing after both excitatory and inhibitory input. Supported by NIH grant HL40959.

315.9

ACTIVITY-DEPENDENT EXTRACELLULAR ALKALINE SHIFTS IN RAT HIPPOCAMPUS ARE SUPPORTED BY EXTERNAL Ba^{2+}.

SE Smith,* II Grichtchenko & M Chesler. Depts. Physiol./Neurosurg. NYU Med. Ctr., 550 1st Ave., NY, NY 10016.

Activity-dependent alkaline shifts in area CA1 are blocked in 0 Ca^{2+} [1,2], suggesting involvement of plasmalemmal Ca^{2+}-H^+ exchange (Ca^{2+} ATPase) [3]. We therefore studied whether external Ba^{2+} could support agonist- and electrically-evoked alkaline shifts. In s. radiatum, AMPA-evoked alkalinizations were blocked in 0 Ca^{2+}, but recovered after addition of 50-500 μM Ba^{2+}. Adding 300 μM Cd^{2+}/100 μM Ni^{2+} reduced both control and Ba^{2+}-dependent alkaline shifts by ~50%. Activation of pyramidal cells (by 10-100 Hz current pulses along the soma-dendritic axis) in 20 μM CNQX/50 μM APV elicited alkalinizations of 0.1-0.2 unit pH in s. radiatum that were blocked by TTX, Cd^{2+}/Ni^{2+} or 0 Ca^{2+}. In 0 Ca^{2+} media, addition of 1-10 mM Ba^{2+} restored the alkaline shifts which could again be blocked by TTX or 300 μM Cd^{2+}/100 μM Ni^{2+}. Both the AMPA and the electrically-evoked Ba^{2+} responses were increased by benzolamide and persisted in HEPES-buffered media. These data demonstrate that Ca^{2+} entry is not an absolute requirement for the alkaline shift. If Ca^{2+}-H^+ exchange mediates these responses, the data suggest that either (**a**) Ba^{2+} is transported by the exchanger, or (**b**) electrical activity induces sufficient release of Ca^{2+} from internal stores. [1] Paalasmaa et al. (1994) *J. Neurophysiol.* 72:2031. [2] Smith et al. (1994) *Neuroreport* 5:2441. [3] Schwiening et al. (1993) *Proc. R. Soc. Lond. B* 253:285. Supported by the NIH (NS32123).

315.10

VOLTAGE ATTENUATION AND INTRACELLULAR RESISTIVITY IN NEOCORTICAL PYRAMIDAL NEURONS.

N.Spruston* and G. Stuart†. Dept. Neurobiology & Physiology, Institute for Neuroscience, Northwestern University, Evanston, IL 60208-3520. †Division of Neuroscience, John Curtin School of Medical Research., Australian National University, Canberra, ACT 0200 Australia.

Direct measurements of voltage attenuation in response to small somatic hyperpolarizing current pulses were obtained with simultaneous patch-pipette recordings from the soma and primary apical dendrite of layer V pyramidal neurons in slices of rat neocortex. Steady-state voltage decayed approximately linearly, with 50% attenuation 333 μm from the soma in control conditions. Addition of 3–5 mM CsCl to the bathing solution blocked the "sag" in voltage responses, presumably due to a block of a hyperpolarization activated conductance (I_h). Attenuation in the presence of CsCl was considerably less than in control, with 50% attenuation at 545 μm.

Neurons were routinely filled with biocytin, and three neurons fully reconstructed and modeled with NEURON. Using the membrane time constant determined from somatic and dendritic voltage responses in the presence of CsCl to constrain the passive membrane properties, an intracellular resistivity of about 150 Ωcm best described the observed voltage attenuation in CsCl.

A hyperpolarization-activated sag conductance (with kinetics modified slightly from Budde et al. J. Neurophysiol. 72:2737–2742, 1994) incorporated into the passive models revealed that the voltage attenuation observed in control conditions could only be reproduced if a large portion of the total I_h conductance was concentrated in the apical dendrites. Consistent with this observation, sag in dendritic voltage responses was greater than at the soma. These data suggest that a nonuniform distribution of I_h channels channels plays an important role in determining voltage attenuation in layer V pyramidal neurons and that its density is substantially higher in the distal apical dendrites than in the soma or basal dendrites.

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315.11

SUMMATION OF SYNAPTIC POTENTIALS IN LAYER V PYRAMIDAL NEURONS.

A.D. Reyes* and B. Sakmann, Max-Planck-Institut for Medizinische Forschung, D-69028 Heidelberg, Germany

The firing of cortical neurons depends on the amplitude of the composite synaptic potential reaching the soma and initial axon segment. The manner in which individual synaptic potentials originating in dendrites summate at the soma is yet unclear.

To examine how unitary excitatory postsynaptic potentials (EPSPs) summate, we performed simultaneous whole-cell recordings from three layer V pyramidal neurons wherein two neurons have a common input to a third neuron. Slices were made from P14 rats and maintained at 34°C. The presynaptic neurons were stimulated individually and simultaneously and the resulting EPSPs recorded in the soma of the third neuron. In most cases (15/20 triplets), the EPSPs summated linearly. Summation was linear: 1) at different subthreshold membrane potentials (-70 to -50 mV); 2) when the EPSPs were evoked at different delays; and 3) when the EPSPs were evoked in trains (10-66 Hz). In 3 cases, summation was supralinear and in 2 cases, sublinear.

To examine how inputs from different dendritic branches summate at the soma, simultaneous recordings were made from the soma, basal dendrites, and apical dendrites of individual neurons. Preliminary results indicate that current injection at either the apical and a basal dendrite or two basal dendrites produce voltage deflections that summate linearly at the soma.

These data suggest that in the subthreshold range, unitary EPSPs of layer V neurons summate linearly at the soma.

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315.12

RESONANCE PROPERTIES MEASURED FROM SOMA AND DENDRITES OF LAYER 5 NEOCORTICAL NEURONS.

B. Hutcheon*, I. Segev, P. Carlen, Y. Yarom. Department of Neurobiology, The Hebrew University, Jerusalem, Israel. 91904

Previous studies of the frequency responses of central neurons have shown that their subthreshold voltage-dependent conductances can generate electrical membrane resonances. These resonances confer band-limited frequency preferences on these neurons which may underlie spontaneous oscillations of the membrane potential or affect the coupling of periodic synatic inputs to firing. Recording sites on the somata and apical dendrites of layer 5 pyramidal neurons in neocortical slices from young rats were identified visually under infrared illumination with the aid of Nomarski optics and a video system. Low-frequency subthreshold resonances were observed from the cell body and also from the apical dendrite at distances greater than 100 μM from the soma. Recordings from the soma and dendrite of the same neuron showed that the resonant frequency was almost unchanged by position along the dendrite. In contrast, the results of simulations using a compartmental model of an idealized neuron with a resonance-generating current in the soma and a single passive dendrite showed that the resonant frequency measured from the dendrite is expected to be up to 30% lower than that measured from the soma depending on the electrotonic length of the dendrite. The discrepancies between our observations and the predictions from the model suggest that the currents generating resonance in neocortical neurons are distributed over both the soma and dendrites. This distribution of resonant properties may contribute to the efficient conduction of resonant-band signals along dendrites and attenuate or amplify post-synaptic potentials depending on their frequency compositions.

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315.13

Roles of Negative Feedback by Potassium Currents and Recurrent Inhibition

Jonghan Shin*, Div. of Biology, Caltech 139-74, Pasadena, CA 91125.

This abstract describes precision improvement of neural coding by internal negative feedback currents such as the delayed potassium current, etc. and external recurrent inhibition. The negative feedback by the delayed potassium current can reduce errors induced in transforminging analog potential of soma into nerve pulses. The errors related on neural coding are filtered by high pass filter which result from the negative feedback mechanism. Thus signal-to-noise ratio of neural coding is increased at low frequency band in which important informations exist. Moreover additional negative feedback via recurrent inhibition, etc. could make the high pass filter higher order, which result in more signal-to-noise ratio than without it. Also this could explain internal biophysical mechanism about power spectral shapes observed from many experimental preparations, which has a dip at low frequences and a positive slope between the low frequency band and high frequency band. In general, we know that the signal-to-noise ratio of neural coding would increase with increasing maximum mean firing frequency. But we cannot explain efficient neural coding of nerve cell completely, using this kind of oversampling and averaging concepts only. As a result, a kind of noise spectral shaping via negative feedback mechanism could address hidden secrets of the efficient neural coding. (Supported by a Colvin Fellowship from the Division of Biology of Caltech.)

315.14

CHARACTERIZATION OF VOLTAGE-GATED K^+ CHANNELS IN THE SOMA AND DENDRITES OF HIPPOCAMPAL CA1 PYRAMIDAL NEURONS Dax Hoffman*, J. Magee, and D. Johnston
Division of Neuroscience, Baylor College of Medicine, Houston, TX 77030

Single potassium channel recordings using cell-attached and outside-out patches were carried out on the somatic and dendritic membranes of CA1 pyramidal neurons from rat hippocampal slices. The three most frequently encountered types of potassium channels were a transient, A-type channel, a delayed-rectifier channel, and a large conductance, calcium-dependent channel. The transient, A-type channel is activated by moderate depolarization from the resting potential and has a single-channel conductance of 7–10 pS. Micromolar concentrations of 4-aminopyridine inhibit the current. The number of channels per patch has ranged from 2 to greater than 100. Inactivation is mostly voltage-independent with time constants of inactivation between 15 and 20 ms. A second, commonly observed potassium channel is a delayed-rectifier type. The delayed-rectifier is not encountered as frequently as the A-type channel. This delayed onset channel has a single-channel conductance of approximately 20 pS and is activated at potentials positive to –40 mV, more depolarized than the A-type channel. Another frequently observed outward current channel is inferred to be a calcium-dependent potassium channel from it's range of activation and single-channel conductance. This channel exhibited large unitary currents and single-channel conductance (90–140 pS) reminiscent of the BK or maxi-K channels. At potentials greater than +60 mV the single-channel conductance saturates, most likely due to magnesium block. (MH44754, MH48432, N511535).

315.15

ELECTROTONIC PROPERTIES OF NON-PYRAMIDAL NEURONS IN THE HIPPOCAMPAL CA3 REGION, **Raymond A. Chitwood* and David B. Jaffe, Div. of Life Sci., Univ. of Texas at San Antonio, San Antonio, TX 78249.**

Inhibitory neurons control firing activity in biological neural networks. We have been studying the properties of non-pyramidal neurons in the four extra-pyramidal subregions, *s. lacunosum-moleculare*, *s. radiatum*, *s. lucidum* and *s. oriens*, of hippocampal area CA3.

Whole-cell current-clamp recordings were made from non-pyramidal neurons in 14-28 day-old rat hippocampal slices. For cells in all four regions, the mean input resistance (R_N) was 521 MΩ ± 218 and the mean slowest membrane time constant, measured within the linear range of the membrane, was 68 ms ± 35 (mean ± S.D., n=96). All though there was large variance between neurons in each sub-field, there was no significant difference in passive membrane properties between the four subregions.

A subset of cells were labeled with biocytin or Neurobiotin for morphometric analysis. Electrotonic analysis of compartmental models constructed from these data was performed using NEURON. R_N of the modeled neurons were within 20% of measured values in 12 of 24 cells. Electrotonic analysis suggests that, in spite of their relatively shorter and more limited branching dendritic trees, there is significant voltage attenuation from most dendritic locations to the soma.

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POSTSYNAPTIC MECHANISMS: Ca^{2+} SIGNALLING

316.1

Focal photolysis of caged Ca using two-photon excitation

Christian Lüscher*, Peter Lipp, Hans-R. Lüscher & Ernst Niggli.
Department of Physiology, University of Bern, Switzerland

Subcellular Ca signals are a common feature of many cell types including neurons and myocytes. Here we describe the use of a Ti-sapphire laser yielding two photon photolysis (TPP) of caged Ca, in a very small volume (subfemtoliter). The energy of the Ti-sapphire laser (wave length 700 nm, pulse duration 75 fs, repetition rate 80 MHz, output energy 150 mW) was reduced by a polarizing filter to 8-70 mW and parfocally, coaxially combined with an argon laser (514 nm, 2 mW) of a confocal laser scanning microscope (CLSM) to detect the released Ca. Cultured hippocampal neurons of the rat and guinea pig cardiac myocytes were dialyzed with Fluo-3 (0.1 mM, 0.25 Ca) and DM nitrophen (1 mM) in the whole-cell configuration of the patch clamp technique. High temporal resolution (500 Hz) was obtained in the line scan mode of the CLSM.-The three-dimensional point spread function was determined in the reflection mode or using fluorescein. *In vitro* full width at half maximum (FWHM) in the two photon mode was 0.6 µm for x-y and 1 µm for z-axes. Photolysis of caged Ca led to an increase of up to 100% of the baseline fluorescence. Diffusion of uncaged Ca could be observed (decay $\tau = 20$ ms, $\lambda = 6.2$ µm).-*In vivo*, in both cell types DM nitrophen could be uncaged without damaging the cell (ΔF/F up to 2). In hippocampal neurons Ca spreading in the cytoplasm was slow (decay $\tau = 90$ ms, $\lambda = 8.7$ µm)-In contrast TPP led to a more confined ($\lambda = 3.2$ µm), rapidly reversible ($\tau = 25$ ms) Ca spreading in cardiac myocytes, and occasionally triggered propagating Ca waves. These findings suggest that uncaged Ca is sufficient to activate Ca-induced Ca release and subject to an active reuptake of Ca in cardiac myocytes, whereas in the neurons investigated uncaged Ca mainly spreads by diffusion only.

Supported by Swiss NSF

316.2

LOCAL DENDRITIC CALCIUM TRANSIENTS CAUSED BY UNITARY SYNAPTIC EVENTS IN HIPPOCAMPAL NEURONS. V. N. Murthy*, T.J. Sejnowski and C.F. Stevens. Computational Neurobiology, and Molecular Neurobiology Laboratories, Salk Institute, 10010 N. Torrey Pines Rd., La Jolla, CA 92037.

Spontaneous, action potential-independent calcium transients have been observed in dendrites of cortical neurons, and are presumed to be caused by spontaneous transmitter release (Science, 263:529). To determine the mechanisms involved in these presumed miniature synaptic calcium transients (MSCTs), we performed high-temporal resolution confocal imaging of cultured hippocampal neurons filled with 50 µM fluo-3 through a patch pipette. MSCTs were observed at resting membrane potentials in the presence of TTX and no added Mg^{++}, and 100 mosM sucrose to increase spontaneous vesicle release. MSCTs persisted when AMPA receptors were blocked by 10 µM DNQX, and calcium channels were blocked by 50 µM cadmium. MSCTs were blocked reversibly by 100 µM D-APV, an NMDA receptor antagonist. In the presence of 3 mM external Mg^{++}, MSCTs could be suppressed by voltage-clamping the soma at -70 mV; transients reappeared when the membrane was depolarized to -30 mV to remove the Mg^{++} block of NMDA receptors. Taken together, these findings indicate that MSCTs are caused by calcium entry through NMDA receptors when spontaneous vesicle release occurs. MSCTs originate at sites 1 - 2 µm along the dendritic axis, and can spread to 10 µm axially. Preliminary analysis of multiple occurrences of MSCTs at a single site indicates that they can vary considerably in amplitude and duration. These experiments could further the understanding of the causes of response variability at single synapses. Support: HHMI and NIH (TJS and CFS); HHWhitney Fellowship (VNM).

316.3

TWO-PHOTON CONFOCAL IMAGING OF CALCIUM SIGNALS IN HIPPOCAMPAL SLICES AND CEREBELLAR GRANULE CELLS. A.C.Hargreaves, B.G.Frenguelli* & G.L.Collingridge. Dept. of Anatomy, University of Bristol, Bristol, UK BS8 1TD.

We have used two-photon laser scanning confocal microscopy (TP-LSCM) to visualise calcium dynamics in acutely-prepared hippocampal slices and in 3-10 day old cultures of cerebellar granule cells. Hippocampal slices were prepared using standard techniques from 3-17 day old rat pups and maintained, submerged, in 4 ml of standard aCSF containing indo-1 AM (10 μM) and pluronic F-127 (0.2-0.4 %) at room temperature. Loading of CA1 pyramidal cells with indo-1 was only obtained in young slices (3-6 days). Older slices showed loading of putative interneurones and superficial dendrites only. Cerebellar granule cells were prepared from 7 day old rats by dissociation in Ca^{2+}-free medium and were loaded with 5 μM indo-1 AM with 0.2 % pluronic F-127. TP-excitation was provided by a Spectra Physics Tsunami Ti-Sapphire laser coupled to a BioRad MRC 1024 LSCM. Excitation of indo-1 was achieved by 50-130 fs pulses of red light (λ=700-750 nm) at 82 MHz. Emission was collected via a 405 nm bandpass filter and a 460 longpass filter. Under TP excitation, the isosbestic wavelength for indo-1, usually around 450 nm, shifted to a shorter wavelength (~410 nm).

CA1 neurones in hippocampal slices from young (~4 day old) rats showed increases in intracellular calcium in response to L-glutamate (L-Glu) (1-10 mM), AMPA (100 μM) and NMDA (100 μM). Stimulation of the Schaffer collateral/commissural pathway caused a rise in intracellular Ca^{2+} ($[Ca^{2+}]_i$) in a few neurones, although the size of the responses and the number of responding cells was greatly increased in medium lacking Mg^{2+}. In cultured cerebellar granule cells, AMPA, NMDA and L-Glu evoked increases in $[Ca^{2+}]_i$. In addition, both acutely-prepared slices and cerebellar cultures showed evidence of spontaneous Ca^{2+} activity.

The financial support of the Wellcome Trust (UK) is gratefully acknowledged.

316.4

CONFOCAL RATIOMETRIC CALCIUM IMAGING IN HIPPOCAMPAL SLICE NEURONS DURING SYNAPTIC FREQUENCY POTENTIATION. O. Thibault*, R.W. Hadley and P.W. Landfield. Dept. Pharmacology, College of Medicine, Univ. Kentucky, Lexington, KY 40536.

Synaptic activation has been shown to induce calcium (Ca) transients in dendritic and somatic compartments of CA1 hippocampal neurons. Both voltage-gated Ca channels and receptor-operated Ca channels contribute to this Ca influx. Although the frequency-dependence of postsynaptic Ca influx has been investigated in several studies, its relation to short-term synaptic plasticity (e.g. EPSP facilitation) has not been assessed in detail. Thus, it is not clear whether the magnitude of the EPSP facilitation determines the degree of Ca influx or conversely, whether large Ca transients in dendrites reduce EPSP conduction to the soma (by activating shunting conductances).

In the present study, we combined whole cell patch clamp recording and Ca imaging to determine the relationship of Ca influx to the magnitude of frequency potentiation (facilitation) of the EPSP during repetitive synaptic activation in adult (3-5 month-old) rat hippocampal slice CA1 neurons. We used the ratiometric dye Indo-1 (potassium salt) to monitor calcium entry with a rapid acquisition, UV-compatible confocal laser scanning microscope (Nikon RCM 8000). The neurons showed characteristic electrophysiological responses in the presence of Indo-1 in the recording pipette, including significant afterhyperpolarizations and large facilitation of the EPSPs during 7-10 Hz stimulation of stratum radiatum. Stimulation of the Schaffer-commissural pathway yielded large and sustained calcium transients in somatic and dendritic compartments, which appear to be related in a complex manner to the degree of frequency potentiation of the EPSP. (Supported by NIH AG04542, AG10836)

316.5

CALCIUM ACTION POTENTIALS IN APICAL DENDRITES OF NEOCORTICAL PYRAMIDAL NEURONS IN RAT BRAIN SLICES. J. Schiller*, Y. Schiller and B. Sakmann. Max-Planck-Institut fur Medizinische Forschung, Heidelberg, Germany, D-69120.

To explore synaptic integration in mature neocortical layer 5 pyramidal neurons in rat brain slices we performed double whole-cell voltage recordings from an apical dendrite and the soma of the same neuron combined with high speed calcium imaging measurements. Synaptic stimulation or dendritic current injection initiated dendritic action potentials in the distal apical dendrites, which in turn evoked a large calcium influx into these dendrites. Initiation of dendritic action potentials resulted from co-activation of dendritic voltage dependent calcium and sodium channels. Dendritic action potentials evoked by current injection were eliminated altogether by the addition of Cadmium, and their initiation threshold markedly increased after the addition of TTX. Initiation of dendritic action potentials by synaptic stimulation was blocked by addition of AMPA and NMDA receptor blockers. We conclude that apical dendrites of neocortical pyramidal neurons contain active conductances, which can support initiation of dendritic action potentials, and thus possibly are important in synaptic integration and amplification of synaptic potentials.

Supported by the Minnerva Foundation

316.6

ACTION POTENTIAL INITIATION IN NEOCORTICAL PYRAMIDAL NEURONS - REVISITED. Greg Stuart* and Bert Sakmann.
Abt. Zellphysiologie, MPI für medizinische Forschung, Heidelberg, Germany.
* Present address: Division of Neuroscience, John Curtin School of Medical Research, Australian National University, Canberra, Australia.

Simultaneous patch pipette recordings were made from the soma and main apical dendrite of neocortical layer V pyramidal neurons in brain slices from 28 day old rats at 35 °C. Synaptic stimulation of the distal apical dendrites at an intensity threshold for somatic action potential initiation usually evoked action potentials first at the soma, whereas high intensity synaptic stimulation often evoked dendritic regenerative responses prior to somatic action potentials (n=23). This apparent shift in the site of action potential initiation was observed with both whole-cell and cell-attached recordings (n=8). Dendritic electrogenesis was not observed during proximal synaptic stimulation and its time to onset relative to that of the somatic action potential was greater the more distal the dendritic recording site from the soma, indicating that this dendritic electrogenesis was generated in the distal apical dendrites. The occurence of this dendritic electrogenesis could be modulated by changes in resting membrane potential following current injection via the somatic or dendritic recording pipettes. To investigate if this dendritic electrogenesis propergates faithfully to the soma and axon, simultaneous patch pipette recordings were made from the soma, dendrite and axon of the same cell. These experiments showed that action potentials were always initiated in the axon before the soma with the same onset latency and time to peak, independent of whether a dendritic regenerative response occurred or not (n=5). In conclusion, these findings suggest that synaptic stimulation can initiate dendritic regenerative responses in the distal apical dendrites of neocortical pyramidal neurons. These regenerative events, however, do not appear to propagate faithfully to the soma and axon, indicating that the final site of synaptic integration and action potential initiation in neocortical pyramidal neurons is always in the axon, even during dendritic electrogenesis.

Supported by the Max-Planck-Gesellschaft

316.7

SPATIALLY LOCALIZED CALCIUM TRANSIENTS IN HIPPOCAMPAL CA3 NEURONS PRODUCED BY MOSSY FIBER SYNAPTIC TRANSMISSION, David B. Jaffe*, Div. of Life Sciences, Univ. of Texas at San Antonio, San Antonio, TX 78249.

The hippocampal mossy fiber (MF) terminal field contains a lower density of NMDA receptors compared to other areas of the hippocampus. High-time resolution fluorescent imaging was used to test the hypothesis that MF synaptic transmission could produce localized increases Ca^{2+} in CA3 pyramidal neurons that is NMDA receptor-independent.

CA3 neurons in rat hippocampal slices were filled via patch pipettes with the Ca^{2+}-sensitive dye Fura-2. In addition, pipettes contained Cs^+ and QX-314 to block K^+ and Na^+ currents. High-frequency stimulation (100 Hz, 1 second duration) of the MFs produced increases in Ca^{2+} in proximal dendrites. Distal dendritic signals were not detected. These transients were not blocked by the NMDA receptor antagonist D,L-APV (100 μM, n=5). Futhermore, signals were detected when the membrane potential was raised above the reversal potential for ionotoropic glutamate receptors (> 0 mV). Finally, in some cases MF-stimulated Ca^{2+} signals propagated approximately 5-15 μm from initial sites of origin. The shape of the delayed transients along the dendrite was not consistent with diffusion of Ca^{2+} or the indicator dye.

These data suggest that tetanus of the MFs can trigger localized increases in Ca^{2+} independent of NMDA receptor activation.

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316.8

OMEGA-AGATOXIN-IVA-INSENSITIVE CALCIUM CHANNELS IN RAT CEREBELLAR PURKINJE NEURONS, S. WATANABE[1,2], T. MIYASHOU[1,3], H. TAKAGI[1], Y. KIRINO[2], H. SUZUKI[3] H. MIYAKAWA[1*] and Y. KUDO[1], [1]Tokyo Univ. of Pharmacy & Life Science, Tokyo 192-03; [2]Faculty of Pharmaceutical Sciences, The Univ. of Tokyo, Tokyo 113; [3]School of Science & Engineering, Waseda University, Tokyo 169, Japan.

Voltage-activated Ca^{2+} channels in mammalian neurons govern many physiological processes, such as transmitter release and neuronal dendritic integration. In rat cerebellar Purkinje neuron, Ca^{2+} influx is largely dependent on the activation of the voltage dependent Ca^{2+} channels induced by the firing of the action potentials. But the spatial distribution of Ca^{2+} channels and their contribution to physiological activity is not well understood. To help delineate the contribution of different channel types to somatic and dendritic functioning, we imaged Ca^{2+} increase in response to the depolarized voltage pulse and studied the effect of several specific Ca^{2+} channel antagonists on Ca^{2+} increase in the soma and dendrites of Purkinje neurons. ω-Agatoxin-IVA (200 nM) blocked Ca^{2+} increase in Purkinje cell somata but did not show significant effect in dendrites, whereas Ni^{2+} (50 μM) reduced Ca^{2+} increase in dendrites. Blockers for other types of Ca^{2+} channels did not show significant effect on Ca^{2+} increase. These data indicate that ω-Agatoxin-IVA-sensitive Ca^{2+} channels (P) are dominant in cell body and Ni^{2+}-sensitive Ca^{2+} channels (T or R?) are localized in dendritic region. These conclusions suggest that the different Ca^{2+} channel types participate in distinct physiological functions: Ni^{2+}-sensitive Ca^{2+} channels likely play a greater role in dendritic integration, whereas ω-Agatoxin-IVA-sensitive Ca^{2+} channels are more important for somatic Ca^{2+}-dependent process.

316.9

Calcium Signals in the Zebrafish Mauthner Cell: Large Size and Potentiation with Repetitive Sensory Stimulation. D.M. O'Malley* and J.R. Fetcho, Department of Neurobiology & Behavior, SUNY @ Stony Brook, NY 11794.

The Mauthner cell, a large reticulospinal neuron found in fish and amphibians, integrates an array of sensory inputs (auditory, visual and lateral line), and triggers a robust escape behavior. Mauthner cells in the hindbrain of intact larval zebrafish were retrogradely labeled with the fluorescent indicator calcium green dextran (CGD) to visualize *in vivo* the cells' activity during escape behaviors (Fetcho & O'Malley, 1995). In response to single sensory stimuli, the Mauthner cell soma often exhibited a large calcium signal (20% to 100% increase in CGD fluorescence). These large signals were evoked repetitively over hours or days, and consistently recovered rapidly and completely. The size of the calcium signals was surprising because the Mauthner cell is known to fire only a single action potential each time it triggers the escape behavior. Antidromic stimulation of the Mauthner cell with single shocks produced just the small fluorescence increases (5 to 10%) typically observed after single action potentials in other neuronal somata (e.g. rat thalamus, Zhou et al., '95; and bullfrog sympathetic neurons, O'Malley, '94). As a rough estimate, a 50% increase in CGD fluorescence corresponds to about a 145 nM calcium increase, while a 5% increase equates to about a 10 nM change. In many fish, the fluorescence increases were initially small, but with periodic stimulation, increased dramatically to the higher levels seen at the outset in other fish. Also, providing 3 sensory stimuli in succession (at 2.5 sec intervals within a trial), often produced one enhanced response, together with smaller responses in that trial. 1 mM caffeine also enhanced the calcium responses, suggesting a role for internal calcium stores. These potentiated calcium signals may represent a novel form of plasticity. Because calcium has powerful influences on cellular activities in the nucleus, soma, and dendrites, including the enhancement of auditory inputs to the Mauthner cell [Wolszon & Faber, 1988], these calcium signals are likely to have physiological consequences. Supported by NSF grant IBN-9514777 to DMO and NINDS grant NS-26539 to JRF.

316.10

IMPROVED SPATIAL RESOLUTION IN GLUTAMATE RECEPTOR MAPPING WITH A NOVEL AND INEXPENSIVE METHOD FOR TWO-PHOTON EXCITATION OF CAGED COMPOUNDS. D.L. Pettit*, S.S.-H. Wang, K.R. Gee^ & G.J. Augustine. Dept. Neurobiology, Duke Medical Center, Durham, NC & ^Molecular Probes, Eugene OR.

'Caged' glutamate has been used to map the distribution of receptors and synaptic connections in brain slices. However, unfocused light causes photolysis of glutamate throughout the slice, limiting spatial resolution (Neuron 15:755). We have devised a simple solution to this problem using a glutamate derivative that has two caging groups, requiring absorption of two UV photons before conversion to active glutamate. Spatial resolution will be improved because the probability of generating active glutamate is high only near the focal point of the light beam. This is similar to two-photon excitation with a pulsed laser (PNAS 91:6629), but has the advantage that it can be performed with an inexpensive arc lamp. We tested the method via whole-cell patch clamp recordings from pyramidal neurons in rat hippocampal slices. Photolysis of either singly- or doubly-caged glutamate was accomplished by varying the focal point of a UV light beam positioned over the dendrites of the neurons. Glutamate-induced currents varied according to the z-axis position of the beam, with maximal responses evoked when the beam was focused on the dendrite. The relationship between axial position and current was well fit by a gaussian function, with a half-width that was significantly smaller for doubly-caged glutamate than for the singly-caged compound. Thus, doubly-caged compounds offer a simple and inexpensive way to improve spatial resolution in experiments requiring local photolysis of caged compounds. Supported by NH15177, NS17771, 09586 and 34045.

316.11

LOCAL CALCIUM RELEASE BY IP_3 IN PURKINJE CELL DENDRITES. E.A. Finch* and G.J. Augustine. Dept. Neurobiology, Duke University Medical Center, Durham, NC 27710.

To characterize the spatial and temporal properties of IP_3-induced Ca release in dendrites, local photolysis of caged IP_3 was used to generate rapid and focal increases in IP_3 within Purkinje cells (PCs) in cerebellar slices. Localized photolysis of caged IP_3 (2-10 μm spots) evoked Ca release, which was monitored with Ca Green using confocal laser-scanning microscopy. In all regions of the dendritic arbor the Ca signals evoked by IP_3 were highly restricted, in no case spreading more than 20 μm, and were thus confined to one or a few dendritic branches. The time course of the Ca signal depended on the amount of IP_3 generated, with the highest [IP_3] producing increases in [Ca] with a short latency (< 10 ms), fast rate of rise (< 10 ms), and [Ca] in the micromolar range. Brief pulses of IP_3 produced transient Ca increases that declined to baseline levels within several seconds, whereas sustained or repetitive generation of IP3 produced longer-lasting increases in Ca. To further assess the effective range of released Ca, we used whole-cell recording to monitor the activation of dendritic Ca-activated K channels, which have a low affinity for Ca. K currents were activated only by IP_3 concentrations that produced near-maximal Ca signals, indicating that Ca released from intracellular stores by IP_3 can reach micromolar levels at the plasma membrane. Thus, in PC dendrites IP_3 can produce large Ca signals that are restricted to small dendritic compartments. These findings are consistent with the idea that rises in postsynaptic IP_3 can locally influence dendritic physiology during synaptic transmission. Supported by NS-09586 and NS-34045.

POSTSYNAPTIC MECHANISMS: GLUTAMATE SIGNALS

317.1

PHARMACOLOGY OF CA1 PYRAMID TO ORIENS ALVEUS INTERNEURONE CONNECTIONS IN HIPPOCAMPUS. Afia Ali and Alex M. Thomson*, Dept. Physiology, Royal Free Hospital School of Medicine, Rowland Hill Street, London NW3 2PF, UK

CA1 pyramid to oriens alveus (O/A) interneurone connections were studied with dual intracellular recordings and biocytin-filling in slices of rat hippocampus. O/A interneurone somata were located at the O/A border with horizontally orientated spiny dendrites. Their axons ramified extensively in stratum lacunosum moleculare. In 3 pairs, trains of 4 presynaptic spikes elicited EPSPs showing strong paired pulse facilitation. To determine whether NMDA receptors contributed to EPSPs and to this facilitation, D-AP5 was applied in one pair. In control conditions the first 3 EPSPs in the train were larger and broader at -72 than at -78 mV (half widths 113, 110 and 112.5 %). However at -69 mV, the EPSPs were also smaller and narrower than at -72mV. Responses to brief current pulses showed a similar sensitivity to membrane potential. D-AP5 (62μM) caused a more rapid decay of the EPSPs, which were up to 10% smaller and significantly narrower than under control conditions (half widths of the 4 EPSPs reduced by <10, 15, 20 and 15 % respectively). Prolonged application of D-AP5 lead to only a small change in membrane time constant (10.8 cf. 11.8msec). Subsequent addition of DNQX (20μM) reduced the EPSP amplitude to <10% of control within 20 minutes.
This work was supported by Sandoz Pharma, Basel.

317.2

SYNAPTIC FACILITATION IN THE DENTATE GYRUS OF THE RAT: EFFECT OF STIMULATION SITE, TARGET SITE, AND MEASUREMENT PROTOCOL J Ferbinteanu, B. Srebro* and N.W. Milgram University of Toronto-Scarborough Campus, 1265 Military Trail, Scarborough, Ontario M1C-1A4, and Department of Physiology, University of Bergen, Norway.

Although synaptic facilitation has been reported in the dentate gyrus, this is not true in all cases. Such inconsistencies could reflect differences in pathway tested, recording site, or possibly in parameter used to measure facilitation. To examine this problem, we studied synaptic facilitation of field excitatory postsynaptic potentials (fEPSPs) evoked by stimulation of either the lateral (LPP) or medial (MPP) perforant path. A three-pulse test procedure was used with a reference pulse followed by a conditioning -test pulse pair. To avoid contamination of fEPSP, the intensity of the reference and test pulses were identical and were set at stimulation levels below threshold for triggering population spikes. We studied recording sites in both the molecular and granular cell layers and measured both the slope and amplitude of the fEPSP.

Consistent with previous literature, we found differences in parametric properties of fEPSPs evoked by LPP and MPP stimulation. In addition, stimulation of the LPP produced more robust facilitation than MPP. Facilitation also varied as a function of the recording site; greater facilitation was recorded in the molecular layer. The results varied as a function of the parameter used to estimate fEPSP, more consistent facilitation was obtained with the amplitude measure. Further analysis confirmed that for the fEPSP the slope and amplitude parameters are not interchangeable. A regression analysis revealed a low correlation between the slope and amplitude measurements. This low correlation was a reflection of non-linearity of the slope function, which contrasts with the assumption commonly made. This non-linearity is probably a reflection of co-activation of excitatory and inhibitory postsynaptic potentials.

This work was supported by a grant to NWM from NSERC

317.3

POST-SYNAPTIC CONDUCTANCES CAUSE NON-LINEAR SUMMATION OF AMPA-MEDIATED EPSPs IN RAT NEOCORTICAL LAYER V PYRAMIDAL NEURONS. J.S. Nettleton* and W.J. Spain, Depts. of Neurology and Physiology and Biophysics, Univ. of Washington and The VA Puget Sound Health Care System, Seattle, WA 98108.

It is hypothesized that voltage sensitive conductances present on the soma and dendrites of neurons, cause non-linear summation of EPSPs. We tested this hypothesis in layer V pyramidal neurons using intracellular recording with sharp electrodes in the presence of 100 μM AP-5 (NMDA blocker) and 3 μM Bicuculline ($GABA_A$ blocker). This isolates the AMPA response but does not completely disinhibit the slice. Subthreshold AMPA EPSPs (1-10mV) were induced by a stimulating electrode in layer I (E1), and another one in the layers II-V (E2). The areas of stimulation were isolated from each other by a cut just below layer I. To block post-synaptic conductances, 50 mM QX-314 was included in the recording electrode in some experiments. We quantified the summation as an area ratio (area of experimentally measured response to area of the mathematical sum of the individual stimulus responses, E1+E2). In approximately 50% of the cells, the response to the two stimuli temporally spaced 5-10 ms apart was >10% larger than the mathematical sum and 20% of all cells had > 25% increase. In contrast, ≈90% of the QX-314 loaded cells summed linearly. The average area ratio was statistically different in the QX-314 vs. non QX-314 loaded cells (two-tailed Student's T-test, $P<0.05$). These results demonstrate that post-synaptic conductances can alter EPSP summation. Preliminary data in which current pulses applied at the soma were summed with a layer I evoked EPSP, suggest that the non-linear boosting occurs in the apical dendrite.

Supported by a VA Merit Review (W.J.S.) and NIH GM07108 (J.S.N.)

317.4

NON-NMDA RECEPTOR OCCUPANCY AND OPEN PROBABILITY DURING TRANSMISSION AT SYNAPSES WITH ONE RELEASE SITE. R. Angus Silver*, Stuart G. Cull-Candy and Tomoyuki Takahashi
Pharmacology, University College London, London WC1E 6BT, UK.
Neurophysiology, Institute for Brain Research, University of Tokyo, Japan.

The fraction of postsynaptic receptors occupied following the release of a quantum of transmitter is a fundamental synaptic parameter. At the neuromuscular junction receptors are not saturated. However, at central synapses receptor saturation has been widely postulated. We have investigated non-NMDA receptor occupancy and open probability (P_o) during transmission at rat cerebellar mossy fibre-granule cell synapses with one functional release site. EPSCs from single site synapses were identified on the basis of their lack of dependence of amplitude on release probability. Release probability was altered by changing the $[Ca^{2+}]/[Mg^{2+}]$ ratio in the external solution, and the mean EPSC amplitude (excluding failures) was measured. We have modelled transmitter release at synapses with different numbers of release sites and have developed a criterion for identifying synapses with one release site. Although synapses identified with this method are likely to have only one release site, the presence of additional release sites with low release probabilities cannot be ruled out. Postsynaptic receptor open probability was calculated in two ways. A lower limit was estimated from the coefficient of variation of the single-site EPSCs, and an upper limit was calculated from the ratio of the mean EPSC amplitude and the largest EPSC amplitude. Postsynaptic receptor occupancy was estimated from P_o by dividing by the maximal open probability. These limits for occupancy (0.45-0.6) suggest that release of a quantum of transmitter does not saturate non-NMDA receptors at the cerebellar mossy fibre-granule cell synapse.

Supported by the Monbusho International Scientific Research Program of Japan, the Wellcome Trust and the Howard Hughes Medical Institute.

317.5

KAINATE-INDUCED CURRENTS IN CA3 HIPPOCAMPAL NEURONS. M. Vignes*, C. Mathiesen and G.L. Collingridge, Dept. of Anatomy, University of Bristol, Bristol BS8 1TD, UK.

We have investigated the postsynaptic effects of kainate receptor activation in CA3 hippocampal neurons. Whole-cell patch clamp recordings were performed on hippocampal slices, prepared from 12-15 day old rats, perfused with a standard medium Mg^{2+}-containing medium at room temperature. GABA receptor-mediated responses, i.e. $GABA_A$ and $GABA_B$, were inhibited respectively by applying picrotoxin (50μM) in the perfusion and by Cs^+ in the recording electrode. The perfusate also included GYKI 52466 (100μM) and AP5 (50μM), in order to block AMPA and NMDA receptors, respectively.

Under these conditions, the application of 200nM kainate evoked inward currents (-81±8pA, n=11) accompanied by a decrease in the input resistance. These effects were reversible and antagonized by the non-NMDA receptor antagonist CNQX (83±6% reversal, 10μM, n=5). In contrast, AMPA was much weaker under these conditions (-30±6pA, 10μM, n=7). Thus, kainate is a potent excitant of CA3 neurons via the activation of kainate receptors. In comparison, kainate is a weaker excitant in area CA1 under similar conditions. For example, inward currents of 10-30pA were induced by 1μM kainate (Chittajallu et al., *Nature*, 379, 78-81,1996).

317.6

ACTIVATION OF A SINGLE PYRAMIDAL NEURON SUPPRESSES EXCITATORY TRANSMISSION IN CA1 STRATUM RADIATUM OF RODENT HIPPOCAMPUS Y.Yanovsky[1], D. Stevens*[1], J.T. Sanz[2], E.H. Buhl[2] and H.L. Haas[1] [1]Institute of Physiology, Heinrich-Heine-University, Düsseldorf, Germany and [2]MRC Anatomical Neuropharmacology Unit, University of Oxford, Oxford, UK

Pyramidal cells in the CA1 area of the rodent hippocampus target with their recurrent collaterals both neighboring pyramidal cells and GABAergic interneurons. We studied the efferent target selectivity of biocytin labeled pyramidal cells (n=2) employing random electron microscopic sampling of pyramidal cell synaptic boutons. We found the recurrent output of CA1 pyramidal cells heavily biased towards GABAergic local-circuit neurons, since 50% (n=10) of the pyramidal cell terminals were found in synaptic contact with dendritic shafts displaying the characteristic electron microscopic features of non-principal cells. Strong intracellular stimulation of single pyramids (10 bursts, 0.5 s, 0.5 nA at 0.1 Hz) but not an equal number of dissipated action potentials, reduced excitatory transmission in the apical dendritic region in slices from mouse hippocampus by 19 ± 7% (SE, n=36) with a delayed onset and for prolonged periods in two phases. The first phase had a duration of ca. 20 min, the second lasted usually longer than 1 hour. EPSPs in the stimulated pyramids were increased for equally long periods but this was not the cause of the field EPSP reduction. Paired pulse facilitation of the field EPSPs was unchanged indicating a postsynaptic effect. At least part of the effect may be mediated by metabotropic glutamate receptor activation.

317.7

REUPTAKE CONTRIBUTES TO THE REMOVAL OF GLUTAMATE FROM THE SYNAPTIC CLEFT OF RAT CEREBELLAR GRANULE AND UNIPOLAR BRUSH CELLS. N.T. Slater* and G.A. Kinney, Department of Physiology, Northwestern University Medical School, Chicago, IL, 60611 U.S.A.

The contribution of transporters to the removal of glutamate from the synaptic cleft during transmission has been controversial; inhibitors of glutamate transport have been observed to prolong the AMPA receptor-mediated synaptic current evoked by single stimuli at some synapses, but are without effect at others. However, the contribution of reuptake to the removal of glutamate following single EPSCs may be masked by rapid receptor desensitization. Moreoever, many of the pathways examined normally fire at high frequencies, during which glutamate transport may play a more prominent role. To examine this issue further we have studied the actions of the glutamate transport inhibitor L-*trans*-PDC on the time course of the AMPA receptor-mediated synaptic current in cerebellar granule (GC) and unipolar brush cells (UBCs; D.J. Rossi et al. J Neurophysiol 74:24,1995) using whole-cell recording in thin slices of rat vestibular cerebellum (nodulus). In GCs, the decay of single, pharmacologically-isolated AMPA EPSCs were best fit as the sum of two exponentials (τ_f=3.8 ms; τ_s=53.1 ms,n=6); the slow component was prolonged by 100 μM L-*trans*-PDC (τ_s=99.7 ms, n=6) and the EPSC peak was reduced (25%). In the presence of 100 μM cyclothiazide (to block desensitization), a similar enhancement of the slow component was observed (cyclothiazide alone: τ_f=13.8 ms; τ_s=139.5 ms; cyclothiazide + L-*trans*-PDC: τ_f=15.7 ms; τ_s=388.4 ms, n=8). With repetitive stimulation (7 stimuli at 40 Hz) a significant enhancement in the net charge was observed (39 ± 17%, n=4) in the presence of L-*trans*-PDC alone. In UBCs the peak amplitude of the fast EPSC was reduced and the net charge enhanced by L-*trans*-PDC. The data are consistent with the notion that reuptake contributes to the removal of glutamate from the synaptic cleft at both small (GC) and large (UBC) diameter contacts. This process primarily shapes the synaptic current when glutamate is present within the cleft for prolonged periods, during repetitive activity at small synapses and the prolonged entrapment of glutamate at the giant UBC synapse. Supported by DC002764 & NS34840 to N.T.S.

317.8

THE SLOW TIME COURSE OF AMPA RECEPTOR-MEDIATED SYNAPTIC CURRENTS IN RAT CEREBELLAR UNIPOLAR BRUSH CELLS REFLECTS AN ENTRAPMENT OF GLUTAMATE IN THE SYNAPTIC CLEFT. G.A. Kinney*, L.S. Overstreet, and N.T. Slater, Department of Physiology, Northwestern University Medical School, Chicago, IL, 60611 U.S.A.

Activation of glutamatergic mossy fibers afferents to unipolar brush cells (UBCs) of mammalian vestibular cerebellum evokes a long-lasting synaptic current (>2 s) with a biphasic AMPA receptor-mediated component displaying a late peak at ~300 ms (D.J. Rossi et al. J Neurophysiol 74:24,1995). It was proposed that this reflects an entrapment of glutamate in the synaptic cleft, and the slow EPSC results from a steady-state current. To further examine this proposal, AMPA receptor-mediated synaptic currents in UBCs were recorded using whole-cell recording in thin slices of rat cerebellar nodulus at 22°C. In double-pulse experiments, the peak of the fast EPSC, which will probe for the fraction of available receptors, was depressed in amplitude compared to the peak of the first stimulus, and recovered with a slow time course ($t_{1/2}$= 200 ms) which mirrored the time course of synaptic currents in the presence of cyclothiazide. The steady-state dose-response curve for glutamate acting at AMPA receptors, measured in excised patches from both UBCs and granule cells, was biphasic, peaking at 50 μM, and declining to 50-70% of this value at 1 mM. When glutamate was slowly washed from patches to simulate the slow decline of [glutamate] in the synaptic cleft, the steady-state current displayed a marked hump in patches from both cell types. In UBCs displaying slow EPSCs, reduction of release probability by lowering extracellular $[Ca^{2+}]$ from 2.5 to 1.0 mM eliminated the slow EPSC, and it was restored at 1 mM $[Ca^{2+}]$ by a brief repetitive stimulus (2-5 stim at 40 Hz). Similarly, slow EPSCs were revealed or enhanced in UBCs by repetitive stimuli. Taken together, these results support the hypothesis that the slow AMPA receptor-mediated EPSC in UBCs reflects a long-lasting entrapment of glutamate in the synaptic cleft, and the time course of this current is sculpted by the kinetic properties of the postsynaptic receptor. Supported by the NIH (NS34840 to N.T.S.).

317.9

CROSS TALK BETWEEN EXCITATORY SYNAPSES IN THE HIPPOCAMPUS. D.E. Bergles* and C.E. Jahr. Vollum Institute, Oregon Health Sciences University, Portland, OR 97201.

Diffusion of transmitter to adjacent synapses has been suggested as a mechanism for heterosynaptic depression at both excitatory and inhibitory synapses. Despite this evidence that transmitters can diffuse for some distance, evidence for the activation of postsynaptic receptors by transmitter diffusing from neighboring release sites, or cross talk, is equivocal (Diamond and Jahr, 1995; Mennerick and Zorumski, 1995), except at calyceal synapses (Trussell et al., 1993) or when transmitter reuptake is reduced (e.g. Isaacson et al., 1993). We investigated whether the concentration profile of glutamate is prolonged at excitatory synapses in acute hippocampal slices when the probability that neighboring sites are releasing glutamate is increased.

The time course of glutamate in the synaptic cleft has been estimated in culture by measuring the non-equilibrium inhibition of NMDA EPSCs by the rapidly-dissociating antagonist D-aminoadipate (D-AA) (Clements et al., 1992). We recorded NMDA EPSCs (V_h = +40mV) from CA1 pyramidal neurons in acute slices prepared from 11-15 day old rats, and compared the inhibition obtained with DL-α - aminopamilate (DL-APA), an antagonist with a ~5x faster unbinding rate than D-AA, under low and high stimulus intensities (a manipulation which changes the number of active fibers, but does not change release probability). At elevated release probability, the amount of inhibition of NMDA EPSCs by DL-APA was less at high stimulus intensity (73±6%) than at low stimulus intensity (82±5%). In contrast, NMDA EPSCs evoked at high and low stimulus intensities were reduced the same amount with D-carboxypiperazin-propyl-phosphonate (D-CPP) (low:85±4%; high:84±3%), an antagonist with a ~1000x slower unbinding rate. These data suggest that increasing the density of active synapses increases the concentration profile of glutamate seen by NMDA receptors at some synaptic sites through intersynaptic diffusion. Supported by NIH NS21419.

317.10

MIDDLE TERM POTENTIATION OF MINIATURE EXCITATORY POSTSYNAPTIC CURRENTS IN CULTURED RAT SUPERIOR CERVICAL GANGLION NEURONS. Shirasaki, T.*[1], Liu, C.[1], Lu, F.[2], Kuba, K.[2] [1]Dept. of Physiol. Saga Med. Sch., Saga 849; [2]Dept. of Physiol., Nagoya Univ. Sch. of Med, Showa-Ku, Nagoya 466, Japan

The amplitude of nicotinic miniature excitatory postsynaptic currents (MEPSC) grows during a high K^+ (40mM) treatment and it lasts for more than 20 min after the treatment in rat cultured superior cervical ganglion neurons. The potentiation of MEPSC was seen in about one third of neurons studied and accompanied by the potentiation of acetylcholine (ACh)-induced currents. These middle term potentiation of MEPSC and ACh-induced current were produced in similar time course by the conditioning application of a nicotinic agonist, dimethylphenylpiperazinium (DMPP) or ACh in the presence of atropine. Intracellular application of BAPTA 5 mM abolished the increases in both intracellular free Ca^{2+} concentration and high K^+-induced MEPSC potentiation. Calmodulin dependent kinase II (CaMKII) inhibitor, KN62, blocked the generation of MEPSC potentiation. However, an inactive analog, KN04, had not such effects. The magnitude of potentiation of ACh-induced currents induced by a high K^+ or ACh treatment was smaller as the test ACh responses became greater. These results suggest that Ca^{2+} entered through nicotinic ACh receptor channels enhances its activation via activation of CaMKII, leading to the middle term potentiation of MEPSC in rat sympathetic neurons.

317.11

MAGNESIUM SENSITIVITY OF SYNAPTIC NMDA RECEPTORS IN RAT SPINAL CORD LAMINA I AND II NEURONS DURING POSTNATAL DEVELOPMENT. R. Bardoni*, P.C. Magherini & A.B. MacDermott. Dept. of Biomedical Sciences, Univ. of Modena, Modena (Italy), I-41100 and Dept. of Physiology and Cellular Biophysics, Columbia Univ., New York, NY 10032.

NMDA receptors have been shown to mediate activity-dependent developmental changes in motoneurons during a critical time in early postnatal development (Kalb & Hockfield 1992) but there is little information concerning the possible roles of NMDA receptors in synaptic transmission and synaptic maturation during postnatal development in the spinal cord dorsal horn. The first two postnatal weeks are the most active time of synaptic maturation in rat dorsal horn (Fitzgerald 1985). Therefore, we have tested for developmental changes in one of the most physiologically important properties of the NMDA receptors, voltage-dependent Mg^{2+} block, as a first step towards understanding the developmental role of NMDA receptor in the dorsal horn. NMDA EPSCs were recorded in spinal cord slices from postnatal rats (P2-P15). Whole cell patch clamp recordings were made from lamina I and II neurons. The neurons were visually identified, using a modification of the infrared video imaging system (Bardoni et al. 1995). EPSCs were evoked by focal stimulation in the proximity of the patch-clamped cell. During the first 2 postnatal weeks, as in the adult (Yoshimura & Jessell 1990), the EPSCs evoked at -70 mV in normal Krebs (1 mM Mg^{2+}) were mediated predominantly by non-NMDA receptors (blocked by 10 μM CNQX). However, a large NMDA receptor-mediated component, monitored at + 50 mV, was present in cells from P2-P15. These NMDA EPSCs were strongly blocked at -70 mV in a Mg^{2+}-dependent manner. For example, at -70 mV, current amplitude increases by 18 times in 0 Mg^{2+} bath in neurons from P3-P4 rats. These results indicate that NMDA receptors are expressed postsynaptically as early as P2 in lamina I and II neurons in rat dorsal horn and that they are strongly Mg^{2+}-sensitive throughout the main period of synaptic maturation.
This work was supported by MURST, NSF, NIH and Whitehall Foundation.

317.12

ATTENUATION OF EPSPS IN THE DENDRITES OF MOTONEURONS FROM ORGANOTYPIC RAT SPINAL CORD CULTURES. M. Larkum, T. Launey, A. Dityatev and H.-R. Lüscher*. Dept. of Physiol., Univ. of Bern, Switzerland CH-3012.

Motoneurons *in vivo* receive a large number of synaptic inputs. To understand how they process this input to generate action potentials, it is necessary to determine the influence of dendritic properties on the integration of synaptic input. Recently we have shown that it is possible to simultaneously record excitatory postsynaptic potentials (EPSPs) in the dendrites and somata of motoneurons from organotypic rat spinal cord slice cultures (Larkum et. al. *J. Neurophysiol.* **75**:154-170, 1996). Here we present the results of experiments with two electrodes patched either on the soma and a dendrite or on two different dendrites allowing us to measure the attenuation of subthreshold signals in the dendritic tree.

Spontaneous signals were detected using a modified version of an algorithm devised by Ankri et. al (*J. Neurosci. Meth.* **52**:87-100, 1994). With two electrodes, the location of the synaptic input could be placed into one of three categories: distal to electrode 1, distal to electrode 2 or between the two electrodes. Regression lines could be fitted through clusters of points representing these different populations by comparing the amplitudes of events at both electrodes. The likelihood that there were one, two or three populations was calculated using Wilks statistics. This method applied to simulated data was found to accurately predict both the number of populations and the attenuation of signals. The data recorded from motoneurons in organtypic cultures indicate that the somato-dendritic trees were quite compact electrically but suggest that the soma can greatly affect the spreading of EPSPs from one dendrite to another.
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317.13

DEVELOPMENTAL CHANGES IN NMDA RECEPTOR-MEDIATED SYNAPTIC TRANSMISSION TO RAT MOTONEURONS. J.H. Singer, M.C. Bellingham*, and A.J. Berger. Department of Physiology and Biophysics, University of Washington, Seattle, WA 98195-7290

We examined the development of the NMDA receptor (NMDA-R) component of glutamatergic excitatory postsynaptic currents (EPSCs) recorded in visualized hypoglossal motoneurons (HMs) in transverse brainstem slices from rats in three age groups: P1-2 (group 1), P8-11 (group 2), P16-26 (group 3). Monosynaptic NMDA-R mediated EPSCs were evoked by extracellular stimulation in the reticular formation just ventrolateral to the hypoglossal motor nucleus (n.XII) in the presence of strychnine (10 μM), bicuculine (10 μM), and DNQX (20 μM) to block glycine, $GABA_A$, and AMPA/kainiate receptor mediated currents, respectively. The remaining current displayed a region of negative slope conductance at depolarized holding potentials (-30 mV) and was blocked by APV (50 μM), indicating that it was, indeed, NMDA-R mediated. At -30 mV, the peak current in group 1 HMs was -316.7±72 pA (n=4). This decreased to -149.9±27 pA in group 2 HMs (n=4) and to -77.1±35 pA in group 3 HMs (n=3).These data support the hypothesis that NMDA-R mediated synaptic transmission decreases dramatically over the first four weeks of postnatal development.

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317.14

DIRECT MEASUREMENT OF COUPLING BETWEEN DENDRITIC SPINES AND SHAFTS MEASURED USING TWO-PHOTON EXCITATION PHOTOBLEACHING AND PHOTOACTIVATION. W. Denk, K. Svoboda*, and D.W. Tank, Lucent Technologies, Bell Laboratories, Murray Hill, NJ 07974.

Dendritic spines are attached to their parent dendrite via a thin neck. Speculation regarding the functional significance of spines has centered on the presumably large diffusional and electrical resistance of the spine neck. We measured the time-course of diffusional exchange between spines and their parent dendrite. CA1 pyramidal cells in slices of rat hippocampus were dialyzed and voltage clamped using whole-cell electrodes containing 200 μM fluorescein dextran (MW = 3 kD) at 22-24° C. Single spine heads were photobleached using two-photon excitation of fluorescence and the fluorescence recovery due to diffusional exchange with the parent dendrite was characterized with two-photon microscopy (line-scan mode, 2 ms temporal resolution). Fluorescence recovered completely with an exponential time course with time constants in the range τ = 20 - 100 ms. Experiments using two-photon uncaging of fluorescein dextran gave consistent results. Spine heads are therefore diffusionally isolated on this time scale. The time constant of fluorescence recovery by diffusion can be expressed as $\tau = lV_h/AD$, where l and A are the length and cross sectional area of the spine neck respectively, V_h is the spine head volume, and D is the dextran diffusion coefficient. The spine neck resistance is then $R_n = \tau D\rho_i/V_h$. Comparing spine head fluorescence intensity distributions to published electron microscopy data provided an estimate of V_h. $D = 1.0 \times 10^{-6}$ cm^2/s was measured *in vitro* using photo-release techniques. Using a cytoplasmic resistivity of $\rho_i \sim 250\ \Omega$ cm, we estimate R_n = 4 - 50 MΩ. The spine neck conductance (R_n^{-1} = 5-30 nS) is large compared to unitary synaptic conductances (< 500 pS), implying that the neck does not attenuate synaptic current, and therefore cannot be used to control synaptic weight. We estimate that during synaptic activation the spine head potential is elevated by at most 3 mV with respect to the dendrite.
(Supported by Bell Laboratories)

317.15

DEMONSTRATION OF N-METHYL-D-ASPARTATE (NMDA) MINIATURE EXCITATORY POSTSYNAPTIC CURRENTS IN RAT MOTONEURONS. J.A. O'Brien and A.J. Berger*, Department of Physiology and Biophysics, University of Washington, Seattle, WA 98195-7290.

Whole-cell patch-clamp recordings were used to study NMDA miniature excitatory postsynaptic currents (mEPSCs) in hypoglossal motoneurons (HMs) from neonatal rats 1-8 days old. HMs were identified using Normarski optics and infrared translumination of transverse brainstem slices, 300 μm thick. NMDA mEPSCs were recorded from HMs bathed in nominally Mg^{2+} free extracellular solution containing TTX (1 μM), bicuculline (20 μM), strychnine (20 μM), and DNQX (10 μM) while voltage clamping the cells at -80 mV (n=4). NMDA mEPSCs were completely abolished by addition of Mg^{2+} (3 mM, n=3) to the bathing solution. Additionally, NMDA mEPSCs were also abolished by the addition of APV (50 μM, n=3) to the bathing solution.

The global mean amplitude and interevent interval of NMDA mEPSCs were -13.0 ± 2.4 pA and 1.37 ± .91 seconds, respectively. NMDA mEPSC kinetics were studied by fitting a single exponential to the decay phase of each mEPSC. The global average τ was 11.9 ± 4.6 ms. Addition of 50 mM sucrose to the bathing solution increased the frequency of mEPSCs without changing the mEPSC amplitude (n=3), suggesting that the NMDA mEPSCs were due to presynaptic release of glutamate. We conclude that it is possible to identify NMDA mEPSCs, based on their pharmacological properties, in HMs from neonatal rats. (Supported by NS 14857)

317.16

THE PHYSIOLOGY OF EXCITATORY SYNAPSES IN THE LATERAL AND BASOLATERAL AMYGDALA. N. K. Mahanty* and P. Sah, The Neuroscience Group, Department of Physiology, Faculty of Medicine, University of Newcastle, Newcastle, NSW 2308, AUSTRALIA.

The amygdala is a part of the limbic system which has been implicated in the acquisition of fear conditioning. Little is known about the synaptic physiology in the various subregions of the amygdala. We investigated the excitatory synaptic physiology of the input from the external capsule (e.c) to the lateral (LA) and basolateral (BLA) amygdala. Experiments were performed on coronal or horizontal slices of the amygdala from 18-22 day old rats at room temperature. Recordings were made from neurones in the amygdala using the "blind" whole cell technique using biocytin containing electrodes. Cells were classified as pyramidal or interneurones, based on their morphology, capacitative transient, and their ability to elicit an AHP.

Synaptic inputs were stimulated using bipolar stimulating electrodes placed over the e.c. Inhibitory inputs were blocked with picrotoxin (50 μM). At a holding potential of -60 mV stimulation of the e.c. elicited short latency inward currents. Depolarisation of the cell revealed an additional slow component to the synaptic current. The fast component had a linear current-voltage relation while the slow component exhibited a region of negative slope conductance. The fast component was selectively blocked by the non-NMDA receptor antagonist CNQX (10 μM). In contrast, the slower component was selectively blocked by the NMDA receptor antagonist d-AP5 (50 μM). Spontaneous synaptic potentials (minis) recorded in the presense of tetrodotoxin (1 μM) at -70 mV had a fast rise time and monophasic decay. At +50 mV a second slower component was seen which was blocked by d-AP5 (50 μM). These results demonstrate that synaptic transmission between the e.c. and the lateral, and basolateral, amygdala is glutamatergic, and that the NMDA and AMPA receptors are co-localised in the post synaptic membrane. Supported by the National Health & Medical Research Council of Australia.

EXCITATORY AMINO ACIDS: EXCITOTOXICITY I

318.1

Cytokine-activated microglia enhance the neurotoxicity of glutamate in primary culture of rat cerebellar granule cells. W-K. Kim* and Y.S. Pae, Dept. of Pharmacol., College of Medicine; Div. of Neurosci., Medical Research Center, Ewha Womans University, Seoul, Korea.

Microglia have been shown to be activated by inflammatory cytokines and produce a number of toxic mediators. Here we report that activated microglia can enhance the glutamate-mediated neurotoxicity in rat cerebellar granule cells in culture. Neurotoxicity was assessed by measuring the leakage of lactate dehydrogenase and/or by counting the cells stained with trypan blue during the 18-24 hour incubation following exposure to glutamate. Cultured microglia were activated by lipopolysaccharides (LPS, 1.0 μg/ml for 2 hour) and γ-interferon (γ-IFN, 100U/ml for 2 hour) and 2 days later were used for co-culture with cerebellar granule cells. Activated microglia induced cerebellar granule cell damage less than 20%. In pure culture of cerebellar granule cells, a 2-min exposure to glutamate (100 μM) resulted in neuronal cell death less than 20%. However, glutamate-mediated neurotoxicity was synergistically potentiated in co-cultures of cerebellar granule cells and activated microglia: over 80% of neuronal cells were killed by the 2-min exposure to 100 μM glutamate). Similar potentiation of glutamate action was also found when cerebellar granule cells were briefly exposed to glutamate and then co-cultured with activated microglia. These observations suggest that during ischemic brain injury cytokine-activated microglia may be implicated in the inflammatory reaction and potentiate the glutamate-mediated neurotoxicity as well. (This work was partially supported by U.Research Grant (for W-K. Kim)).

318.2

IMAGING SWELLING OF THE NEOCORTEX DURING ACUTE EXCITOTOXICITY. Nicole G. Rolfe* and R. David Andrew, Department of Anatomy and Cell Biology, Queen's University, Kingston, Ontario K7L 3N6.

Neuronal swelling is considered an early excitotoxic event, the result of excessive release of glutamate that leads to receptor mediated Na^+ influx followed by Cl^- and water. Until recently, studies of excitotoxicity have investigated cell body swelling in culture or in histological sections that are sampled hours or days post-trauma. Swelling of CA1 dendritic regions in the hippocampal slice is inducible within minutes by glutamate agonists or ischemia as measured by real time increases in light transmittance (LT) (Andrew and colleagues, this meeting). Regional elevation of LT is an indirect measure of increased cell volume in the hippocampal slice (Andrew and MacVicar, 1994). Because swelling of the cerebral cortex can be a lethal consequence of trauma/ischemia, we hypothesized that frontal and parietal neocortical slices from the rat might display swelling, detectable as elevated LT, in response to glutamate agonists. Bath application of 100 μM NMDA for 1 min at 35°C increased LT in layers I-V by 40 to 60% but not in underlying white matter (n=6). The NMDA response was completely blocked by pre-application of 100 μM of the NMDA antagonist AP-5 (n=4). A 1 min exposure to 100 μM kainate evoked a temporally and regionally similar response that was blocked by 10 μM of the non-NMDA antagonist CNQX. Preliminary spectrometry revealed that the LT increased across the 400 to 800 nm spectrum which further supports cell swelling rather than narrow band activation of chromophores (e.g. cytochromes).

We conclude that a 1 min application of a NMDA or non-NMDA glutamate agonist evokes pronounced and reversible swelling in neocortex similar to that seen in dendritic regions of the hippocampal CA1 region. Whether longer exposure times lead to neuronal death (as in CA1) is currently being examined. *Supported by the Heart & Stroke Foundation of Ontario and the Canadian MRC*

318.3

THIOPENTAL REDUCES NMDA AND AMPA INDUCED NEURONAL DAMAGE IN THE RAT HIPPOCAMPAL SLICE. Hechen Zhu, Ira S. Kass* and James E. Cottrell, Departments of Anesthesiology and Pharmacology, SUNY Health Science Center at Brooklyn, Brooklyn, NY 11203

Thiopental is a common anesthetic agent that shows protection against cerebral anoxia. To examine whether such protection involves the attenuation of glutamate excitotoxicity, the effect of thiopental on NMDA and AMPA induced neuronal damage was investigated. Extracellular responses in CA1 pyramidal cells due to Schaffer collateral stimulation were quantified by measuring the amplitude of the post-synaptic evoked population spike (P.S.). The percentage recovery after thiopental and/or NMDA/AMPA was calculated by dividing the amplitude of the P.S. after the treatment by its pre-treatment value. The duration of NMDA (25μM) or AMPA (15μM) application was 10 minutes. Thiopental (600μM) was present 15 min before, during and 10 min after the NMDA or AMPA treatment. The recovery of the P.S. in both thiopental groups is significantly better than that with NMDA or AMPA alone (see Table). This demonstrates that thiopental can improve recovery of rat hippocampal slices from NMDA and AMPA induced neuronal damage, if it is present before, during and shortly after the insult. Hence, blockade of the NMDA and AMPA mediated glutamate excitotoxicity may be one mechanism by which thiopental protects against anoxic damage. (Supported by funding: NIGMS GM38866)

Table Effect of thiopental on the percentage recovery of the P.S.

	1 hr	2 hr	3 hr	4 hr	5 hr
NMDA (n=8)	49 ± 7	47 ± 6	44 ± 6	——	——
NMDA+Thiopental(n=10)	91 ± 7*	85 ± 8#	79 ± 10!		
AMPA (n=6)	3 ± 3	4 ± 4	7 ± 6	14 ± 9	15 ± 9
AMPA+Thiopental (n=6)	20 ± 8	39 ± 6*	52 ± 3*	54 ± 5#	50 ± 6#

All values are mean ± SEM. * P<0.001 # P<0.01 ! P<0.05

318.4

LOW-AFFINITY INDICATORS DISTINGUISH LETHAL CALCIUM ENTRY DURING GLUTAMATE RECEPTOR ACTIVATION IN CULTURED NEURONS. K.L. Hyrc*, S.D. Handran, S.M. Rothman, and M.P. Goldberg, Center for the Study of Nervous System Injury, Washington Univ. School of Med., St. Louis, MO 63110.

Although abundant evidence suggests that calcium entry participates in excitotoxic neuronal death, studies using fluorescent calcium indicators have failed to show a relationship between intracellular free calcium concentrations ($[Ca^{2+}]_i$) and subsequent neuronal injury. One explanation may be that conventional indicators, such as fura-2, are limited by their high affinity for Ca^{2+} ($K_d \approx 0.2$ μM) and can not assess potentially toxic $[Ca^{2+}]_i$ levels above 1-2 μM. We examined this hypothesis using fluorescence videomicroscopy with the ratiometric, low-affinity Ca^{2+} indicator, benzothiazole coumarin (BTC; $K_d \sim$ 7-14 μM; Iatridou et al., *Cell Calcium*, 15:190, 1994).

Murine cortical neuronal cultures were loaded with either fura-2/AM or BTC/AM (6 μM). Confocal microscopy showed that labeling with BTC/AM or BTC free acid (microinjected by patch electrode) was uniformly distributed throughout the neuronal cytoplasm. Cultures were exposed for 10-15 min to 100-300 μM NMDA, 100-300 μM glutamate, 300-500 μM AMPA, or 50 mM KCl (the latter two with MK-801). Each treatment produced a large rise in $[Ca^{2+}]_i$ detected by fura-2. However, only the NMDA and glutamate exposures resulted in neuronal death one day later. When $[Ca^{2+}]_i$ was measured by BTC (400 nm/480 nm ratio), AMPA and K^+ depolarization elicited only a small transient rise in $[Ca^{2+}]_i$, whereas NMDA and glutamate induced much larger, sustained elevations in $[Ca^{2+}]_i$. There was a positive correlation between $[Ca^{2+}]_i$ determined by BTC ratio, and increasing toxicity with NMDA exposure (50 - 300 mM). Other low-affinity indicators, including mag-fura-5 and calcium green-5N, also demonstrated $[Ca^{2+}]_i$ elevation after NMDA application; however, BTC may be especially useful because of its selectivity for Ca^{2+} over Mg^{2+}, and ability to provide ratiometric data. These data support the hypothesis that micromolar elevations of intracellular calcium may be critical for glutamate receptor-mediated cell death. Supported by NIH NINDS grants NS32140 (MPG), NS19988 (SMR). and T32NS07071 (SDH).

318.5

INHIBITION OF TUMOR NECROSIS FACTOR-α (TNF-α) ATTENUATES NMDA-INDUCED STRIATAL NEUROTOXICITY IN PERINATAL RATS. J.M. Galasso, P. Wang, D. Martin, and F.S. Silverstein*. Neuroscience Program and Depts. of Pediatrics and Neurology, Univ. of Michigan, Ann Arbor, MI 48109 and Dept. of Inflammation, Amgen Boulder Inc., Boulder, CO 80301.

Recent observations implicate pro-inflammatory cytokines including TNF-α and IL-1β as mediators of acute brain injury. In 7 day old (P7) rats, direct intra-cerebral administration of NMDA elicits a rapid increase in TNF-α gene expression (Stroke 26:1093); however, the pathogenetic role of TNF-α is unknown. To test the hypothesis that TNF-α production contributes to the severity of excitotoxic injury *in vivo*, we sought to determine the efficacy of a TNF-α inhibitor, a dimeric polyethylene glycol linked form of the type I soluble receptor of TNF [TNF binding protein (bp)] in protecting against NMDA neurotoxicity. Methoxyfluorane-anesthetized P7 rats received a right intra-striatal co-injection of 10 nmol of NMDA and 3.75 μg of TNFbp (volume:1 μl), followed 15 min later with an i.p. injection of 50 μg of TNFbp (n=13). Litter-mate controls received heat-treated (100°C, 30 min) TNFbp (n=11). Severity of brain injury was quantified on P12 by measuring intact Nissl staining in the striatum bilaterally in 20 μM cresyl-violet-stained coronal sections (≥14/brain), using NIH Image; a summation formula was used to estimate striatal volumes and lesion volumes were calculated as the inter-striatal volume differences. Treatment with the active form of TNFbp attenuated NMDA-induced damage by 26% in comparison with controls [lesion volume (mean±SEM) 12.3±5 vs 16.7±3, p<0.012, t-test]. These results suggest that TNF-α may be an important mediator of NMDA receptor-induced neurotoxicity *in vivo*. Thus, pharmacological antagonism of endogenous TNF-α may represent an effective neuroprotective strategy against acute brain injury.

[Supported by a grant from the Hearst Foundation to FSS].

318.6

ANALYSIS OF ACUTE RESPONSES TO IBOGAINE AND A PRIMARY METABOLITE IN CEREBELLAR PURKINJE CELLS. L.J. Larson-Prior* and P.L. Hawkins. Dept of Neuroscience & Anatomy, Penn State University College of Medicine, Hershey, PA 17033.

Ibogaine, an indole alkaloid under investigation as an anti-addictive agent, has been shown to be neurotoxic in the cerebellar cortex at high doses (>100 mg/kg i.p.). Ibogaine may exert its anti-addictive effects via a recently identified long-lasting metabolite, 12-hydroxyibogamine (noribogaine). The acute toxicity (1 - 48 hr) of these agents on cerebellar Purkinje cells was investigated using immunocytochemical markers for glial activation (GFAP), Purkinje cell (PC) morphology (calbindin-28), and cell stress (HSP-70).

Adult male Sprague-Dawley rats were injected with ibogaine or noribogaine (5 mg/ml in distilled water) at a final dose of 40 mg/kg i.p. Injections were given in 4 equal doses at 15 minute intervals over the course of an hour. One, 6 and 48 hrs after administration of the final dose, animals were sacrificed and the brain immunostained using antibodies to GFAP, calbindin-28 (C-28) or HSP-70.

No differences from controls were seen in staining for C-28 or GFAP at any of the time points examined for either treatment group. PCs continued to stain strongly and completely for C-28 and no changes in the astroglial population were noted. Increased synchrony of firing in the inferior olivary nuclei has been suggested as a mechanism mediating ibogaine cytotoxicity in the cerebellum. No changes in C-28 or GFAP staining, and no differences between drug treated animals and controls were noted in these cells. However, 48 hrs after ibogaine treatment, cerebellar Purkinje cells were strongly stained for HSP-70 while animals treated with noribogaine showed no staining for this marker. Immunostained PCs were found only in the vermal cortex. The inferior olivary nuclei were equally immunoreative for HSP-70 protein in both ibogaine and noribogaine treated animals, while control animals showed no immunoreactivity for this marker. Thus, ibogaine produces a stress reaction in cerebellar vermal PCs within 48 hrs of application. Noribogaine, which does not mirror this potentially toxic stress reaction may thus prove a more useful antiaddictive agent. *Supported in part by NS 30759 and IBN-9514844 (LLP).*

318.7

AChE Reactivity in the Basal Lateral Amygdala: A Post-Mortem Index of Seizure Severity Following Soman J. Forster, F. Cann, G. Ballough[1], B. Hackley*, M. Filbert. U.S. Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground 21010. [1]LaSalle University Philadelphia, PA 19141

The possibility that the loss of AChE reactivity in discrete brain regions may have differential importance for induction of status epilepticus was investigated. Rats were infused with monosialoganglioside, GM1, through a permanent cannula implanted intracerebroventricularly (i.c.v.) and connected to an osmotic minipump. Controls received saline infusions or were sham operated. Rats received soman or saline injections 4.0 ± 0.5 days following GM1 infusions. ECoG recordings were monitored via indwelling cortical electrodes. Twenty-seven hours after soman administration, rats were euthanized via transcardial perfusions with brains removed and processed for AChE histochemistry. An Image Analysis System assessed optical densities of AChE staining in the piriform cortex (Pir), basal lateral amygdaloid nucleus (BL) and caudate putamen (CPu). All soman rats showed evidence of seizure initiation within 10 min. However, 6 out of 11 rats infused with GM1 did not develop status epilepticus. All animals receiving soman showed statistically significant reductions in staining for AChE. However, in those rats infused with GM1 that did not develop status epilepticus, the AChE staining in the BL was significantly higher than in rats not receiving GM1. These observations suggest that the loss of AChE reactivity in the BL may be a postmortem index of seizure severity following soman administration.

Funding: Department of Defense

318.8

EFFECT OF BCL-2 EXPRESSION ON GLUTAMATE EXCITOTOXICITY AND APOPTOSIS IN IMMORTALIZED RAT HIPPOCAMPAL CELLS. C. A. Sherman, E. M. Evest, M. R. Rosnert, J. A. Izzo*, J. W. Kusiak. Molecular Neurobiology Unit, NIA, Baltimore, MD 21224; †The University of Chicago, Chicago, IL 60637.

Excitotoxicity plays a role in neuronal death seen after acute neurological insults (ischemia, stroke) and may be involved in neurological disorders such as Parkinson's disease and Alzheimer's disease. Whether or not this phenomenon leads to a necrotic or apoptotic cell death pathway is controversial. In order to study the molecular mechanisms involved in glutamate excitotoxicity, two SV-40^{ts}-immortalized rat embryonic hippocampal cell lines were utilized. These were a parent line, H19-7, and a Bcl-2 transfected line, H19-7/Bcl-2. These cells were differentiated by a temperature shift to 39°C with 10ng/ml bFGF, N2 supplements in serum free media. By RT-PCR, they were found to express the GluR-3, GluR-6 and KA-2 glutamate receptor subunit mRNAs. The H19-7 and H19-7/Bcl-2 cell cultures were differentiated and treated with 1 mM glutamic acid for 24 hours. H19-7 cultures exhibited a 30% cell loss determined by cell counts and MTS assay while the H19-7/Bcl-2 cultures did not. LDH release was not increased in either line compared to untreated cells. Glutamate treatment of H19-7 cultures led to increased DNA laddering which did not occur in the similarly treated H19-7/Bcl-2 cultures. Based on the lack of LDH release, DNA laddering and the protection from cell loss by Bcl-2, we conclude that the glutamate excitotoxicity and cell loss exhibited in the H19-7 cell line is apoptotic in nature. This work was supported by the NIH and the Aluminum Association.

318.9

RESISTANCE OF NEONATAL CA1 NEURONS TO GLUTAMATE-INDUCED INJURY IN VITRO IS ASSOCIATED WITH MINIMAL INCREASES IN $[Ca^{++}]_i$. J.D. Marks*. Department of Pediatrics, University of Chicago, Chicago, IL 60637.

Glutamate receptor activation can increase free intracellular calcium ($[Ca^{++}]_i$), alter neuronal morphology, and cause cell death. In the rat, glutamate receptor subtype expression changes markedly during the first 3 weeks postnatal life. We have shown that CA1 neurons from 3 week old rats respond to glutamate stimulation with somal swelling, dendrite retraction, and loss of membrane integrity, whereas neurons from newborn animals show none of these effects. We hypothesized that this neonatal resistance occurs because glutamate increases $[Ca^{++}]_i$ to lower levels in neurons from newborns compared with neurons from older animals. We measured $[Ca^{++}]_i$ and changes in neuronal morphology by obtaining alternating differential interference contrast and epifluoresence images of single, Fura-2 loaded, hippocampal CA1 neurons that had been acutely dissociated from mature (P21-P25) and newborn (P0-P5) Sprague-Dawley rats. Images were obtained every 20 s at baseline, during glutamate perfusion (100 μM), and during washout. Mean somal $[Ca^{++}]_i$ at baseline was significantly lower in neonatal neurons (80 ± 9 nM, mean ±SEM , n=10) than in mature neurons (120 ± 9 nM, n=12; p<.01). Glutamate increased $[Ca^{++}]_i$ abruptly in all neurons. However, mean peak $[Ca^{++}]_i$ attained during glutamate stimulation was significantly lower in neonatal neurons (343 ± 53 nM) compared with mature neurons (2066 ± 338 nM, p<.0001). Following glutamate stimulation, mature neurons demonstrated marked swelling and dendrite retraction, followed by sudden loss of dye and cell death. Morphology of neonatal cells did not change. We conclude that glutamate causes smaller $[Ca^{++}]_i$ increases in neonatal neurons than in mature neurons. This decreased $[Ca^{++}]_i$ response may underlie the lack of morphologic change and subsequent death seen in newborn neurons.

Supported by the Biological Sciences Division, University of Chicago

318.10

NEUROPROTECTIVE ROLE OF *c-fos* ANTISENSE OLIGONUCLEOTIDE AGAINST GLUTAMATE/NMDA INDUCED NEUROTOXICITY IN THE RAT HIPPOCAMPUS. X.-C. M. Lü*, J. R. Dave, M. Laskosky, H. S. Ved and F. C. Tortella. Divi. of Neurosci., Walter Reed Army Inst. Res., Washington, DC 20307

Excitatory amino acids (EEA) have been shown to stimulate the expression of *c-fos* proto-oncogene through the glutamate/NMDA receptor complex. Previous studies in our lab showed that treatment of hippocampal cultures with 5 μM *c-fos* antisense oligonucleotide (ASO) partially blocked glutamate-induced neuronotoxicity (Dave, et al., Neurosci Abst, 1993). To further understand the role of the *c-fos* oncogene in the mechanisms of neuronal death, we investigated the dose-response and the time-course of *c-fos* ASO after *in vitro* and *in vivo* glutamate or NMDA treatments. Primary cultures enriched in neurons dissociated from embryonic rat hippocampus were treated in serum-free media with vehicle or 80 μM glutamate. Addition of 10 or 25 μM *c-fos* ASO to the culture for 4 hr promoted the survival of hippocampal neurons in a dose-dependent manner; 23% survival at 10 μM and 97% survival at 25 μM. *In vivo*, rats received stereotaxic bilateral injections of either 25 nM NMDA alone (n=3) aimed at CA1 of the hippocampus or injections of 25 μM (n=6) or 125 μM (n=6) *c-fos* ASO 30 min or 4 hr prior to the NMDA injection at the same hippocampal site. During the next 24 hr, NMDA treatment alone produced extensive neuronal degeneration along the CA1 axis rostral and caudal to the injection site. Cell density measurements revealed that 30 min pretreatment with either 25 μM or 125 μM *c-fos* ASO resulted in significant survival of CA1 neurons. However, no significant effect was observed with 4 hr pretreatment at both doses. Collectively, these *in vitro* and *in vivo* results demonstrate a protective role of *c-fos* ASO against glutamate or NMDA induced neurotoxicity in a dose- and time-dependent manner and suggest a causative role of the expression of *c-fos* proto-oncogene during EEA induced neuronal death. (Research was funded by the U.S. Army Medical Research and Materiel Command.)

318.11

Neuroprotective effect of ocular hypotensive α_2 adrenoceptor agonists in primary rat hippocampal cultured neurons. R. K. Lai*, D. Hasson and L. A. Wheeler. Dept. of Biological Sciences, Allergan, INC., Irvine, CA 92715.

α_2 adrenoceptor agonists are a class of compounds that have shown promising therapeutic efficacy for the treatment of ocular hypertension associated with glaucoma and post-ocular surgical procedures. These compounds effectively lower intraocular pressure in rabbits, monkeys and humans. Recent studies suggest that this class of compounds are also neuroprotective in the focal ischemia and the optic nerve crush models. We examined the effect of novel Allergan α_2 adrenoceptor agonists, brimonidine and AGN 191103, on NMDA and kainic acid induced toxicity in primary rat hippocampal cultured neurons. By monitoring the lactate dehydrogenase release and number of cell death, we found that these α_2 compounds are neuroprotective against the kainic acid (10^{-4}M) induced cytotoxicity but not the NMDA (10^{-4}M) induced cytotoxicity. Radioligand binding studies demonstrated no significant interaction by these compounds at the NMDA, kainate, glycine or PCP sites of the glutamate receptor, suggesting that the neuroprotective effect is probably downstream. Exposure to these compounds for 24 hr upregulated the expression of bFGF 2-fold in these hippocampal neurons. We further found that mRNA expression of bFGF increased 3-fold in retina obtained from rats that were i.p. injected with brimonidine or AGN 191103 24 hours earlier at 1 mg/kg dose using both RT-PCR and Northern analyses. The combined data suggest a mechanism by which α_2 adrenoceptor agonists brimonidine and AGN 191103 are effective ocular hypotensive agents with neuroprotective properties.

(Supported by Allergan, INC.)

318.12

NEUROPROTECTIVE EFFECTS OF 1,25(OH)$_2$ VITAMIN D$_3$ IN HIPPOCAMPAL NEURONS SUBJECTED TO NMDA INSULT. N.M. Porter*, V. Thibault and P.W. Landfield. Dept. Pharmacology, Coll. Medicine, Univ. Kentucky, Lexington, KY 40536.

It is well established that vitamin D (VitD) plays an important role in Ca^{2+} homeostasis in the periphery. Although VitD receptors are present in hippocampal neurons, much less is known regarding the function of VitD in the CNS. Because VitD regulates Ca^{2+} homeostasis in the periphery, we hypothesized that it may play a similar role in the CNS. The present study was undertaken to examine the effects of VitD in an experimental paradigm of neuronal injury involving Ca^{2+} influx, NMDA-induced excitotoxicity.

Primary rat hippocampal neurons, maintained in serum, were chronically treated (days 3, 6 and 8) with the active metabolite of VitD, 1,25(OH)$_2$ vitamin D$_3$ (0.1-1000 nM). Moderate insults were induced on day 8 by treatment of cultures with NMDA (100-300 μM). Cell survival was quantitated 24 and 96 hr later by counting viable cells using phase-contrast microscopy.

The number of surviving hippocampal neurons was significantly greater in cultures pretreated with VitD, particularly at the lower concentrations of VitD. NMDA induced 70% cell death in cultures pretreated with ethanol vehicle (0.05%) but only a 37% reduction in cells pretreated with VitD. The results suggest that as in the periphery, VitD may regulate Ca^{2+} homeostasis in the CNS, and exert protective effects against certain (Ca^{2+}-dependent) types of neuronal insults. ***(Supported by NIH AG10836)***

318.13

GLUTAMATE RELEASE DURING GLOBAL CEREBRAL ISCHEMIA IS ATTENUATED BY THE Na^+-CHANNEL BLOCKER LAMOTRIGINE. A. Bacher and M. H. Zornow*. Department of Anesthesiology, University of Texas Medical Branch, Galveston, TX 77555-0830

The excitatory amino acid glutamate is known to play an important role in ischemia-induced neuronal injury (1). Recently, it was demonstrated that Na^+-channel blockers can improve neuronal survival rate after ischemia (2,3). The present study was performed to investigate the effects of the Na^+-channel blocker lamotrigine on glutamate release during transient cerebral ischemia.

After IRB approval, 28 New Zealand white rabbits were randomly assigned to 4 groups: control (n=8), 30°C hypothermia (n=7), and lamotrigine 20 mg/kg (n=8) or 50 mg/kg (n=5). Animals were anesthetized (1% halothane), intubated, and mechanically ventilated to maintain normocarbia. Microdialysis catheters were stereotactically placed in the dorsal hippocampal region and perfused with synthetic cerebrospinal fluid. Two 10 minute-periods of global cerebral ischemia were produced by systemic hypotension (trimethaphan 5 mg i.v.) and inflation of a neck tourniquet to obtain an isoelectric EEG. Lamotrigine was intravenously administered 90 minutes before the onset of the first ischemic period. Glutamate concentrations in the microdialysate were determined by HPLC. Levels after ischemia and reperfusion were compared by ANOVA and Dunnett's test. Data are presented as means±SD.

Increases in glutamate concentration were minimal during the first ischemia and reperfusion. During the second ischemia, there was an approximately five-fold increase in glutamate concentration compared to baseline in the control group (peak=5.48±2.11 μM). Signficantly lower values ($p<0.008$) were found in the hypothermia (0.97±0.94 μM) and the lamotrigine 50 mg/kg (2.16±1.49 μM) groups. Values of the lamotrigine 20 mg/kg group (3.5±1.03 μM) were signficantly lower ($p<0.008$) than those of the control group (5.92±1.98 μM) during second reperfusion.

We conclude that lamotrigine is comparable to hypothermia in attenuating hippocampal glutamate increases associated with transient global ischemia.

1. J Cereb Blood Flow Metab 1989;9:629-639 2. Brain Res 1992;593:1-6
3. J Neurosci 1989;9:3720-3727

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318.14

NEUROPROTECTIVE EFFECTS OF DEEP HYPOTHERMIA ON EXCITOTOXIC INJURY IN CULTURED CORTICAL NEURONS. M. Tymianski*[1], R. Sattler[1], R.F. Spetzler[2] and J.M. Zabramski[2]. [1]Playfair Neuroscience Unit, University of Toronto and [2]Barrow Neurological Institute, Phoenix, AZ 85013.

Temperature reductions to 10-20°C are used clinically to protect brain tissue against ischemic injury. However, the mechanisms by which deep hypothermia protects neurons are unknown. We therefore investigated the survival of cultured cortical neurons under a range of combinations of temperature (12-37°C), excitatory amino acid (EAA) receptor agonists (glutamate, NMDA, AMPA, kainate and tACPD, each at 0,30,100,300 and 1000μM) and antagonists (APV 50,100μM, MK-801 50μM, CNQX 10μM). The time-course and extent of excitotoxicity was gauged in a multiwell fluorescence scanner as described by Sattler et al., (abstract, this meeting). All temperatures were well tolerated by control cultures. Neuroprotective efficacy of hypothermia against EAA insults was U-shaped, with maximal protection achieved at 20°C and 30°C, but less so at 12°C. Equimolar insults with glutamate and NMDA were equally toxic at 37°C, but glutamate toxicity was temperature resistant- while NMDA toxicity was considerably lessened by the temperature reduction. The temperature-insensitive component of glutamate toxicity was still due to NMDA receptor activation as it was abolished by NMDA antagonists, and was not reproduced by non-NMDA agonists or the mGluR agonist tACPD. Although hypothermia may protect neurons by attenuating endogenous EAA release (Bruno et al., J Neurochem 63, 1398), the present data show that a significant component of neuroprotection by deep hypothermia can also occur postsynaptically, though these postsynaptic effects are sensitive to the specific agonist used. (Supported by an MRC clinician-scientist award to MT, a National Centers of Excellence of Canada Studentship to RS, and an Ontario Technology Fund grant with Allelix Biopharmaceuticals to MT).

318.15

ON THE NEUROPROTECTIVE AND NEUROTOXIC POTENTIAL OF INTRACELLULAR CALCIUM CHELATION IN ORGANOTYPIC HIPPOCAMPAL SLICES EXPOSED TO OXYGEN/GLUCOSE DEPRIVATION (OGD). Khaled. M. Abdel-Hamid* and Michael Tymianski. Playfair Neuroscience Unit, University of Toronto, Ontario, Canada, M5T 2S8.

Excessive endogenous glutamate release has been implicated in ischemia-induced central neuronal injury, mainly by derangement of intraneuronal Ca^{2+} homeostasis. An attractive neuroprotective strategy is to enhance neuronal capacity to buffer excess Ca^{2+} influx associated with ischemic events, thus reducing Ca^{2+} mediated neurotoxicity. Here we present evidence that neuronal pre-loading with the artificial Ca^{2+} buffer, BAPTA, is associated with early partial protection of cultured organotypic hippocampal slices against OGD (*in vitro* ischemia). Hippocampal slices from 7d postnatal rat pups were maintained *in vitro* (Stoppini *et al*, 1991) for 12 days prior to 60 minutes of OGD at 37°C (>90% neuronal loss at 24 hours). NMDA antagonists (MK801 and APV) produced a sustained protection (60-70% protection at 3 hours, 40-50% protection at 24 hours). Pre-treatment with BAPTA-AM (60 min) was associated with neuroprotection at 3 and 5 hours after OGD (50%, 40% for 10μM BAPTA-AM, and 60%, 50% for 100μM BAPTA-AM pre-treatments) which did not persist at 24 hours. Analogous effects were observed with the low affinity Ca^{2+} chelator APTRA-AM. The protection time-course of BAPTA was parallelled exactly by the time-course of BAPTA retention in the slice tissue as measured by ^{14}C-BAPTA autoradiography. Neuroprotection was absent when 100μM BAPTA-AM was used prior to injury with 100μM NMDA, suggesting that protection from OGD may be mediated at the pre-synaptic level, possibly by attenuating transmitter release. Furthermore, with submaximal neuronal injury using 40μM NMDA, 100μM BAPTA-AM pretreatment caused severe neuronal loss. These results illustrate the complexity of the role of Ca^{2+} in neuronal damage, and that chelation of intraneuronal Ca^{2+} may have both a protective and neurotoxic potential. (Supported by an Ontario Technology Fund grant with Allelix Biopharmaceuticals to MT).

318.16

PREGNENOLONE SULFATE MODULATES EXCITOTOXIC AND APOPTOTIC NEURONAL INJURY IN CELL CULTURE J.K. Muir*, L.M.T. Canzoniero, S.L. Sensi and D.W. Choi. Center for the Study of Nervous System Injury and Dept. of Neurology, Washington Univ. School of Medicine, St. Louis, MO 63110.

The neurosteroid pregnenolone sulfate (PS) enhances NMDA receptor-gated current while reducing AMPA, kainate and GABA receptor-gated currents (Wu et al., Mol. Pharmacol. 40:33, 1991). Thus, we hypothesized that PS would enhance NMDA-induced, and reduce AMPA/kainate-induced, excitotoxic neuronal injury.

As predicted by the electrophysiology, neuronal death in mixed murine cortical cell cultures (DIV 14-16) exposed to 10 μM AMPA or 40-50 μM kainate for 20-24 hr was reduced by concurrent addition of 50-100 μM PS. Further, the submaximal injury induced by 3-12 μM NMDA was potentiated. Application of 100 μM PS alone for 24 hr was not toxic. Interestingly, the apoptotic neuronal injury induced by exposure of cultures to 100 nM staurosporine for 24-48 hr (Koh et al., Exp. Neurol. 135:153, 1995) was concentration-dependently reduced by 1-100 μM PS. This protective effect was blocked by the NMDA antagonist MK-801. Cycloheximide (1 μg/ml) also attenuated staurosporine-induced neuronal death, however the protective effect of cycloheximide was unaffected by MK-801. We hypothesize that the ability of PS to reduce neuronal apoptosis may be mediated by elevations of intracellular free calcium, due to enhancement of NMDA receptor-gated current as well as perhaps reduction in GABA receptor-mediated current.

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318.17

PREGNENOLONE SULFATE NEUROTOXICITY IN CULTURED CHICK CORTICAL NEURONS. J.M. Fahey* and D.G. Lindquist. Department of Pharmacology and Experimental Therapeutics, Tufts University School of Medicine, Boston, MA 02111.

Pregenenolone sulfate (PS) has been reported to selectively augment glutamate-induced depolarizations mediated by the N-methyl-D-aspartate (NMDA) subtype of the glutamate receptor. Previously, this laboratory has demonstrated that NMDA-mediated increases in intracellular calcium in primary cortical neurons are potentiated in the presence of PS. The present study examines the effect of this neurosteroid on neuronal cell death alone and in combination with NMDA/glycine. Primary cortical chick neurons were exposed to PS and subsequently assayed for [^{3}H]ouabain binding to quantify cell loss. Neuronal survival was significantly decreased in the presence of PS at 24 h (500 μM), 48 h (500 μM) and 72 h (250 μM and 500 μM) relative to controls. In a second study, co-application of non-toxic concentrations of NMDA/glycine (500 μM/50 μM) and PS (250 μM) for 48 h significantly reduced neuronal survival in these neurons relative to either compound alone. These data indicate that higher concentrations of the neurosteroid PS alone are toxic to cortical neurons *in vitro*. In addition, this study demonstrates a synergistic toxic effect of PS and NMDA/glycine, providing additional evidence of the possible involvement of PS in excitotoxicity and cell death.

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318.18

Gene Transfer of Calbindin D28k cDNA by HSV Amplicon Vector Decreases Calcium Ion Mobilization Following Hypoglycemic Challenge And Enhances Neuronal Survival. T. J. Meier*, D. Y. Ho and R. M. Sapolsky. Dept. of Biological Sciences, Stanford University, Stanford, CA 94305-5020.

Maintenance of intracellular calcium ion homeostasis plays an important role in the cellular well-being of hippocampal neurons. Disruption of that homeostasis often leads to cell death. In recent years the function of calcium-binding proteins as calcium ion (Ca^{2+}) buffers has been proposed. We hypothesized that calbindin D28k (CB) may function as an intracellular Ca^{2+} buffer and constructed herpes simplex virus vectors to deliver rat CB cDNA to hippocampal neurons *in vitro*. These vectors co-express bacterial *lacZ* as a reporter gene. Cells were infected with vectors delivering the CB cDNA, or the CB gene inactivated by insertion of stop codons in all three reading frames near the 5' end of the cDNA (*cbs*). Infected cells were subjected to 12 or 24 hours of hypoglycemia (0.5 mM glucose), and then examined by calcium ratiometric imaging utilizing the fluorescent calcium indicator fura-2. Following hypoglycemic incubation, cells infected with vectors carrying the CB gene exhibited lower overall amounts of Ca^{2+} mobilized in response to aglycemic challenge, as compared to uninfected cells. Cells infected with the *cbs* vector exhibited significantly higher Ca^{2+}. Cells infected with these constructs were assayed for survivorship after 12-or 24-hour hypoglycemic challenge *in vitro*. Infected neurons which survived the treatment were visualized using the chromogenic substrate X-gal. Cells receiving the CB gene gained a significant survivorship advantage over cells receiving the cbs vector at 12 but not at 24 hours post hypoglycemic challenge. Our work previously indicated that decreased Ca^{2+} mobilization via CB overexpression did not enhance neuronal survival following glutamate exposure. In contrast, we demonstrate here, in response to hypoglycemia, both decreased Ca^{2+} mobilization and increased survivorship of neurons infected with CB vectors.

318.19

ELEVATION OF ENDOGENOUS BRAIN KYNURENIC ACID LEVELS BY NOVEL ANALOGUES OF NICOTINYLALANINE, A.F. Miranda*, K. Jhamandas, R. Beninger and R.J. Boegman. Departments of Pharmacology & Toxicology, Psychology, and Medicine, Queen's University, Kingston, Ontario, Canada K7L 3N6.

Endogenous excitotoxins have been implicated in several neurodegenerative disorders. One such agent quinolinic acid (QUIN) is derived from the kynurenine pathway which also yields kynurenic acid (KYNA), an endogenous excitatory receptor antagonist. Previously, we have demonstrated that nicotinylalanine (NICALA), an inhibitor of kynureninase and kynurenine hydroxylase, in combination with probenecid, an inhibitor of organic acid transport, and kynurenine, the precursor of KYNA, elevates brain KYNA and protects against QUIN toxicity *in vivo (Soc.Neurosci.Abstract 21:74, 1995)*. NICALA however, is not a potent inhibitor of kynureninase or kynurenine hydroxylase. This study examined the ability of novel analogues of NICALA to increase endogenous brain KYNA levels. Male Sprague-Dawley rats (125-150 g) received equivalent doses (5.6 nmoles/5 μl i.c.v.) of An#1 (2-amino-4-hydroxy-4-(3-pyridyl) butyric acid), An#2 (2-amino-1,4-dihydroxy-4-(3-pyridyl) butane dihydrochloride salt),NICALA or saline in combination with probenecid (200 mg/kg, i.p.) and kynurenine (450 mg/kg, i.p.). Brain KYNA levels were measured at 1.5, 3, 6 and 9hr following treatment. NICALA, An#1 and An#2 produced elevations of brain KYNA that were at least 3-fold greater than those produced by saline 3hrs post treatment. An#1 produced elevations of brain KYNA levels that were approximately 1.5-fold greater than those produced by NICALA 3hrs post treatment. KYNA levels following administration of An#2 were not significantly different than those produced by NICALA at all time points. This study demonstrates that An#1 and An#2 elevate endogenous brain KYNA levels and thus, have the potential to be effective neuroprotective agents. (Supported by MRC Canada, The Huntington's Society of Canada and Neurochem Inc.)

318.20

NEUROPROTECTIVE INTERACTION EFFECTS OF NMDA AND AMPA RECEPTOR ANTAGONISTS IN AN IN VITRO MODEL OF CEREBRAL ISCHEMIA. R.L. Arias* and J.R.P. Tasse. CNS Disorders Division, Wyeth-Ayerst Research, CN-8000, Princeton, NJ 08543-8000.

An in vitro model of ischemia was developed in the rat hippocampal slice preparation. Ischemia was defined as a thirty minute aglycemic period ending in five minutes of concurrent anoxia. Slice viability was determined before and after ischemia by testing for the presence of a population spike in CA1 upon electrical stimulation of the Schaffer collaterals. Exposure of slices to an ischemic period resulted in a nearly complete loss of viability. This effect was prevented by withholding calcium from the buffer during ischemia. The glutamate subtype antagonists, MK-801 (NMDA noncompetitive), CGS-19755 (NMDA competitive), GYKI-52466 (AMPA noncompetitive) and YM90K (AMPA competitive) were bath applied to slices, alone and in combination, during ischemia. AMPA antagonists applied alone had no neuroprotective effect in this model. NMDA antagonists applied alone had a modest neuroprotective effect. Co-application of MK-801 and GYKI-52466 resulted in complete neuroprotection, comparable to that obtained with calcium withholding. The effect of this combination treatment on slice survival rate was synergistic, that is greater than the sum of the individual drug effects. Results indicate that neuroprotection against ischemia may require a combination therapy approach.

EXCITATORY AMINO ACIDS: EXCITOTOXICITY II

319.1

THE MAPK/ERK KINASE (MEK) INHIBITOR PD-98059 REDUCES NEURONAL CELL DEATH FOLLOWING COMBINED OXYGEN AND GLUCOSE DEPRIVATION IN PRIMARY NEOCORTICAL CULTURES. A.W. Probert*, P.A. Boxer, S.S. Basu, T.K. Brabham and R.G. MacKenzie. Parke-Davis Pharmaceutical Research, Division of Warner-Lambert Co., Ann Arbor, MI 48105.

The neuroprotective effect of the MAPK/ERK kinase (MEK) inhibitor, PD-98059, was evaluated in primary neocortical neuronal cultures exposed to increasing intervals of combined oxygen (1%) and glucose (1 mM) deprivation (COGD). Neuronal injury (increased lactate dehydrogenase levels in culture supernate), which first appears following 135-150 minutes of COGD and peaks after 240-255 minutes of deprivation, is preceded by a similar pattern of $^{45}Ca^{++}$ uptake approximately 15 minutes prior to injury onset. This initial injury sequence is primarily driven by glutamatergic mechanisms, since it can be abolished in the presence of 100 μM R(-)-3-(2-Carboxypiperazine-4-yl)-propyl-1-phosphonic acid (CPP), a competitive NMDA antagonist. Treatment with PD-98059 [30 μM] prolonged deprivation intervals required to initiate both neuronal injury and $^{45}Ca^{++}$ uptake by 45-60 minutes, but failed to inhibit either parameter following excitotoxic challenges with either NMDA [30-500 μM], glutamate [100 μM] or AMPA [200 μM]. MAP kinase activation was found to be maximized in both normoxic and hypoxic control samples but was decreased by approximately 75% in cultures treated with PD-98059. Present data suggests PD-98059 may affect injury outcome through presynaptic influences on MAP kinase activity.

(Supported by Warner-Lambert Co.)

319.2

INDUCTION OF HEME OXYGENASE-1 (HO-1) IN RAT BRAIN FOLLOWING ACUTE PHENCYCLIDINE ADMINISTRATION. S Rajdev* and F.R. Sharp, Department of Neurology, V A Medical Center (127) and University of California, San Francisco, CA 94121.

Acute administration of NMDA receptor antagonist phencyclidine induces neuronal necrosis in cingulate and retrosplenial cortices in adult rats. However, the exact cellular mechanism of this toxicity is not completely understood. In this study we show that doses of PCP which produce pathomorphological changes in neurons, also significantly increase the expression of HO-1, a small heat shock protein (HSP32) known to be induced by oxidant stress, in rat brain. Since previous studies have shown heat shock protein 70 (HSP70) induction in brain regions vulnerable to PCP, we also studied the relationship between HO-1 and HSP70 expression. Unlike HSP70 which is primarily induced in pyramidal neurons, HO-1 increase was mainly observed in microglia and astrocytes. The increase in HO-1 protein was dose-dependent (5-50 mg/kg, PCP, i.p.) and was found to be maximum at 24 hrs during time course studies. HO-1 positive cells could be detected within 7 hrs and were still present at 5 days following a single dose of PCP. Interestingly, these HO-1 positive cells mostly presented themselves as a layer surrounding the HSP70 positive neurons in cingulate and retrosplenial cortices, though considerable staining was observed throughout the cortex especially piriform cortex and hippocampus. The increase in HO-1 was also observed in striatum, an area where no HSP70 positive neurons have been detected. These results suggest that HO-1 may be a sensitive indicator of neuronal stress and injury. Treatment of animals with 1,3-dimethylthiourea, an antioxidant, significantly blocked the increase in both HO-1 and HSP70 positive cells, suggesting an involvement of oxidative stress in PCP toxicity. Whether this dramatic increase in HO-1 levels in response to PCP *in vivo* is a protective mechanism or plays a role in PCP toxicity is being investigated.

This work was supported by a grant from the National Alliance for Research on Schizophrenia and Depression (NARSAD).

319.3

EXCITOTOXIC STRIATAL LESIONS INDUCED BY THE ANTIOXIDANT ENZYME INHIBITOR MERCAPTOSUCCINATE, W.F. Maragos*, R.A. Fox, O. Ben-Joseph and B. Ross, Dept. Neurology, Univ. Kentucky, Lexington, KY, 40536 and Dept. Radiology and Biol. Chem., Univ. Michigan, Ann Arbor, MI 48109

Reactive oxygen species (ROS) such as superoxide and hydrogen peroxide have been implicated in the pathogenesis of a number of neurodegenerative disorders. The accumulation of ROS beyond antioxidant defenses (i.e., oxidative stress) stimulates a number of processes including lipid peroxidation, enzyme inactivation and damage to DNA. ROS also increases glutamate release and impairs glutamate uptake, however it is not known whether this is functionally significant. In this study, we have used the antioxidant enzyme inhibitor mercaptosuccinate (MS) as a novel method by which to examine in vivo oxidative toxicity. MS is a competitive inhibitor of glutathione peroxidase (GPx). The GPx pathway is the principle route for the degradation of hydrogen peroxide. Although not highly toxic itself, H_2O_2 can react with transition metals such as iron and undergo the Fenton reaction to produce hydroxyl radicals, which are among the most highly toxic ROS. A total of 24 rats received intrastriatal injections of 2 µmol of MS. Twelve of the animals were pretreated with 3 mg/kg of the NMDA antagonist MK-801 and 12 with saline. Five days following surgery, brains were processed for histopathological assessment. Although somewhat variable in size (17.6 ± 4.5 mm^3), lesions in the control group demonstrated a necrotic core surrounded by a region of neuronal loss and gliosis. In the MK-801 group, lesion volumes were markedly attenuated (1.3 ± 0.35 mm^3, $p<0.01$) and the morphology of the striatum preserved. Six animals injected with mercaptoethanol displayed almost no tissue damage (0.38 + 0.11 mm^3). The results of this study indicate 1) that inhibition of antioxidant enzymes may provide a method by which to create oxidative tissue damage and 2) ROS toxicity may, in part, be mediated by activation of NMDA receptors.

Supported by the Univ. Kentucky Physician Scientist Award (W.F.M.)

319.4

EFFECTS OF PYRROLOQUINOLINE QUINONE ON GLUTAMATE INDUCED PRODUCTION OF REACTIVE OXYGEN SPECIES IN RAT FOREBRAIN NEURONS J. Scanlon*, E. Aizenman, and I.J. Reynolds. Depts. of Pharmacology and Neurobiology, University of Pittsburgh School of Medicine, Pittsburgh, PA 15261.

Pyrroloquinoline quinone (PQQ), a water soluble redox cofactor and essential vitamin, may act as a free radical scavenger and also as a modulator of the N-methyl-D-aspartate (NMDA) receptor associated redox modulatory site. With the use of the oxidation sensitive dye dihydroethidium (DHE), we examined the effects of PQQ on free radical production in cultured forebrain neurons following glutamate receptor activation. Both glutamate (100 µM) and hydrogen peroxide (30 mM) produced a rapid increase in DHE fluorescence indicating dye oxidation. PQQ (5-200 µM) effectively inhibited DHE fluorescence induced by glutamate but not by hydrogen peroxide. To determine if this effect of PQQ was due to free radical scavenging or to modulation of the redox site, we examined the effects of various reductants and oxidants of the NMDA redox site in combination with PQQ. Glutamate induced DHE fluorescence was enhanced by the thiol reductant dithiothreitol (5 mM DTT) and was inhibited by the thiol oxidant dithiolnitrobenzoic acid (100 µM DTNB). PQQ (50 µM) was capable of significantly ($p<.001$) reversing the effects of DTT. However, PQQ did not further decrease the response to glutamate in DTNB treated neurons. These results suggest that PQQ was indeed inhibiting the response to glutamate by oxidizing the NMDA receptor redox site and not by free radical scavenging. Thus, the previously reported neuroprotective action of PQQ may be solely mediated by an action of this drug on the NMDA receptor.

Support Provided By: American Heart Association (IJR) and its Western Pennsylvanian Affiliate (EA).

319.5

INCREASED EXTRACELLULAR CYSTEINE DURING ANOXIA/AGLYCEMIA IN VITRO IS REDUCED BY γ-GLUTAMYL TRANSPEPTIDASE INHIBITION Li, X., Revesjö, C., and Sandberg, M.* Inst. of Anatomy and Cellbiology, Univ. of Göteborg, S-413 90 Göteborg, SWEDEN and Weber S.G. Dept of Chem., Univ. of Pittsburgh, PA 152 60, USA

Anoxia/aglycemia (N_2/CO_2; 0 mM glucose; 10 min) evoked efflux of amino acids and γ-glutamyl peptides was studied from incubated rat hippocampal slices. Determination of amino acids and peptides was performed by reversed phase HPLC of OPA-derivatives and fluorescence detection. Anoxia/aglycemia for 10 min increased the net efflux of glutamate, γ-glutamyl glutamate, cysteine and cysteine sulfinate. Preincubation with acivicin, an enzyme blocker of γ-glutamyl transpeptidase, increased the net efflux of glutathione (GSH) while the apparent efflux of cysteine, cysteine sulfinate and γ-glutamyl glutamate were reduced. The net efflux of glutamate was not affected by acivicin. The results implicate that cysteine, a neurotoxic amino acid, increase extracellularly during anoxia/aglycemia as a result of transpeptidation of GSH and extracellular amino acids by γ-glutamyl transpeptidase.

Supported by Swedish Nat. Sci. Res. Council (1905-310) and NIH grant #GM 44842.

319.6

DEPLETION OF BRAIN GLUTATHIONE ASSOCIATED WITH BLOCKADE OF GLUTAMATE-INDUCED HIPPOCAMPAL APOPTOSIS IN MICE. R. S. Bitner* and G. E. Isom. Dept. of Medicinal Chemistry and Molecular Pharmacology, Purdue University, West Lafayette, IN 47907

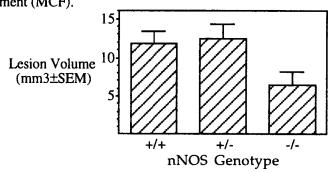
In vitro studies show that glutamate-induced apoptosis involves the depletion of glutathione (GSH), which leads to oxidative stress (Ratan *et al.*, J. Neurochem. 62:376, 1994). The purpose of the present study was to determine if GSH depletion would exacerbate apoptosis *in vivo* in the hippocampus following intracerebroventricular (i.c.v.) administration of glutamate. TUNEL (TdT dUTP nick end labeling) staining, characteristic of apoptosis, was seen in the CA1 region of the hippocampus of mice that received 48 hrs earlier a single i.c.v. injection of glutamate (3.2 µmol). However, in mice that also received the GSH synthesis inhibitor L-buthionine sulfoximine (BSO) (13.4 µmol i.c.v.) 24 hrs earlier, glutamate was completely ineffective in producing any type of hippocampal damage. Contrary to our initial hypothesis, these results indicate that *in vivo* GSH depletion is neuroprotective against glutamate-induced hippocampal apoptosis. It is possible that BSO excitotoxic blockade involves an unknown mechanism independent of GSH depletion. But, this unexpected finding may also suggest that depleting GSH triggers compensatory systems, absent from *in vitro* preparations, that offer protection against subsequent excitotoxic insults. (Supported in part by NIEHS grant 04140)

319.7

REDUCED NMDA TOXICITY IN TYPE I NITRIC OXIDE SYNTHASE GENE KNOCKOUT MICE, *IN VIVO*. C. Ayata, G. Ayata, H. Hara, R. Christie, P.L. Huang, M.C. Fishman*, M.A. Moskowitz. Stroke and Neurovascular Regulation, Depts. Neurosurgery and Neurology; Cardiovascular Res. Center, Dept. of Medicine, Massachusetts General Hosp., Harvard Medical School, Charlestown, MA, 02129

We investigated the lesions produced by intrastriatal NMDA microinjection in wild type and type I NOS-/- mice. Littermate wild type (nNOS+/+, n=8), heterozygote (nNOS+/-, n=12) and homozygote (nNOS-/-, n=7) knockout mice (20-28g) were anesthetized by halothane (2.5% for induction and 1-1.5% for maintenance). NMDA (67 mM in 0.1M phosphate buffered saline, 0.3 µL, over 2 min) or vehicle was injected stereotaxically into the striatum 0.7 mm anterior, 1.7 mm lateral and 3 mm ventral from bregma. The needle was kept in place for an additional 8 min. Mice were sacrificed after 48 hr and frozen sections (20µ thick, every 200µ) were stained by H&E. The volume of the lesion was measured by an image analysis system and expressed as percent of wild type (±SEM). NMDA caused reproducible and well delineated lesions. Morphological changes (e.g., pyknosis, eosinophilic cytoplasm, TUNNEL positive) suggestive of apoptosis were observed in all strains. The volume of the NMDA lesion was significantly smaller in nNOS-/- (53±10%) than both nNOS+/+ (100±8%) and nNOS+/- (101±15%). A delayed increase in [3H]-L-NA binding was observed in the lesion by autoradiography. This increased binding reached a peak 24 hr after NMDA injection and was absent in nNOS-/- mice. Our results suggest that NO synthesized by nNOS plays a role in NMDA toxicity *in vivo*, and that targeted disruption of type I NOS confers resistance to excitotoxicity.

Supported by NS10828, NS33335 (PLH) and a Bristol-Myers Squibb Company sponsored research agreement (MCF).



319.8

STRIATAL NEURONAL NITRIC OXIDE SYNTHASE POSITIVE NEURONS INCREASE AFTER CEREBRAL HYPOXIC-ISCHEMIC DAMAGE IN IMMATURE RAT. S. Ishiwa, M.E. Blue, W.H. Trescher and M.V. Johnston*. Neuroscience Laboratory, Kennedy Krieger Research Institute and Johns Hopkins Medical Institutions., Baltimore, MD 21205

Nitric oxide has been implicated as a mediator of glutamate excitotoxicity in response to cerebral ischemia. In the present study, we investigated the sequential changes of neuronal nitric oxide synthase (nNOS) expression in immature rat striatum after hypoxic-ischemic damage. Postnatal day 7 rats underwent unilateral carotid artery ligation, followed by exposure to hypoxia in 8% oxygen for 75 minutes at 37°C. At time points 6 hours (n=5), 3 days (n=9), 7 days (n=7) and 30 days (n=6) after hypoxic-ischemic injury, brains were perfused and processed for immunocytochemistry using nNOS specific antibodies (Transduction Labs). The cross-sectional areas of ipsilateral hypoxic-ischemic and contralateral non-ischemic sides of the striatum were measured in coronal sections. nNOS positive neurons were counted in a standard area comprising 36% of the striatal area within a section. Measurements were made automatically with a microcomputer-based video image analysis system (Imaging Research Inc.) and the average counts from the ipsilateral hypoxic-ischemic side were compared to the contralateral non-ischemic side and to that of untreated controls (n=5). The results showed that the number of nNOS positive neurons declined with age. At each timepoint after injury studied, the number of nNOS positive neurons on the side ipsilateral to the hypoxic-ischemic damage was significantly increased ($p<0.05$) compared with the contralateral non-ischemic side. These results suggest that in the immature brain, hypoxic-ischemic damage produces a long-term induction of nNOS activity.

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319.9

POTENTIATION OF GLUTAMATE NEUROTOXICITY BY SERUM ALBUMIN. ROLE OF FREE RADICALS. E.G. Sorokina, V.P. Reutov, N.P. Vinskaya, B.I. Khodorov, and V.G. Pinelis*. Lab. of Membranology, Inst. of Pediatrics, RAMS, Moscow, 117963, Russia, Lab. of Gen. Pathology of Nervous System, Inst. of Gen. Pathology and Pathophysiology, RAMS, Moscow, 125315, Russia.

S. Eimerl and M. Schram (Brain Research, 1991, 560, 282-290) found that serum albumin (SA) when applied simultaneously with glutamate (GLU) greatly potentiates delayed neuronal death (DND) in rat cerebellar granule cell cultures.The objective of the present work was to clarify the origin of this SA effect. Experiments were performed with cultured rat cerebellar granule cells. Cytoplasmic Ca^{2+} concentration was measured in individual fura-2-loaded neurons. DND was evaluated by counting of cerebellar granule cells containing and excluding trypan blue 4hr after the termination of the neurotoxic treatment. The principal findings of the study are as follows. (1) The bovine SA (4mg/ml) does not enhance Ca^{2+} influx through GLU-activated channels. (2) SA caused no toxicity in absence of GLU. (3) DND enhanced by a combined 15-min application of SA (4 mg/ml) and GLU (100 μM, 10 μM glycine, Mg^{2+}-free solution) to nerve cells can be effectively prevented by the NO synthase inhibitor Nω-nitro-L-arginine methyl ester (100 μM) applied to the medium prior to and during a neurotoxic challenge. (4) Along with GLU neurotoxicity SA also greatly enhances DND induced by a 15-min exposure of cell cultures to NO donors $NaNO_2$ (10μM) or sodium nitroprusside (10 μM) as well as of the prooxidant $FeCl_2$ (10 μM). The results obtained suggest that SA-induced potentiation of GLU neurotoxicity results from exacerbation of the toxic effects of NO and oxygen free radicals on the neuronal membranes. (Supported by RFFI and ISF).

319.10

SOD2 MAY ACCOUNT FOR THE RESISTANCE OF nNOS NEURONS TO EXCITOTOXICITY. M. Gonzalez-Zulueta[1], L.M. Ensz[1], V.L. Dawson*[1,2,3], and T.M. Dawson[1,2]. Departments of Neurology[1], Neuroscience[2] and Physiology[3], The Johns Hopkins University School of Medicine, Baltimore, MD 21287.

Neurons containing neuronal nitric oxide synthase (nNOS) activity liberate nitric oxide (NO) and mediate, in part, the toxicity associated with derangements of glutamate neurotransmission via the NMDA subtype of glutamate receptors. These neurons have the remarkable ability to preferentially survive in Huntington's Disease as well as the neurotoxicity induced by ischemia or exposure to excessive concentrations of glutamate. The mechanisms of neuroprotection in these nNOS neurons are unknown.

We have obtained PC12 cells which differ from the parental cell line in that they are resistant to NO-induced toxicity. We have used this *in vitro* model of resistance to NO toxicity and an antisense approach to investigate the role that manganese superoxide dismutase (SOD2) may play in neuroprotection. Exposure of NO-resistant PC12 cells to specific SOD2 antisense oligonucleotide restored sensitivity to sodium nitroprusside (SNP), a NO releasing agent, with SNP-induced cell death equivalent to that seen in the parental cell line. This effect was not observed in cells exposed to control sense oligonucleotide and was not due to bare antisense oligonucleotide-associated toxicity. These results suggest that SOD2 downregulation potentiates NO-induced cell death. Our findings may be relevant to the mechanisms of neuroprotection of nNOS neurons in neuronal degenerative disorders caused by impaired regulation of cellular levels of NO and superoxide.

Supported by AFAR and AHA

319.11

NITRIC OXIDE AND AMPA-INDUCED EXCITOXICITY IN ORGANOTYPIC HIPPOCAMPAL SLICE CULTURE. J. Chan* Dept. of Neurology, SUNY Health Science Center, Brooklyn, NY 11203.

Brief (30 minute) exposure of organotypic cultured rat hippocampal slices to 5 μM AMPA results in Ca^{2+}-dependent toxicity 24 hours later, preferentially in CA1 of the pyramidal cell layer. Slices were exposed to AMPA in sucrose buffer containing 10 mM Na^+, 2 mM $CaCl_2$, 5 μM MK-801 (to block NMDA receptors) and 3 μM cyclothiazide (to inhibit desensitization of AMPA receptors), with or without antagonist. Twenty four hours later, toxicity was quantified by propidium iodide fluorescent microscopy within fixed areas of each slice pre-defined as CA1 or CA3. N=6 for each condition.

Delayed pyramidal cell death was prevented (dose-dependently) by the selective AMPA receptor antagonists 6-nitro-7-sulphamoylbenzo[f] quinoxaline-2,3-dione (NBQX;10 μM), LY293558 (Lilly; 10 μM) and Joro spider toxin (10 μM), but not by the NMDA receptor antagonist MK-801 or the free radical scavenger α-tocopherol. The nitric oxide synthase (NOS) inhibitor L-Nitro-arginine methyl ester (L-NAME) and the nitric oxide scavengers oxyhemoglobin and attenuated up to 80% of the delayed damage to CA1. Differing inhibition of toxicity by NOS inhibitors selective for neuronal (7-nitroindazole and S-methyl-L-thiocitrulline), inducible (S,S'-1,3-phenylene-bis[1,2-ethanediyl]-bis-isothiourea and S-ethylisothiourea) and endothelial (Tannin) isoforms of NOS will be presented.

EXCITATORY AMINO ACIDS: EXCITOTOXICITY III

320.1

DOMOIC ACID-STIMULATED RELEASE OF GLUTAMATE AND ASPARTATE FROM CULTURED RAT CEREBELLAR GRANULE NEURONS OCCURS VIA THREE ROUTES: SWELLING-INDUCED EFFLUX, REVERSAL OF SODIUM-DEPENDENT TRANSPORT AND VESICULAR RELEASE. F.W. Berman and T.F. Murray* College of Pharmacy and Toxicology Program, Oregon State University, Corvallis Oregon, U.S.A.

We have shown that domoate causes cerebellar granule neuron toxicity by stimulating the release of excitatory amino acids (EAAs) into the exposure buffer, thereby indirectly activating NMDA receptors. Domoate-stimulated EAA release was characterized by exposing cerebellar granule neurons (3.5 - 4.0 × 10^6 neurons per 35 mm culture dish) to 10 μM domoate in a 1 ml volume and assaying for glutamate and aspartate at specific timepoints by HPLC analysis. Glutamate and aspartate concentrations increased rapidly between 2 and 5 minutes, reaching a maximum of 10.0 ± 0.9 and 5.3 ± 0.8 μM, respectively (N = 5), at 15 minutes. When neurons were pre-incubated for 1 hr with 200 μM L-trans-2,4-pyrrolidine dicarboxylate, a competitive and transportable inhibitor of the high affinity glutamate transporter, glutamate and aspartate efflux were reduced by 47 ± 10 and 55 ± 15 %, respectively. EAA efflux was reduced further, by 89.6 ± 4.1 and 94.3 ± 5.0 % for glutamate and aspartate, respectively, when swelling of PDA pre-exposed neurons was prevented with 100 mM sucrose. Cyclopentyl-adenosine had no further effect on EAA release, whereas the adenosine receptor antagonist 8-p-sulpho-phenyl-theophylline (8-pSPT) facilitated glutamate efflux, to 136 ± 4 % of domoate-stimulated controls, suggesting that vesicular release is inhibited by the tonic activation of adenosine receptors. 8-pSPT had no effect on aspartate release. Tetrodotoxin reduced the rapid phase of EAA release and completely prevented 8-pSPT-induced increases in glutamate efflux. These results suggest that cultured cerebellar granule neurons exposed to domoate release EAAs by two primary routes, reversal of the sodium-dependent glutamate transporter and via osmotically driven swelling with attendant EAA efflux, while vesicular release of EAAs is largely prevented by the tonic activation of adenosine receptors.

320.2

CHRONIC ADMINISTRATION OF THE GLUTAMATE UPTAKE INHIBITOR L-TRANS-PYRROLIDINE-2,4-DICARBOXYLATE PRODUCES STRIATAL EXCITOTOXIC LESION. J-C. Lievens, M. Dutertre, C. Forni, P. Salin* and L. Kerkerian-Le Goff. Laboratoire de Neurobiologie Cellulaire et Fonctionnelle, CNRS, 31 chemin Joseph Aiguier, 13402 Marseille Cedex 20.

This study examined the morphological effects of chronic intrastriatal infusion of L-*trans*-pyrrolidine-2,4-dicarboxylate (PDC) via osmotic minipumps. PDC was found to induce dose- and time-dependent striatal lesion. Administration of PDC at 5 10-2M (25 nmoles/0.5μl/hr) for 3 or 14 days produced neuronal damage of quite similar extent. Histological evaluation on frontal sections at the injection site showed that the primary neuronal loss was circumscribed to a circular area of about 0.6 mm radius and represented 25-30% of the striatal surface; anteroposterior examination showed that the lesion extended over 2.5-3mm. The lesion area shared a total loss of glutamate decarboxylase, enkephalin and substance P mRNA hybridization signals, suggesting degeneration of the two populations of striatal efferent neurons. In the surrounding apparently spared striatal areas, the levels of glutamate decarboxylase or substanceP mRNAs did not differ from control values, whereas enkephalin mRNA expression decreased by an average 28%, suggesting a higher sensitivity of enkephalin neurons to glutamate-uptake-mediated alterations. Combined systemic treatment with dizocilpine maleate (0.25mg/kg/i.p.) did not prevent the PDC-mediated neuronal injury, suggesting that this neurodegenerative process may not primarily involve NMDA receptors. PDC at 10-2M did not produce neuronal damage when administered over 14 days (5nmoles/0.5μl/hr), but elicited restricted degeneration when administered over 27 days (25 nmoles/2.5μl/hr).

This study provides the first in vivo evidence that chronic inhibition of glutamate uptake is sufficient for inducing neuronal damage, and supports the view that defective transport is one of the critical condition that may give rise to delayed toxicity of an endogenous transmitter system in the striatum.

320.3

POTENTIATION OF L-GLUTAMATE-INDUCED NEUROTOXICITY *IN VITRO* BY INHIBITION OF GLUTAMATE UPTAKE. C.L. Willis*, C.S. Esslinger, A.R. Chamberlin*, R.J. Bridges, Pharm. Sci., Univ. of Montana, Missoula, MT 59812, Chemistry, Univ. of California*, Irvine, CA 92717

Transport of glutamate is thought to be a key step in the regulation of extracellular glutamate levels and prevention of excitotoxic damage. Previous studies have identified L-*trans*-2,4-pyrrolidine dicarboxylate (L-*trans*-2,4-PDC), L-*anti-endo*-3,4-methanopyrrolidine dicarboxylate (L-*anti-endo*-MPDC), *meso*-2,4-MPDC and DL-threo-β-hydroxyaspartate (β-THA) as potent competitive inhibitors of the Na-dependent synaptosomal glutamate transporter. In the present study we have used neuronal cortical cultures to assess the excitotoxic properties of these compounds in the presence and absence of added glutamate. L-Glutamate and transport inhibitors were added to 19 day old cultures prepared from fetal (E16) rats. Neuronal lysis was quantified 24 hr later by measuring the levels of extracellular lactate dehydrogenase and expressed as a percentage of total neuronal lysis induced by addition of NMDA (100μM) and kainate (100μM). L-*trans*-2,4-PDC, L-*anti-endo*-MPDC, *meso*-MPDC or β-THA alone, induced little or no neuronal lysis at 100μM. At 200μM significant neuronal lysis was observed with both L-*trans*-2,4-PDC (30%) and β-THA (45%). When 50μM L-glutamate was co-applied with L-*trans*-2,4-PDC and L-*anti-endo*-MPDC, neuronal lysis was increased in a concentration-dependent manner to 100%. In contrast, co-application of *meso*-MPDC had no effect on glutamate toxicity. This lack of effect may reflect its activity as an excellent substrate of the transporter. The potentiated neuronal damage induced by transport inhibition mimicked the injury induced by high levels of glutamate (200μM) in that the neuronal loss could be almost completely prevented by MK-801 (10μM). These studies identify a series of conformationally constrained transport inhibitors and should prove useful in elucidating the neuropathological consequences of impaired glutamate uptake. This work was supported in part by NIH NS30570, NS27600 and RR10169.

320.4

BARBITURATES CAN IMPAIR GLUTAMATE UPTAKE AND POTENTIATE EXCITOTOXIC NEURONAL DEATH

R.A. Swanson*, L.L. Seid, K. Farrell, and R.G. Giffard
Dept. of Neurology, Univ. of California and VAMC, San Francisco, CA 94121.

Barbiturates can reduce brain metabolic rate and antagonize neuronal depolarization induced by excitatory amino acids. Barbiturates have failed, however, to produce consistent reductions in brain injury resulting from stroke or trauma. In this study, rat cortical astrocyte cultures exposed to ≥ 200μM seconal showed increased glucose utilization and slowing of glutamate uptake. These findings were accentuated at low medium glucose and mimicked by azide, suggesting a barbiturate effect on oxidative ATP production (Chance and Hollunger, 1963). Barbiturates had a biphasic effect on the sensitivity of rat cortical neurons to 5 minute NMDA exposures: toxicity was markedly potentiated at barbiturate concentrations between 50μM and 300μM, but antagonized at levels above 1mM. These findings suggest that neurons are particularly sensitive to the cyanide-like effect of barbiturates. The cyanide-like effect of barbiturates may counter or outweigh NMDA antagonistic effects at barbiturate concentrations employed in vivo. Supported by NIH RO1 NS31914

320.5

CAPILLARY ELECTROPHORESIS DETERMINATION OF GLUTAMATE IN RAT BRAIN DIALYSATE - IMPROVEMENTS IN DETECTION AND ANALYSIS TIME USING CYCLODEXTRINS - W. H. Church* and K. Dranchak, Dept. of Chemistry/Neuroscience Prog., Trinity College, Hartford, CT 16106, C. S. Lee, Dept.of Chemistry , East Carolina Univ., Greenville, NC 27858

Glutamic acid and aspartic acid were derivatized to fluorescent products using naphthalene-2,3-dicarboxyaldehyde (NDA) in the presence of cyanide and separated using capillary zone electrophoresis (CZE). Quantitation and separation performance was evaluated using separation buffers containing α, β, γ, and hydroxypropyl β-cyclodextrins (CD). β- and hydroxypropyl β-cyclodextrin enhanced the fluorescent signal of standard 5 μM CBI-GLU by factors of 1.73 and 1.69 and CBI-ASP by factors of 1.72 and 1.53, respectively. A CD-concentration dependent decrease in resolution was observed. Separation times were dependent on CD concentration. CD:CBI-amino acid binding constants calculated using changes in migration time were compared to those calculated by the steady-state fluorescence enhancement technique. Analysis of 10 μL of rat brain dialysate by CZE using 4 mM β-cyclodextrin in borate buffer resulted in base-line resolution of CBI-GLU and CBI-ASP in 3.6 minutes with signal enhancement of 1.11 and 1.23, respectively. Preliminary studies of rats subjected to isolation stress illustrate the usefulness of this method in quantifying the effects of stress on striatal and hippocampal glutamate levels.

320.6

On-line Monitoring of Extracellular Glutamate: a Novel Capillary Biosensor

Rebecca J. Olson Cosford and Werner G. Kuhr, University of California, Riverside, CA 92521-0403.*

We have developed a novel flow-injection based biosensor for glutamate. The characteristics of this sensor, including low micromolar sensitivity and sub-second time response, make its application to *in vivo* neurochemical measurements particularly attractive. The biosensor exploits the biological selectivity of glutamate dehydrogenase (GDH) to convert glutamate to a detectable signal. In the presence of nicotinamide adenine dinucleotide (NAD^+), GDH converts glutamate to α-ketoglutarate while simultaneously reducing NAD^+ to NADH. Detection of NADH may be accomplished using laser induced fluorescence (325 nm excitation wavelength) or by electrochemistry. By attaching GDH to the inner surface of a small diameter fused silica capillary (40 μm i.d.; 150 μm o.d.) and including NAD^+ in the perfusion buffer, it is possible to transduce glutamate to a detectable signal during transport from the sampling site to the detection window of the capillary.

Response time for the biosensor is 450 milliseconds with detection limits of 3 μM (s/n = 3). However, improvements in sensitivity are expected with higher laser power and optimization of enzyme efficiency within the capillary. Efficiency varies between 2% and 92% with optimal performance at low perfusion rates and high glutamate concentrations. The biosensor has been successfully applied to the measurement of extracellular glutamate at the neuromuscular junction of the *musca domestica* demonstrating the feasibility of use its *in vivo*. Future work will involve the incorporation of this enzyme-based sensor as the outlet line for a microdialysis probe to allow on-line monitoring of dialysate glutamate. Also, efforts have been initiated to use this sensor on-line with capillary electrophoresis allowing the separation of NADH from other potentially fluorescent components in the cerebrospinal fluid.

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320.7

Glutamate Monitoring with Enzyme-Modified Electrochemical Probe

Mark A. Hayes† and Werner G. Kuhr, University of California, Riverside, CA 92521-0403. †Arizona State University, Tempe, AZ 85287*

The monitoring of glutamate in a biologically relevant time-course, sample size and sensitivity is a very difficult task. Enzyme-modified microelectrodes (with glutamate dehydrogenase) have been developed to accomplish this goal. Quality of fast scan voltammetry of β-nicotinamide adenine dinucleotide- reduced form (NADH) is critical for sensitivity of enzyme-modified electrodes based on dehydrogenases. While NADH voltammetry is sluggish at solid electrode surfaces, several schemes have been developed to minimize overpotential at carbon fiber electrodes. Unfortunately, this improved voltammetry is reduced upon covalent modification of the carbon fiber surface with enzyme attachment schemes. In this study, strategies for improved pretreatment of carbon fiber electrodes and identification of factors which lead to a reduction of voltammetric quality were investigated. An improved electrochemical pretreatment was developed for 32 μm carbon fiber electrodes. Manufacture techniques for enzyme-modified electrodes were investigated which minimize solution exposure time and provide alternative attachment sites. These attachment schemes include a direct electrochemical amine reaction and an electrochemically reductive hydrazide reaction to form a bond to the surface. Fluorescence and electrochemical evidence is presented confirming surface attachment and improved retention in voltammetric quality, respectively. These probes are presently capable of 1 μM detection limits with DC detection and 50 μM by cyclic voltammetric measurements.

Supported by: NIH

320.8

IMAGING EXCITOTOXIC CELL SWELLING AND NEURONAL DEATH IN THE HIPPOCAMPAL SLICE: CONFIRMATION WITH SPECTROMETRY AND ELECTROPHYSIOLOGY. *Trevor M. Polischuk* and R. David Andrew.* Department of Anatomy & Cell Biology, Queen's University, Kingston, Ontario, Canada, K7L 3N6.

Excitotoxicity involves an acute, early phase of neuronal swelling which may presage cell death. Increased light transmittance (LT) through the brain slice indirectly measures cell swelling. We imaged changes in LT across hippocampal slices induced by three distinct glutamate agonists. At 37°C, a 1 min superfusion of 100 μM NMDA, 10 μM domoate (DOM), or 10 μM AMPA induced a *reversible* increase in LT, primarily in the dendritic regions of area CA1 (strata radiatum and oriens, i.e. RAD and OR). The peak response measured in the CA1 RAD ($n \geq 5$ slices each) was not significantly different among agonists ($p<0.01$). Spectrometry revealed that the increased LT occurred across 400-800 nm spectrum for each agonist (n=5 slices each), confirming generalized swelling rather than narrow band activation of chromophores (e.g. cytochromes). Agonist exposures at 37°C for 1 min resulted in immediate loss of the orthodromically evoked field potential recorded in the CA1 cell body region (PYR) but the response returned to pre-exposure levels once swelling in the dendritic area dissipated. In contrast, increasing exposure times resulted in *irreversible* changes in LT. A 4 min application of 100 μM NMDA (n=7) or a 10 min application of 10 μM DOM (n=12) or 10 μM AMPA (n=6) each caused a characteristic "swell/shrink" event in the CA1 RAD where the LT increase was followed by a pronounced irreversible *decrease* in LT below baseline levels. At the same time, LT irreversibly *increased* in the cell body layer of CA1 (PYR). Again, spectral analyses of RAD and PYR revealed wide band increases in LT indicative of cell swelling. However, the evoked field potential was irretrievably lost following 4 min of 100 μM NMDA (n=7) or 10 min of 10 μM DOM (n=3) coinciding with the irreversible dendritic shrinkage and cell body swelling. We conclude that glutamate-induced excitotoxicity involves dynamic volume changes in both the cell body *and* dendritic regions which coincide with electrophysiological cell death.

Supported by the Canadian MRC and the Heart & Stroke Foundation of Ontario

320.9

USE OF A MULTIWELL FLUORESCENCE SCANNER WITH PROPIDIUM IODIDE TO ASSESS NMDA MEDIATED EXCITOTOXCICITY IN CORTICAL NEURONAL CULTURES

J.G. Rudolph*, J.J. LeMasters, and F.T. Crews. Center for Alcohol Studies and Department of Cell Biology. University of North Carolina, College of Medicine, Chapel Hill, NC 27599.

Previous studies using cortical neuronal cultures have used LDH release as a measure of cell death due to NMDA excitotoxcicity. This method is labor intensive and therefore does not easily allow for detailed time course experiments. We have adapted the use of propidium iodide flourescence in microtiter plates read by a multiwell flourescence scanner. Propidium iodide (PI) intercalates itself into cell DNA once the plasma membrane become permiant due to cell death. Primary cortical neuronal cultures 10-15 days post ARA-C treatment were used. To study NMDA excitotoxcicity cells were incubated with glutamine free DMEM media and 30 µm PI for 10 minutes after which a baseline reading was taken. The PI containing media was removed and replaced with 25 mM HEPES buffer with or without variuos NMDA agonists and antagonists. Cells were washed once with 25 mM HEPES and replaced with glutamine free DMEM media containing 30 µM PI. Flourescent readings were then taken at specified intervals to assess cell death over 24 hours. Following NMDA exposure, when glutamine was present in the DMEM media a more rapid increase of PI flourescence as well as greater tmax values were seen, compared to media without glutamine. Using either a 25 minute or continuous NMDA exposure, maximum flourescence was reached within 6-9 hours. The ED_{50} for NMDA induced excitotoxcicity was 48 µM. This excitotoxcicity could be blocked with 1 µM MK-801, 10 µM Ifenprodil, and Ethanol (IC_{50} = 37.1 µM). Cell death elicited by 25 minute Glutamate exposure was blocked by the NMDA antagonists MK-801 and Ifenprodil, but not the non-NMDA antagonist CNQX. Results seen with PI flourescence following 25 minute exposure to 100 µM NMDA were replicated using the LDH release assay. (Funded by AA06069)

EXCITATORY AMINO ACIDS: EXCITOTOXICITY IV

321.1

EFFECTS OF ACUTE ISCHEMIA ON THE MODULATION OF K^+ CURRENTS BY METABOTROPIC GLUTAMATE RECEPTORS IN CA3 CELLS IN RAT HIPPOCAMPAL SLICE CULTURES.

M. Tanabe, B. H. Gähwiler and U. Gerber*
Brain Research Institute, University of Zurich, CH-8029 Zurich, Switzerland

We were interested whether metabotropic glutamate receptors (mGluRs) are activated as a result of the excessive release of glutamate during cerebral ischemia. Intracellular recordings were made from CA3 pyramidal cells in organotypic hippocampal slice cultures. Brief chemical ischemia (2 mM 2-deoxyglucose and 3 mM NaN_3 for 4 min) reversibly induced outward currents and reduced the slow calcium-dependent afterhyperpolarization current I_{AHP}. Since the activation of mGluRs is also known to reduce I_{AHP}, we examined whether mGluRs mediate the inhibition of this current during ischemia. The reduction in I_{AHP} induced by 1*S*,3*R*-ACPD (10 µM), a selective mGluR agonist, was antagonized by the specific mGluR antagonist MCPG. In contrast, MCPG did not modify the effect of ischemia on the I_{AHP} response. Furthermore, MCPG did not alter the ischemia-induced outward current. We have previously shown that the activation of mGluRs by 1*S*,3*R*-ACPD, or synaptic stimulation in the presence of ionotropic blockers, induces a slow inward current due to the suppression of membrane K^+ conductance. Interestingly, this response was potentiated following the ischemic period (for up to 10 min). Thus the enhancement of mGluR responses after acute ischemia may contribute to glutamate excitotoxicity.

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321.2

ANTISENSE OLIGODEOXYNUCLEOTIDES DIRECTED AGAINST mGluR5 METABOTROPIC GLUTAMATE RECEPTOR PROTECT AGAINST MALONIC ACID LESIONS IN RAT STRIATUM.

J. J. Cha*, A. Talati, J. A. Kerner, and A. B. Young, Dept. Neurology, Massachusetts General Hospital, Boston, MA 02114.

Glutamate receptors linked to phosphoinositide metabolism may play a permissive role in excitotoxicity (Beal et al., *Neurodegeneration* 2:81-91, 1993). We have explored the role of the mGluR5 subtype of metabotropic glutamate receptors in striatal toxicity using antisense oligodeoxynucleotides (ODN's). Phosphorothioate-modified antisense ODN's, scrambled ODN controls, or saline were injected into the right lateral ventricle of rat brain at various intervals prior to receiving a left intrastriatal injection of the mitochondrial toxin malonic acid. Rats were sacrificed 48h later, and the lesion sizes were determined by 2,3,5-triphenyltetrazolium chloride (TTC) staining of brain slices.

Pretreatment of rats with i.c.v. injection of antisense ODN directed against mGluR5 protected against malonic acid-induced striatal lesions in a time- and dose-dependent manner. Maximal protection was achieved when antisense ODN was injected 30h prior to the malonic acid injection. With single dose injections of antisense ODN, protection was dose dependent, with doses of 12 and 24 nmols being more protective than doses of 1 and 4 nmols.

Analysis of mGluR5 mRNA expression by *in situ* hybridization revealed no change in expression following treatment with antisense ODN directed against mGluR5. However, a measure of protein function, [^{3}H]glutamate receptor binding under metabotropic receptor preferring conditions, showed a dose dependent decrease with pretreatment with antisense ODN.

These data argue that mGluR5 plays a permissive role in certain types of striatal toxicity. Further, in this system, the mechanism by which antisense ODN's exert their action appear to be at the level of translation into protein.

Supported by 1RO1 AG13617

321.3

MECHANISMS UNDERLYING THE PROTECTIVE EFFECT OF GROUP-II METABOTROPIC GLUTAMATE RECEPTORS (mGluRs) IN CULTURED CORTICAL NEURONS.

V. Bruno[1,2]*, M. Storto[1] and F. Nicoletti[1,3]. [1]I.M.N. "Neuromed", Pozzilli, Italy; Institute of Pharmacology, [2]School of Medicine or [3]Pharmacy, Catania University, Italy.

Activation of group-II mGluRs by DCG-IV protects mouse cultured cortical neurons grown over a monolayer of astrocytes against NMDA toxicity. The neuroprotective action of DCG-IV has been ascribed to multiple mechanisms, which include the inhibition of (i) cAMP formation, (ii) voltage-sensitive Ca^{2+} channels, and (iii) glutamate release. All these mechanisms should efficiently reduce neuronal excitability and synaptic transmission only during the exposure time to DCG-IV. Surprisingly, however, DCG-IV was neuroprotective when transiently applied to the cultures 6 hours before the NMDA pulse. This effect of DCG-IV could be prevented by applying the protein synthesis inhibitor, cycloheximide (CHX) either in combination with DCG-IV or during the interval between the DCG-IV- and the NMDA pulse. A delayed exposure to CHX also prevented the neuroprotective activity of DCG-IV applied in combination with NMDA. We suggest that activation of group-II mGluRs induces the expression of "neuroprotective genes", which oppose to NMDA toxicity during the progression of neuronal damage.

321.4

INDUCTION OF LIMBIC SEIZURES BY INTRACERBRAL INJECTIONS OF 3,5-DIHYDROXYPHENYLGLYCINE (DHPG), A SELECTIVE METABOTROPIC GLUTAMATE RECEPTOR AGONIST, PRODUCES NEURONAL INJURY IN MICE.

W. H. Jordan*, J. A. Johnson, and J. P. Tizzano, Lilly Research Laboratories, Eli Lilly and Company, Greenfield, IN 46140.

Metabotropic glutamate receptors (mGluRs) are a heterogeneous family of receptors coupled to multiple second messenger systems via G-proteins. We have previously described that the mGluR selective agonist 1S,3R-1-aminocyclopentane-1,3-dicarboxylic acid (ACPD) induces limbic seizures and selective neuronal injury in mice. In the present study we further evaluate the potential for limbic seizures and neuronal injury of the highly selective mGluR agonist 3,5-dihydroxyphenylglycine (DHPG), which like ACPD activates phosphoinositide (PI) coupled mGluRs (mGluR1 and/or mGluR5), however, is devoid of cAMP formation when expressed in non-neuronal cells. DHPG (800 nmol; 5ul) injected into the thalamus of mice induced limbic seizures that were similar to ACPD injections. At two days post-injection, neuronal necrosis and increased staining of glial fibrillary acidic protein occurred in the hippocampus. Necrosis was observed in the subiculum and in CA1-3, but not in the dentate gyrus. These data suggest that induction of mGluR mediated limbic seizures and neuronal injury in mice are a result of activating PI linked in situ mGluRs.

321.5

METABOTROPIC GLUTAMATE RECEPTOR AGONIST 1S,3R-ACPD INDUCES INTERNUCLEOSOMAL DNA FRAGMENTATION and CELL DEATH in RAT STRIATUM.

Y. Wang, Z.-H. Qin, Streicher Eugene *and T. N. Chase. ETB, NINDS, NIH, Bethesda, MD 20892.

To characterize some biochemical features of 1S,3R-ACPD-induced neurotoxicity, DNA fragmentation and striatal cell death were assessed after the acute unilateral administration of this drug into rat striatum. 1S,3R-ACPD induced internucleosomal DNA fragmentation in a dose-dependent manner. TUNEL and propidium iodide staining showed DNA fragmentation and profound nuclear condensation in striatal cells. Internucleosomal DNA fragmentation induced by 1S,3R-ACPD was attenuated by a protein synthesis inhibitor and was partially blocked by non-selective and selective metabotropic glutamate receptor antagonists. Receptor autoradiography showed that 1S,3R-ACPD (900 nmol) caused a loss of about 20%, 10% and 13% of striatal D_1 and D_2 dopamine receptors and NMDA receptors, respectively. 1S,3R-ACPD-induced striatal cell death was confirmed by in situ hybridization histochemistry which revealed significant decreases in GAD and proenkephalin mRNAs. Somatostatin mRNA was also decreased, indicating some associated interneuron degeneration. These results indicate that apoptotic mechanisms may contribute, at least in part, to the cellular neurotoxicity of 1S,3R-ACPD. Cell death caused by 1S,3R-ACPD may include GABAergic projection as well as interneurons.

321.6

DIFFERENTIAL ALTERATIONS in TRANSCRIPTION FACTOR BINDING ACTIVITIES DURING EXCITOTOXIN-INDUCED APOPTOSIS in RAT STRIATUM.

Z.-H. Qin, Y. Wang and T. N. Chase*. ETB, NINDS, NIH, Bethesda, MD 20892.

To understand cellular mechanisms contributing to neuronal death, we measured changes in transcription factor binding activities during apoptosis induced by the NMDA receptor agonist quinolinic acid (QA). QA (120 nmol) induced rapid and robust increases in AP-1 and NF_kB binding activities. The increase in AP-1 binding activity was relatively transient. NF_kB binding dramatically increased from 2-12 h and then declined but remained elevated 24 h after QA injection. E_2F-1 binding activity was only increased 6 h after QA injection. OCT-1 and SP-1 binding gradually decreased following QA injection. Myc-Max and CREB were not significantly altered. Increasing intensity of internucleosomal DNA fragmentation was detected from 12 h to 24 h after QA injection. Alterations in AP-1, NF_kB, E_2F-1, OCT-1 and SP-1 binding activities were blocked by the NMDA receptor antagonist MK-801. The increases in AP-1 and NF_kB binding activities were also attenuated by the protein synthesis inhibitor cycloheximide. These studies demonstrated a complex pattern of changes in transcription factor binding activities during QA-induced apoptosis and a temporal correlation of these alterations with the appearance of internucleosomal DNA fragmentation, suggesting that some genes may be turned on while others may be turned off during apoptosis.

321.7

METABOTROPIC GLUTAMATE RECEPTORS MODULATE STRIATAL QUINOLINIC ACID TOXICITY

L.R. Orlando*, D.G. Standaert, J.B. Penney, Jr., and A.B. Young. Program in Neuroscience, Harvard University and Dept. of Neurology, Massachusetts General Hospital, Boston, MA 02114.

Metabotropic glutamate receptors (mGluRs) appear to play a role in excitotoxicity. We have found that two mGluR agents, (S)-4-carboxy-3-hydroxyphenylglycine ((S)-4C3HPG) and (RS)-1-aminoindan-1,5-dicarboxylic acid, protect the rat striatum against quinolinic acid lesions. Both of these compounds are group 1 mGluR antagonists, while (S)-4C3HPG is also a group 2 mGluR agonist.

Another way of examining the effects of mGluRs on excitotoxicity is via restoration of striatal lesions after decortication, which has been shown to block toxicity of not only quinolinic acid but also NMDA, AMPA, and kainate. We have tried a number of mGluR agents, including (±)-1-aminocyclopentane-trans-1,3-dicarboxylic acid (*t*-ACPD) and the more selective group 1 agonists *trans*-azetidine-2,4-dicarboxylic acid (*t*-ADA) and (RS)-3,5-dihydroxyphenylglycine (DHPG), none of which were toxic alone in sham or decorticated animals. These agents were co-injected with quinolinic acid into the striatum of male rats that had been decorticated two weeks prior, and 2,3,5-triphenyltetrazolium chloride (TTC) was used for lesion analysis. None of these agonists restored toxicity at doses of 1000 nmoles co-injected with either 100 or 200 nmoles quinolinic acid. Only at extremely high doses of quinolinic acid (400 nmoles) and mGluR agonist (2000 nmoles) is toxicity partially restored (average lesion by TTC = 11.78 mm^3).

Interestingly, DCG-IV, an NMDA receptor and group 2 mGluR agonist, restores toxicity in decorticated animals at a more moderate dose of 100 nmoles (average lesion by TTC= 13.81 mm^3). The same dose in normal animals creates a very large lesion extending beyond the striatum into the cortex. We are investigating whether this is due to the NMDAR or mGluR actions of this compound.

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321.8

ZINC CHELATING AGENTS INHIBIT EXCITOTOXIC ACTION OF QUINOLINIC ACID ON BASAL FOREBRAIN CHOLINERGIC NEURONS.

P.J. Lee, K. Jhamandas, R.J. Boegman*, and R.J. Beninger[1], Dept. of Pharmacology and Toxicology and [1]Psychology & Psychiatry, Queen's Univ. Kingston, Ontario, Canada K7L 3N6.

Picolinic acid, which chelates zinc, attenuates the excitotoxic action of quinolinic acid (QUIN). To determine if zinc chelation underlies picolinate action, we evaluated potential of two known zinc chelators to inhibit the neurotoxic action of QUIN on cholinergic neurons of the rat nucleus basalis magnocellularis (nbM). The neurotoxicity was assessed by measuring loss of choline acetyl- transferase (ChAT) activity in the cortex or amygdala 6 days after an intra-nbM injection of QUIN (60, 120 nmol) with or without the zinc chelators - diethyldithiocarbamate (DCA; 120-480 nmol) or N-N-N'-N'-tetrakis (2-pyridylmethyl) ethylene-diamine (TPEN; 120 nmol). Injection of QUIN (60, 120 nmol) reduced ChAT activity in both regions. Combination of DCA or TPEN with QUIN partially prevented this reduction of ChAT activity. DCA failed to influence the reduction in ChAT activity produced by injections of NMDA (30 nmol) or AMPA (1 nmol). The results show that known zinc chelators produce a selective attenuation of cholinergic neurotoxicity produced by QUIN and mimic the action of picolinic acid. [Supported by MRC Canada].

321.9

EFFECT OF NITRIC OXIDE SINTHASE (NOS) INHIBITORS-ON QUINOLINATE-INDUCED NEUROTOXICITY.

D. Santamaría, F. Pérez, A. Santamaría, B. Escalante and C. Ríos*. Dept. of Neurochemistry, I.N.N.N. and Dept of Pharmacology and Toxicology, CINVESTAV, MEXICO 14269.

Quinolinate (QUIN), a potent excitotoxin acting on NMDA receptors, is able to increase circling behavior (CB) and lipid peroxidation(LP) in rats, two expressions of neurotoxicity. It has been recently proposed a role of nitric oxide as a mediator of QUIN injury. Both in vivo and in vitro QUIN toxicity was tested in the presence of inhibitors of NOS activity, such as L-nitro arginine methyl ester (L-NAME). QUIN-induced CB (observed after apomorphine challenge) and LP (evaluated by lipid fluorescent products) were measured in striatally-lesioned rats and brain synaptosomes, respectively. QUIN (240 nmol/uL)-mediated-CB (265 turns/hr) was blocked by 5, 10 and 15 mg/kg of i.p. L-NAME (-25, -49 and -52 %, respectively, as compared vs QUIN-treated rats). QUIN (10 mM)-induced LP was 156 % vs control and L-NAME (10, 20 and 40 mM) prevented LP by -52, -42 and -51 % vs QUIN, respectively. Similar results were obtained with other NOS inhibitors. These findings suggest a contribution of NOS activity on QUIN induced brain injury.

321.10

THE NMDA RECEPTOR AGONIST, QUINOLINIC ACID, INDUCES *hsp70* GENE EXPRESSION AND APOPTOSIS IN THE DENTATE GYRUS.

J.W. Sharp*, Dept Anatomy & Physiology, College of Veterinary Medicine, Kansas State University, Manhattan, KS 66506-5602.

The NMDA receptor agonist, quinolinic acid, was hypothesized to generate apoptosis in the rat brain. Quinolinic acid should induce apoptosis in the same hippocampal areas with heat shock gene, *hsp70,* induction.

Female Sprague-Dawley rats (200-250gm) were placed under halothane anesthesia and stereotaxically injected with 5μl of 4mg/ml quinolinic acid into the right lateral cerebral ventricle. After 72 hours survival, rats were anesthetized with halothane and perfused. Alternate frozen brain sections (40μm) were processed for evidence of apoptosis or heat shock protein, HSP70, induction. For apoptosis, DNA fragmentation was determined by binding digoxigenin-11-dUTP to 3'-OH ends of DNA using the terminal deoxynucleotidyl transferase enzyme (TdT). An anti-digoxigenin antibody conjugated with a peroxidase enzyme was then used to detect the digoxigenin complexed to DNA fragments. HSP70 induction was determined by the avidin-biotin/horseradish-peroxidase immunocytochemical technique utilizing a monoclonal antibody (Amersham).

Quinolinic acid HSP70 induction was found predominately in CA3 pyramidal cells, and cells of the polymorph and granule cell layer of the dentate gyrus. Evidence of apoptosis was seen only in dentate granule cells. There was no evidence of apoptosis in areas where HSP70 was not induced. 5μl of 4mg/ml quinolinic acid induced HSP70 in more than 90% of dosed rats. Rats without HSP70 induction did not show evidence of apoptosis.

Results suggest that apoptotic-like cell death may be an important consequence of NMDA receptor mediated excitotoxicity. This however, may not be as widespread as necrosis or heat shock induction.

American Heart Association

321.11

RECOVERY OF HYPOTHALAMIC NMDA-INDUCED C-*FOS* EXPRESSION FOLLOWING NEONATAL MSG LESIONS. M. Natarajan and M. Wilkinson* Depts. of Obst. and Gynaecol., Physiol. and Biophys., Dalhousie U., Halifax, N.S., Canada.

We have previously reported that a single, neonatal injection of MSG (4mg/g; day 2) induces precocious puberty in female rats (Neuroendocr. 52 143, 1990). At this dose MSG exerts neurotoxic damage to the ARC nucleus. We hypothesize that some degree of recovery or reorganization must occur to sustain the emergence of normal ovulatory function approximately 4 weeks later. To test this we have used c-*fos* expression as a developmental marker of ARC response to GLU.R stimulation with NMDA (Dev. Br. Res. 73 193, 1993). Neonatal (d2) female pups were injected with MSG (4mg/g, s.c.). Controls received saline. Groups of pups (n = 4) were given injections of NMDA, known to stimulate ARC c-fos (d3 - d29). Pups were sacrificed 2hr after NMDA injection. Control (saline) pups responded to NMDA with a robust age-dependent induction of FOS-like immunoreactivity (FLI). MSG treatment severely attenuated the FLI response immediately following the lesion (d2 - d10). Subsequently, FLI recovered up to 75% of control values by d16. Full recovery was seen by 29 days. In contrast, pups given chronic MSG treatment (d2,4,6,8; 4mg/g s.c., known to delay puberty and induce severe endocrinopathies) followed by NMDA as above (d16 - d29), revealed only a 50% recovery of FLI by d29. In both treatment groups, the zone of recovery (i.e. re-appearance of FLI) began close to the third ventricle and with time, radiated outwards towards the periphery of the ARC. FLI-positive neurons which reappeared in the ARC following MSG lesions presented a highly irregular nuclear morphology. Nissl staining indicated both condensed nuclear and cytoplasmic areas. We conclude that recovery of ARC function (i.e. onset of puberty) after a neonatal MSG lesion is coincident with the reappearance of a normal FLI response to NMDA. Supported by Canadian MRC and the Atlee Endowment Fund.

321.12

RAPID INDUCTION OF NF-κB IN ADULT BUT NOT JUVENILE RAT LIMBIC STRUCTURES FOLLOWING SEIZURE ACTIVITY. Y. Rong, M. Baudry.* Neuroscience Program, Univ. of Southern California, Los Angeles, CA 90089

Previous studies have indicated that increased formation of oxygen free radicals is likely to participate in the cascade of events leading to neuronal damage following kainate (KA)-induced seizure activity. As reactive oxygen species are involved in signal transduction pathways leading to nuclear factor kappa B (NF-κB) activation, we examined the effects of KA treatment on the activation of NF-κB in adult and juvenile rat brain. For comparison, changes in two other transcription factors, activator protein-1 (AP-1) and Sp1, were also determined. In adult rat piriform cortex and hippocampus, significant induction of NF-κB was observed at 4 h after KA injection, and maximal increase was reached at 8-16 h post-treatment. NF-κB binding activities returned to control levels by 5 days after injection. NF-κB binding activities were slightly decreased in adult rat cerebellum at 8 and 16 h after KA treatment. In the juvenile rat, no significant changes in NF-κB binding activity were observed in piriform cortex, hippocampus and cerebellum after KA injection. Changes in AP-1 binding activity were qualitatively similar to those observed with NF-κB in adult but not juvenile rat brain, as AP-1 was significantly induced in juvenile piriform cortex and hippocampus following KA injection. On the other hand, little or no changes in Sp1 activity were detected in adult and juvenile rat brain. Our results provide further evidence that oxidative stress participates in neuronal damage resulting from KA-induced seizure activity.

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321.13

LOW DOSE ADMINISTRATION OF DOMOIC ACID IN THE NEONATAL RAT: C-FOS EXPRESSION AND SPATIAL LEARNING. S.M. Strain, G.V. Allen[a] and R.A.R. Tasker*. Dept. Anat. & Physiol. Atlantic Vet College, UPEI, Charlottetown PEI, C1A 4P3 and Dept. Anat. and Neurobiology, Dalhousie Univ., Halifax, NS, B3H 4H7, Canada.

Our objective was to assess the effects of administration of equitoxic but subconvulsive doses of domoic acid (DOM) on central patterns of C-Fos expression and spatial learning in developing female Sprague Dawley rats. Injections (i.p.) of various doses of DOM or saline (n=6) were administered to different groups on postnatal days 0, 5, 14, 22 and 30. Dose response curves for behavioural toxicity were generated to determine equitoxic doses of DOM for each age. C-Fos experiments were conducted in groups of rats (n= 3-5) from P5 to adult receiving either saline or an equitoxic but subconvulsive dose of DOM. Assessment of spatial learning in the Morris water maze began at postnatal day 60 (n=8 per dose). Two hours post-injection animals were euthanized, perfused transcardially with 4% paraformaldehyde and coronal sections (40μm) of whole brain were incubated in the presence of C-fos antibody. Image analysis revealed widespread cellular C-Fos expression that intensified with age following subconvulsive doses of DOM, although, little or no label was detected in P5 pups. The most pronounced elevations in FOS-LIR occurred in the nucleus gracilis, area postrema, NTS of the medulla, the parabrachial nucleus, central hypothalamic nuclei, the amygdala, and various cortical regions. Preliminary analysis of the maze data imply alterations between groups in spatial learning although full analysis is incomplete at time of submission. We conclude that there are differences in both C-Fos expression and spatial learning following exposure to DOM at different ages. (Supported in part by New Brunswick Heart & Stroke Foundation)

321.14

PRIMING OF CULTURED NEURONS WITH SABELUZOLE RESULTED IN LONG LASTING INHIBITION OF NEUROTOXIN-INDUCED TAU EXPRESSION AND CELL DEATH. Rizzini, D. Uberti, F. Sinelli, M. Pizzi, M. Grilli, P.F. Spano and M. Memo* Div. Pharmacol., Dep. Biomed. Sci. & Biotechnol., Brescia University, School of Medicine, 25123 Brescia, Italy

Sabeluzole is an experimental drug originally developed for its anti-ischemic. anti-epileptic and cognitive enhancing properties. Recently, it has been reported that repeated treatments with sabeluzole protect cultured rat hippocampal neurons aginst NMDA and glutamate-induced neurotoxicity. We evaluated the possibility that Sabeluzole may elicit neuroprotection by acting, either directly or indirectly, on tau proteins in primary neurons from rat brain and in differentiated human neuroblastoma cell line. The repeated treatments of primary cultures of cerebellar granule cells with nanomolar concentration of sabeluzole result in mature cells that are resistent to the excitotoxicity induced by glutamate. Sabeluzole treatment prevents glutamate elicited increase of tau expression, as determinated at two hours after the neurotoxic pulse by immunocytochemestry analysis. Moreover, sabeluzole treatment did not modify the pattern of expression of tau proteins, as shown by measurement of the mRNA levels during the cell maturation by PCR analysis and of tau proteins at DIV 12 by westernblot analysis.We then performed a second set of experiments using the human neuroblastoma cell line SH-SY5Y differentiated in neuron-like cells after treatment with retinoic acid. Since these cells do not express functional glutamate receptors doxorubicin exposure was chosen to induce cell degeneratin. Doxorubicin was neurotoxic and, like glutamate, increased tau immunoreactivity two hours after the treatment. Sabeluzole prevents cell death and the increase of tau induced by doxorubicin. Sabeluzole was indeed able to prevent neurotoxicity induced by agents acting with different mechanisms and at different cellular levels.

This work is supported by C.N.R.

EXCITATORY AMINO ACID RECEPTORS: PHYSIOLOGY, PHARMACOLOGY, AND MODULATION—mGluR II

322.1

A NOVEL SELECTIVE ANTAGONIST OF METABOTROPIC GLUTAMATE RECEPTORS COUPLED TO PHOSPHOLIPASE D. D.E. Pellegrini-Giampietro*, S. Albani Torregrossa, R. Pellicciari[1], M. Marinozzi[1] and F. Moroni. Dipartimento di Farmacologia Preclinica e Clinica, Università di Firenze, 50134 Firenze, and [1]Istituto di Chimica e Tecnologia del Farmaco, Università di Perugia, 06123 Perugia, Italy.

Activation of phospholipase D (PLD) represents an important mechanism leading to a long-lasting and abundant formation of diacylglycerol in brain tissue. Metabotropic glutamate receptors (mGluR) coupled to PLD appear to be distinct from any known mGluR subtype linked to phospholipase C or adenylyl cyclase (Pellegrini-Giampietro et al., *Br. J. Pharmacol.*, in press). Unfortunately, selective ligands for these receptors have not yet been identified. mGluR agonists activate PLD with the following rank order of potencies: quisqualate > ibotenate > L-CCG-I > (1S,3R)-ACPD > L-cystein sulfinate > L-AP3 > L-aspartate > L-glutamate. (+)MCPG (at 1 mM) and DHPG (IC_{50} = 60 μM) inhibit the PLD response induced by agonists, but their antagonist effect is not selective for PLD-coupled mGluRs. With this in mind, we examined the effects of a series of sixteen stereoisomers of phenylcarboxycyclopropylglycine (PCCGs, Pellicciari et al., *J. Med. Chem.*, in press) on the PLD-specific transphosphatidylation reaction resulting in the formation of [^{3}H]phosphatidyl-ethanol ([^{3}H]Pet) in rat hippocampal slices. The most interesting compound appeared to be the (2S,1'S,2'R,3'S)-PCCG stereoisomer (PCCG-16), which antagonized the formation of [^{3}H]Pet induced by (1S,3R)-ACPD in a concentration-dependent manner (IC_{50} = 1 μM) and was unable to elicit a PLD response up to 1 mM. PCCG-16 had no effect (up to 300 μM) on phosphoinositide hydrolysis or adenylyl cyclase activity in BHK cells expressing mGluR1a, mGluR2 or mGluR4. Moreover, PCCG-16 failed to displace (up to 200 μM) radioligand binding to AMPA, NMDA, and kainate receptors, as well as to mGluR1 and mGluR4 in rat cortical homogenates. Selective antagonists of PLD-coupled mGluRs may be of help in elucidating their physiological and pathological functions in the CNS. *Supported by the European Commission (Biomed 1 BMH1-CT93-1033 project)*

322.2

SELECTIVITY OF THE EFFECTS OF *N*-ACETYLASPARTYLGLUTAMATE (NAAG) ON mGluR3 METABOTROPIC GLUTAMATE RECEPTORS. B.Wroblewska*[1], A.Surin[2], S. Pshenichkin[2], J.T. Wroblewski[2], J.H. Neale[1], Dept. Biology[1], and Pharmacology[2], Georgetown University, Washington, DC 20057.

We have shown previously that in cerebellar granule and glial cells NAAG, like glutamate, trans-ACPD, and AP4, decreases forskolin-stimulated cAMP levels in a dose-dependent manner. This inhibition is blocked by pertussis toxin, and is not due to the activation of phosphodiesterase. These data strongly suggested that NAAG interacts with metabotropic glutamate receptor(s). Using clones of metabotropic glutamate receptors, we developed lines of Chinese hamster ovary cells expressing different mGluRs. Our results indicated that only in the cells expressing mGluR3 receptor, NAAG had an inhibitory effect on forskolin-stimulated cAMP formation. NAAG did not inhibit cAMP in the cells expressing mGluR2, mGluR4, mGluR6, and mGluR7, and did not affect PI hydrolysis in the cells expressing mGluR1, and mGluR5. To further investigate this selectivity of NAAG on subtypes of metabotropic glutamate receptors we constructed chimeric receptor molecules in both CHO and A293 cells. Replacement of the extracellular N-terminus of mGluR1a with the corresponding portion of mGluR2 or mGluR3 preserved signal transduction mechanism characteristic for mGluR1a receptor, while generated agonist selectivity characteristic for mGRlu2 or mGluR3. In the cells expressing chimeric receptors glutamate, trans-ACPD, and L-CCG-I increased inracellular Ca (measured by Fura-2 fluorescence). However, NAAG caused increase in $[Ca^{++}]_i$ only in the cells expressing chimeric receptor mGluR3/mGluR1a, but not in mGluR2/mGluR1a, and had no effect on mGluR1a receptor. Our results support the hypothesis that NAAG is a very highly selective, endogenous agonist of type 3 metabotropic glutamate receptor, and interacts with the N-terminal binding domain of the receptor.*(Supported by NIH grants NS28130 and NS01720.)*

322.3

PHARMACOLOGICAL CHARACTERIZATION OF mGluR6 METABOTROPIC GLUTAMATE RECEPTORS EXPRESSED IN CHO CELLS

J.T. Wroblewski*[1], S. Pshenichkin[1] and B. Wroblewska[2]. [1]Dept. of Pharmacology and [2]Dept. of Biology, Georgetown University, Washington D.C. 20007

The metabotropic glutamate receptor mGluR6 belongs to group III mGluRs (together with mGluR4, 7 and 8) characterized by high sensitivity to L-2-amino-4-phosphonobutyric acid (AP4) and has been shown by RNA blot hybridization and *in situ* hybridization to be localized exclusively in the retina. In our studies, using PCR amplification we detected the message for mGluR6 in whole rat brain, in the retina, cerebellum, as well as in the spinal cord and in primary cultures of cerebellar granule neurons and cerebellar astrocytes. To study its pharmacological properties we prepared Chinese hamster ovary (CHO) cells stably transfected with mGluR6 cDNA. This cell line showed a high level of receptor expression and the maximal inhibition of forskolin-stimulated cAMP formation induced by AP4 reached 95%. Dose-response studies performed with several putative agonists indicated that the most potent were serine-O-phosphate (SOP, EC_{50} = 0.1 μM), AP4 (0.5 μM) and glutamate (3 μM), but also (2*S*,1'*S*,2'*S*)-2-(carboxycyclopropyl)glycine (L-CCG-I, 0.5 μM) and less potent *trans*ACPD (50 μM), quisqualate (100 μM) and 4-carboxyphenylglycine (4CPG, 100 μM). The action of 1 μM AP4 was not antagonized by the following compounds (at 1 mM concentration): 4-carboxy-3-hydroxyphenylglycine (4C3HPG), α-methyl-4-carboxy phenylglycine (MCPG), α-methyl-cyclopropylglycine (MCCG), and α-methyl-aminophosphonobutyrate (MAP4). A 50% inhibition of the AP4 effect was observed with 1 mM α-methyl-4-phosphonophenylglycine (MPPG). These results indicate that in addition to the retina mGluR6 may be significantly expressed in other brain stuctures and that its pharmacological profile, as determined in transfected CHO cells, may be distinct from that of other group III metabotropic glutamate receptors. *(Supported by NIH grants NS28130 and NS01720.)*

322.4

DESENSITIZATION OF HUMAN mGLUR1α INVOLVES BOTH TRANSCRIPTIONAL AND TRANSLATIONAL MECHANISMS.

M.A. Desai*, N.G. Mayne, J.P. Burnett, and D.D. Schoepp. Central Nervous System Research, Eli Lilly & Co., Indianapolis, IN 46285.

We have studied desensitization of a human mGluR1α (HmGluR1α) receptor coexpressed with a rat glutamate/aspartate transporter (GLAST) in a novel cell line (AV12-664; ATCC CRL 9595). Desensitization of HmGluR1α-mediated phosphoinositide (PI) hydrolysis is maximal after 12 hour pretreatment with the mGluR agonist, 3,5-dihydroxyphenylglycine (DHPG). This desensitization was blocked in a concentration-dependent manner upon pretreatment with increasing concentrations of the protein synthesis inhibitors, cycloheximide (CHX) or puromycin (PUR). Concentrations of CHX that maximally blocked desensitization inhibited protein synthesis by 88 ± 1.3%, as determined by measurement of [^{3}H]leucine incorporation. DHPG-induced desensitization of HmGluR1α-mediated PI hydrolysis was also fully blocked by the transcription inhibitor, actinomycin D (ActD). Reversal of DHPG-induced desensitization required washout of agonist from the culture medium for 12 hours before subsequent measurement of glutamate-stimulated PI hydrolysis. CHX, at a concentration that maximally inhibited protein synthesis, had no effect on reversal of HmGluR1α desensitization. These data suggest that desensitization of HmGluR1α involves synthesis of an inducible protein. In contrast, reversal of agonist-induced desensitization does not appear to require synthesis of new HmGluR1α protein. Funded by Eli Lilly & Co.

322.5

PHARMACOLOGICAL CHARACTERIZATION OF hmGluR2 AND hmGluR4 EXPRESSED IN CHINESE HAMSTER OVARY (CHO) CELLS USING [^{35}S]GTPγS BINDING.

D. M. Kowal, C. Hsiao[1], S. Leinbach[1], A. Ge, J. Wardwell-Swanson, and J. R. Tasse*. Wyeth-Ayerst Research, CNS Disorders, CN 8000, Princeton, NJ 08852 and [1]Wyeth-Ayerst Research, Core Biotechnology, Radnor, PA 19087.

To date, the functional assessment of cloned human metabotropic glutamate receptor (hmGluR) subtypes negatively coupled to adenylate cyclase has been identified by use of agonist inhibition of forskolin-stimulated cAMP accumulation. In the present study, mGluR agonists have been evaluated in at least two cell lines expressing hmGluRs using a functional [^{35}S]GTPγS binding assay. Comparison of mGluR agonist rank order of potency of EC_{50} values in the hmGluR2 clone showed good correlation with published results for the inhibition of forskolin-stimulated cAMP formation assay (Flor *et al*, Eur. J. Neurosci. **7**:622, 1995). Interestingly, it appeared that both L-quisqualate and L-AP4 possessed weak partial agonist effects in the [^{35}S]GTPγS binding assay which were not observed in the cAMP accumulation assay. Evaluation of agonist activity with the hmGluR4 clone in [^{35}S]GTPγS binding also correlated well with published cAMP EC_{50} determinations (Flor *et al*, Neuropharmacol. **34**:149, 1995). Unexpectedly, L-quisqualate exhibited weak partial agonism at concentrations >10 μM in [^{35}S]GTPγS binding which was not detected in the forskolin-stimulated cAMP assay. The hmGluR7 is presently being expressed in a CHO cell line for evaluation in the [^{35}S]GTPγS binding assay. The results of the present study suggest that [^{35}S]GTPγS binding could be used as a functional assay for hmGluRs expressed in CHO cells. In the case of the determination of partial agonist activity, it appears that the [^{35}S]GTPγS binding assay could be more sensitve than the forskolin-stimulated cAMP assay.

322.6

pA_2 DETERMINATIONS OF THE COMPETITIVE hmGluR4 ANTAGONIST MAP4: A DIRECT COMPARISON OF [^{35}S]GTPγS BINDING AND FORSKOLIN-STIMULATED cAMP ACCUMULATION ASSAYS.

J.R. Tasse, E.A. Muth*, C. Hsiao[1], A. Ge, J. Wardwell-Swanson and D.M. Kowal. Wyeth-Ayerst Research, CNS Disorders, CN 8000, Princeton, NJ 08543 and [1]Wyeth-Ayerst Research, Core Biotechnology, Radnor, PA 19087.

To validate use of the [^{35}S]GTPγS binding assay as a means of assessing functional hmGluR activity, the pA_2 of the selective mGluR4 antagonist MAP4 was determined using a CHO cell line stably expressing cloned hmGluR4. Agonist mediated responses evoked by the Group III mGluR selective agonist L-AP4 were determined in the absence and presence of MAP4 in both the [^{35}S]GTPγS binding assay and the forskolin-stimulated cAMP accumulation assay. L-AP4 produced concentration-related effects in each assay both in the absence and presence of MAP4. L-AP4 responses were shifted to the right in a parallel manner in the presence of increasing concentrations of MAP4. Schild analysis of the dose ratio versus the concentration of MAP4 revealed a linear regression in both assays whose slopes did not differ statistically from unity, indicative of a competitive antagonism. pA_2 values determined for MAP4 between assays were not statistically different. The results of the present study correlate with *in vitro* electrophysiological experiments using the neonatal rat spinal cord preparation (Jane *et al*, Br. J. Pharmacol. **112**:809, 1994) and confirm the suggestion by Johansen and Robinson (Eur. J. Pharmacol.- Mol. Pharmacol. Sect. 290, R1, 1995) that MAP4 is a competitive antagonist of hmGluR4 agonist mediated responses. Furthermore, the finding of similar MAP4 pA_2 values in the [^{35}S]GTPγS binding and the forskolin-stimulated cAMP accumulation assays suggests that the activity in these assays is mediated via a common receptor and that the phamacological activities of mGluR4 ligands, when determined in these assays, are likely to be equivalent.

322.7

METABOTROPIC GLUTAMATE RECEPTORS MODULATE TRANSMITTER OUTPUT: A MICRODIALYSIS STUDY IN THE RAT CORTEX AND CAUDATE.

A. Cozzi, G. Lombardi*, P. Leonardi, M. Carfì, F. Moroni. Dept. of Pharmacology, Univ. of Florence, Viale Morgagni, 65, 50134 Firenze, Italy.

A number of *in vitro* studies have shown that mGluR1 agonists potentiate transmitter release from cortical slices or synaptosomes, and that mGluR2/3 agonists reduce the depolarization-induced transmitter output from striatal slices (see: *Br. J. Pharmacol.* **110**; 1407; 1993, *Neurochem. Int.* **24**; 525; 1994 and *Br. J. Pharmacol.* **117**; 189; 1996). Thus, the stimulation of mGluRs may either decrease or increase transmitter release depending on which receptor subtype is present. In the present study, utilizing microdialysis in freely moving rats, we tested the effects of 1S,3R-ACPD, (RS)-3,5-dihydroxyphenylglycine (DHPG a selective agonist of mGluRs of the first group) and (2S,3S,4S)-α-(carboxycyclopropyl)glycine (L-CCG-1 as a preferential agonist of mGluRs of the second group) on the extracellular concentration of L-Glu and L-ASP in the cortex and in the caudate. In the cortex, the addition of 1S,3R-ACPD (100 μM-1 mM) to the microdialysis fluid caused a concentration-dependent increase of L-Glu in the dialysate. The maximum increase was approximately 4 fold; reached within 5 min of 1S,3R-ACPD application and rapidly returning to basal level. Similarly, DHPG (300 μM - 1 mM) caused a significant increase of L-Glu concentration in the dialysate. L-CCG1 did not change L-Glu output up to a concentration of 100 μM, but slightly increased this output when its concentration was 1 mM. The potentiating effect of 1S,3R-ACPD (300 μM) on glutamate output was reduced by (S)-4-carboxyphenylglycine (4-CPG, 300 μM), a mGluR1 antagonist. When the dialysis probe was placed in the caudate, 1S,3R-ACPD (100 μM-1 mM) or L-CCG-1 (3-10 μM) significantly decreased the appearance of L-Glu in the dialysate. In conclusion, these experiments support the idea that mGluR agents modulate the function of the glutamatergic synapses by increasing or decreasing transmitter release depending on the subtype of the receptor that prevails in the neurons under study.

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322.8

ASTROCYTE INTRACELLULAR pH IS INCREASED BY ACTIVATION OF METABOTROPIC GLUTAMATE RECEPTORS.

BJ Amos, A Mathie†, CD Richards‡ and M Chesler.* Depts.Physiol./Neurosurg. NYU Med. Ctr., 550 1st Ave., NY, NY 10016, Depts.Physiol/Pharmacol. RFHSM, Rowland Hill St. London NW3 2PF.† ‡

We studied the effect of metabotropic glutamate receptor (mGluR) activation upon the intracellular pH (pH_i) of primary cultured (7-21d) rat astrocytes using BCECF fluorescence. Application of the mGluR agonist 1s,3R-ACPD (25-100 μM) evoked a rapid pH_i increase of up to 0.3 unit pH [1]. This response could be evoked by s-3HPG but not by I-CCG-1 or L-AP4 suggesting involvement of a group-I receptor (mGluR1 or mGluR5). The alkalinization was only seen in solutions buffered by CO_2/HCO_3^- and was not blocked by SITS (500 μM), DIDS (750 μM) or EIPA (200 μM). The response persisted in the absence of Na^+, whereas depolarization-induced alkaline shifts (40 mM K^+) were blocked, indicating that the metabotropic pH shift was not due to electrogenic Na^+-HCO_3^- cotransport. The mGluR-evoked response was reduced in 0 Ca^{2+} media and abolished by BAPTA-AM pretreatment, suggesting a Ca^{2+}-dependent mechanism of activation. Our data suggest that *in vivo* alkalinization of astrocytes [2] can have a mGluR-mediated component. Supported by the NIH (NS32123), BBSRC† ‡ and the Wellcome Trust‡. [1] Amos, Mathie & Richards *J. Physiol.* 491:136P [2] Chesler & Kraig (1987). *Am. J. Physiol.* 253:R666.

322.9

STRIATAL METABOTROPIC GLUTAMATE RECEPTORS REGULATE ACUTE AMPHETAMINE-STIMULATED NEUROPEPTIDE GENE EXPRESSION IN THE RAT STRIATUM. J. Q. Wang* and J. F. McGinty, Dept. of Anatomy and Cell Biology, East Carolina Univ. Sch. of Med., Greenville, NC 27858-4354, USA.

Metabotropic glutamate receptors (mGluR) are expressed by medium spiny neurons in the rat striatum. In order to detect possible involvement of mGluRs in regulation of tonic and phasic expression of neuropeptide genes contained by these neurons, a competitive mGluR receptor antagonist, (+)-α-methyl-4-carboxyphenylglycine [(+)-MCPG], was unilaterally injected into the right dorsal striatum (caudoputamen). The effects of pharmacological blockade of striatal mGluRs by (+)-MCPG on basal and amphetamine-stimulated neuropeptide mRNA expression in the striatum on the injection side were evaluated with quantitative *in situ* hybridization. Intrastriatal injection of (+)-MCPG at doses of 0.4, 2 and 10 μg/1 μl had no influence on basal levels of preprodynorphin, substance P and preproenkephalin mRNA in the dorsal striatum. However, (+)-MCPG, at doses of 0.4 and 2 μg/1 μl, significantly attenuated induction of striatal preprodynorphin and substance P mRNA induced by acute injection of amphetamine (2 mg/kg). Moderate induction of preproenkephalin mRNA by acute amphetamine was also attenuated by intrastriatal pretreatment with (+)-MCPG at doses of 0.4 and 2 μg/1 μl, although the effect did not reach significance. Intrastriatal vehicle infusion had no effect on basal or amphetamine-stimulated neuropeptide mRNA expression. These results indicate that activation of (+)-MCPG-sensitive mGluRs is necessary for preprodynorphin and substance P and, to a lesser extent, preproenkephalin mRNA induction in striatal projection neurons in response to indirect dopamine agonist administration. However, activity of (+)-MCPG-sensitive mGluRs may not be important for maintaining constitutive expression of these mRNAs in the striatum (supported by.N.C. Governor's Institute).

322.10

GROUP III mGLURs MODULATE CALCIUM CURRENTS IN THE ADULT RAT NEOCORTEX. A.Stefani*, F. Spadoni and G. Bernardi, IRCCS Ospedale S.Lucia and Uni. Tor Vergata, Via Ardeatina 306 00139 Rome,Italy

The contribution of l-AP4-sensitive mGluRs to the physiology of the corticostriatal projection is a crucial matter with regard to the potential capability of group III agonists to act as neuroprotective agents, to modify the seizure threshold, to influence both short- and long-term changes of synaptic transmission.We have studied, with standard whole-cell techniques, l-AP4- and l-SOP-mediated modulation of HVA Ca2+ currents of pyramidal neurons freshly isolated from the adult rat neocortex. Ba2+ was the charge carrier, 180 NMG and 150 TEA were the main ion species of internal and external solutions. Group III agonists reduced HVA Ca2+ currents in 80% of the recorded cells; significantly, l-SOP was active at nanomolar concentrations. A reliable dose-response curve was hard to define because of the extreme variability of the response. The modulation was partially voltage-dependent (about 1/3rd was relieved by facilitation protocols). Interestingly, MAP4 antagonised the l-AP4-induced inhibition. Each of the calcium channel blockers we have tested (conotoxin GVIA, Agatoxin and nifedipine) blocked part of the responses, supporting the interaction of agonists at group III mGluRs with multiple channel subtypes. Finally, the observed modulation was strictly age-dependent, being negligible in the first 3 postnatal weeks.Funding source: IRCCS Ospedale S.Lucia

322.11

4-HYDROXYNONENAL, A PRODUCT OF LIPID PER-OXIDATION, IMPAIRS SIGNAL TRANSDUCTION OF METABOTROPIC RECEPTORS IN HIPPOCAMPAL NEURONS. E. M. Blanc*, J. F. Kelly, R. J. Mark, and M.P. Mattson Sanders-Brown Center on Aging and Dept. of Anatomy & Neurobiology, Univ. Kentucky. Lexington KY 40536. Gerontology Research Center, NIA, Baltimore, MD 21224.

Amyloid β-peptide (Aβ) can disrupt ion transport systems and kill neurons. We recently showed that subtoxic levels of Aβ can impair muscarinic cholinergic signal transduction, apparently by "uncoupling" the receptor from its GTP-binding (G) protein (Kelly et al, PNAS, in press). Neurotoxic actions of Aβ are mediated by generation of oxyradicals and lipid peroxidation. 4-hydroxynonenal (HNE) is a prominent aldehydic product of lipid peroxidation that may play a key role in the mechanism of Aβ neurotoxicity (Mark et al, this meeting). HNE conjugates to cysteine, lysine and histidine residues of proteins and can impair their function. We now show that HNE can impair coupling of metabotropic acetylcholine and glutamate receptors to their G proteins in cultured rat hippocampal neurons. Carbachol and 3,5-dihydroxyphenylglycine (DHPG) were employed to selectively activate metabotropic receptors for acetylcholine and glutamate, respectively. HNE caused a concentration-dependent suppression of carbachol- and DHPG-induced accumulation of inositol phosphates. Antioxidants, (vitamine E and propyl gallate) were unable to reverse the impairment of metabotropic receptor signaling induced by HNE consistent with HNE's action being downstream of lipid peroxidation. Calcium imaging studies indicated that HNE abolished metabotropic agonist-induced calcium responses. The molecular target of HNE-induced metabotropic signal transduction disruption may be the G protein because GTPase activity induced by metabotropic agonists was abolished in membranes isolated from neurons treated with HNE. Our data indicate that HNE is a mediator of oxidative stress-induced disruption of signaling via GTP binding protein-linked receptors. Oxyradical-induced alteration of metabotropic receptors could contribute to neuronal dysfunction and death in an array of neurodegenerative disorders including Alzheimer's disease. (supported by the NIH, the Alzheimer's Association, and DRET).

322.12

METABOTROPIC GLUTAMATE RECEPTOR-MEDIATED G-PROTEIN ACTIVATION IN RAT CEREBRAL CORTICAL MEMBRANES. N. Nishi*, Y. Odagaki, T. Koyama, *Department of Psychiatry, Hokkaido University School of Medicine, North 15 West 7, Sapporo 060, Japan.*

Glutamate receptors are widely distributed in neurons and glia of the CNS. They are divided into two major classes; ionotropic and metabotropic glutamate receptors (mGluRs). The mGluRs are G-protein coupled receptors which can be further subdivided into three subgroups, namely, Group I ($mGluR_1$, $mGluR_5$), Group II ($mGluR_2$, $mGluR_3$) and Group III (others).

In the present study, we measured high-affinity GTPase activity of G-proteins activated through mGluRs in rat cerebral cortical membranes. The following mGluR-related compounds elevated the high-affinity GTPase activity in a concentration-dependent manner with the mean EC_{50} values indicated in the respective parentheses (μM): (2S,1'S,2'S)-2-(carboxycyclopropyl)glycine (L-CCG-I) (0.90), L-glutamate (4.8), trans-(1S,3R)-1-amino-cyclopentane-1,3-dicarboxylate (t-ACPD) (11), (±)-ibotenate (390). On the other hand, none of the ionotropic glutamate receptor agonists, *N*-methyl-D-asparate (NMDA), kinate and α-amino-3-hydroxy-5-methyl-isoxazole-4-propionate (AMPA) elevated the activity. In addition, quisqualate, (2S,1'R,2'R)-2-(carboxycyclopropyl)glycine (L-CCG-II), L-2-amino-4-phosphonobutyrate (L-AP4) and L-serine-*O*-phosphate (L-SOP) were also ineffective. Some of the phenylglycine derivatives with agonistic and antagonistic properties for $mGluR_2$ and $mGluR_1$, respectively, such as (S)4-carboxy-3-hydroxyphenylglycine ((S)4C3HPG) and (S)3-carboxy-4-hydroxy-phenylglycine ((S)3C4HPG), activated GTP hydrolysis with EC_{50} value of ca. 30-200 μM. These pharmacological profile indicates that the Group II mGluRs, especially $mGluR_2$ receptors, are responsible for the agonist-induced high-affinity GTPase activity in rat cerebral cortical membranes. This method provides a useful strategy for investigation of functional coupling between Group II mGluRs and their coupled G-proteins in native brain membranes. Supported in part by a grant from the Ministry of Education, Science and Culture of Japan.

322.13

INTRACELLULAR CALCIUM RESPONSES TO mGluR-ACTIVATION IN GUINEA-PIG HIPPOCAMPAL SLICES. S.R. Young,* R. Bianchi, and R.K.S. Wong. Dept. of Pharmacology, SUNY-HSCB, Brooklyn, NY 11203.

Activation of metabotropic glutamate receptors (mGluRs) has been reported to have multiple effects in hippocampal pyramidal cells including suppression of K^+ currents, Ca^{2+} release from intracellular stores, and modulation of calcium channels (Pin & Duvoisin, Neuropharmacology 34: 1, 1995).

We studied the effects of the specific mGluR agonist 1S,3R-1-aminocyclopentane-1,3-dicarboxylate (ACPD) on the electrical activity and intracellular calcium ($[Ca^{2+}]_i$) of pyramidal cells in hippocampal slices. Calcium Green-1 was injected into neurons with intracellular electrodes. Cell membrane potentials together with fluorescence of the Ca^{2+}-sensitive dye were recorded. At resting membrane potentials (-59/-73mV), bolus application of ACPD (estimated concentration 100-200 μM) induced depolarization (6-18 mV) and firing associated with $[Ca^{2+}]_i$ increase of similar duration. In tetrodotoxin (0.4 μM) the spike firing was abolished, but the ACPD-induced depolarization (5-22 mV) and the concomitant $[Ca^{2+}]_i$ increase persisted. Hyperpolarizing current pulses showed that the depolarization was associated with a conductance increase. Furthermore, the hyperpolarization reduced the $[Ca^{2+}]_i$ increase caused by ACPD.

The data suggest that mGluR activation induces prolonged depolarization associated with a $[Ca^{2+}]_i$ rise. The $[Ca^{2+}]_i$ rise is, at least in part, caused by an influx through voltage-sensitive channels.

(Supported by NIH)

322.14

METABOTROPIC GLUTAMATE RECEPTOR AGONISTS EVOKE A HYPERPOLARISING RESPONSE IN LABELLED, ACUTELY DISSOCIATED RAT TRIGEMINAL MOTONEURONES, S.J. Harris, K. Appenteng and T.F.C. Batten*. Department of Physiology, University of Leeds, Leeds, LS2 9NQ, UK.

We have developed a preparation to make patch clamp recordings from acutely dissociated, identified, rat V motoneurones. Following dissociation of a block of tissue from the area containing the motornucleus it is difficult to identify V motoneurones on the grounds of morphology or electrophysiology. Therefore motoneurones are prelabelled by retrograde transport of fluorescent latex beads (FluoSpheres 0.04μM, Molecular Probes). Latex beads were injected into the left masseter muscle of Wistar rats on postnatal day 4 (10μl/muscle). Neurones were acutely dissociated from a tissue block taken from the area of the V motornucleus on postnatal day 8. Following an enzymatic incubation period of one hour in 0.5% protease (Sigma type XIV) the neurones were dissociated and plated out in a recording chamber. Bright fluorescence was observed in a population of the neurones and recordings were made directly from labelled neurones. Whole cell patch clamp recordings were made at room temperature (18-20 °C) in oxygenated ACSF containing (mM): NaCl 140, KCl 3, $CaCl_2$ 1.5, $MgCl_2$ 1, Glucose 25, HEPES 10. Drugs were applied locally from micropipettes using pressure pulses. The metabotropic glutamate receptor agonists 1S, 3R-ACPD (aminocyclopentane dicarboxylic acid) and L-CCG-I ((carboxycyclopropyl)glycine) (both 100μM in pipette) evoked a hyperpolarising response with an associated increase in neurone input resistance in labelled neurones (n=5). We have previously reported this response on unlabelled brainstem neurones. These results confirm its presence on trigeminal motoneurones.

Supported by the Medical Research Council

322.15

INWARD CURRENT IN MICE CEREBELLAR PURKINJE CELLS INDUCED BY 1S,3R-ACPD. M. Hirono[1], S. Konishi[2], M. Sakakibara[3] and T. Yoshioka[1]. [1]Dept. of Mol. Neurobiol. Waseda Univ. Tokyo, JAPAN. [2]Mitubishi Kasei Inst. Life Sci. Tokyo, JAPAN. [3]Tokai Univ. Sch. of High-technology for human welfare, Numazu, JAPAN.

Inward current induced by the application of 1S,3R-ACPD to cerebellar Purkinje cells were studied mainly by Knöpfel et al.. The tentative conclusion proposed by them is that the inward current seen after application of 1S,3R-ACPD may be generated by Ca^{2+}-activated non specific cation channel or an electrogenic Na^+/Ca^{2+} exchanger, where Ca^{2+} may be mobilized by IP_3.

We examined these hypothesis by combination of patch clamp and Ca^{2+} imaging recording and eventually we reached to the different conclusions never proposed yet on the basis of experimental results.

(1) Injection of neomycin, spermin and spermigin, selective inhibitors of PIP_2-specific phospholipase C, demonstrated no effect on the production of inward current.

(2) Extracellular application of MCPG, non-specific antagonist of metabotropic glutamate receptor, diminished the response, while specific inhibitor (L-AP3) did not.

(3) Purkinje cells of cerebellar of mice lacking mGluR1 induced no inward current by the application of 1S,3R-ACPD (Conquet et al. 1994).

Therefore it is reasonable to assume that the inward current can be induced by the activation of mGluR1 receptor without hydrolysis of PIP_2 by phospholipase C. In this case mGluR1 receptor can function as ionotropic receptor.

322.16

CROSS-TALK BETWEEN TRANSDUCTION SYSTEMS COUPLED TO PHOSPHOINOSITIDE (PI) HYDROLYSIS AND ADENYLYL CYCLASE (AC) S.Miller*, R.Balazs, Y.Chun and C.W.Cotman, Inst.Brain Aging,UCI, CA 92717

Astrocytes express mGluRs which mediate PI hydrolysis (group I) or are negatively coupled to AC (group II/III). Culturing cells in a chemically defined medium (with EGF and bFGF) results in a marked increase in the amount and function of mGluR5, which is the only group I receptor detectable in these cells. Concurrently, mGluR agonists (one of the exceptions, L-AP4), potentiate rather than attenuate cAMP formation evoked by stimulation of β-adrenergic receptors (isoproterenol,Iso) or directly activating AC (forskoline,FK). It seems that the potentiating effect is due to the influence of some product of the mGluR5-coupled transduction system. 1) mGluR5 agonists had only a marginal effect, if any, on basal cAMP generation. 2) The rank order of agonist potencies for augmenting the evoked cAMP accumulation was similar to that for activating mGluR5. In particular, the most potent agonist was quis, a weak mGluRII/III agonist, followed by DHPG, a selective group I mGluR agonist. 3) L-SOP, which inhibits in other systems ACPD-induced potentiation of evoked cAMP accumulation, had no effect in astrocytes. 4) Potentiation, as mGluR5 function, was PTX-sensitive. mGluR5 stimulation leads *via* IP3 to the release of stored Ca2+, which may activate Ca2+dependent AC isoforms. However, chelating Ca2+ with BAPTA-AM did not abolish DHPG potentiation of cAMP accumulation. In fact, 'Ca2+-free' conditions resulted in a substantial increase in the Iso/FK-evoked cAMP formation, that was further augmented by the mGluR5 agonists. Finally, mGluR5 inhibition abolished DHPG-potentiation of the cAMP response. MCPG, a group I/II inhibitor, attenuated and TPA, which severely depresses mGluR5 activity, abolished the DHPG-evoked potentiation of the cAMP response. It seems, therefore, that the mGluR agonist-induced potentiation of evoked cAMP accumulation is mediated through mGluR5 activation. In astrocytes, similar potentiating effect was exerted by agonists (CCh and methoxamine) activating other PI hydrolysis-coupled receptors. The potentiation through different receptors may involve AC isoenzymes, which are activated by the β,γ subunits of G protein .

$GABA_A$ RECEPTORS: NATIVE FUNCTIONS

323.1

OPTICAL ANALYSIS OF SPATIO-TEMPORAL PATTERNING OF GABA RESPONSES INVOLVING A POSSIBLE NOVEL RECEPTOR IN EARLY EMBRYONIC AVIAN BRAINSTEM. Y. Momose-Sato, K. Sato, A. Hirota, T. Sakai & K. Kamino* Dept. Physiol., Tokyo. Med. & Dent. Univ. Sch. Med., Tokyo 113, Japan

To examine the embryonic functional expression of γ aminobutyric acid (GABA) receptors, we studied the inhibitory effects of GABA on the EPSPs evoked by vagal stimulus in 7- to 10-day old embryonic chick brainstem slice preparations, applying a multiple-site optical recording technique with a voltage-sensitive merocyanine-rhodanine dye (NK2761). First, in the GABA response, we pharmacologically identified three components: (1) component 1 related to $GABA_A$ receptors; (2) component 2 related to $GABA_B$ receptors; and (3) component 3 which is insensitive to $GABA_A$ and $GABA_B$ antagonists, but is stimulated by both $GABA_A$ and $GABA_B$ agonists. Subsequently, we investigated the embryogenesis and early development of the three components, and we have constructed developmental maps of regional distribution patterns of the three components. Components 1 and 3 have already emerged in the 7-day old embryo, and subsequently, component 2 appeared in the 8-day old preparations. No component related to $GABA_C$ receptors was observed, during the 7- to 10-day old embryonic stages. From the pharmacological properties of component 3, we suggest that component 3 is related to a new subtype, $GABA_D$ receptor.

323.2

DEVELOPMENTAL DIFFERENCES IN SYNAPTIC GABAERGIC CURRENTS IN HIPPOCAMPAL AND CEREBELLAR CELLS OF MALE RATS. E. J. Cooper, G. A.R. Johnston* and F. A. Edwards, Dept.of Pharmacology, University of Sydney, NSW 2006, Australia.

Whole-cell patch-clamp electrophysiology was used to record inhibitory synaptic currents (IPSCs) in dentate granule cells (HG) and cerebellar Purkinje cells (CP) in brain slices of male rats. These cell types exhibit IPSCs with different kinetics [1,2] and also feature different $GABA_A$ receptor subunits [3]. Miniature IPSCs (mIPSCs) from HG and CP cells were compared from rats of two age groups (denoted as 10 and 20 postnatal days). The inhibitory neuroactive steroid, 5α-pregane-3α,21-diol-20-one (THDOC) selectively increased the decay rate constant of IPSCs. This effect was stereo-specific and not voltage-dependent. Developmental differences in IPSC sensitivity to low concentrations (0.05 and 0.1μM) of THDOC were found in HG cells as those of 10 day group were prolonged by 24.3% ± 8.4% (n=9) in response to 0.05μM whereas those of the 20 day group were unaffected by concentrations less than 1μM. In contrast, both age groups of CP cells were sensitive to low concentrations of THDOC. However, there were developmental differences in the amplitude and frequency of control mIPSCs of CP cells. A further effect of THDOC was noted in that the degree of potentiation of IPSCs often covered a broad range within the same cell. These differences are most likely due to developmental changes in $GABA_A$ receptor subunits expressed synaptically in these cell types.

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323.3

FAST AND SLOW SPONTANEOUS IPSCs IN CA1 PYRAMIDAL CELS. MI Banks* and RA Pearce, Dept. Anesthesiology, Univ.Wisconsin-Madison 53706.

Two kinetically distinct $GABA_A$ IPSCs evoked in rat hippocampal CA1 neurons ($GABA_{A,fast}$ and $GABA_{A,slow}$) are differentially modulated by anesthetics and may have distinct functional roles. However, the synaptic basis of $GABA_{A,slow}$ is unclear, since only a single population of rapidly decaying spontaneous IPSCs (fast sIPSCs) has been described. We investigated the kinetics of sIPSCs in CA1, and studied the modulation of IPSCs by the benzodiazepine 4'-Chlorodiazepam (ClDZ). ClDZ is known to differentially modulate GABA receptors with different subunit compositions, but its effect on IPSCs in CA1 has not been reported previously.

Whole-cell recordings were obtained from pyramidal cells at 35°C in slices prepared from 2 to 5 week old rats. >90% of sIPSCs recorded in CNQX and APV decayed rapidly as measured by the mean time to 63% decay ($t_{0.63}$). The best Gaussian fit to the $t_{0.63}$ histogram had μ=5.4 ms and σ=2.93 ms (n=5129 events, 6 cells), but 3% of the events decayed with $t_{0.63}$>20 ms (= μ+5σ) and as long as 103 ms. The kinetics of these slow sIPSCs were strikingly similar to $GABA_{A,slow}$ ($t_{0.63}$=41.7±4.8 ms; n=9 cells), evoked by stimulating in *s. lacunosum-moleculare*, while the fast sIPSCs were similar to $GABA_{A,fast}$ ($t_{0.63}$=10.5±1.1 ms), elicited by stimulation in *s. pyramidale*, implying that these spontaneous events arise from the same two groups of synapses giving rise to $GABA_{A,fast}$ and $GABA_{A,slow}$. Consistent with this hypothesis, ClDZ reversibly reduced peak amplitude of evoked $GABA_{A,fast}$ (-75±2%; n=4) and fast sIPSCs (-58±10%; n=3367 events from 3 cells) with little effect on the amplitude of evoked $GABA_{A,slow}$ (+14 ± 43%) or slow sIPSCs (-6±10%; control n=31, ClDZ n=51 events, 3 cells). Thus, we have found that slow sIPSCs are present in CA1 neurons, and that ClDZ is a selective blocker of $GABA_{A,fast}$. These data suggest that $GABA_{A,fast}$ and $GABA_{A,slow}$ arise from different groups of synapses apposed by receptors with distinct properties.

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323.4

KINETIC PROPERTIES OF EXCISED $GABA_A$ RECEPTORS AND SPONTANEOUS IPSCs IN CA1 HIPPOCAMPAL PYRAMIDAL NEURONS. T-B Li, MI Banks and RA Pearce*, Dept. Anesthesiology and Neuroscience Training Program, University of Wisconsin, Madison, WI 53706.

Although a single population of receptors can generate currents with multiexponential decay kinetics, there is evidence that fast and slow components of evoked inhibitory currents in CA1 neurons reflect the kinetic properties of distinct and segregated receptor populations. We have used rapid agonist application methods to study $GABA_A$ receptors in outside-out patches excised from somata of CA1 pyramidal cells in brain slices that were prepared from 16-30 day old rats, and compared their kinetics at room temperature with spontaneous IPSCs.

Outside-out patches were exposed to 1 mM GABA for 1-2.5 ms using a "liquid filament" technique. Currents elicited by 7 to 70 pulses of GABA were obtained from six patches with stable properties. In all cases, averaged currents were fit well by biexponential functions, with time constants of 15.5 ± 4.9 ms and 92.6 ± 24 0 ms (mean ± s.d.). The absolute amplitude of the currents varied considerably between patches, from 3.1 to 78.7 pA, but the proportion of fast and slow components was similar in different patches (42.8 ± 4.4% fast), suggesting that these kinetic properties reflect gating characteristics of a single receptor population.

In contrast to rapid application responses, spontaneous IPSCs recorded from the same population of CA1 pyramidal cells were optimally fit by monoexponential functions. The average decay rate of 8 isolated sIPSCs from a cell with too high a spontaneous rate to analyze all events was 22.1 ms, and of 1062 events from a cell with a low spontaneous rate (17.7 Hz) was 17.4 ms. This decay rate is similar to the fast component of excised receptors.

We have found that differences exist between kinetic responses of receptors excised from somata of pyramidal neurons and IPSCs that are somatic in origin. These results may reflect a change in receptor properties caused by excision of the membrane patch, or differences between subsynapatic and extrasynaptic receptors.

Supported by NIH NS01548 and UW Dept. Anesthesiology.

323.5

GABA CURRENTS IN DEVELOPING RAT DENTATE GYRUS. Y-B Liu*, J. F. Pasternak, B.L.Trommer Division of Pediatric Neurology, Evanston Hospital, Evanston, IL 60201 and Dept. of Pediatrics and Neurology, Northwestern University Medical School, Chicago, IL 60611

Granule cells (GC) at different stages of maturation defined by cell properties and morphologic criteria co-exist in rat dentate gyrus during the first postnatal month. We studied GABA neurotransmission in GC in response to medial perforant path stimulation using whole cell patch clamp recordings in hippocampal slices from 8-32 d rats. GABA postsynaptic currents (PSCs) consist of both monosynaptic (from direct activation of interneurons) and polysynaptic (requiring an intervening glutamatergic synapse) components in GC of all maturities. Monosynaptic GABA PSCs (evoked in 50 μM AP5 and 10μM CNQX) were >96% antagonized by 10μM bicuculline (BMI), reversed at -69 mV, and had slight outward rectification, as expected for GABA$_A$ current. When PSCs were evoked in AP5 (50 μM) and BMI (10 μM), we detected a late current with marked outward rectification that followed the AMPA current. We concluded that this, too, was a GABA current because: it reversed at -61 mV with standard internal solution but near 0 mV when $[Cl^-]_{int}$ and $[Cl^-]_{ext}$ were equimolar; was blocked by picrotoxin 100 μM, but was unchanged by strychnine (5μM), BAPTA (10 mM), and saclofen (500 μM). This was a polysynaptically evoked GABA, since the monosynaptic GABA was blocked by 10 μM BMI. When the combined mono- and polysynaptic GABA PSC was evoked in AP5 (50 μM) at $V_{holding}$=0, the portion resistant to 10 μM BMI (i.e., polysynaptic) was significantly higher in immature (31±14%, n=6) than mature (8±5%, n=7) GC ($p < 0.003$). These data suggest the existence of a unique GABA current that is developmentally regulated, mediated predominantly by polysynaptic pathways, and less sensitive to BMI than the monosynaptic GABA$_A$.

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323.6

"COLD SHOCK" INCUBATION INCREASES ZOLPIDEM SENSITIVITY OF GABA$_A$ IPSCs IN RAT HIPPOCAMPAL CA1 NEURONS. C. Gu*, J.L. Weiner, and T.V. Dunwiddie. University of Colorado Health Sciences Center, Depts. of Pharmacology & Neuroscience and VA Medical Center, Denver, CO 80262.

We previously identified a slice incubation protocol (COLD SHOCK) that decreased extracellular GABA$_A$ IPSPs and increased ethanol, but not flunitrazepam or pentobarbital, sensitivity of GABA$_A$ IPSCs in rat hippocampal CA1 neurons. To further understand the mechanism(s) mediating the effect of COLD SHOCK incubation on ethanol potentiation of GABA$_A$ IPSCs, we characterized the effects of zolpidem, a selective type I benzodiazepine (BZ I) agonist that has been postulated to share the same GABA$_A$ receptor subunit requirements as ethanol. In control slices, zolpidem had no effect on GABA$_A$ IPSCs at 200 nM (5 ± 1%), but did produce significant potentiation at concentrations of 1 μM and higher. In contrast, GABA$_A$ IPSCs evoked from COLD SHOCK slices were significantly potentiated by zolpidem at concentrations as low as 200 nM (53 ± 10 %) ($p < 0.001$ vs control). These results suggest that COLD SHOCK incubation may result in an increase in the contribution of BZ I receptors at GABAergic synapses in hippocampal CA1 neurons or alternatively, that zolpidem enhancement of GABA$_A$ IPSCs, like that observed with ethanol, can be regulated by PKC. Supported by NS 29173 and the VA Medical Research Service.

323.7

SUBUNIT COMPOSITION AND FUNCTIONAL PROPERTIES OF ZOLPIDEM-INSENSITIVE GABA$_A$ RECEPTOR CHANNELS OF EMBRYONIC RAT HIPPOCAMPAL CELLS. *R. Serafini*, I. Maric, X.L. Wen, R. Somogyi, J.L. Barker and D. Maric. Lab. Neurophysiology, NINDS, NIH, Bethesda MD 20892.* Studies on recombinant GABA$_A$ receptors demonstrate that the presence of α_5 and γ_3 subunits confers low affinity to the allosteric modulator zolpidem. A flow-cytometry assay with the voltage-sensitive dye oxonol was performed to identify and sort a population of cells with low zolpidem-induced potentiation of GABA-evoked depolarization. When 5 μM zolpidem was added to a threshold GABA concentration, cells exhibited heterogeneous membrane potential changes ranging from no effect to nearly 0 mV. Cells with no voltage response and cells with the most depolarized membrane potential value were then sorted. PCR analysis demonstrated the mRNA encoding a wide variety of subunits in the zolpidem-sensitive cells, whilst, in the zolpidem-insensitive cells only the transcripts for the α_3 α_5 β_3 γ_3 subunits could be shown. In both populations, analysis of the current fluctuations induced by GABA and recorded whole-cell yielded spectral density plots composed of three components. In the zolpidem-insensitive population, the longest-lasting component exhibited a longer τ value (98±9 msec *vs* 76±7 msec; $p<0.05$) and a lower contribution to the overall power in the fluctuating signal (47±4 % *vs* 62±3 %; $p<0.01$). The data illustrate a novel strategy to sort distinct subpopulations of cells and demonstrate properties of native GABA$_A$ receptor channels which are likely related to the presence of α_5 or γ_3 subunits.

323.8

CALCINEURIN REGULATES DESENSITIZATION OF GABA$_A$ RECEPTORS IN ACUTELY DISSOCIATED RAT HIPPOCAMPAL NEURONS. J.W. Mozrzymas, M. Martina, F. Ruzzier* and E. Cherubini. Biophys Lab, Int Sch Adv Studies (SISSA), 34013 Trieste, Italy.

GABA-evoked currents were recorded in the whole-cell configuration of the patch-clamp technique in acutely dissociated hippocampal neurons obtained from postnatal (P) days P2-P7 old rats. In control conditions, the decay of the currents evoked by GABA (100 μM) could be fitted with a biexponential function having time constants of 0.65 ± 0.24 s and 3.75± 2 s, for the fast and slow component, respectively. The plateau to peak ratio was 0.087 ± 0.034 (n=13). A complete recovery from desensitization could be obtained after at least 150 s. When the cells were dialysed with the cyclosporin A- ciclophilin A complex (CC complex, 50 nM cyclosporin and 20 nM cyclophilin, gift of Dr. H. Boddeke, SANDOZ, Basel), a potent and highly selective calcineurin inhibitor, the decay of GABA-evoked currents could be fitted with a biexponential function having time constant values similar to those obtained in control conditions (0.81± 0.47 and 3.62 ± 2.1 for the fast and slow component, respectively). However the area of the fast component was significantly ($p<0.05$, n=8) smaller. Moreover, the plateau to peak ratio was significantly ($p<0.05$) larger than controls, being 0.185 ± 0.07. With CC complex in the patch pipette, recovery from desensitization was faster, being completed in about 40 s. In order to ascertain the specificity of action of CC complex, in five experiments, a cyclosporin A derivative PSC 833 (which does not affect calcineurin) was used instead of cyclosporin A. In these conditions the desensitization and recovery kinetics were indistinguishable from those seen in controls. We conclude that, in acutely dissociated hippocampal neurons, desensitization processes of GABA$_A$ receptors are strongly regulated by calcineurin. Supported by grants from CNR (95.01664.CT04) and HCMP.

323.9

PROPERTIES OF GABA$_A$ RECEPTOR-MEDIATED CHLORIDE CURRENTS IN RAT NEOCORTICAL PYRAMIDAL CELLS. J.F.M. van Brederode* and W. J. Spain. VA Puget Sound Health Care System, Seattle, WA 98108 and Depts. of Neurology, and Physiology & Biophysics, University of Washington, Seattle, WA 98195.

Previously we described a post-synaptic mechanism as underlying some of the observed differences between evoked GABAA mediated IPSPs recorded from layer 2/3 (L2/3) vs. large layer 5 (L5) pyramidal cells. Here we compare GABAA receptor-mediated currents recorded in outside-out patches excised from the somata of visually identified pyramidal cells located in those layers (fronto-parietal cortex; 2-4 week old rats). Cells were identified in 300 μm thick slices using infrared videomicroscopy. In layer 5 we targeted only pyramidal cells with the largest cell bodies, which was confirmed in a few cells by including biocytin (0.5%) in the patch electrode. The patch solution contained (in mM): 140 CsCl, 1 $CaCl_2$, 3.45 BAPTA, 10 HEPES and 5 Mg_2ATP and pipettes had resistances between 10 and 20 MΩ when filled with this solution. The bath contained CNQX (10 μM), AP5 (50 μM) and TTX (1 μM). After seal formation and patch rupture the pipette was slowly withdrawn from the cell to form an outside-out patch and exposed to long pulses of GABA (10-20 μM), pressure-ejected from a nearby pipette. In the absence of GABA, channel openings were infrequent and brief. In the presence of GABA longer, burst-like openings were observed. Both spontaneous and GABA-induced openings were sensitive to blockade by bath-applied bicuculline (100 μM). Single channel currents reversed near the calculated chloride equilibrium potential. Current-amplitude histograms suggest a main channel conductance of about 30 pS. Supported by a VA Merit Review (W.S) and NIH EY 01208 (J.v.B.)

323.10

N-ETHYLMALEIMIDE ALTERS THE INTRINSIC MEMBRANE PROPERTIES AND INHIBITORY SYNAPTIC POTENTIALS OF NEURONS IN A RAT SEPTAL SLICE PREPARATION. K.D. Phelan* and H.R. Mahler. Dept. of Anatomy, Univ. of Arkansas for Medical Sciences, Little Rock, AR 72205.

N-ethylmaleimide (NEM) has long been used to investigate receptor coupling to G-proteins in membrane preparations. Although it has recently been applied to cultured neurons and brain slice preparations, the effects of NEM on neurons have not been well characterized. In the present study, intracellular recordings were used to determine the effects of NEM on intrinsic membrane properties and synaptic potentials of rat dorsolateral septal nucleus neurons using a submerged slice preparation.

Superfusion of NEM (100 μM, 10-30 minutes) consistently produced an irreversible decrease in neuronal input resistance. It also blocked the low threshold calcium spikes that occur during rebound depolarization, calcium spikes evoked during depolarizing current steps, and slow calcium-dependent afterhyperpolarizations. Cells typically displayed a marked increase in spontaneous excitatory synaptic events and less consistent changes in the frequency of action potential firing. NEM pretreatment selectively reduced the amplitude of GABAb IPSPs, while increasing the amplitude of GABAa IPSPs. NEM completely blocked low intensity stimulus evoked GABAb IPSPs and baclofen (<30 μM) induced membrane hyperpolarizations. However, a low amplitude, slow component of GABAb IPSPs evoked at high stimulus intensity and a small amplitude baclofen (100 μM) induced membrane hyperpolarization was more resistant to NEM. At the same time, baclofen activation of presynaptic GABAb receptors was blocked by NEM pretreatment suggesting a differential sensitivity of presynaptic and postsynaptic GABAb receptors to NEM alkylation. These findings indicate that although NEM can be used in slice preparations to investigate the presumed G-protein coupling of receptors in single neurons, it can also produce significant changes in the intrinsic membrane properties and excitability of neurons.

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323.11

INFLUENCE OF HIGH FREQUENCY STIMULATION ON INHIBITORY POSTSYNAPTIC POTENTIALS RECORDED FROM NEURONS OF THE ROSTRAL NUCLEUS OF THE SOLITARY TRACT. G. Grabauskas and R. M. Bradley*. Sch. of Dentistry, Univ. Michigan, Ann Arbor, MI 48109-1078.

During gustatory stimulation of the tongue afferent taste fibers are capable of responding with impulses frequencies up to 100 Hz. We have examined the consequence of this high frequency afferent stimulation (HFS) on IPSPs elicited in 58 neurons of the rostral nucleus of the solitary tract (rNST) in a brain slice preparation. IPSPs were evoked by stimulation of the solitary tract (ST) after elimination of excitatory synaptic activity with blockers of NMDA and AMPA/kainate glutamate receptors. Whole cell recordings were made from second order rNST neurons and the solitary tract was stimulated at frequencies that mimic the *in vivo* firing rate of afferent taste fibers (5 - 50 Hz). We found that HFS induced membrane hyperpolarization and increased conductance that could be blocked by bicuculline indicating involvement of GABA. When compared to single shock stimuli HFS altered the characteristics of the evoked IPSPs. In most neurons (n = 42) HFS prolonged the decay time of the IPSP. Depending on the frequency, duration and magnitude of the ST stimulation, the decay time of the IPSP was lengthened several hundred orders of magnitude compared to single shock stimuli. Thus, HFS can potentiate the IPSPs thereby increasing the time of inhibition. In some neurons (n = 10) HFS elicited a biphasic response with an initial hyperpolarization which then became depolarizing and elicited action potentials. The depolarizing amplitude and the number of action potentials was dependent on the frequency, duration and magnitude of the stimulus. Thus GABA activation which is normally inhibitory can becomes excitatory at these high stimulation frequencies. For a few neurons (n = 6) there was no difference between the IPSPs initiated by HFS and a single shock. This short-term change in synaptic activity induced by afferent frequencies normally resulting from taste stimulation can alter the transmission of taste information in rNST and illustrates the importance of inhibitory activity in the gustatory relay nucleus. Supported by NIDCD, NIH Grant DC 00288 to RMB.

323.12

REGULATION OF GABA$_A$ RECEPTOR δ SUBUNIT mRNA EXPRESSION IN CULTURED RAT CEREBELLAR GRANULE NEURONS. L.M. Gault and R.E. Siegel*. Dept. of Pharmacology, Case Western Reserve University, Cleveland, OH 44106-4965.

The distribution of GABA$_A$ receptor subunit mRNAs in the cerebellum has been well-characterized, but the factors regulating their expression are largely unknown. Previous studies in cultured rat cerebellar granule neurons have shown that the δ subunit mRNA is barely detectable in neurons maintained in a defined nondepolarizing medium (5 mM KCl). In contrast, the δ transcript increases six-fold between 2-4 days in neurons maintained in a depolarizing medium (25 mM KCl). When calcium influx through L-type channels is reduced by the addition of 1 μM nifedipine, levels of the δ transcript decrease by 75%. The possibility that gene expression can similarly be altered by calcium entry through the NMDA receptor was also investigated. Although HPLC measurements indicate that comparable amounts of glutamate, the neurotransmitter of granule neurons, are present in the medium of neurons maintained in nondepolarizing or depolarizing conditions, activation of NMDA receptors in nondepolarizing medium may be less effective due to a magnesium block. In fact, addition of 50 μM NMDA to increase activation of this receptor subtype results in a significant rise in the level of the δ transcript to about 40% of that attained in neurons maintained in depolarizing medium. These results suggest that activation of NMDA receptors plays a role in initiating δ subunit mRNA expression and raise the possibility that synaptic contact between glutamatergic mossy fibers and granule neurons *in vivo* regulates levels of this subunit mRNA. Supported by NIH grant NS 31266.

323.13

BIDIRECTIONAL REGULATION OF GABA$_A$ RECEPTOR α1 AND α6 SUBUNIT EXPRESSION BY A cAMP-MEDIATED SIGNALLING MECHANISM IN CEREBELLAR GRANULE CELLS IN PRIMARY CULTURE

Christopher L. Thompson, Simon Pollard and F. Anne Stephenson (SPON: Brain Research Association). Department of Pharmaceutical and Biological Chemistry, School of Pharmacy, University of London, London, UK.

Forskolin treatment of rat cerebellar granule cells in culture resulted in bidirectional regulation of the expression of GABA$_A$ receptor α1 and α6 subunits. Thus, forskolin applied at 2 days in vitro (2 DIV) increased expression of the α1 subunit but decreased the expression of the α6 subunit. Values with respect to vehicle-treated control cultures, both assayed at 9 DIV by immunoblotting, were 310 ± 48% for α1, and 25 ± 16% for the α6 subunit. Similar effects were evoked following chronic treatment with both dibutyryl cAMP (1 mM) and 3-isobutyl-1-methylxanthine (IBMX, 250 μM). Dideoxyforskolin (10 μM) had no effect on the level of expression of either the α1 or the α6 GABA$_A$ receptor subunits. The changes in subunit expression were accompanied by a 1.7-fold increase in total specific [^{3}H]Ro15-4513 binding sites expressed by intact cerebellar granule cells. This increase in total binding sites was accommodated by a 2.7-fold increase in diazepam-sensitive (DZ-S) [^{3}H]Ro15-4513 binding sites in accordance with the observed increase in α1 subunit expression. The diazepam-insensitive (DZ-IS) subtype of binding sites were not significantly changed. These results suggest that GABA$_A$ receptor subtype expression can be differentially regulated by intracellular cAMP concentration.

This work was supported by the MRC (UK).

323.14

EXPOSURE OF PRIMARY GRANULE CELLS TO POTASSIUM DEPOLARIZATION OR TO N-METHYL-D-ASPARTATE ALTERS THE STABILITY OF THE α1 GABA$_A$ RECEPTOR SUBUNIT mRNA. Snezana Ikonomovic*, William Paljug, and Dennis R.Grayson. Neurosciences Research Center and Department of Psychiatry, Medical College of Pennsylvania and Hahnemann University, Pittsburgh, PA 15212.

K^+-induced depolarization or NMDA treatment promotes the survival and maturation of cultured cerebellar granule cells *in vitro* and increases the amount of functional NMDA receptors present. We have previously shown that NMDA and K^+ depolarization of primary cultures of cerebellar granule cells increases the mRNA levels of the α1 and α5, but not the α6 GABA$_A$ receptor subunits as compared to cultures maintained in low KCl. These alterations are accompanied by increases in the α1 and α5 receptor subunit proteins, and increases in the Bmax associated with ^{3}H flunitrazepam binding. Whole cell voltage clamp recordings show that the NMDA treatment increases the affinity of the receptors for GABA and increases flunitrazepam potentiation of GABA-mediated Cl^- currents. We have also shown that the mRNA increases occur with a concomitant increase in the transcription rates of the corresponding genes. In the present study, we have used the transcriptional inhibitor actinomycin D (Act-D) to block mRNA synthesis 24 hr after adding NMDA to granule cell cultures maintained in low KCl containing media. Total RNA was isolated from control and treated cultures at various times (0, 1, 2 and 3 hrs) after adding Act-D (10 μg/ml) and steady state levels of mRNA were examined. Absolute amounts of α1 and α6 mRNAs were quantitated using an RT PCR based assay with subunit specific primer pairs and internal standards. The half life of both the α1 and α6 mRNAs was comparable and longest in granule cells maintained in high KCl. The stability of the α1 mRNA decreased in granule cells treated with NMDA for 24 hr. The half life ($t_{1/2}$) of this mRNA decreased from 13 hr to 2.7 hr after the addition. In contrast, no change was observed in the stability of the α6 mRNA in cells maintained in treated cultures as compared to the low K^+ maintained controls. This suggests that NMDA alters patterns of GABA$_A$ receptor subunit expression at multiple levels. Supported by NIH grants NS 30537 and K04 NS01647 to D.R.G.

323.15

GABA$_A$ RECEPTORS IN DOPAMINERGIC NEURONS OF RAT SUBSTANTIA NIGRA PARS COMPACTA.

D. Eugène, L. Cathala and D. Paupardin-Tritsch*. Neurobiologie Cellulaire, Institut des Neurosciences, CNRS - Université Pierre et Marie Curie, 9 Quai Saint Bernard, 75005 Paris, France.

We have studied the effect of isoguvacine, a specific agonist of γ-aminobutyric acid$_A$ (GABA$_A$) receptors, on dopaminergic (DA) neurons. These neurons were acutely dissociated according to I. Mintz's procedure (J. Neurosci. 1994, 14, 2844-53) from slices containing only substantia nigra pars compacta of 7-12 day old rats. Seventy-five percent of the neurons with neurites (n = 114) showed positive staining for tyrosine hydroxylase determined by immunocytochemistry.

Using the whole cell patch-clamp configuration, bath application of isoguvacine (1-200 μM) induced a Cl^- current in these DA-containing neurons. Concentration-response curves of peak current amplitude could not be fitted by a single exponential but display two components.

Since molecular biological and binding studies have demonstrated that GABA$_A$ receptors with different subunit composition may coexist in the same cell, single channel patch-clamp currents were recorded from outside-out patches of DA neurons. At a holding potential of -70 mV, 1 μM isoguvacine evoked single channel currents of small amplitude, brief duration openings that usually occurred as single events. The amplitude histogram showed that this channel had a main conductance level of about 8 pS. In addition, 2-5 μM isoguvacine produced single channel openings that were longer in duration and larger in conductance (22 pS) and occurred in bursts of opening and closing. Both single channel currents reversed at E_{Cl} and were blocked by bicuculline (10 μM). These results might account for the two components of the concentration-response curve and suggest that DA neurons contain two types of GABA$_A$ receptors.

323.16

GABA MEDIATED DENDRITIC PLATEAU POTENTIALS IN RAT NEOCORTICAL PYRAMIDAL NEURONS. R. Cerne* and W. J. Spain. Depts. of Neurology and Physiology & Biophysics, Univ. of Washington, and The VA Puget Sound Health Care System, Seattle, WA 98108

Last year we reported that continuous intense activation of GABA$_A$ receptors in slices of rat somatosensory cortex resulted in a plateau potential (PP) following repetitive firing of action potentials. Whole-cell current-clamp recordings were made from somata of visually identified layer V pyramidal cells in slices of from 3-5 week-old rats. Internal solution contained Cl^-= 9 mM. Slices were superfused with ACSF containing APV, CNQX and CGP-35348 ($[Cl^-]_o$=141 mM). Somatic Nernst equilibrium potential for Cl^-=-73 mV. In the presence of GABA (>50 μM) or muscimol (100 μM) an evoked train of action potentials was followed by a voltage and time dependent PP that does not depend on calcium entry. The PP was associated with low amplitude sodium dependent spikes which continued and arose directly from the baseline even after the soma was repolarized by means of constant current injection to -70 mV suggesting a dendritic origin. In addition, in neurons with surgically amputated apical dendrites the PP was absent. The PP was enhanced with increased intracellular Cl^- but was blocked by furosemide (1 mM) or bumetanide (100 μM) but not by acetazolamide (100 μM) suggesting that it depends on a high dendrite to low soma $[Cl^-]_i$ gradient.

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324.1

DEHYDRO-PIRLINDOLE IS A CLOZAPINE-LIKE PARTIAL GABA$_A$ RECEPTOR BLOCKER AS WELL AS A POTENT SELECTIVE INHIBITOR OF MONOAMINE OXIDASE-A. A.E. Medvedev[1], V.I. Shvedov[2], T.M. Chulkova[1], O.A. Fedotova[2], E. Saederup[3] and R. F. Squires[3*]. [1]Institute of Biomedical Chemistry, Russian Academy of Medical Science, Moscow, Russia, [2]Centre of Drug Chemistry-Institute of Pharmaceutical Chemistry, Moscow, Russia, [3]Nathan Kline Institute, Orangeburg, NY 10962.

Pirlindole is clinically effective in the treatment of depression as well as some forms of schizophrenia. Dehydro-pirlindole (DHP) is an active metabolite possibly formed by the action of MAO on pirlindole. DHP, but not pirlindole, partially reversed the inhibitory effect of 1 μM GABA on ^{35}S-TBPS binding (ΔB_{opt} = 42%, EC_{50} = 12 μM). DMCM (methyl 6,7-dimethoxy-4 ethyl-β - carboline-3-carboxylate) and Ro5-4864, acting on largely separate populations of benzodiazepine binding sites, both partially reverse the inhibitory effect of 1 μM GABA (ΔB_{opt} 40% and 49%, EC_{50} 9 and 710 nM, respectively) and together they reverse ~75%, with 14% overlap. Like clozapine, the reversing effect of DHP was partially additive to the reversing effects of DMCM (100 nM) and Ro5-4864 (5 μM). Thus, clozapine and DHP both preferentially block a population of GABA$_A$ receptors (~25%) that does not appear to be coupled to benzodiazepine binding sites. In addition, DHP selectively inhibits MAO-A from rat brain and human placenta with IC_{50} values of 6.4 and 4.5 nM, respectively, in a slowly reversible way. Pirlindole is also a selective, but weaker, inhibitor of MAO-A with IC_{50} values of 320 and 90 nM, respectively. The MAO-A inhibitory and GABA antagonistic effects of DHP may be involved in the clinical antipsychotic/ antidepressant effects of long term treatment with pirlindole. Funding: Russian State Program "Design of New Drugs" (Grant No. 040103) and New York State, OMH.

324.2

ANTIDEPRESSANT AND BICUCULLINE ACTION AT THE GABA$_A$ RECEPTOR CHLORIDE-IONOPHORE COMPLEX; SCHILD REGRESSION ANALYSIS. E. Malatynska*. Evansville Center for Medical Education, University of Indiana, Evansville, IN 47712

It was shown that antidepressants inhibit GABA-stimulated $^{36}Cl^-$ uptake in rat cerebral cortex. The site of antidepressant action at the benzodiazepine-GABA$_A$ receptor chloride ionophore complex was subjected to study. The allosteric modulation of the GABA$_A$ receptor mediated chloride flux by antidepressants through the benzodiazepine receptor site was previously excluded. However, it is still not clear whether antidepressants are competitive antagonists or non-competitive (allosteric modulators) of the GABA$_A$ receptors. To study this problem the Schild regression analysis was used. The GABA concentration-response curves for $^{36}Cl^-$ uptake in rat cerebral cortex were generated in the absence and presence of increasing concentrations of antidepressants: amitriptyline (AMI), amoxapine (AMX) mianserine (MIA) and known GABA$_A$ receptor antagonist, bicuculline (BIC). The concentrations of inhibitors were 2μM, 8μM, 32 μM 128 μM and 512 μM. The Emax value of GABA for stimulation of $^{36}Cl^-$ uptake was reduced only by the highest antidepressant concentration used while it was reduced by all BIC concentrations. Dose ratios were calculated at the GABA EC-50 values at each concentration of drug. The pA_2 values for AMI, MIA, AMX and BIC were 3.9, 4.4, 5.4 and 6.2 respectively. Respective slope values were 0.7, 0.6, 0.7 and 1.0. The slopes for antidepressants were significantly different from unity. It is concluded from our study that neither antidepressants nor BIC are pure competitive antagonists of GABA at the GABA$_A$ receptors in the rat cerebral cortex. Supported by NARSAD

324.3

CaM KINASE II-DEPENDENT PHOSPHORYLATION OF SYNAPTIC PLASMA MEMBRANE INCREASES MUSCIMOL BINDING. S.B. Churn*, and R.J. DeLorenzo. Department of Neurology, Medical College of Virginia. Richmond, VA 23298.

Calcium/calmodulin-dependent kinase II (CaMKII) is an important, neuronally enriched calcium effector enzyme. However, many pathological states such as seizure activity and stroke result in decreased CaMKII activity. CaMKII-dependent phosphorylation has been shown to increase Cl- currents in rat spinal dorsal horn neurons (J. Neurophys. 1995. 73:2099). In addition, GABA receptor subunits have been shown to be substrates for CaMKII (J.Neurochem. 1993. 61:375). Since CaMKII activity may alter GABAergic function, this study was designed to determine the effects of phosphorylation on muscimol binding on synaptic plasma membrane fractions isolated from rat whole brain homogenates. P-2 fractions were isolated by standard techniques, lysed in hyposmotic buffer, washed 3X and the resultant synaptic plasma membrane (SPM) resuspended into buffer (50 mM Tris-HCl, pH = 7.4). The SPM fractions were subjected to CaMKII-dependent phosphorylation (J. Neurosci. 1995. 15:3200), and then tested for muscimol binding. For muscimol binding, samples were equilibrated with ^{3}H-muscimol (100 nM) ± 100μM GABA for 1 hr on ice. Binding reactions were terminated by rapid filtration under vacuum and washing with iced-cold buffer. Addition of CaMKII enriched from rat brain resulted in 60% increase in muscimol binding when compared to sham-treated P-2 fractions (CaMKII reactions without addition of kinase). The significant increase in muscimol binding was observed in 3 different SPM preparations ($p < 0.01$, Student's t test, $n = 16$). In addition, the increase in muscimol binding could be reversed by co-incubation with W-7, H-7, KN-93 and peptide inhibitors specific for CaMKII. The results demonstrate CaMKII-dependent regulation of muscimol binding in SPM. R01-NS23350, P01-NS25630.

324.4

KINETICS OF ENDOGENOUS PHOSPHATE INCORPORATION INTO THE GABA$_A$ RECEPTOR SUGGESTS A CASCADE OF KINASES AND A RECEPTOR ASSOCIATED PHOSPHATASE ACTIVITY. Michel Bureau*, Jacques Laschet, Frédéric Minier, Bertrand Evrard and Patrick Chauvel, Laboratory of Biochemistry, School of Pharmacy and Medicine, University of Rennes I, Rennes, France, F-35043.

The phosphorylation of either the GABA$_A$ receptor itself or of a closely associated protein may be required to maintain receptor function (Stelzer et al., *Science*, 241: 339-341. 1988). The receptor « run down » is accelerated by alkaline phosphatase. The molecular identification of the phosphatase and kinase protein(s) involved in these mechanisms is presently unknown, although we recently showed that endogenous kinase activities are associated with the purified receptor (Bureau and Laschet, *J. Biol. Chem.* 270, 26482-26487, 1995). We have studied the effects of incubation time on endogenous phosphorylation in the presence of several activators, i.e. Mg^{2+}, Mn^{2+} and Zn^{2+}. At short incubation time we observed the phosphorylation of two copurified bands of about 38 and 16 kDa: this occured before any incorporation of phosphate into the receptor. Phosphorylation and dephosphorylation of the 38 kDa band took place within the first few minutes of incubation. The 16 kDa band was phosphorylated in the following minutes. The dephosphorylation rate of the latter was coincident with the phosphorylation of an upper band of 19 kDa and of the receptor itself. At longer incubation time, a slow dephosphorylation took place after one hour. Taken together these results suggest 1) that the endogenous phosphorylation of the GABA$_A$ receptor could be under the dependence of a cascade of copurified kinases 2) the presence of an endogenous phosphatase activity.

324.5

IONIC DEPENDENCE OF THE ENDOGENOUS PHOSPHORYLATION AND DEPHOSPHORYLATION OF GABA$_A$ RECEPTOR PROTEIN. Jacques Laschet[1,2]*, Frédéric Minier[1], Bertrand Evrard[1], Guy Dandirfosse[2], Patrick Chauvel[1] and Michel Bureau[1], Lab. of Biochemistry, Univ. of Rennes I, Rennes, France, F-35043 and Lab. of Biochemistry and Physiology, ULg, Liège, Belgium, B-4000.

GABA$_A$ receptors are ligand gated ion channels that mediate most inhibitory synaptic transmission in the central nervous system. Recently we have identified an endogenous phosphorylation on distinct purified polypeptides by Ser/Thr and Tyr kinase activities (Bureau and Laschet, *J. Biol. Chem.* 270, 26482-26487, 1995). We studied the ionic modulations of this phosphorylation that predominates on the 51 kDa-polypeptide corresponding to α1 subunit. Each ion alone produced a dose-dependent activation with different efficiencies (Zn^{2+} > Mn^{2+}, Ca^{2+} > Mg^{2+} > Spermine). However, in the presence of low concentrations of Mg^{2+} (30 μM) or Zn^{2+} (10 μM), the dose-dependent activation of the other ions (except Ca^{2+}) was replaced by a biphasic modulation i.e. an activation followed by a inhibition. In the case of calcium, a dose-dependent inhibition was directly observed (IC_{50} = 100 μM), suggesting that the inhibition of endogenous phosphorylation, or the activation of phosphatase activity by Ca^{2+} requires the presence of another divalent cation. By increasing the temperature of incubation from 30°C to 40°C, the activations by Zn^{2+} and Ca^{2+} were specifically lost whereas the modulations by Mn^{2+}, Mg^{2+} and Spermine were thermoresistant. These results suggest not only the presence of multiple binding sites for these ions with possible competition, but are also consistent with the modulation of multiple kinase and phosphatase activities associated with the purified receptor.

324.6

MODULATION OF GABA$_A$ RECEPTORS BY ALUMINUM. PAUL Q. TROMBLEY* Dept. of Bio. Science, Florida State Univ., Tallahassee, FL. 32306.

The effects of aluminum (Al^{3+}) on several classes and subtypes of amino acid receptors were examined using whole-cell voltage-clamp electrophysiology on rat olfactory bulb mitral/tufted (M/T) cells in primary culture. Elevated Al^{3+} in the CNS has been associated with cognitive impairment and implicated in Alzheimer's disease. Several laboratories have demonstrated that inhaled Al^{3+} results in uptake into the brain and distribution along olfactory pathways. The effects of Al^{3+} on amino acid receptors were examined because synaptic pathways that use amino acid transmitters are often involved in neuropathology associated with Al^{3+}. Under voltage-clamp at -60 mV, 100 μM Al^{3+} was co-applied during the middle of the current evoked by amino acid receptor agonists. Al^{3+} had no effect on membrane currents evoked by 500 μM glutamate, 100 μM kainate, 100 μM NMDA, or 100 μM glycine. In contrast, 10-100 μM Al^{3+} potentiated GABA (30 μM) mediated currents by as much as several hundred percent. However, in most M/T neurons, at concentrations ≥ 300 μM, Al^{3+} blocked the GABA-evoked current. In a subpopulation of neurons, Al^{3+} inhibited the current and potentiation was never observed. These results may indicate that GABA receptors express two binding sites for Al^{3+}: one potentiates the response to GABA, and one inhibits the response. GABA receptors in some cells may only express the inhibitory site. Neither effect of Al^{3+} was voltage-dependent, suggesting an allosteric binding site(s). Zinc and copper are endogenous to the olfactory bulb and can alter the function of amino acid receptors. Carnosine is a dipeptide contained in and released by olfactory sensory neurons. Whereas carnosine can reduce or eliminate the modulatory effects of zinc and copper on amino acid receptors, carnosine dramatically potentiated the effects of Al^{3+} on GABA mediated currents. These results suggest that Al^{3+} may contribute to neuropathology by affecting GABAergic pathways.

This research was supported in part by the NIH.

324.7

ORGANOCHLORINE PESTICIDES REGULATE PRENATAL EXPRESSION OF GABA$_A$ RECEPTOR SUBUNITS *IN VITRO* AND *IN VIVO*. J. Liu[1*], A. L. Morrow[2], L. Devaud[2], D. R.Grayson[3] and J. M. Lauder[1]. [1]Dept. of Cell Biology and Anatomy and [2]Psychiatry and Center for Alcohol Studies, Univ. of North Carolina School of Medicine, Chapel Hill, NC 27599, [3] Dept. of Psychiatry, Med. Coll. of Penn. and Hahnemann Univ., Pittsburgh, PA 15212

In order to test the hypothesis that the ability of organochlorine pesticides to specifically target GABA$_A$ receptor may make the developing nervous system especially vulnerable to these neurotoxins, we used *in vitro* and *in vivo* models to determine whether exposure to these pesticides alters developmental expression of GABA$_A$ receptor subunits. For the *in vitro* model, dissociated cultures were prepared from embryonic day 14 (E14) brainstem and cultured in BME + 10% NuSerum for 1 day *in vitro* (DIV) , then treated for 48 hrs with 10 μM of the pesticide dieldrin in serum-free medium. For the *in vivo* model, timed pregnant rats were injected i.p. with 2.5 mg/kg dieldrin or with vehicle beginning on day 12 of gestation (E12) and injections continued until E17. After pregnant rats were sacrificed by rapid decapitation under ether anesthesia, fetuses were rapidly removed and their brainstems dissected. Absolute amounts of α1, β3, γ1 mRNA transcripts were quantified in cultured and fetal brainstem by competitive RT/PCR using internal standards. Expression of α1 and β3 mRNA were up-regulated, but γ1 mRNA was down-regulated by dieldrin *in vitro*, whereas α1, β3, γ1 mRNAs were down-regulated by dieldrin *in vivo*. These results suggest that *in utero* exposure to organochlorine pesticides may pose a risk to the developing brain by virtue of their ability to alter expression of GABA$_A$ receptor subunits. Supported by NIEHS grant to JML.

324.8

THE INTERACTION OF ORGANOCHLORINE INSECTICIDES WITH RECOMBINANT HUMAN GABA$_A$ RECEPTORS. L.S. ASPINWALL[1], K.A. WAFFORD[2*], I. BERMUDEZ[1] AND L.A. KING[1]. [1]Oxford Brookes University, Oxford. OX3 0BP. [2]MSD Research Laboratories, Harlow, Essex CM20 2PT. UK.

The effect of convulsant and non-convulsant hexachlorocyclohexane (HCH) isomers on recombinant gamma-aminobutyric acid (GABA$_A$) receptors were studied, using a variety of expression systems, the baculovirus system, stably expressing mammalian cells and *Xenopus oocytes*. Using human α_1, β_3 and γ_2 subunits, all three systems were shown, by ligand binding assays and electro-physiological studies, to produce functional, triple subunit receptors that bound both [^{3}H]GABA and [^{3}H]flunitrazepam (FNZ). It was observed that the mammalian cell system produced four-fold higher levels of the GABA$_A$ receptor, than the baculovirus system, and was therefore more practical, for use in the radioligand binding studies.

γ-lindane and two of its isomers, the α and δ forms, were applied to recombinant GABA$_A$ receptors, in the presence of [^{3}H]muscimol, an analogue of GABA, in an optimised radioligand binding assay. The three insecticides produced no displacement of [^{3}H]muscimol suggesting that they do not interact with the GABA binding site on the GABA$_A$ receptor. Similar binding assays were performed using [^{3}H]FNZ, showing no displacement, demonstrating no interaction with the benzodiazepine binding site. Chloride [^{36}Cl] flux experiments on this cell line, in the presence and absence of the three insecticides, clearly showed that γ-lindane inhibited the influx of chloride ions through the channel, while the α and δ isomers potentiated the influx, the δ isomer exhibiting the largest effect. This phenomenon was substantiated by electro-physiological studies on the stably expressing mammalian cells, using the whole cell patch clamp technique. Further studies involving [^{35}S]t-butylbicyclophosphorothionate (TBPS) binding, a known chloride channel blocker, and expression of different combinations of the human GABA$_A$ receptor subunits in *Xenopus oocytes* should enable identification of the site of binding and which subunit(s) influence this interaction.

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324.9

LOW LEVEL HYPERBARIC EXPOSURE: SELECTIVE UNCOUPLER OF ALLOSTERICALLY MODULATED RECEPTORS. R.L. Alkana*, M.B. Bolger, R.D. Brinton and D.L. Davies. Dept. of Molecular Pharmacology and Toxicology, School of Pharmacy, University of Southern California, Los Angeles, CA 90033.

Exposure to helium-oxygen (heliox) gas, at 12 times normal atmospheric pressure (ATA), antagonized the behavioral effects of diazepam and pentobarbital, but not those of THIP, a GABA prodrug, and picrotoxin in C57 mice. These findings suggest that pressure acts selectively by uncoupling allosteric modulation of GABA$_A$ receptor function. We tested this hypothesis further by investigating the effects of pressure versus GABA$_A$ receptor drugs in LS mice. Exposure to 12 ATA heliox antagonized the anticonvulsant effects of the allosteric modulators diazepam and pentobarbital. Pressure also antagonized flunitrazepam- and pentobarbital-potentiation of GABA-activated ^{36}Cl$^-$ uptake in LS mouse brain microsacs. In contrast, pressure did not alter GABA stimulation or picrotoxin blockade in this system. Further, pressure did not alter the behavioral or *in vitro* effects of the neuroactive steroid 3α,5β-P. These results support the hypothesis that pressure selectively antagonizes drugs by uncoupling allosteric modulation of receptor function. Moreover, the lack of pressure antagonism versus 3α,5β-P suggests that there is heterogeneity among allosteric coupling mechanisms that can be identified by pressure. Since pressure is a physical manipulation, this selectivity among allosteric modulators cannot derive from pressure's chemical or molecular structure. Rather, sensitivity to pressure antagonism likely reflects pressure acting on similar physico-chemical changes that underlie a subset of coupling mechanisms among allosteric modulators. Further studies are necessary to test this hypothesis and to determine whether pressure can be used as a tool to link coupling mechanisms within and across allosterically modulated receptor systems (e.g., GABA$_A$ and NMDA). (Supported by NIAAA, NIH grants R01AA03972, AA05234, F31AA05436 and the USC School of Pharmacy).

324.10

NAAG modulates expression of the GABA-A α 6 subunit mRNA via activation of a mGluR in cultured cerebellar granule cells. S. Ghose*, B. Wroblewska, D.R. Grayson[1], J.H. Neale. Dept.of Biology, Georgetown University, Washington D.C. and [1]Neuroscience Research Center, Department of Psychiatry, Medical College of Pennsylvania and Hahnemann University, Pittsburgh, PA .

We have demonstrated that treatment of cultured cerebellar granule cells with N-acetylaspartylglutamate (NAAG) transiently alters the expression of the GABA-A α 6 subunit mRNA. Using a quantitative PCR, we determined the increase to be up to 170% of control levels. The first increase in expression is seen within 30 minutes of addition of NAAG returning towards control levels after two hours before showing a second peak at 4 hours. Similar effects of equal potency are seen on treatment with trans-ACPD and glutamate. In addition, the response is pertussis-toxin sensitive, implying the involvement of a metabotropic glutamate receptor. L-AP 4 , a group III mGluR agonist, had no effect. Stimulating the muscarinic and alpha adrenergic receptors (negatively linked to adenylate cyclase) in these cells reproduced the effects seen with NAAG. Forskolin abolished the response. This suggests that the effect of NAAG is mediated via a decrease in intracellular cAMP. This is in accordance with previous data (Wroblewska et al. 1995) that NAAG activates mGluR3. We measured the effect of NAAG on GABA-A α 6 mRNA stability by treating the cells with D-actinomycin. We found that NAAG decreases the half-life of the message which could explain the rapid return of the mRNA levels to the baseline levels. These data indicate that short-term treatment with NAAG stimulates a group II mGluR inducing transient increases in GABA-A α 6 mRNA of a shorter half-life. (Supported by NIDA)

324.11

SUBSTANCE P DOWN-REGULATES THE FUNCTION OF GABA$_A$ RECEPTORS VIA PROTEIN KINASE C IN BULLFROG SENSORY NEURONS. K. Yamada[1], T. Tokimasa*[2], J. P. Gallagher[3] and T. Akasu[1]. [1]Dept. Physiol., Kurume Univ. Sch. Med., Kurume 830, [2]Dept. Physiol., Tokai Univ. Sch. Med., Isehara 259-11, Japan and [3]Dept. Pharm. & Tox., Univ. of Texas Med. Br., Galveston, TX 77555, U.S.A.

Effects of substance P (SP) and related tachykinins on the function of γ-aminobutyric acid-A (GABA$_A$) receptors were examined in acutely dissociated neurons of bullfrog dorsal root ganglia (DRG) by using whole-cell voltage-clamp techniques. Application of SP (10 nM-1 μM) depressed inward currents produced by GABA$_A$ receptor activation (I_{GABA}) in a non-competitive manner. Neurokinin A (NKA) and neurokinin B (NKB) also depressed the I_{GABA}; the rank order of agonist potency was SP > NKA > NKB. [D-Arg[1], D-Trp[7,9], Leu[11]]SP (spantide, 1 μM) and L-703,606 (1 μM), antagonists for NK$_1$ receptors, blocked the SP-induced depression of the I_{GABA}. SP irreversibly depressed the I_{GABA}, when DRG neurons were intracellularly dialyzed with GTPγS (200 μM). Intracellular GTPβS (500 μM) prevented the SP-induced depression of the I_{GABA}. The SP-induced depression of the I_{GABA} was blocked by H-7 and PKC(19-36), protein kinase C (PKC) inhibitors but not by H-9 and HA-1004, PKA inhibitors. The I_{GABA} was blocked by 1,2-sn-di-octanoylglycol (DOG, 10-50 μM), a PKC activator, but not by inositol trisphosphate (IP$_3$, 50 μM). Okadaic acid (2 μM) and adenosine 5'-O-3-thiotriphosphate (ATPγS, 5 mM) prolonged the SP-induced inhibition of the I_{GABA}. It is concluded that SP down-regulates the function of GABA$_A$ receptor in bullfrog DRG neurons through an NK$_1$ receptor that links to a G-protein-PKC pathway. (Glant-in-Aid (A) 07308054, Japan)

324.12

GABA-STIMULATED CHLORIDE UPTAKE TO RAT BRAIN SYNAPTONEUROSOMES IS INHIBITED BY THYROID HORMONE. D.B. Williams, K. Lee, D. M. Azzarano, H. Lee, and J. V. Martin*. Biology Department, Rutgers University, Camden, NJ 08102.

We recently showed that submicromolar concentrations of triiodothyronine (T_3) and thyroxine (T_4) stereospecifically stimulate the binding of [^{35}S]t-butylbicyclo-phosphorothionate (a convulsant ligand for the GABA$_A$ receptor complex) to highly washed rat brain membranes, while higher concentrations of the hormones inhibited radioligand binding. Further studies investigated the effect of L-T_3 on GABA-stimulated uptake of ^{36}Cl$^-$ into synaptoneurosomes. Whole forebrains from male Sprague-Dawley rats were homogenized in 7 volumes (w/v) of ice-cold buffer (118 mM NaCl, 4.7 mM KCl, 1.18 mM $MgSO_4$, 2.5 mM $CaCl_2$, and 20 mM HEPES-Tris, pH 7.4), diluted with 30 more ml of buffer, and gravity-filtered through three layers of nylon mesh. The filtrate was gently pushed through a 10 μm filter and centrifuged at 1,000 x g for 15 minutes. The final pellet was resuspended and used as the synaptoneurosomal preparation. An aliquot of the preparation was preincubated at 30°C for 20 minutes. GABA and/or various doses of L-T_3 were added with 0.5 μCi of ^{36}Cl$^-$. In five seconds, 5.0 ml of ice-cold buffer including 100 μM picrotoxin was added, and the suspension was filtered under vacuum. After the filters were washed twice, the retained radioactivity was determined. L-T_3 inhibited GABA-stimulated flux of ^{36}Cl$^-$ into synaptoneurosomes in a dose-dependent fashion, with a half-maximally effective dose of 100 nM. Recent estimates of the concentrations of thyroid hormones in the synaptic cleft of the CNS (Mason et al., *Neuropsychopharmacology* 8, 253-258, 1993) are within the range which should exert the effects on GABA$_A$ receptor binding and activity found in our studies. Our observations of the effects of thyroid hormones on the GABA$_A$ receptor complex therefore suggest a potential non-genomic mechanism for thyroid hormone actions in adult brain. This study was supported by grants from the Rutgers University Busch Fund and the National Science Foundation (IBN 94-12109).

324.13

CONFOCAL IMAGING OF INTRACELLULAR CHLORIDE ($[Cl^-]_i$) IN ACUTELY PREPARED SLICES: MEASUREMENT OF GABA$_A$ RECEPTOR ACTIVITY. Jon R. Inglefield* and Rochelle D. Schwartz-Bloom. Dept. Pharmacol., Duke Univ. Med. Ctr., Durham, 27710

We have developed an optical recording technique, using UV laser-scanning confocal microscopy (LSCM) and the fluorescent Cl^- ion indicator, 6-methoxy-N-ethylquinolinium chloride (MEQ), to image changes in $[Cl^-]_i$ in individual neurons of acute brain slices (12-21 d.o. rats). MEQ is quenched collisionally by increased $[Cl^-]_i$. After bath-loading slices (27°C) with the cell-permeant form (reduced) of MEQ, there was intense fluorescence (F) within numerous neurons in the hippocampus, neocortex and cerebellum. MEQ fluorescence was localized to the cytosol of both the somata and dendrites of neurons but was typically lower (higher $[Cl^-]_i$) in dendrites compared with the soma, suggestive of an intraneuronal $[Cl^-]_i$ gradient. There was no autofluorescence. When MEQ-loaded neurons were exposed to the UV laser continuously (> 1200 sec), photobleaching proceeded with a rate constant of k = 0.0041 ± 0.0007 sec^{-1} and a $t_{1/2}$ = 173.8 ± 24.6 sec. Thus, illumination time never exceeded 20 sec in all experiments to minimize the photobleaching to ~6%. In hippocampal pyramidal cells, superfusion of GABA produced a time- and concentration-dependent increase in $[Cl^-]_i$ (EC_{50} = 40 μM) that was blocked by picrotoxin (1 mM). Pentobarbital (1mM) and diazepam (50 μM) in the perfusate each enhanced the GABA-mediated relative change in MEQ fluorescence (ΔF). Also, the GABA (40 μM)-induced ΔF varied ($P<0.05$) among cell types and brain regions: cerebellar Purkinje cells (70.6±4.7%) > cerebellar granule cells (38.5±5.7%) > pyramidal neurons of the hippocampus (30±1.7%) and neocortex (19.5±2.1%) = hippocampal interneurons (20.7±3.6%). Local application (via picospritzer) of the GABA$_A$ agonist, muscimol (10 μM), to hippocampal neurons led to a rapid (2-5 seconds) ΔF of 25%. This response was fully reversible and was blocked by the GABA$_A$ antagonist, bicuculline (100 μM). Optical imaging of $[Cl^-]_i$ using MEQ and UV-LSCM is a useful new method to assess GABA$_A$ responses in neurons of defined morphology within living brain slices. Supported by NS28791 and American Heart Assoc., NC Affiliate, Inc.

324.14

BIOCHEMICAL AND IN VIVO EFFECTS OF A SELECTIVE INVERSE AGONIST FOR α_5 SUBUNIT-CONTAINING GABA$_A$ RECEPTORS. J.R. Atack, R. McKernan, K. Wafford and G.R. Dawson* Merck Sharp & Dohme Research Laboratories, Neuroscience Research Centre, Harlow, CM20 2QR, U.K.

The GABA$_A$ receptor is now generally accepted to be a pentameric chloride ion channel composed of a combination of the 14 GABA$_A$ receptor subunits (α_{1-6}, β_{1-3}, γ_{1-3}, δ and ρ). It contains a benzodiazepine binding site, the pharmacology of which is dictated by the combination of α and γ-subunits within the receptor. Radioligand binding studies of recombinant GABA$_A$ receptors have identified FG8094 as being 50 fold selective for α_5- compared to α_2- or α_3-containing GABA$_A$ receptors whilst electrophysiological studies in oocytes expressing $\alpha_5\beta_3\gamma_2$ receptors showed that FG8094 is an inverse agonist. Displacement of *in vivo* binding of [^{3}H]FG8094 or [^{3}H]Ro15-1788 was used to establish doses of FG8094 which occupy α_5 and α_1, α_2 and α_3-containing GABA$_A$ receptors, respectively. Doses of FG8094 that were relatively selective for α_5-containing GABA$_A$ receptors did not induce seizures in mice nor did they potentiate pentylenetetrazole-induced seizures. However, at higher doses, which gave appreciable occupancy of α_1, α_2 and α_3-, as well as α_5-containing GABA$_A$ receptors, FG8094 blocked the anticonvulsant effects of benzodiazepine agonists. These data suggest that α_5-containing GABA$_A$ receptors do not mediate the anticonvulsant effects of benzodiazepine agonists nor do they appear to be involved in GABA$_A$ receptor-mediated seizures.

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GABA$_A$ RECEPTORS: MICROANATOMY

325.1

CLATHRIN-COATED VESICLES FROM BOVINE BRAIN CONTAIN UNCOUPLED AND PARTIALLY DEGRADED GABA$_A$ RECEPTORS. M.H. Jalilian Tehrani* and E.M. Barnes, Jr. Dept. of Biochem., Baylor Col. of Med., Houston, TX 77030.

Both sequestration and uncoupling of GABA$_A$ receptors are thought to represent early events in use-dependent receptor down-regulation. We have examined a possible relationship between these processes using clathrin-coated vesicles (CCVs), a preparation which may include vehicles important for GABA$_A$ receptor sequestration (Tehrani and Barnes, 1993). The level of ^{3}H-muscimol and ^{3}H-flunitrazepam (^{3}H-flu) binding to CCVs represents 12.3 ± 1.8% and 7.9 ± 1%, respectively, of that in crude synaptic membranes. The parameters of ^{3}H-flu binding for CCVs (K_D = 12.2 ± 3.5 nM; B_{max} = 225 ± 45 fmol/mg) also differ from those for synaptic membranes (K_D = 1.38 ± 0.2 nM; B_{max} = 1060 ± 30 fmol/mg). As expected, addition of GABA (EC_{50} = 2.2 μM) to synaptic membranes enhanced ^{3}H-flu binding (E_{max} = 122%), an effect completely blocked by SR95531. In contrast, neither GABA nor SR95531 had any significant effect on ^{3}H-flu binding to CCVs. Immunoblotting of synaptic membranes with an antibody against a GABA$_A$ receptor β2S(317-428) fusion protein revealed major a 59-kDa and a minor 53-kDa polypeptide. For CCVs, in addition to these proteins, a range of smaller peptides (32-45 kDa) was also detected. Our results indicate that, during transit from the surface to coated vesicles, GABA$_A$ receptors undergo both structural and pharmacological modifications. These may reflect a common proteolytic step which occurs early in the process of GABA$_A$ receptor down-regulation.

Supported by NIH grants NS34253 and NS11535.

325.2

VARIABILITY IN SYNAPTIC GABA$_A$ RECEPTOR CONCENTRATION IN CEREBELLAR NEURONS. Z. Nusser[1], W. Sieghart[2]* and P. Somogyi[1]. [1]MRC Anatomical Neuropharmacology Unit, Mansfield Road, Oxford, OX1 3TH; [2]Dep. of Biochemical Psychiatry, Psychiatrische Universitätsklinik, Vienna, Austria

The postsynaptic response to the release of a single quantum of GABA varies greatly in central neurons. There is also a large variability in postsynaptic responses to GABA in a single cell as revealed by the skewed amplitude distribution of miniature GABA$_A$ receptor mediated inhibitory postsynaptic currents (IPSCs). Our aim was to test whether these variabilities can be explained by quantitative differences in postsynaptic GABA$_A$ receptor concentration in synapses of different types of neurons. To address this question a postembedding immunogold method was employed on ultrathin sections from freeze-substituted and Lowicryl-embedded rat cerebellar tissue. The α1 subunit of the GABA$_A$ receptor was visualised using a subunit-specific polyclonal antibody and silver intensified immunogold as marker. Quantitative comparison of immunolabeling at different synapses is possible under postembedding conditions, because there is no difference between different synapses in their access to antibodies. Golgi cell to granule cell synapses contain the lowest level of synaptic immunolabel followed by GABAergic synapses on Purkinje cells. The highest level of immunolabeling is observed in GABAergic synapses on interneurons in the molecular layer. Similarly to our previous observation of variability in α1 subunit content between different Golgi cell to granule cell synapses, we have found that GABAergic synapses on Purkinje cell somata contain much lower immunolabeling than those on Purkinje cell dendrites. These results show that the differences in postsynaptic GABA$_A$ receptor concentration may underlie the variability of postsynaptic responses to synaptically released GABA.

325.3

EXPRESSION OF GABA$_A$ RECEPTOR (GABA-R) β2 SUBUNIT mRNA AND [^{3}H]MUSCIMOL BINDING IN THALAMUS DO NOT CHANGE DURING THE COURSE OF ABSENCE-LIKE SEIZURES INDUCED BY γ-HYDROXYBUTYRATE (GHB). P.K.Banerjee*[1], N.J.K.Tillakaratne[4], S. Brailowsky[7], R.W.Olsen[3,5,6] O.C. Snead[1] and A.J.Tobin[2,3,4,5,6] [1]Dept. of Neurology, Hosp. for Sick Children, Toronto; Depts. of [2]Neurology, [3]Pharmacology and [4]Physiol. Sci, [5]Mol. Biol .Inst. and [6]Brain Res. Inst., UCLA; [7]Inst. de Fisiol.,U.N.A.M., Mexico .

In thalamic relay nuclei neurosteroids fail to modulate GABA-R binding during the course of GHB-induced absence seizures, and as the seizures terminate GABA-R α4 subunit mRNA levels in relay nuclei decrease and the effect of neurosteroids is restored (Banerjee et. al., Soc. Neurosci. Abstr. 1995). GABA-R β2 subunit is abundant in the thalamus and β subunits possess binding sites for [^{3}H]muscimol which are prevalent in the thalamus. In the present study we compared the expression of GABA-R β2 mRNA and [^{3}H] muscimol binding in thalamus during GHB-seizures. We used in situ hybridization with ^{35}S-labeled cRNAs to determine the expression of mRNAs for rat β2 and GAD_{67} form of glutamate decarboxylase (as control). [^{3}H]Muscimol (5 nM) binding was performed to specifically label the high affinity sites. GABA-R β2 subunit mRNA was highly expressed in thalamus and its distribution in relay nuclei correlated with that of high affinity [^{3}H]muscimol sites. In contrast, GAD_{67} cRNA hybridized strongly to reticular nucleus of thalamus (nRT) but not to relay nuclei. During GHB-seizures [^{3}H]muscimol binding in the thalamus did not change. Likewise neither β2 nor GAD_{67} mRNA levels in relay nuclei or in nRT changed during GHB-seizures. These results suggest that the loss of neurosteroid modulation of GABA-R binding in thalamus during GHB-seizures and/or restoration of neurosteroid effect with the cessation of GHB-seizures is not influenced by GABA-R β2 subunits.

325.4

GABA$_A$ RECEPTOR SUBUNIT mRNA EXPRESSION IN SINGLE CULTURED HIPPOCAMPAL NEURONS. A.R. Brooks-Kayal, D.E. Pleasure* and H. Jin. Depts. of Pediatrics and Neurology, Univ of Pennsylvania School of Medicine and Children's Hospital of Philadelphia, Philadelphia, PA 19104.

GABA is the major inhibitory neurotransmitter in the vertebrate forebrain. Alterations in GABAergic transmission have been implicated in some forms of epilepsy, and developmental changes in GABA$_A$ receptor expression may play an important role in both normal development and certain developmental neurological disorders. The relative abundances of mRNAs encoding 12 different GABA$_A$ receptor subunits were examined in cultured embryonic rat hippocampal neurons using the technique of single-cell mRNA amplification. Hippocampal neurons from embryonic day 17 rat pups grown in dissociated neuronal culture were analyzed on days 2, 7, 14 and 28 *in vitro* (DIV2-28). To determine the variability of GABA$_A$ receptor subunit expression between neurons of similar age in culture, eight individual neurons were examined at DIV 28. Prominent expression of α2, α5, α6, β1 and γ2 mRNAs were detected, and the results demonstrate a high degree of consistency in levels of subunit expression between these same-age neurons. Preliminary studies examining a single neuron each at DIV 2, 7, and 14 suggest that levels of expression of several GABA$_A$ receptor subunit mRNAs undergo an increase in relative expression as cultured rat embryonic hippocampal neurons differentiate *in vitro*. To ascertain that GABA$_A$ receptor subunit expression changes in a consistent fashion during *in vitro* development, multiple neurons will be evaluated at each timepoint. (Supported by NIH 5P30HD28815 & McCabe Fund of UPa)

325.5

THE ANATOMICAL LOCALISATION OF THE mRNAs FOR THE α1- α6 SUBUNITS OF THE GABA-A RECEPTOR IN POST-MORTEM HUMAN BRAIN. DJS Sirinathsinghji*, M Rigby, RP Heavens, B LeBourdelles, P Wingrove, R McKernan, G Dawson, R Hill, I Ragan, JH Xuereb, and P Whiting. Merck Sharp & Dohme Research Laboratories, Neuroscience Research Centre, Harlow, Essex CM20 2QR, UK; Department of Psychiatry, University of Cambridge and MRC Brain Bank Laboratory, Cambridge CB2 2QR,UK

cDNAs encoding the α1- α6 subunits of the human GABA-A receptor have been isolated in our laboratories. These sequences were used to generate specific oligonucleotide probes (^{35}S-labelled) for the localisation of the mRNA transcripts in normal human brain. The α1 mRNA was widely expressed with high levels in frontal, occipital and temporal cortex, hippocampus, caudate, putamen and cerebellum (granule, molecular and Purkinje layers). The α2 and α4 mRNAs were also present in these areas but expression in the cerebellum was restricted to the Bergmann glia for α2 and to the granule cell layer for α4. The α3 mRNA was less widely distributed with expression mainly in the cortical areas and hippocampus. The α5 mRNA was strongly expressed in the frontal cortex, nucleus accumbens and medial temporal lobe (hippocampus, entorhinal, perirhinal and temporal cortex). The α6 mRNA was expressed exclusively in the granule cell layer of the cerebellum. None of these subunits were detectable in brain stem nuclei. Studies are in progress to map in greater detail the distribution of the above mRNAs as well as those for the β, γ and δ subunits in human brain. (Supported by Merck).

325.6

GABA$_A$ RECEPTOR SUBUNIT MESSENGER RNA DETECTION IN SUPERIOR CERVICAL GANGLIA. Z. F. Liu and D. R. Burt. Department of Pharmacology and Experimental Therapeutics, University of Maryland School of Medicine, Baltimore, MD 21201.

Gamma-aminobutyric acid (GABA), the major inhibitory neurotransmitter in the brain, mediates fast synaptic inhibition by opening the chloride channel intrinsic to the GABA$_A$ receptor. GABA$_A$ receptors are composed of subunits belonging to different families (α, β, γ, δ, ρ) according to degree of sequence homology. At present, the subunit composition of native GABA$_A$ receptors in the central nervous system is not known and there are relatively few reports of GABA$_A$ receptors in peripheral tissues, where the subunit composition may be simpler and easier to determine. Considerable functional data indicates the presence of GABA$_A$ receptors in superior cervical sympathetic ganglia (SCG). We studied GABA$_A$ receptor subunit mRNA levels in rat SCG, using subunit-specific oligonucleotide primers and reverse transcriptase polymerase chain reaction (RT-PCR) amplification. Qualitative data indicated that, of those tested, GABA$_A$ receptor α4, α6, β1, β3, and γ2 subunit mRNAs were found in rat SCG; only the β2 subunit was negative. This result differs from the adrenal medulla, which reportedly contains β2 subunit mRNA as well as β1 and β3 (Ymer et al., 1989, EMBO J. 8:1665). Quantitative RT-PCR was performed to determine actual GABA$_A$ receptor subunit mRNA levels in rat SCG by using mouse GABA$_A$ receptor subunit clones as internal standards. We started quantitative measurements with the α4 subunit, reportedly very low in brain. The α4 mRNA levels in SCG correspond to about 2000 α4 mRNA molecules per SCG (or only about one molecule per twenty principal neurons). The very low messenger level of the α4 subunit may correspond to "leakage" of transcription in the rat SCG. This shows the importance of quantitative measurements in interpreting positive PCR results. (Supported in part by NIH grant HD16596 to DRB)

325.7

AUTORADIOGRAPHIC LOCALIZATION OF α5-SUBUNIT CONTAINING GABA$_A$ RECEPTORS. S.M. Cook, K. Quirk, R. McKernan and J.R. Atack* Merck Sharp & Dohme Research Labs., Neuroscience Research Centre, Harlow, CM20 2QR, U.K.

The pharmacology of the benzodiazepine binding site of the GABA$_A$ receptor is a function of the α and γ-subunits contained within the receptor. Non-selective radioligands, such as [^{3}H]Ro 15-1788, label a combination of α1, α2, α3 and α5 subunit-containing receptors. Recently, however, FG 8094 has been identified as a benzodiazepine possessing greater than 50-fold selectivity for α5- over α1-, α2- or α3-containing receptors. In the present study, [^{3}H]FG 8094, which was found to have an affinity for post-mortem human cortical α5-containing receptors of 1.5 nM, has been used as a radioligand to identify α5-containing receptors in sections of human and monkey brain. For autoradiographic studies, 30 μM sections of human and monkey brain were incubated with 1 nM [^{3}H[FG 8094 (in the presence of 1 uM zolpidem to block α1-, α2- and α3-containing receptors) for 1 hr. at room temp. with non-specfic binding defined using 1 μM flunitrazepam. Specific binding was greater than 95% of total binding and showed marked regional variations in the distribution of binding sites. Thus, there was negligible binding in cerebellum whereas binding was most pronounced in the hippocampus and lower layers of the cortex, particularly the temporal cortex. Moreover, there were distinct variations within the hippocampus with, for example, a particularly high abundance of α5 binding sites in the dentate gyrus whereas there was low expression of binding sites in the subiculum.

MSD Ltd.

325.8

DISTRIBUTION IN THE RABBIT CENTRAL NERVOUS SYSTEM OF A mRNA ENCODING A PORTION OF THE α1 GABA$_A$ RECEPTOR SUBUNIT. Yunxing Wu[1], Adam A. Book[2], E. Hazel Murphy[2] and Dennis R.Grayson[1,2]. Neuroscience Research Center and Department of Psychiatry[1,2], Medical College of Pennsylvania and Hahnemann University (MCPHU), Pittsburgh, PA 15212 and Department of Anatomy and Neurobiology[2], MCPHU, Philadelphia, PA 19129.

A rabbit subfragment of the α1 receptor subunit was generated using primers corresponding to the intracellular loop region of the rat sequence (positions 1091 bp to 1321 bp). We used RT-PCR under reduced stringency annealing conditions (55°C) to isolate a homologous region of the rabbit mRNA. The amplification product was sequenced and over the 230 bp subclone, the rat and rabbit DNA sequences were 95% identical. However, all sequence substitutions were in third bp positions with respect to the coding frame resulting in a 100% amino acid concordance. This subclone was used to assess the distribution of the α1 mRNA in sections prepared from adult and developing rabbit brain using *in situ* hybridization with an ^{35}S-cRNA labeled riboprobe. Hybridized brain sections were exposed to film, emulsion coated and counterstained. Both film exposure optical density and silver grain distribution were analyzed to determine localization. Analysis of coronal sections showed that at P20, the α1 receptor subunit mRNA was most abundantly expressed in large neurons at the border of the stratum oriens and the hippocampal pyramidal cell layers and within the polymorphic layer of the olfactory tubercle. α1 mRNA was moderately abundant in layers II and V/VI of the frontal cortex, layers II and V of the anterior cingulate cortex (ACC), pyramidal cells of the CA1 and CA2 hippocampal fields, pyramidal layer of the olfactory tubercle, dentate gyrus granule cells, medial septum and diagonal band. Silver grain density was lower in layer IV of the neocortex, layers III and VI of the ACC, hippocampal CA3 field, lateral septum, and caudate-putamen. Analysis of adult sections indicates that many of these patterns are maintained once the brain is fully developed. Supported by NIH grants DA06871 P01 to E.H.M, NIH Training Grant NS07287, and K04 NS01647 to D.R.G.

325.9

Temporal and Laminar-Specific Expression of 10 GABA$_A$ Receptor Subunit mRNAs in Fetal Monkey Visual and Sensorimotor Cortex. M. M. Huntsman*, T. Tran and E. G. Jones, Dept. of Anatomy and Neurobiology, University of California, Irvine, CA 92717.

The GABA$_A$ receptor is the primary target for fast inhibitory neurotransmission in the adult mammalian brain and functions as a ligand-gated chloride-ion selective channel. Thirteen subunit genes have been cloned and appear to co-exist in preferred combinations or subtypes consisting of at least one subunit from each of the three major classes, known as alpha, beta and gamma. The identified subunit genes are subclass variants of these classes grouped by their sequence identity and likely assembling into heteromeric combinations of five where it is presumed the subtype determines the physiological and pharmacological functions of GABA$_A$ receptors in vivo. In the adult monkey cerebral cortex, the major receptor subtype likely contains the α1, β2 and γ2 subunits as the transcripts which encode these subunits are expressed at extremely high levels that exceed levels observed for other subunits like α2, α3, α4, α5, β1, β3 and γ1 transcripts. Subcloned cDNAs for the α1, α2, α3, α4, α5, β1, β2, β3,γ1 and γ2 subunits were transcribed and localized in fetal and neonatal monkey visual and sensorimotor cortex in order to determine the likely predominant GABA$_A$ receptor subtype in cortical development. In situ hybridization histochemistry of these labeled transcripts identify a completely different aggregation of potential GABA$_A$ receptor subtypes in comparison with adults, with dramatic increases in levels, especially for α2, α3, α5, β1, β3 and γ2 transcripts. The laminar specification of α5 transcripts reaches peak levels at embryonic day 137 and resolves the unique distribution pattern of adult animals after postnatal day 125. The presence of subunit mRNAs in regions of the developing cortex probably before the likely onset of inhibition raises the possibility that specific GABA$_A$ receptor subtypes may be involved in synaptogenesis and the establishment of topographic maps in pre- and early post-natal development. Supported by NIH grant 5-T32-NS07357.

325.10

DISTRIBUTION OF THE GABA$_A$ RECEPTOR COMPLEX, BETA SUBUNIT IN THE ENCEPHALON OF FROG (*Rana pipiens*) **M. I. Aller[1], S. Jeneonis[2] K. V. Fite[2] and A. Fernández-López[1]*, [1]Dpt. Biologia Celular y Anatomia. Universidad de León, León, 24071, Spain. [2]Dpt. Psychology. University of Massachusetts at Amherst. MA 01003, USA.**

This work reports an immunocytochemical, semi-quantitative distribution of the beta subunit GABA$_A$ receptor complex throughout the encephalon of frog (*Rana pipiens*) by using a specific monoclonal antibody against this subunit (Boehringer, clone bd 17). Several different fixatives (4% paraformaldehyde, 4% paraformadehyde-0.1% glutaraldehyde and 4% paraformaldehyde- 0.1M L-lysine- 0.01M sodium m-periodate) were used. Results obtained with all fixatives were consistent both with regard to the labelled structures and their relative densities of labelling.

The highest labeling with this antibody was found in striatum and cerebellum and was defined as 100%. Very high densities of labeling (>75%) were found in the olfactory tubercle, lateral pallium, torus semicircularis and some nuclei of the medulla. Regions of the thalamus, some tegmental nuclei and some strata of tectum opticum show intermediate levels of labeling (50-75%) while structures as septum, medial pallium or nucleus isthmi appeared to have little immumoreactivity for this antibody.

These data are consistent with those obtained from autoradiographic binding of 3H flunitrazepam receptor complex in frog. These findings indicate that this monoclonal antibody raised against the mammalian beta subunit of the GABA$_A$ complex permits characterization of this subunit in the frog.

Acknowledgement: This work has been financed by DGICYT P92-883. MI Aller is a fellowship of the University of Leon.

325.11

THE GABA$_A$ RECEPTOR SUBUNITS IN THE MAMMALIAN RETINA. A.K. Mehta*, A. Gutiérrez, Z.U. Khan, C.P. Miralles and A.L. De Blas, Div. Mol. Biol. and Biochem., Univ. Missouri, Kansas City, MO 64110.

Subunit-specific antibodies (α_{1-6}, β_{1-3} and γ_2) were used to immunoprecipitate detergent-solubilized [^{3}H]muscimol (^{3}H-MUS) and [^{3}H]flunitrazepam (^{3}H-FNZ) binding sites from bovine retina. The ^{3}H-MUS and ^{3}H-FNZ immunoprecipitation values obtained with each anti-subunit antibody were α_1 (56% and 58%), α_2 (24% and 37%), α_3 (28% and 15%), α_4 (13% and 0%), α_5 (16% and 3%), α_6 (8% and 0%), β_1 (9% and 1%), β_2 (75% and 49%), β_3 (19% and 20%) and γ_2 (76% and 60%) respectively. Therefore I) α_1, β_2 and γ_2 are the most abundant GABA$_A$ receptor subunits in retina and II) a relatively high proportion of GABA$_A$ receptors have benzodiazepine binding site(s). The ^{3}H-FNZ immunoprecipitation values obtained with the various anti-subunit antibodies were similar to the diazepam-sensitive (DS) [^{3}H]Ro15-4513 immunoprecipitation values. In addition, the bovine retina has about 13% of the [^{3}H]Ro15-4513 binding sites that are diazepam-insensitive (DI). However, the DI binding sites from bovine retina could not be precipitated by either anti-α_6 or anti-α_4 antibodies, which contrasts with the high proportion of cerebellar DI binding sites that could be precipitated with anti-α_6. The cellular localization of some of these subunits has been revealed by immunocytochemistry in rat retina. Supported by grant NS17708 from NINDS.

325.12

THE α_4 SUBUNIT OF THE GABA$_A$ RECEPTORS FROM RAT BRAIN AND RETINA. Z.U. Khan, A. Gutiérrez, A.K. Mehta, C.P. Miralles and A.L. De Blas,* Div. Mol. Biol. and Biochem. Univ. Missouri, Kansas City, MO 64110.

A novel anti-α_4 antibody has been used for the purification and characterization of the α_4-containing GABA$_A$ receptors in the rat brain and for studying the immunocytochemical distribution of the α_4 subunit peptide in rat brain and retina. The anti-α_4 antibody recognized a 66 KDa peptide in brain membranes and immunoprecipitated 10-28% of the brain GABA$_A$ receptors in various brain regions as determined by [^{3}H]muscimol binding. The highest immunoprecipitation values were obtained in the thalamus and the lowest in the cerebellum. Surprisingly, the receptors immunoprecipitated by anti-α_4 showed little or no diazepam-insensitive or diazepam-sensitive [^{3}H]Ro15-4513 binding sites in any brain region. In the cerebellum, where 25% of the [^{3}H]Ro15-4513 binding is diazepam-insensitive, much of the latter was immunoprecipitated by an anti-α_4 antibody but not by the anti-α_4 antibody. Immunoblots of immunoaffinity-purified GABA$_A$ receptors from the cerebral cortex on immobilized anti-α_4 revealed molecular colocalization of α_4 and γ_2. However, the absence of significant benzodiazepine binding in these GABA$_A$ receptors suggests that the assembly of the α_4 and γ_2 subunits in the cerebral cortex and in other brain regions is such that they do not normally form diazepam-insensitive [^{3}H]Ro15-4513 binding sites. This result contrasts with the presence of diazepam-insensitive [^{3}H]Ro15-4513 binding sites in the GABA$_A$ receptors expressed in heterologous systems resulting from the combination of α_4, γ_2 and β_2 subunits. Immunocytochemistry has revealed the abundance of α_4 peptide immunoreactivity in the thalamus and dentate gyrus (mainly in the hilar neurons and the inner 1/3 of the granule cell layer). The α_4 immunoreactivity is also present in the external plexiform layer of the olfactory bulb and in all layers of the neocortex and pyriform cortex. In the retina, α_4 is concentrated on ganglion cells (including some giant ganglion cells), the inner plexiform layer and to a lesser extent in the outer plexiform layer. Supported by grant NS17708 from NINDS.

GABA$_A$ RECEPTORS: RECOMBINANT STUDIES AND MOLECULAR MAPPING

326.1

THE GABA$_A$ RECEPTOR α_6 SUBUNIT GENE IS TIGHTLY LINKED TO THE α_1-γ_2 SUBUNIT CLUSTER IN MOUSE CHROMOSOME 11. K.M. Garrett*, A. Rotter, J. Gan and T.W. Seale, Departments of Physiology and Pediatrics, University of Oklahoma Health Sciences Center, Oklahoma City, OK 73190.

The GABA$_A$ receptor is a ligand-gated channel and is the site of action for several sedative-hypnotic drug classes. This receptor is a multimeric complex composed of varying combinations of α, β, γ and δ subunits. The subunits are found in clusters in both the human and mouse genome. We have identified RFLPs for the α_1 (Hind III) and α_6 (BstE II) subunit genes in C57BL/6J and DBA/2J inbred mice. We have determined that these two genes are tightly linked on mouse chromosome 11 by analyzing the strain distribution patterns of the two RFLPs and microsatellite markers flanking these gen$\checkmark$ in 26 BXD recombinant inbred lines. These results further demonstrate clustering of the GABA$_A$ receptor subunit genes on mouse chromosomes and the synteny between the mouse and human genome. The physiological and pharmacological functions of the GABA$_A$ receptor α_6 subunit are not well understood. Mapping of the murine *Gabra6* gene and identification of an RFLP for the α_6 subunit in the mouse provides a means for quantitative trait loci analysis for probing behavioral functions of the α_6 subunit. Supported by grants from Presbyterian Health Foundation to KMG and TWS, NIMH (MH49791) to TWS and NINDS (NS18089) to AR.

326.2

GABA$_A$ RECEPTOR DESENSITISATION: THE ROLE OF THE γ2 SUBUNIT AND ITS PHYSIOLOGICAL SIGNIFICANCE. C. Dominguez-Perrot*, P. Feltz, M. O. Poulter, Laboratoire de physiologie générale, Université Louis Pasteur, 67084 Strasbourg, France.

To examine the role of the γ2 subunit in GABA$_A$ receptor desensitisation, the relaxation of GABA-induced whole cell currents was compared for two different recombinant GABA$_A$ receptors ($\alpha1\beta3$ and $\alpha1\beta3\gamma2$). The $\alpha1\beta3\gamma2$ receptor desensitised faster ($\tau1$=41.6±8.3ms, $\tau2$=2.4±0.6s; two exponential model at EC100) than $\alpha1\beta3$ ($\tau1$=145±12ms, $\tau2$=6.3±2.1s). For both receptors, the Hill slopes ≥ 1, implying two steps in the activation. For $\alpha1\beta3$ receptor, the fast desensitisation rate of GABA current were unaltered in range from EC50 to EC100 (10-20μM) and the slow desensitisation was observed above ~EC20 whose rate was dependant on GABA concentration (maximum at ~EC80). Voltage-dependency of the desensitisation rate was only seen for $\alpha1\beta3\gamma2$ receptor ($\tau2$ increased by ~15-20 fold when the holding potential was elevated from -60 to +40 mV). This indicates that the γ2 subunit is responsible for the voltage-dependency of GABA$_A$ receptor desensitisation. Recovery from desensitisation was also biphasic: the first phase was faster for $\alpha1\beta3\gamma2$ receptor, the rate of the second phase being the same. Isoguvacine (EC50≈10μM) induced biphasic relaxation and taurine (EC50≈7mM) induced monophasic relaxations for both. The computer simulation of receptor state kinetics showed that γ2 subunit prolongs the time course of a synaptic potential by fastening the desensitisation. The physiological role of the γ2 subunit might be to prolong the time course of synaptic current through modifying desensitisation kinetics and its voltage-dependency.
Supported by MRT grant.

326.3

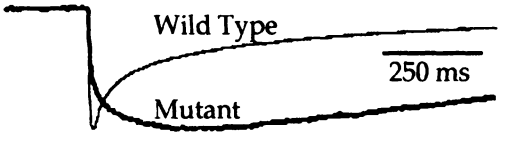
COMPARISON OF THE DECAY KINETICS OF RECOMBINANT GABA$_A$ RECEPTOR CURRENTS FOLLOWING TRANSIENT AGONIST APPLICATION
K. F. Haas*[1], E. C. Burgard[1] and R. L. Macdonald[1,2]. Departments of Neurology[1] and Physiology[2], University of Michigan, Ann Arbor, MI 48104-1687.

Synaptic GABA$_A$ receptor (GABAR) currents likely reflect a response to high GABA concentrations available in the synaptic cleft for at most a few msecs, yet IPSCs decay over a much longer period of time. Studies from native GABARs now suggest that entry into and out of multiple desensitized states can explain the prolongation of current following brief GABA application. We have addressed the mechanisms underlying desensitization with rapid GABA application to outside-out patches containing recombinant GABARs of known subunit composition. cDNAs encoding rat αX (X = 1,5,or 6), β3, and γ2L subunits were individually subcloned into the plasmid vector pCMVNeo. L929 fibroblasts were transiently transfected with various combinations (1:1:1) of each, resulting in expression of functional $\alpha X\beta3\gamma2L$ GABARs. Outside-out patch-clamp recordings (E_{Cl} = 0 mV) were obtained from positively transfected cells. GABA (1 mM) was applied to the patch using a piezo-driven application system. Prolonged (200 msec) GABA application to patches (Vm = -75 mV) produced multiple-channel ensemble currents that exhibited both rapid (τ = 5-20 msec) and prolonged (τ = 50-200 msec) components of desensitization. Brief (5 msec) applications produced ensemble currents that deactivated with two time constants similar to those of desensitization. Single-channel responses to brief applications were characterized by instantaneous channel opening followed by multiple bursts of openings lasting 20 - 100 msec. Although different GABAR isoforms exhibited characteristic burst patterns, all isoforms eventually entered prolonged (50-100 msec) closed (desensitized) states prior to reopening. These data suggest that similar to native receptors, recombinant GABARs enter desensitized states which prolong GABAR current decay following brief agonist applications. R01-NS33300 (RLM).

326.4

A POINT MUTATION OF THE GABA$_A$ RECEPTOR DESTABILIZES ITS CLOSED AND DESENSITIZED STATES.
I. D. Clements*, L. Tierney, B. Birnir, P. Pillai, P. Gage and G. Cox
Australian National University, Canberra, ACT 0200, Australia.

The hypothetical structure of the GABA$_A$ receptor has the M2 membrane spanning region lining the channel pore. Consistent with its critical location, the M2 region is highly conserved across the ACh receptor family, and a leucine residue near the center of this region is 100% conserved. In the ACh channel, substituting this leucine with a more hydrophilic residue dramatically slows and decreases desensitization (Revah et al., Nature 353:846, 1991). In the present study, the homologous leucine residue in the GABA$_A$ receptor alpha subunit was substituted with a hydrophilic threonine. Alpha and beta subunits were co-expressed in Sf9 cells and formed functional heteromeric GABA$_A$ receptors. Drugs were applied using a fast solution exchange system. A step into GABA (10 mM) produced a strongly desensitizing response from wild type, but not from mutant channels. Intriguingly, the mutant's slow activation rate was very similar to the fast desensitization rate of the wild-type response. Mutant channels exhibited a tonic leak current in the absence of GABA that was selectively blocked by picrotoxin (500 μM). These finding suggest that the mutation enables the channel to open in the absence of agonist, and converts the desensitized state into a conducting state. The highly conserved leucine residue plays a crucial role in closing the GABA$_A$ channel. (Dept. of Health, Australia)



326.5

POINT MUTATIONS OF THE GABA$_A$ RECEPTOR AFFECTING MODULATION BY AND BINDING AFFINITIES OF BENZODIAZEPINE SITE LIGANDS. A. Buhr, R. Baur, P. Malherbe* and E. Sigel. Dept. of Pharmacology, Univ. Bern, 3010 Bern, Switzerland: *Pharma Divison, Preclinical Research, F. Hoffmann-La Roche Ltd. 4002 Basel, Switzerland.

Mutants of the GABA$_A$ receptor have been described in the α and γ subunits which increase modulation by diazepam (Buhr *et al.*, 1996, Mol. Pharmacol. 49, in press). Rat wild type or mutated α1, β2, and γ2S subunits were coexpressed in Xenopus oocytes and investigated with electrophysiological techniques. The response to zolpidem was increased in receptors containing the αT206A mutation but was nearly absent in channels containing γF77L. Competition experiments indicated that Ro 15-1788 similarly to diazepam still interacts with the mutated γ subunit, while zolpidem lost this ability. To quantify these data, radioligand binding experiments were performed using membranes of transiently transfected HEK 293 cells. Receptors containing γF77L displayed an 8 to 28-fold reduced affinity for diazepam and [^{3}H]Ro 15-1788 and a 130-, 290- and 920-fold reduced affinity for CI 218872, zolpidem and DMCM, respectively. αT206A resulted in a 4 to 5-fold decreased affinity for diazepam and zolpidem. Interestingly, for the same mutant receptors the affinities for CI 218872 and DMCM were 16- and 5-fold increased. Results on double mutants will also be discussed. The residues αT206 and γF77 are directly homologous to residues involved in GABA binding. Therefore, our results support the postulate that the benzodiazepine site is located in a homologous position to the agonist binding site. Supported by Grant 31-37192.93 from the Swiss National Science Foundation.

326.6

GABA$_A$ RECEPTOR HETEROGENEITY IN THE IMR-32 CELL LINE. D. W. Sapp* and H. H. Yeh. Dept. Pharmacology & Program in Neuroscience., Univ. of Conn. Health Center, Farmington, CT 06030.

An analysis of GABA$_A$ receptors expressed in a variety of neuronal and non-neuronal cell lines may provide insight into the relationship between functional assembly of the receptor and subunit composition. Here, we employed whole-cell patch clamp electrophysiology to examine the modulatory effects of zinc (Zn) and diazepam (DZ) on GABA responses elicited in a human neuroblastoma cell line, IMR-32. Preliminary PCR-based expression profiling of GABA$_A$ receptor subunit mRNAs from IMR-32 cultures revealed prominent expression of the α3, β3, γ3 subunit transcripts with comparatively low levels of α5 and γ2. Electrophysiologically, 3 profiles of GABA responses emerged from recordings of >70 IMR-32 cells. <20% displayed a GABA response that was potentiated by DZ but was insensitive to Zn, similar to that found in WSS-1 cells stably transfected with the α1β1γ2 subunit combination. In the remaining cases, an approximately equal proportion of cells showed GABA responses that either were completely blocked by Zn and resistant to potentiation by DZ or were "mixed", with Zn partially blocking the GABA response that could be shown to be potentiated by DZ.

That clear-cut reciprocal sensitivity to DZ and Zn can be observed in individual IMR-32 cells suggests heterogeneity in functional GABA$_A$ receptors within this cell line and that some IMR-32 cells may express a γ-less GABA$_A$ receptor. Other IMR-32 cells may express both γ-containing and γ-less receptors, as reflected by a "mixed" sensitivity to Zn and DZ. Ongoing studies are addressing these issues using combined patch clamp recording and single-cell RT-PCR.

Supported by PHS grants NS24830 and AA08361(HHY) and T32 AA07290(DWS)

326.7

FUNCTIONAL CHARACTERISTICS AND ALLOSTERIC MODULATION OF HUMAN γ-AMINOBUTYRIC ACID TYPE A RECEPTORS CONTAINING THE α4 SUBUNIT. K.A. Wafford, S.A. Thompson, D. Thomas, S. Pollack* & P.J. Whiting, Merck Sharp & Dohme Research Laboratories, Neuroscience Research Centre, Terlings Park, Eastwick Road, Harlow, Essex, CM20 2QR.

Recombinant human α4β1γ2s and α4β3γ2s GABA$_A$ receptors were expressed in *Xenopus* oocytes and the pharmacology of these receptors investigated using the two-electrode voltage clamp method. The GABA EC$_{50}$ for α4β1γ2 was similar to α1β1γ2 (33.1μM), but slightly lower than α6β1γ2 (14.4 μM). The efficacy of the GABA partial agonists THIP and P4S were markedly reduced on α4β1γ2 compared to α1β1γ2 and α6β1γ2. GABA responses on human α4β1γ2 receptors were sensitive to the competitive antagonists, bicuculline (100μM) and SR95531 (1μM), and the non-competitive antagonist picrotoxin (100μM). Several compounds, which act via the benzodiazepine site, were examined at a concentration of 1μM. The effects of these compounds on α4β1γ2 when compared to α1β1γ2 and α6β1γ2 revealed a unique profile, showing most similarity to α6β1γ2. Similar to α1 and α6 containing receptors, α4β1γ2 receptors were potentiated by the steroid, 5α-pregnan-3α-ol-20-one, (367 ± 26%). Marked potentiation of the GABA response (706 ± 82%) was observed with pentobarbital (100 μM). This compared to 288 ± 32% on α1β1γ2s and 719 ± 52% on α6β1γ2. The direct activation by pentobarbital on α4β1γ2s receptors was extremely small (6% of maximum GABA at 1mM) compared to 31% and 147% on α1β1γ2 and α6β1γ2 respectively. α4β1γ2 also showed no direct activation by propofol, unlike the other combinations studied. The receptor was potentiated by propofol to a greater degree on α4β1γ2 and α1β1γ2 than α6β1γ2. Furosemide, which shows selectivity for the α6β3γ2 subtype, also showed some selectivity for the α4β1γ2 subtype over α1β3γ2. These experiments reveal a unique pharmacology for α4 containing receptors with similarities to both α6 and α1. Funded by M.S.D. Research Laboratories.

326.8

LOCATING THE ANION-SELECTIVITY FILTER IN THE GABA$_A$ RECEPTOR CHANNEL. M. Xu, J. Horenstein, and M. H. Akabas*. Ctr for Mol. Recog., Columbia Univ.., NY, NY 10032.

We previously identified 9 residues in the M2 segment of the α_1 subunit that are exposed in the channel lumen of the GABA$_A$ receptor using the scanning-cysteine-accessibility method (J. Gen. Physiol. 107:195-205). In this anion-selective channel, cationic sulfhydryl reagents, applied extracellularly, reacted with the mutant α_1T261C but not with the more cytoplasmic mutant α_1V257C, whereas, anionic reagents reacted with both mutants. In the gene superfamily of ligand-gated ion channels, there is an alanine at the position aligned with the intermediate ring of charge (α_1A254) in the anion-selective channels and an anionic residue in the cation-selective channels. To probe the role of the charge at position 254 on the ability of cationic sulfhydryl reagents to penetrate into the channel, we made a double mutant α_1A254E/V257C. The cationic reagent, MTSET, reacts with this mutant but the rate at which the anionic reagent, MTSES, reacts is markedly reduced. This suggests that the residue at the intermediate ring position may be an important determinant of charge selectivity. Support NIH NS30808, Klingenstein Foundation., AHA, NYHA.

326.9

IDENTIFICATION A SINGLE AMINO ACID ON THE GAMMA SUBUNIT OF THE GABA-A RECEPTOR REQUIRED FOR HIGH AFFINITY BINDING OF [3H]Ro15-1788. P.B.Wingrove, K.A.Wafford and P.J.Whiting*, Merck Sharp and Dohme Research Laboratories, Neuroscience Research Centre, Terlings Park, Eastwick Road , Harlow, Essex, CM20 2QR.

It is known that both an α and a γ subunit are required for the formation of a benzodiazepine binding site on the GABA-A receptor; the pharmacology being dependent on the subunit isoforms present. When expressed transiently in HEK293 cells in combination with α1 and β1 subunits, GABA-A receptors containing a γ2 (or γ3) subunit have a much higher affinity for [3H]Ro15-1788 than receptors containing γ1. This criterion was used to assay chimeric γ1/γ2 subunits and subsequently γ1 subunit mutants to identify a single amino acid, γ2Phe77, which conferred a high affinity binding site for [3H]Ro15-1788.

Saturation curves for the binding of [3H]Ro15-1788 were constructed for the recombinant receptors α1β1γ2 and α1β1γ1ΔIle79Phe and the Kd values calculated by Scatchard analysis to be 0.91±0.22 nM and 3.13±0.20 nM respectively (mean ± s.e.mean). Displacement of this [3H]Ro15-1788 binding by β-CCM and CL218-872 indicated that they also have a high affinity for α1β1γ1ΔIle79Phe. The affinities for flunitrazepam and zolpidem, however, were unaffected by this mutation.

Thus, Phe77 of γ2 (numbering of mature peptide) is necessary for high affinity binding of [3H]Ro15-1788, CL218-872 and β-CCM to the GABA-A receptor but is either not necessary or not sufficient for binding flunitrazepam and zolpidem. It is of interest to note that Phe77 is at a homologous position to Phe92 of the α1 subunit which has been implicated as a critical residue at the GABA binding site suggesting some structural homology between binding sites for different ligands.

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326.10

MEASUREMENT OF FUNCTIONAL EFFICACY OF COMPOUNDS ACTING AT THE BENZODIAZEPINE SITE OF α1 SUBUNIT-CONTAINING GABA$_A$ RECEPTORS STABLY EXPRESSED IN LTK CELLS, USING THE MICROPHYSIOMETER. A.J. Smith, R. McKernan* & J.R Atack. Merck, Sharp and Dohme Research Laboratories, Terlings Park, Eastwick Rd., Harlow, UK.

The Cytosensor Microphysiometer measures changes in extracellular acidification rates resulting from alterations in energy demand made on cells in response to receptor or ion channel activation or from alterations in Na^+/H^+ exchange across the membrane during homeostatic control of intracellular pH.

Chloride conductance through GABA$_A$ receptors can be modulated by compounds acting at the benzodiazepine site. Cells expressing receptors with subunit composition α1β3γ2 were seeded at 10^5 cells per cup, with 4-5 days induction and experiments conducted in DMEM containing 44mM NaCl, pH 7.4. Responses to GABA, which were specifically blocked by bicuculline and picrotoxin and were maximal at 30μM, were characterized by an initial alkalinisation followed by a peak of acidification, typically from 100% up to a maximum of 140-150% after normalization with respect to baseline. The alkalinisation was more evident at low agonist doses and became progressively masked at higher concentrations. Agonist time-courses were performed to optimise the acidification response. Increasing agonist exposure times from 15 to 25 secs prior to rate determination eliminated the initial alkalinisation and maximised the acidification component.

From the GABA dose-response curve, a dose of GABA was selected which produced an increase equivalent to 20% of the maximum response (10μM; 11±0.7% increase over baseline, n=13) and the modulatory effects of benzodiazepine agonists were examined. The benzodiazepine full agonist flunitrazepam (1μM) significantly potentiated the response to 10μM GABA by 125±11% (n=12). Benzodiazepine partial agonists with varying efficacy could also be detected. The weak partial agonist bretazenil, for example, produced a significant potentiation (32±4%) of the response to 10μM GABA. In addition, the benzodiazepine full inverse agonist DMCM (10μM) inhibitied the response to 20μM GABA up to a maximum of 50%. In summary, the Microphysiometer can be used to determine functional efficacy at the benzodiazepine site of the GABA$_A$ receptor for both agonists and inverse agonists and the degree of resolution is sufficient for detection of weak partial agonists.

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326.11

INFLUENCE OF THE α SUBUNIT ON INTERACTION OF PICROTOXIN-SITE LIGANDS WITH RECOMBINANT GABA$_A$ RECEPTORS. G.H. Dillon* and Cathy Bell-Horner. Dept. of Pharmacology, Univ. of North Texas Health Science Center at Fort Worth, Fort Worth, TX 76107.

We have defined previously the kinetic interactions of picrotoxin-site ligands with the α1β2γ2 configuration of recombinant GABA$_A$ receptors, and the influence of GABA on these interactions. Here we report the influence of the α subunit on the interactions of picrotoxin (PX) and U-93631 [4-dimethyl-3-t-butylcarboxyl-4,5-dihydo (1,5-a) quinoxaline], a novel ligand to the picrotoxin site, with recombinant GABA$_A$ receptors. Whole-cell patch clamp recordings were obtained from HEK293 cells stably expressing α6β2γ2 or α3β2γ2 receptors. Concentration-response curves yielded EC_{50} and Hill coefficient values of 1.6 μM and 1.5 respectively, for α6β2γ2 receptors, and 20 μM and 1.3, respectively, for α3β2γ2 receptors. EC_{25} concentrations of GABA were applied to α6β2γ2 or α3β2γ2 configurations in the presence of varying [U-93631] or [PX]. The IC_{50} for current inhibition by U-93631 on α6β2γ2 receptors in the absence of GABA, determined from its effect on initial peak current, was 42 μM. The presence of GABA for 10 sec. shifted the IC_{50} for U-93631 approximately 10-fold (to 3.5 μM). GABA similarly influenced the interaction of PX with the α6β2γ2 receptor (IC_{50} shifted from 99 to 8.5 μM). These results are comparable to those seen in α1β2γ2 receptors. In α3β2γ2 receptors, shifts in IC_{50} values due to GABA were significantly smaller (2-fold) for both U-93631 and PX. This effect was due to a more marked inhibition of initial peak current by both compounds, and is at least partly attributable to slower GABA binding/channel gating in the α3β2γ2 configuration. This may permit faster U-93631 and PX binding, relative to GABA binding, in α3-containing receptors, compared to α1- or α6-containing receptors. (Support: NIH R29 ES07904 and TX Advanced Research Program # 009768-027).

326.12

DO GABA$_A$ RECEPTORS IN WSS-1 CELLS USE AN HEK-293 β3 SUBUNIT? P. A. Davies*, E. Hoffmann‡, H. J. Carlisle, R. F. Tyndale‡ and T.G. Hales. Dept. Anesthesiology, UCLA. LA, CA 90025 and ‡Addiction Research Foundation and Dept. Pharmacology, University of Toronto, Ontario M5S1A8, Canada.

WSS-1 cells, produced by transfecting human embryonic kidney (HEK) cells with rat GABA$_A$ receptor α1, β2 and γ2 subunit cDNAs, bind [^{3}H]flunitrazepam (Wong *et al.*, Mol. Pharmacol., 42, 996, 1992). However, β2 mRNA is undetectable in Northern and Southern blots. This implies that α1 and γ2 subunits combine to form receptors in WSS-1 cells. However, HEK cells transiently transfected with α1 and γ2 cDNAs do not have functional GABA$_A$ receptors. We used patch-clamp and RT-PCR analyses to study the pharmacology and subunit composition of GABA$_A$ receptors in WSS-1 cells.

GABA activated currents recorded from WSS-1 cells reversed at the Cl^- equilibrium potential. The anesthetics propofol (3μM), and pentobarbital (100μM), the anxiolytic diazepam (1μM) and the neurosteroid 5α-pregnan-3α-ol-20-one (100nM) all potentiated submaximal GABA-activated (100μM) currents by 310% (n=3), 278% (n=2), 296% (n=7) and 247% (n=7) of control, respectively. GABA-evoked currents were inhibited to 8% (n=2) of control by picrotoxin (10μM). Interestingly, the β2/3 subunit selective anticonvulsant loreclezole (10μM) also transiently potentiated GABA-evoked currents by 255% (n=6) of control. In the absence of GABA, both propofol (100 μM) and loreclezole (100 μM) activated currents in WSS-1 cells. RT-PCR analysis revealed that WSS-1 cells have both α1 and γ2 mRNAs, HEK cells have neither. The β2 RT-PCR product was barely detectable in WSS-1 cells only, and was smaller than the expected band size. However, both WSS-1 and untransfected HEK cells had detectable levels of human β3 mRNA.

Our data suggest the presence of human β3 and/or rat β2 subunits in GABA$_A$ receptors of WSS-1 cells. Antisense knock out of native HEK β3 subunit may identify its role, if any, in the function of these receptors. (Supported by NIH grant GM48456).

326.13

EXPRESSION OF ENDOGENOUS GABA TYPE A RECEPTOR IN HEK293 CELLS. S. Ueno*[a,c], C. Zorumski[b], J. Bracamontes[a] and J.H. Steinbach[a]. [a]Dept. Anesthesiology and [b]Dept. Psychiatry, Washington Univ. Sch. of Med., St. Louis, MO 63110. [c]Present address, Div. of Pharmacol. National Institute of Health Sciences,Tokyo, Japan 158.

The human embryonic kidney cell line (HEK293) has been utilized for the expression studies of GABA-A receptors. We have compared the properties of GABA-A receptor subunits expressed in HEK293 and quail fibroblast (QT6) cell lines. cDNAs of GABA receptor subunits were transfected and expressed in the HEK293 and QT6 cells with the expression vector pcDNA3 (Invitrogen). Recordings of GABA-activated responses were made using standard whole cell methods, 24-72hr. after transfection. The properties of the GABA-elicited responses in transfected HEK293 cells differ from expectations from previous work. In addition, some untransfected HEK 293 cells respond to applications of GABA and transfection of cells with α1 or γ2L subunits alone can result in the expression of GABA-elicited currents. Further, expression of a mutated β2 subunit (β2(Y205S)) in combination with α1 and γ2L results in cells showing appreciable responses to GABA. Finally, transfection of HEK293 cells with α1 plus γ2L subunits results in responses to GABA which are potentiated by the drug loreclezole, suggesting that a subunit resembling the β2 or β3 subunit had assembled with the α1γ2L subunits. On the contrary, when QT6 cells are used as the expression system, no responses are seen in untransfected cells, or in cells transfected with α1 or γ2L subunits alone, or with α1γ2L subunits. In addition, no response to GABA is seen from QT6 cells transfected with α1β2(Y205S)γ2L subunits. These observations indicate that caution should be taken in interpreting the results of studies of the properties of GABA-A receptors transfected in HEK293 cells, most likely because HEK293 cells express low levels of endogenous GABA-A subunits. Grant NIH PO1 GM47969, RSDA MH00964 and Funds from the Anesthesiology Dept. of Washington Univ.

326.14

STUDY OF THE GABA$_A$ RECEPTOR ASSEMBLY PATTERNS IN XENOPUS OOCYTES. M.L.Gordey* and R.W.Olsen. Department of Molecular and Medical Pharmacology, UCLA Sch. of Med., Los Angeles, CA 90095.

Both γ2S and β2 subunits are capable of "rescuing" intermediate GABA$_A$ receptor combinations from endoplasmic reticulum (ER) and thus promote the expression of the full (αxβ2γ2S) heteropentameric receptors on the cell surface. We used the Xenopus oocytes expression system to study patterns of the GABA$_A$ receptor assembly, as these cells maintain their vitality long enough during expression of the GABA$_A$ receptors to allow injections of cRNAs for subunits separately at different time points. Mixture of α1 and γ2S cRNAs produced no detectable Cl^- currents upon application of 1 mM GABA after 6 days of expression. When the same cells were coinjected with β2 cRNA, 24 hr later we recorded GABA (10 mM)-evoked Cl^- currents;these were not inhibited by 100 μM $ZnCl_2$ and were enhanced by flurazepam (10 μM) by approx. 69%. The same paradigm was used to express [α1β2] and [α4β2] combinations for 6 days and then coinject γ2S cRNA. GABA (100 μM)-evoked Cl^- currents recorded 24 hr later were increased 6 times over that of [α1β2] alone and 50 times for [α4β2]. In both cases the currents were inhibited by 100 μM $ZnCl_2$ (20% for [α1β2] and 50% for [α4β2]). [α1β2γ2S currents were increased by 10 μM flurazepam by 50%. The zinc inhibition of the Cl^- currents was not observed 48 hr after γ2S cRNA coinjection. The increase in the currents could not be accounted for expression of β2, αxγ2S or β2γ2S subunit combinations on the cell surface. We propose that both γ2S and β2 subunits can "rescue" intermediate GABA$_A$ receptor combinations trapped in the ER and thus produce full heteropentamer receptor on the cell surface. Moreover changes in zinc sensitivity during this "rescue" may be ascribed to different stoichiometry of the pentamers. We are now testing α1 and α4 subunits of the GABA$_A$ receptor in the same paradigm as well as involvement of the cytoskeleton in the "rescue" mechanism. Funded by NS 28772.

326.15

PROTEINS COPRECIPITATING WITH GABA$_A$ RECEPTORS FROM CALF BRAIN

K. Kannenberg, R. Baur and E. Sigel*. Department of Pharmacology, University of Bern, CH-3010 Bern, Switzerland.

GABA$_A$ receptors are nonrandomly distributed over the neuronal membrane, with a high density at postsynaptic sites. How such GABA$_A$ receptor clusters are generated and maintained is unknown. By analogy to the anchoring of other ion channels it seems likely that specific proteins are associated with GABA$_A$ receptors. In an attempt to isolate these linker proteins, we precipitated GABA$_A$ receptors from Triton X-100 solubilised calf brain membranes with the mAb bd24 coupled to Protein A-Sepharose. This antibody recognizes the human and bovine, but not the rat α1-subunit. The precipitates were analysed for the presence of additional proteins. Apart from considerable amounts of tubulin and somewhat lower amounts of actin other proteins have been observed. The cellular prion protein, that was speculated to interact with GABA$_A$ receptors, and the molecular chaperonin GRP78 could not be detected in the immunoprecipitate. Specific association of proteins with GABA$_A$ receptor complexes was shown in several negative controls, including immunoprecipitation from SDS solubilised membranes and use of unmodified Protein A-Sepharose or a nonspecific mAb. Furthermore, these proteins were also lacking in bd24-precipitates from rat brain membranes. Analysis of peptides generated from trypsin cleavage of the different proteines by microsequencing is under way. Supported by Grant 31-37192.93 from the Swiss National Science Foundation.

326.16

PROTEASE MAPPING AS A METHOD FOR STUDYING LIGAND-INDUCED STRUCTURAL ALTERATIONS OF THE GABA$_A$ RECEPTOR AND MAPPING THE GABA BINDING SITE. C. A. E. Hauser, F. Gerner, and F. Holsboer*. Max Planck Institute of Psychiatry, Clinical Institute, 80804 Munich, Germany.

The γ-aminobutyric acid (GABA) type A receptor complex interacts with GABA at the large extracellular region of the protein. This region contains the N-terminus and what is termed the cysteine loop, an important structural feature of several binding sites. We utilized limited proteolysis of rat GABA$_A$ receptor α_1, β_2 or γ_2 subunits synthesized by *in vitro* translation with microsomes from canine pancreas to detect structural alterations induced by GABA. GABA binding changes receptor conformation, giving rise to a fragment resistent to proteolysis. Digestion of β_2 without ligand was complete, whereas digestion of the GABA-occupied subunit with subtilisin yielded a 31-kDa fragment and with trypsin a slightly larger fragment of approximately 38 kDa. For α_1 and γ_2 GABA provided weak protection. This finding might explain the postulated location of the two GABA binding sites on the receptor pentameric complex, thought to be situated at the interface of the β_2 subunit. Thus, protease mapping is a suitable method for determining alterations in receptor configuration.

supported by the Max Planck Society

326.17

IDENTIFICATION OF CELLULAR COMPONENTS ASSOCIATED WITH GABA$_A$ RECEPTORS BY THE YEAST TWO HYBRID SCREEN. H. Wang and R.W. Olsen*. Molecular Biology Institute, and Department of Pharmacology, UCLA, Los Angeles, CA 90095.

By using the modified yeast two hybrid system, Interaction Trap, we have identified several cellular proteins interacting with GABA$_A$ receptor α1 subunit and γ2L subunit. Binding sites for agonists and antagonists as well as other modulatory ligands are located on the N-terminal extracellular domain of GABA$_A$ receptor proteins. In addition to this extracellular regulation, a large body of evidence suggests that the GABA$_A$ receptors can be regulated by cellular components presumably through its intracellular loop between TM3 and TM4. In this work, we used Interaction Trap to screen for cellular proteins which can physically associate with GABA$_A$ receptors. We generated two yeast strains. One harbors the intracellular loop of α1 subunit as bait. Another contains the intracellular loop of γ2L subunit as bait. Then a human fetal brain cDNA library was transformed into these two strains. Candidate prey were selected on the basis of reporter gene expression requiring bait and prey protein interaction. The prey plasmids in the positive yeast clones were rescued and tested for their specificity. The prey screened for α1 subunit interaction only interact with α1 but not γ2L and vice versa. The sequence information of the positives suggests relationship to interesting known proteins. Their ability to interact with GABA$_A$ receptors in vitro is under investigation.

This work is supported by NIH grant NS28772(RWO) and training grant GM07185(HW).

326.18

EXPRESSION OF AN EXTRACELLULAR DOMAIN OF GABA$_A$ RECEPTOR. H. Xue*, H. Zheng, X.L. Zhong, P. Lee and J.T. Wong. Department of Biochemistry, Hong Kong University of Science and Technology, Clear Water Bay, Hong Kong.

A 16 Kd fragment of the extracellular part of bovine GABA$_A$ receptor α1 subunit has been expressed in *E. coli* as a GST-fusion protein. With a single anion exchange column (Sepharose Cl$^-$ 6B, Pharmacia), the fusion product has been purified to near 90% purity. After thrombin digestion, the 16 Kd product is being characterized for stability and secondary structure by calorimetry and circular dichroism, respectively. It will then be subjected to NMR solution structure investigation. Previous mutational studies have identified this part of the receptor as the binding sites of ligands, GABA and Benzodiazapine. The success of structural characterization of the protein produced in this study will eventually facilitate structure-based drug design for GABA$_A$ receptors.

Supported by Research Grant Council of Hong Kong and Hong Kong University of Science and Technology Direct Allocation Grant.

326.19

SUBCELLULAR LOCALISATION OF GABA$_A$ RECEPTORS IS DETERMINED BY RECEPTOR B SUBUNITS. C.N.Connolly[1], J.R.A. Wooltorton[2], T.G. Smart*[2] and S.J.Moss.[1] [1]MRC LMCB, Dept. of Pharmacology, University College London, London WC1E 6BT, England. [2]Dept. of Pharmacology, The School of Pharmacy, London WC1N 1AX, England.

γ-aminobutyric acid type A (GABA$_A$) receptors are the major sites of fast synaptic inhibition in the CNS. They are constructed from 4 subunit classes with multiple members: α(1-6), β(1-4), γ(1-4), and δ(1). The contribution of subunit diversity in determining receptor subcellular targeting was examined in HEK293 and polarised Madin-Darby canine kidney (MDCK) cells using α1, γ2S, β1, β2 and β3. Significant detection of cell surface homomeric receptors was found only for the β3 subunit in both cell types. Expression of all α/β binary combinations resulted in cell surface staining in both cell types. However, a non-polarised distribution was evident for α1β1 complexes with specific basolateral targeting of both α1β2 and α1β3 complexes. The distribution of these receptors were unaffected by the presence of the γ2S subunit. Interestingly, delivery of receptors containing the β3 subunit (β3, α1β3 and α1β3γ2S) to the basolateral domain occurs via the apical surface. These results demonstrate that β subunits can selectively target GABA$_A$ receptors to distinct cellular locations. Changes in the spatial and temporal expression of β subunit isoforms may therefore provide a mechanism for relocating GABA$_A$ receptor function between distinct neuronal domains. Given the critical role of these receptors in mediating synaptic inhibition this process may provide a novel means of generating synaptic plasticity.

This work was supported by the MRC.

326.20

STOICHIOMETRY OF RECOMBINANT α1β2γ2 GABA$_A$ RECEPTORS. D. S. Weiss*, Y. Chang, R. Wang, and S. Barot. Neurobiology Research Center and the Department of Physiology and Biophysics, University of Alabama at Birmingham, Birmingham AL 35294-0021.

The GABAA receptor is presumed to be a pentamer comprised of α, β, and γ subunits, although the stoichiometry remains controversial. We have addressed the stoichiometry of the GABA receptor by site-directed mutagenesis of a conserved leucine (to serine) in the putative second membrane-spanning domain of the α1, β2, and γ2 subunits. This point mutation increased the GABA sensitivity of the receptor in proportion to the number of incorporated mutant subunits. Coexpression of both wild type (e.g. α) and mutant (e.g. α_m) subunits, along with their wild type counterparts (e.g. β and γ) in *Xenopus* oocytes, resulted in a mixed population of receptors with distinct GABA sensitivities. We determined the number of each subunit type from the number of components comprising these compound GABA dose-response relationships. We conclude that the α1β2γ2 GABA receptor is a pentamer comprised of two α subunits, two β subunits, and one γ subunit. (Grant # NS35291.)

326.21

GABA$_A$-RECEPTOR β2 SUBUNIT IS SUFFICIENT FOR MODULATION OF THE RECEPTOR BY TYROSINE PHOSPHORYLATION. Q. Wan, H.Y. Man, J.L. Braunton, G.M. Brown* and Y.T. Wang., Hosp. Sick Children and Clark Insti., Univ. Toronto, Toronto, M5G 1X8 Canada

GABA$_A$ receptors are the major receptor mediating fast inhibitory synaptic transmission in the brain. We have previously demonstrated that in cultured CNS neurons the β2 subunit of the GABA$_A$ receptor is tyrosine phosphorylated and that the function of GABA$_A$ receptors is modulated by changes in tyrosine phosphorylation states within the cells. In the present study, we have investigated the contribution of β2 subunit to the functional modulation of GABA$_A$ receptors by protein tyrosine kinases (PTKs) in human embryonic kidney cells (HEK 293) expressing recombinant GABA$_A$ receptors. GABA$_A$ receptor-mediated currents were induced by pressure-ejection of GABA (100 μM) and recorded with a CsCl-filled pipette under whole-cell patch-clamp configuration. In cells transiently transfected with cDNAs encoding α1, β2 and γ2 subunits, the amplitude of GABA currents was reversibly reduced by extracellular application of genistein (100 μM), a membrane-permeable inhibitor of PTK, whereas the currents were unaffected by daidzein (100 μM), an inactive analogue of genistein, consistent with an ongoing modulation of the receptor function by basal activity of endogenous PTKs observed in the cultured CNS neurons. As the γ2, but not the α1 subunit has previously been shown to be tyrosine-phosphorylated by co-transfection of a constitutive PTK, we next examined the modulation in cells transiently expressing only α1 and β2, and lacking the γ2 subunit. In these cells, we observed similar inhibitory effects of genistein on the GABA-induced response. Thus, our results suggest that the β2 subunit of GABA$_A$ receptor is sufficient for supporting modulation of the receptor function by protein tyrosine phosphorylation and consequently tyrosine phosphorylation of this subunit may be an important mechanism for regulating GABA$_A$-receptor function in the CNS. (Supported by Canadian MRC)

327.1

LOSS OF SPECIFIC GABA$_A$-RECEPTOR SUBPOPULATIONS IN MICE LACKING THE α5-SUBUNIT GENE. J.-M. Fritschy[1]*, D. Benke[1], D.K. Johnson[2], H. Mohler[1], U. Rudolph[1], [1]Inst. of Pharmacology, Univ. of Zurich, 8057 Zurich, Switzerland; [2]Oak Ridge National Laboratory, Oak Ridge, TN 37831, USA.

The mechanisms governing the assembly of functional GABA$_A$-receptors remain unresolved. In mutant mice lacking the γ2-subunit, GABA$_A$-receptors are present in unchanged numbers and distribution, being comprised of α- and β-subunits. The lack of γ2-subunit does thus not appear to interfere with receptor assembly. To determine whether the presence of an α-subunit is required for the formation of GABA$_A$-receptors, we analysed mutant mice carrying a chromosomal deletion encompassing both the α5- and γ3-subunit genes, or the γ3-subunit gene only (Culiat et al., 1994, Proc. Natl. Acad. Sci. USA 91:2815). The latter mice served as controls. Autoradiographic analysis in α5$^-$/γ3$^-$ mutant mice revealed a nearly complete loss of zolpidem-insensitive benzodiazepine-binding sites, corresponding to GABA$_A$-receptors containing the α5-subunit. Likewise, a 20% decrease in the number benzodiazepine-binding sites was measured in the hippocampus, in line with the prevalence of receptors containing the α5-subunit. Immunofluorescence with subunit-specific antibodies confirmed the absence of α5-subunit staining in α5$^-$/γ3$^-$ mutant mice. In addition, a decreased β2,3- and γ2-subunit staining was apparent in neurons which normally express the α5-subunit (e.g., olfactory bulb granule cells, hippocampus pyramidal cells), but not in other brain regions (e.g., thalamus, inferior colliculus, cerebellum). These results indicate that the absence of the α5-subunit gene results in the disappearance of a subpopulation of GABA$_A$-receptors in the adult mouse brain, thereby suggesting that the α5-subunit is required for the formation of these receptor complexes. Thus, the α-subunit, unlike the γ2-subunit, might play a major role in assembly or targeting of GABA$_A$-receptors. Support: Swiss National Science Foundation, grant 31-32624.91.

327.2

IMPAIRMENT OF GABA$_A$ RECEPTOR FUNCTION BY SUBUNIT GENE TARGETING IN MICE RESULTS IN NEOPHOBIA. F. Crestani, D. Benke, G. Reyes, J.-M. Fritschy, B. Lüscher* and H. Mohler, Institute of Pharmacology, University of Zurich, CH-8057 Zurich.

Clinical and experimental observations support the hypothesis of functional alterations in the GABA$_A$ receptor complex in anxiety-related disorders. Using gene targeting, the GABA$_A$ receptor γ2 subunit was shown to be necessary for the formation of benzodiazepine (BZ) sites and normal single channel conductance of GABA$_A$ receptors (Gunther *et al.*, PNAS 92, 7749, 1995). In heterozygous (γ_2+/0) mice, the γ2 subunit protein was reduced by about 50%. The structure of a small population of GABA$_A$ receptors were affected, as evidenced by a reduction of the number of BZ sites (25%). The impairment of GABA$_A$-receptor function manifested itself behaviorally. The γ_2+/0 mice exhibited neophobia in a free-choice exploratory paradigm and novelty-dependent hypolocomotion in an openfield in contrast to wildtype littermates. These behavioral changes were specific since spontaneous locomotion, rearing behavior, learning capacity (Morris test) and neuronal plasticity (LTP) were unaltered. A pretreatment with flumazenil before exposure to novelty prevented neophobia in γ_2+/0 mice. Thus, emotional reactivity toward novelty appears to be under stringent GABA-ergic control. Support: Swiss National Science Foundation.

327.3

IN VIVO ALTERATION OF GABA RECEPTOR COMPOSITION AND FUNCTION USING ADENO-ASSOCIATED VIRUS (AAV) VECTOR GENE TRANSFER. T.J. McCown*, H.E. Criswell, X. Xiao, G.R. Breese, A.L. Morrow and R.J. Samulski, UNC Neuroscience Center, Gene Therapy Center and Center for Alcohol Studies, University of North Carolina, Chapel Hill, NC 27599 and Somatix Therapy Corp, Alameda, CA 94501

Because AAV vectors are capable of gene transfer and expression in the CNS (McCown et al., Brain Res. 713:99, 1996), we sought to alter composition and function of an important therapeutic target, the GABA$_A$ receptor. Seven days after AAV vectors containing a CMV promoter and a truncated human GABA$_A$ α1 cDNA were microinjected into the rat inferior colliculus, GABA α-like immunoreactivity was localized to neurons, far exceeding endogenous levels. The glomerular layer of the olfactory bulb is an area devoid of GABA α1 protein, and patch clamp recordings on cells acutely dissociated from this brain region do not exhibit zolpidem enhancement of GABA-induced chloride currents. However, 7 days after infusion of the AAV vector, 2 cells dissociated from this brain region exhibited high affinity zolpidem facilitation of GABA responses, and single cell RT-PCR demonstrated the presence of the AAV vector. Thus, it may be possible to alter the composition and function of receptors *in vivo* using AAV vector-mediated gene transfer. (supported by AA09122 and HL51818, HL48347 and AA-09013)

327.4

AN ANTISENSE OLIGODEOXYNUCLEOTIDE TO THE GABA-A RECEPTOR γ2 SUBUNIT DECREASES BENZODIAZEPINE BINDING WITH LITTLE EFFECT ON GABA BINDING. T.-J. Zhao, H.C. Rosenberg, M. Li and T.H. Chiu*, Dept. of Pharmacology, Medical College of Ohio, Toledo, OH 43699.

The presence of a γ_2 subunit in the GABA-A receptor is important for optimal binding of benzodiazepines. Previously, we found a significant increase in convulsive threshold for β-CCM, a benzodiazepine "inverse agonist", but no change in picrotoxin threshold, following unilateral intracerebroventricular (ICV) infusion of γ_2 antisense oligo in rats. In the present study we have evaluated the effect of this treatment on GABA-A and benzodiazepine receptor binding. Unilateral ICV infusion of the 17-base phosphorothioate-modified antisense oligo (18 μg in 2 μl saline) was performed every 12 hr for 3 days. Control rats were treated the same way with a sense oligo. Brains were frozen and para-sagittal sections used for quantitative autoradiographic analysis of the binding of 5 nM [^{3}H]flunitrazepam (FNP) and 50 nM [^{3}H]GABA (in the presence of baclofen). Compared to rats treated with a sense oligo, the rats that had received γ_2 antisense oligo had a 15-25% decrease of specific [^{3}H]FNP binding in most brain areas, and this was significant in several brain regions including frontal cortex, cerebellum molecular layer, zona reticulata of substantia nigra, and CA 3 of hippocampus (n=5). In contrast, [^{3}H]GABA binding was not changed, except for a significant 10% decrease in cerebellar granule cell layer (n=5). The results show that the antisense oligo to the GABA-A receptor γ_2 subunit reduced benzodiazepine binding with little effect on GABA-A receptor binding in vivo. This may indicate a change in the subunit composition of GABA-A receptors in this experiment. Supported by DA02194.

327.5

INCREASED NEUROPEPTIDE Y (NPY) IMMUNOREACTIVITY FOLLOWING ANTISENSE KNOCK-DOWN OF GABA$_A$ RECEPTOR γ2 SUBUNIT IN RAT HIPPOCAMPUS. J. Karle*[1], J. D. Mikkelsen[2] and M. Nielsen[1]. 1. The Research Institute of Biological Psychiatry, St. Hans Hospital, DK-4000 Roskilde, 2. Department of Neurobiology, H. Lundbeck A/S, Denmark.

Antisense oligodeoxynucleotides (ODNs) can be used as selective inhibitors of gene expression in the central nervous system of rodents *in vivo*. Antisense ODNs are short strands of synthetic DNA which presumably work via a nucleotide specific action at the level of mRNA. We have previously shown that continuous intrahippocampal infusion of a phosphorothioate ODN antisense to the rat GABA$_A$ receptor γ2 subunit mRNA induces a substantial decrease in benzodiazepine receptor binding in rat hippocampus. This effect is parallelled by a decrease in the number of GABA$_A$ receptors and by an extensive loss of hippocampal neurones. Control experiments with sense and mismatch ODNs have demonstrated the specificity of the effect. Immunocytochemical analyses have been carried out in order to further characterize the effect induced by the antisense ODN. Following unilateral infusion of antisense ODN, but not mismatch control ODN, a marked increase in NPY-immunoreactivity (NPY-ir) in hilar neurones of the fascia dentata was observed bilaterally. Also, intense NPY-ir was visualized bilaterally in the mossy fibers and in perikarya of the entorhinal cortex. The findings are reminiscent of reported changes following seizures induced by e.g. kainic acid and support the hypothesis that blocked synthesis of GABA$_A$ receptors in the hippocampus leads to selective neuronal cell death via diminished GABAergic inhibitory neurotransmission.

327.6

ANTISENSE INHIBITION OF STRIATAL GABA$_A$ RECEPTOR PROTEINS DECREASES GABA-STIMULATED CHLORIDE UPTAKE AND INCREASES COCAINE SENSITIVITY IN RATS. J. Peris*, B. J. Jung, P. Walker, O. Malakhova, Y. Bokrand and D. Wielbo, Depts. Pharmacodynamics and Pharmaceutics, Univ. Florida, Gainesville FL 32610.

The functional status of GABA$_A$ receptors in striatum is inversely related to behavioral sensitivity to cocaine. Exposure of striatum to antisense oligodeoxynucleotides (ASODNs) targeted to the mRNAs for the α_2 and the β_3 subunits of the GABA$_A$ receptor should decrease expression of receptor proteins. A combination of these ASODNs (25 μg each), scrambled ODNs or saline was injected into right lateral ventricle of rats and striatal GABA$_A$ receptor function was measured 24 hrs later. There was significantly less GABA-stimulated Cl uptake in right striatum compared to left striatum of ASODN treated rats with no significant difference between sides in control animals. ASODN injection increased behavioral stereotypy and contraversive turning caused by cocaine when tested 18-20 hrs after ODN treatment ($F(2,26) = 3.8$; $p < 0.05$). Nissl staining indicated there was no difference between the density of stained cells in the left and right edges of striatum in control rats (left: 63±7, right: 65±8) or in ASODN treated rats (left: 69±4, right: 67±5). When fluorescein-conjugated ASODNs were injected and rats were killed 24 hrs later, fluorescence signal was present up to 1.5 mm from the cannula tract and was densest near the most ventral portion of right lateral ventricle. There was penetration into both striatum and lateral septum with evidence of uptake into individual cells. Thus, ASODN is present in striatum at times during which behavioral and neurochemical indices of GABA receptor function are decreased without causing obvious neurotoxicity. Supported by the Alcoholic Beverage Medical Research Foundation.

328.1

DIFFERENTIAL AGONIST REGULATION OF μ OPIOID RECEPTOR IN HEK 293 CELLS.
A. D. Blake*, G. Bot, J. C. Freeman and T. Reisine
Dept. of Pharmacology, University of Pennyslvania, Philadelphia, PA 19104

Mu opioid receptors mediate the analgesia induced by morphine. Prolonged use of morphine causes tolerance development and dependence. To explore the molecular basis of tolerance and dependence, the cloned mouse μ receptor with an N-terminal epitope tag, was stably expressed in human embryonic kidney cells (HEK 293 cells) and the effects of prolonged agonist treatment on receptor regulation were examined. In HEK 293 cells, the expressed μ receptor showed high affinity, specific, saturable binding of radioligands and a pertussis toxin sensitive inhibition of adenylyl cyclase. Pretreatment of cells with 1μM morphine or DAMGO resulted in no apparent receptor desensitization, as assessed by opioid inhibition of forskolin-stimulated cAMP levels. However, the morphine and DAMGO pretreatments resulted in a 3-4 fold compensatory increase in forskolin-stimulated cAMP. The opioid agonists, methadone and buprenorphine, are used in the treatment of addiction, due to a markedly lower abuse potential. Pretreatment of μ expressing HEK 293 cells with methadone or buprenorphine abolished the ability of opioids to inhibit adenylyl cyclase. No compensatory increase in forskolin-stimulated cAMP accumulation was found with methadone of buprenorphine, however these opioids blocked the compensatory effects observed with morphine and DAMGO. These results indicate that methadone and buprenorphine interact differently with the mouse μ receptor than either morphine or DAMGO. The ability of methadone and buprenorphine to desensitize the μ receptor and block the compensatory rise in forskolin-stimulated cAMP accumulation may be an underlying mechanism for the therapeutic efficacy of these agents in treating addiction. Supported by DA08951 and DA05636.

328.2

TYR-W-MIF-1 ATTENUATES MORPHINE-INDUCED DOWN-REGULATION OF MU AND DELTA OPIATE RECEPTORS IN SH-SY5Y CELLS.
L.M. Harrison*, A.J. Kastin and J.E. Zadina. VA Medical Center and Dept. of Medicine and Neuroscience Training Program, Tulane University Sch. of Med., New Orleans, LA 70146.

SH-SY5Y cells have been used as a model system for studying acute and chronic opiate effects. Morphine down-regulates, and naloxone up-regulates, both μ and δ receptors after chronic exposure (JPET265:254; 270:1086). We tested the ability of Tyr-W-MIF-1 (Tyr-Pro-Trp-Gly-NH_2), a peptide from brain (Peptides 13:632) that binds to the μ opiate receptor (Life Sci 55:461), to antagonize morphine down-regulation of receptors. SH-SY5Y cells were initially cultured in serum-containing medium and switched to serum-free medium to avoid peptide metabolism. The change decreased μ receptors and increased δ receptors such that their ratio approached 1:1, but morphine and naloxone retained their abilities to down- and up-regulate receptors in the serum-free medium. Met-enkephalin at 10μM decreased μ (32%) and δ (60%) receptors, but Tyr-W-MIF-1 at doses of 30μM and 100 μM did not affect the number of either μ or δ receptors. Tyr-W-MIF-1 remained intact after 24h in the serum-free medium. In combination with 3μM morphine, Tyr-W-MIF-1 attenuated morphine-induced down-regulation of receptors in a dose-dependent manner. Morphine-induced down-regulation of μ receptors was significantly attenuated by 30μM ($p<0.05$) and 100μM ($p<0.01$) doses of Tyr-W-MIF-1. Down-regulation of δ receptors was significantly attenuated by 30μM Tyr-W-MIF-1 ($p<0.05$), with a statistical tendency at the 100μM dose. These results indicate that the endogenous peptide Tyr-W-MIF-1 can attenuate morphine-induced changes in receptor number and thus may be part of a compensatory opiate-modulating system activated during tolerance.
Supported by the VA and NIDA predoctoral training grant DA05645.

328.3

CYCLIC AMP DEPENDENT PROTEIN KINASE INDUCES PHOSPHORYLATION OF MORPHINE STIMULATED BUT NOT DAMGO STIMULATED MU OPIOID RECEPTORS
S. Chakrabarti, A. M. Babey*, P. Y. Law, and H. H. Loh. Department of Pharmacology, University of Minnesota Medical School, MN 55455, USA.

Receptor phosphorylation was examined using a functional, hemagglutinnin epitope-tagged μ-opioid receptor stably expressed in murine neuroblastoma Neuro2a cells. Receptor density and affinity were 1.87 pmoles/mg, and 0.3 nM respectively for [^{3}H]Diprenorphine binding. In a cell-free system, catalytic subunits of cAMP-dependent protein kinase (PKA) profoundly increased μ-opioid receptor phosphorylation in the presence of morphine. This effect of morphine was dose-dependent from 1-1000 nM, and was completely blocked in the presence of naloxone, suggesting a receptor mediated effect. Further, morphine analogs or other alkaloids similarly stimulated *in vitro* receptor phosphorylation induced by PKA. However, it was interesting to observe that a potent μ-opioid selective peptide [D-Ala^2, MePhe^4, Glyol^5]enkephalin (DAMGO) was incapable of stimulating this PKA-induced *in vitro* phosphorylation of μ-opioid receptors. Moreover, following chronic morphine pretreatment, morphine stimulation of PKA-induced phospborylation of the receptor decreased drastically. In contrast, chronic DAMGO pretreatment did not affect morphine stimulation of PKA-induced receptor phosphorylation. Furthermore, this *in vitro* phosphorylation of μ-opioid receptors induced by PKA in presence of morphine, was completely abolished in pertussis toxin pretreated cell membrane suggesting effective receptor G-protein coupling was essential for this phosphorylation. Taken together all these results suggest that PKA-induced phosphorylation of μ-opioid receptors *in vitro* demonstrated a distinct difference between morphine and DAMGO occupied forms of the receptor. It is therefore possible to speculate that this *in vitro* receptor phosphorylation induced by catalytic subunits of PKA in the presence of morphine may be useful for future studies of agonist-receptor interactions and determination of agonist-induced desensitization. (Supported by NIH grants DA-01583, DA-00564, DA-08131, DA-05695, DA-07339 and KO5-DA 70554).

328.4

TRAFFICKING OF MU OPIOID RECEPTOR (MOR) IN ENTERIC NEURONS *IN VIVO*.
C. Sternini*, M. Spann, B. Anton, D.E. Keith Jr., N.W. Bunnett, M. Von Zastrow, C. Evans, and N.C. Brecha. CURE, Opioid Res. Ctr. & Depts. of Med., Neurobiol. & Psych., UCLA, & WLA VAMC, Los Angeles, CA 90073; & Depts. Surg., Physiol., & Psych., UCSF, San Francisco, CA 94143.

Opioids influence multiple functions in the nervous system by activating G protein-coupled receptors. We examined the trafficking of MOR in enteric neurons of the guinea pig ileum, which naturally express this receptor, in response to opiate alkaloids that are used for pain control using an affinity-purified antibody to rat MOR C-terminus and confocal microscopy. MOR immunoreactive (IR) neurons are located in the myenteric plexus and have the morphology of motor neurons with broad dendrites and a long axonal process. In control animals, MOR-IR is primarily confined to surface membrane. After 30 min of exposure to the opiate agonist, etorphine (Et) (0.06-0.1 mg/kg), MOR-IR is present in cytoplasmic and perinuclear vesicles. MOR internalization occurs by 15 min and persists up to 120 min. By 180 min, MOR recycles to the cell surface. The antagonist, naloxone (10 mg/kg), inhibits Et-induced MOR endocytosis. Morphine (5-20 mg/kg), a high affinity MOR agonist, does not cause detectable endocytosis, and it partially inhibits Et-induced MOR internalization. This study provides evidence that enteric motor neurons have functional MOR. The occurrence of agonist-induced endocytosis of MOR in enteric motor neurons *in vivo* at the soma, dendrites and axons, suggests pre- and post-synaptic regulation of intestinal motility by opioids and alkaloids. Supported by DK41301, DA 05010, & VA Res. Funds.

328.5

A CONSTITUTIVELY RECYCLING MUTANT OF THE μ OPIOID RECEPTOR
Veronica Segredo*, Neil T. Burford, Jelveh Lameh, Wolfgang Sadée, University of California, San Francisco, CA 94143-0446

To determine the domains of the μ opioid receptor involved in internalization we constructed two C-tail truncation mutants bracketing a 4-residue domain (354TSST357). An epitope tag (EE) was added to the extracellular N-tail of the wild-type and the two mutant receptors (μEE, T354EE and T358EE, respectively). The three μ receptor constructs stably expressed in HEK cells did not markedly differ in ligand binding affinities or G-protein coupling. Both μEE and T358EE resided largely at the plasma membrane and internalized upon DAMGO, but not morphine, stimulation. In contrast, T354EE was already concentrated in intracellular vesicles before agonist exposure, and recycled to the plasma membrane following treatments to block endocytosis through clathrin-coated vesicles. T354EE therefore internalized and recycled constitutively. Despite agonist-independent internalization of T354EE, there was no evidence of elevated basal G-protein coupling. Colocalization of μEE and T354EE with the transferrin receptor in intracellular vesicles indicated that the cellular pathway of μ receptor internalization is similar to that of the transferrin receptor. These results strongly suggest that the TSST domain in the C-tail suppresses receptor internalization, and that suppression can be reversed either by stimulation with a permissive agonist (*i.e.*, DAMGO) or by selective truncation of the inhibitory region.

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328.6

AGONIST-INDUCED FUNCTIONAL DESENSITIZATION OF THE MU OPIOID RECEPTOR IS MEDIATED BY LOSS OF MEMBRANE RECEPTORS RATHER THAN UNCOUPLING FROM G-PROTEIN.
YS. Pak, A. Kouvelas, M.A. Scheideler, J. Rasmussen, B.F. O'Dowd and S.R. George*. Dept. Pharm. and Med, Univ. of Toronto, Add. Res. Fdn, Toronto, ONT, Canada, Novo Nordisk A/S, Denmark.

The effects of acute exposure of the opioid peptide, [D-Ala^2, N-Me-Phe^4, Gly-ol^5]-enkephalin (DAMGO) on the mu opioid receptor were examined in CHO K-1 and BHK stable transfectants. In the CHO cell line, acute 1 hour treatment with DAMGO decreased the density of receptors without affecting the affinity or proportion of agonist-detected sites and attenuated the agonist ability to inhibit forskolin-stimulated cAMP accumulation. In contrast, acute treatment of BHK cells did not affect receptor density or agonist ability to inhibit cAMP accumulation but longer duration of agonist exposure resulted in a reduction of membrane receptor, identical to the CHO cells. These results suggested that for the mu opioid receptor, alteration in receptor density was the major determinant for the observed agonist-induced desensitization. Consistent with this notion, the ratio of the DAMGO concentration yielding half-maximal occupation of the mu receptor to that yielding half-maximal functional response was less than 1. This suggests the necessity for a high mu receptor occupancy rate for maximal functional response, so that loss of cell surface opioid binding sites was a critical determinant in reducing the functional response. This hypothesis was further supported by the observation that irreversible inactivation of fixed proportions of mu opioid receptors using β-chlornaltrexamine demonstrated that there were few spare receptors, in contrast to what had been reported for other G-protein coupled receptors, including the delta opioid receptor. Taken together, these data suggest that the opioid agonist, DAMGO has a high affinity for the mu receptor but has to occupy more than 70 percent of the available receptors to generate the maximal second messenger-linked response. (Supported by the Addiction Research Fdn, Medical Research Council of Canada & National Institute of Drug Abuse).

328.7

NITRIC OXIDE IS INVOLVED IN DESENSITIZATION OF MU OPIOID INHIBITION OF ADENYLYL CYCLASE ACTIVITY BUT NOT DOWN-REGULATION OF MU RECEPTORS AFTER CHRONIC MORPHINE EXPOSURE IN SH-SY5Y CELLS. J. Y. Xu* and J. M. Bidlack. University of Rochester, Rochester, NY 14642.

Co-administration of nitric oxide (NO) synthase inhibitors, such as N$^{\omega}$-nitro-L-arginine methyl ester (L-NAME), along with morphine prevents morphine tolerance (Babey et al., 1994; Elliot et al., 1994). The cellular mechanisms of this prevention were investigated using the human neuroblastoma SH-SY5Y cell line differentiated with retinoic acid. This cell line expresses both μ and δ opioid receptors in a ratio of 5:1, respectively. Culturing of cells with 1 μM morphine for 18 hours, followed by extensive washing, produced a significant reduction in the B_{max} value for the binding of μ-selective peptide [^{3}H]DAMGO. Incubation of cells with 100 μM L-NAME, together with 1 μM morphine for 18 hours did not affect this down-regulation caused by the chronic morphine treatment. Culturing of cells with 10 μM morphine for 18 hours, followed by extensive washing, also produced a desensitization of DAMGO induced-inhibition of forskolin-stimulated adenylyl cyclase activity. In contrast to the ineffectiveness in the binding assay, 100 μM L-NAME, when given along with chronic morphine treatment, prevented the desensitization to DAMGO in the inhibition of forskolin-stimulated adenylyl cyclase activity. Culturing of cells with 100 μM L-NAME alone for 18 hours, followed by extensive washing, neither affected the B_{max} value for [^{3}H]DAMGO binding, nor DAMGO-induced inhibition of adenylyl cyclase activity. Our results suggest that NO is involved in the desensitization of μ opioid inhibition of adenylyl cyclase activity, but not down-regulation of μ opioid receptors during chronic morphine exposure in SH-SY5Y cells. (Supported by USPHS grant DA03742 and DA07232.)

328.8

BASAL μ OPIOID RECEPTOR PHOSPHORYLATION: MODULATION BY RECEPTOR LIGANDS AND KINASE INHIBITORS. Z. Wang, M. Kassack, N. Burford, & W. Sadée*. Departments of Biopharm.Sci. and Pharmac.Chem., University of California San Francisco, CA 94142-0446.

We have proposed the hypothesis that agonist stimulation of the μ opioid receptor enhances μ phosphorylation, and thereby, causes sensitization or constitutive activation, rather than desensitization. Moreover, the kinase inhibitor H7 prevented naloxone induced withdrawal jumping and antinociceptive tolerance in morphine treated mice (Z. Wang, E.J. Bilsky, F. Porreca, & W. Sadée, Life Sci. PL. 54,339-350, 1994). We studied μ receptor phosphorylation in HEK293 cells transfected with a rat μ opioid receptor (Y. Chen, A. Mestek, J. Liu, J.A. Hurley, & L. Yu, Mol.Pharmacol. 44, 8-12, 1993), epitope tagged at the C-terminus. The μ receptor was found to be phosphorylated at a basal rate which was further enhanced by acute stimulation with morphine (J.R. Arden, V. Segredo, Z. Wang, J. Lameh, & W. Sadée, J.Neurochem. 65, 1636-1641, 1995). The kinase inhibitor H7 indeed suppressed basal and morphine-stimulated μ phosphorylation (Z. Wang, J.R. Arden, & W.Sadée, FEBS Let., in press). We therefore studied the regulation of basal μ receptor phosphorylation by kinase inhibitors and μ receptor ligands. Basal μ receptor phosphorylation was mediated by a unique kinase activity, and it was slowly modulated by an apparent positive feedback loop between receptor and kinase. The ability of μ agonist and antagonist ligands to affect μ phosphorylation did not correlate with their ability to stimulate G protein coupling. Our results support the hypothesis that basal μ receptor phosphorylation plays a role in narcotic tolerance and dependence. Supported by NIDA, DA 04166.

328.9

SELECTIVE DOWN-REGULATION OF *DELTA* OPIOID RECEPTOR SUBTYPES IN MOUSE CORTICAL NEURONS *IN VITRO* AND IN NG108-15 CELLS. A. Ambrosini, L. Bresciani, C. Manzoni^, P. Petrillo^, G. Dondio^, F. Fidone, A. Barassi, G. Racagni* and G.D. Clarke^. Center of Neuropharmacology, Univ. of Milan; ^SmithKline Beecham, Baranzate, Milan (Italy).

The effects of preferential agonists and antagonists for *delta1* (DPDPE/BNTX) and *delta2* (deltorphin II/NTB) opioid receptors on cAMP formation have been studied in various cell systems, namely: CHO cells stably expressing the cloned mouse *delta* opioid receptor cDNA, NG108-15 cell line, and mouse cerebrocortical neurons *in vitro*. In CHO cells the responses to DPDPE and deltorphin II were similarly blocked by BNTX or NTB, and to a lesser extent, by the general antagonist naloxone. In contrast, in NG-108-15 cells and in cortical neurons a distinct *delta1* and *delta2* pharmacology was observed, the rank order of potency of the antagonists on the DPDPE effect being BNTX≥naloxone>>NTB, and on the deltorphin II effect NTB>BNTX>>naloxone. A chronic (48 h) treatment of cortical neurons with DPDPE or deltorphin II (both 100 nM) lowered basal cAMP levels by 15% and completely abolished deltorphin II (100 nM) but not DPDPE (0.1-1 μM) acute response, suggesting that in cortical neurons *delta2* subtype is more susceptible to down-regulation than *delta1* subtype. A 48 h pretreatment of NG108-15 cells with DPDPE (1 nM) selectively reduced acute DPDPE (10 nM) but not acute deltorphin II (10 nM) effect. Chronic treatment with deltorphin II (1 nM) abolished almost completely both responses; lower acute and chronic concentrations of deltorphin II are now being tested. Overall, our data strongly support the evidence for *delta* opioid receptor subtypes both in NG108-15 cells and in cortical neurons.

Study supported by SmithKline Beecham (Italy)

328.10

PROTEIN KINASE A REGULATION OF DELTA OPIOID RECEPTOR GENE EXPRESSION IN NG108-15 CELLS

Beata Buzas, and Brian M. Cox*, Dept. of Pharmacology, Uniformed Services University, Bethesda, MD, 20814.

The effects of activation of adenylyl cyclase-protein kinase A pathway on delta opioid receptor (DOR) gene expression has been investigated. Competitive polymerase chain reaction assays were used to compare the relative levels of DOR transcripts in treated and control cells. Activation of prostaglandin E1 (PGE1) receptors, which stimulate adenylyl cyclase, resulted in a reduction in DOR mRNA levels in NG108-15 cells. Direct stimulation of adenylyl cyclase by forskolin or treatment of cells with dibutyryl-cAMP (db-cAMP) mimicked the effect of PGE1. Receptor protein levels underwent a similar degree of downregulation, as measured by loss of radioligand binding sites. D-Ser2-Leu-enkephalin-Thr6 inhibition of adenylyl cyclase was also diminished after db-cAMP treatment. Protein kinase A inhibitors partially reversed the transcriptional repression by PGE1 or db-cAMP. Cycloheximide, a protein synthesis inhibitor, did not prevent cAMP mediated DOR mRNA downregulation, suggesting modification of preexisting transcription factors in this process.

Supported by a grant from the National Institute on Drug Abuse.

328.11

REGULATION OF DELTA OPIATE RECEPTOR INTERNALIZATION AND DOWN-REGULATION. S. Cvejic, N. Trapaidze, C. Cyr† and L. A. Devi*. Dept. of Pharmacology, NYU Medical Center, New York, NY 10016 and † Dept. of Pharmacology, Univ. of Washington School of Medicine, Seattle, WA 98195

It is well known that prolonged exposure to opiates causes reduction in the number of opiate receptors. To study internalization and down-regulation of delta opiate receptor (DOR), we generated Chinese Hamster Ovary (CHO) cell lines that stably express "flag" epitope tagged wild type DOR or mutant DOR with deletion or point mutations of the C-terminal tail. Both wild type and mutated receptor exhibit similar binding and functional coupling properties. Short time treatment (2-30 minutes) of CHO cells with wild type DOR with 100 nM DADLE causes rapid internalization of the receptors as determined by the reduction in mean surface receptor fluorescence intensity measured by flow cytometry. Long term exposure (1 - 48 hours) to 100 nM DADLE causes loss of diprenorphine binding both in the whole cell binding assay and in cell homogenates indicating down-regulation of the receptor. Unlike wild type DOR, mutant receptors with deletions of C-terminal tail or with point mutations within the C-terminal tail show impaired internalization and/or down-regulation. These results suggest that C-terminal tail has an important role in regulation of internalization and down-regulation of DOR. A possibility that regulation of internalization and down-regulation is mediated through interaction with other protein(s), is supported by specific coimmunoprecipitation of proteins with DOR from the CHO cell lysates using anti "flag" antibody.

This work is supported by grant DA08863.

328.12

CEREBROCORTICAL δ-OPIOID RECEPTOR mRNA EXPRESSION IN MORPHINE-ADDICTS. P. Schmidt[1,2]*, A. Wevers[3], K. Maderspach[4], E. Cserpán[4], I. Cserpán[5], M. Staak[2], H. Käferstein[2], G. Sticht[2], H. Schröder[3], [1]Inst. f. Rechtsmed., Univ. Bonn, 53111 Bonn, FRG, [2]Inst. f. Rechtsmed. u. [3]Inst.II f. Anat., Univ. zu Köln, 50823 Köln, FRG, [4]Inst. of Biochem. and [5]Genetics, BRC, Hung. Acad. Sci., 6701 Szeged, H.

Animal experiments have shown a simultaneous "up"- and "down" regulation of different opiate receptor types following chronic agonist/antagonist treatment. By means of non-isotopic in situ hybridization we had shown the localization of the δ-OR mRNA in the human frontal cortex. The present postmortem study of the morphine-related fatalities of drug addicts versus a non-addicted control group is intended to elucidate whether chronic opiate exposure affects the distribution of δ-OR mRNA expressing neurons in the human neocortex (area 10). Morphine-related fatalities (n=7, 21 to 33 yrs with blood unconjugated morphine levels from 0.15 to 2.49 mg/kg and a total morphine from 0.15 to 3.21 mg/kg) were compared to non-addicted controls (n=7, 19 to 42 yrs). For non-isotopic in situ hybridization digoxigenin 3'end labeled oligo-nucleotide probes were applied. Hybrids were visualized by alkaline phosphatase coupled anti-digoxigenin antibodies and incubation with NBT/BCIP. Numerical densities of mRNA containing and Nissl-stained neurons were assessed morphometrically. In the drug related fatalities 28815 ± 1642 / mm^3 expressing δ-OR mRNA were found, in the control group 30741 ± 1905 / mm^3, accompanied by an increase of Nissl-stained neurons. The differences did not reach statisticial significance. These findings go along with the binding behavior of opioid ligands in postmortem brains of heroine addicts (Gabilondo et al., Psychopharmacol 115:135, 1994) revealing similar receptor densities and affinities in the control subjects and addicts.

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328.13

IN UTERO EFFECTS OF BUPRENORPHINE ON OPIOID RECEPTORS. L. B. Jefcoat[1], M. M. Belcheva[1], M. T. Ho[1], F. E. Johnson*[2], J. Yanai[3], S. Barron[4], and C. J. Coscia[1], Depts. [1]Biochem. & Mol. Biol. and [2]Surg, St. Louis Univ. Sch. Med., St. Louis, MO, [3]Dept Anat. & Emb., Hebrew Univ.-Hadassah Sch. Med., Jerusalem, Israel. [4]Dept. Psychol., Univ. of Kentucky, Lexington, KY.

Previous *in vivo* studies revealed that buprenorphine can down-regulate μ and up-regulate δ_2 and κ_1 opioid receptors in rat brain. In order to assess gestational effects of buprenorphine on offspring, pregnant rats were administered buprenorphine by i.p. injection. Opioid receptor binding parameters (K_d and B_{max}) were measured in P1 brain membranes. At 2.5 mg/kg, buprenorphine evoked an up-regulation of κ_1 opioid receptors (98%>controls) as assessed with the agonist ^{3}H-U69593. Homologous binding assays with the μ-selective agonist [D-ala^2,mephe^4,gly-ol^5] enkephalin revealed a buprenorphine-induced decrease in receptor density at 0.3, 0.6 and 2.5 mg/kg drug treatments. A greater down-regulation of μ receptors for males (85%,73% resp.) than for their female counterparts (43%,57%, resp.) was observed. These data provide evidence for an opioid response to buprenorphine in pup brains treated prenatally wherein μ receptors were down-regulated and κ_1 receptors were up-regulated. Since buprenorphine is being evaluated in clinical trials for the treatment of heroin abuse, the *in utero* actions of the drug have ramifications for its use in maternal drug abuse. Supported by NIDA grant DA 05412 and NIH training grant H207050.

328.14

MULTIPLE MECHANISMS ACCOUNT FOR CHRONIC MORPHINE INDUCED RECEPTOR REGULATION IN RAT CNS. M. Szűcs, B. Bozó, B. Tombor, G. Fábián, M. Szikszay. Biol. Res. Center Hung. Acad. Sci., Szeged, Hungary **(SPON: European Neuroscience Association)**

Analgesic tolerance was induced by s.c. injections of increasing doses of morphine-HCl twice daily for 5 days in Wistar rats. Neither the K_d nor the B_{max} values of the μ opioid agonist [^{3}H]DAMGO binding were changed in whole brain P_2 or highly purified synaptic plasmamembrane fractions upon chronic morphine treatment. Affinity labeling with [^{3}H]DAMGO-chloromethyl ketone followed by SDS-PAGE and fluorography revealed specific incorporation of the label into the ≈50 kD_a binding subunit to a similar extent in tolerant and naive rat brain membranes. Measuring Gpp(NH)p inhibition of [^{3}H]DAMGO binding, or opioid agonist stimulation of [^{35}S]GTP-γ-S binding revealed no significant alterations in the coupling of μ opioid receptors to G-proteins. Thus, desensitization and down-regulation are not evident in rat whole brain membranes. In spinal cord P_2 membranes [^{3}H]DAMGO binding site number decreased in female, but was up-regulated by 164% in male rats after morphine tolerance developed. We conclude that chronic morphine *in vivo* induces diverse molecular events which are influenced by several factors such as those shown in the present study.

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328.15

EXPRESSION OF BRAIN OPIOID RECEPTORS IN MICE WITH TARGETED ABLATION OF PITUITARY POMC CELLS. E.M. Unterwald*[1,2], J. Kreuter[1], M.J. Sirianni[3], R.G. Allen[3], and M.J. Kreek[1]. [1]The Rockefeller University, New York, NY 10021; [2]New York University Medical Center, New York, NY 10016; [3]Oregon Health Sciences University, Portland, OR 97201.

Proopiomelanocortin (POMC) is a peptide precursor that gives rise to ACTH, α-, β-, and γ-MSH, and β-endorphin. The role of POMC-derived peptides in the regulation of opioid receptors was investigated using a line of transgenic mice that express a fusion gene composed of the pituitary expression-specific promoter region of the POMC gene, driving the herpes simplex viral-1 thymidine kinase (Allen et al., *Mol Endo* 9:1005, 1995). Three week old mice were treated with the antiherpes agent ganciclovir, 90 mg/kg daily for 14 days. One day following the treatment, 98% of the pituitary intermediate lobe melanotropes and anterior lobe corticotropes were ablated and greater than 98% of ACTH, β-endorphin, and α-MSH was lost. Binding of [^{3}H]DAMGO to μ opioid receptors as measured by quantitative autoradiography was significantly reduced in several brain regions of the mice with POMC cell ablations including the central grey (-37%), superficial grey layer of the superior colliculus (-33%), and median raphe (-23%). No significant differences were found in the binding of [^{3}H]bremazocine (in the presence of DAMGO and DPDPE) to κ opioid receptors or in the binding of [^{3}H]naltrindole to δ opioid receptors. These results suggest that POMC-derived peptides play a role in the regulation of central μ opioid receptor expression, possibly mediated through glucocorticoid receptors. (Supported by P50-DA05130 & K05-DA00049 (MJK), DA08267 (EMU), and NSF 9108426 (RGA).)

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MU OPIOID RECEPTOR GENE KNOCKOUT STRATEGIES. L Sora*, N. Takahashi , H.F. Liu , R. Revay , N. Goodman , D.M. Donovan , L Sharp , L.L. Miner , & G.R. Uhl # Mol. Neurobiology, NIH/NIDA, IRP; #Neurol. Neurosci. JHUSM, Balto., MD 21224

The mu opioid receptor is thought to be the opiate receptor subtype that is important for morphine mechanism, analgesia, reward, tolerance and dependence, cardiovascular and respiratory functions, and several neuro-endocrine-immune effects. The endogenous opioid peptides as well as morphine and other opioid alkaloids, are known to bind the mu-receptor to produce many physiological effects, including the modulation of neurotransmitter release and the alteration of ionic conductance and cell firing. To investigate the function of the mu-receptor, we have identified an ~16.5kb clone from a murine 129SvJ genomic library that contained the first exon and ~8kb of 5' and ~8kb of 3' flanking sequence. Targeting vectors replaced a putative promoter region and the first exon with the pgk neomycin resistance gene cassette. 800 Neo resistant embryonic stem (ES) cell lines were screened by Southern blotting, and eight ES cell lines showed homologous recombination at the mu-receptor locus. Heterozygous and homozygous knockout mice is helping to define mu receptor specific feature of normal and morphine-modulated behavior and neurochemistry.

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DIFFERENTIAL REGULATION OF μ RECEPTORS BY METHAMPHETAMINE IN p53 KNOCK-OUT MICE.
Bruce Ladenheim*, Hiroshi Hirata, and Jean Lud Cadet, Molecular Neuropsychiatry Section, NIH/NIDA, IRP, Baltimore, MD 21224

p53 is an important regulator of cell death and neuro-degeneration. We have shown recently that p53 is a mediator of methamphetamine (METH)-induced degeneration of striatal dopamine (DA) terminals since p53 knock-out mice are protected against the toxic effects of the drug. We showed, in addition, that METH caused increases in p53 in the striata of wild-type mice. Because p53 is a known transcription factor, we reasoned that absence of or overexpression of p53 might alter the expression of some striatal proteins after administration of METH. As a first step towards testing this idea, we have used quantitative autoradiographic techniques to measure striatal μ opioid receptor density after METH in wild-type as well as in heterozygous and homozygous p53 knock-out mice. In wild-type mice, METH caused no changes in striatal μ receptors at either 1, 3 or 15 days post-treatment. However, in both heterozygous and homozygous mice METH caused marked time-dependent decreases in striatal μ receptors. These results suggest that p53 might participate in maintaining the steady state levels of opiate receptor after METH administration. These data identify yet another role for p53 in the central nervous system.

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EXPRESSION OF OPIOID RECEPTOR PROTEIN IMMUNOREACTIVITY IN THE DEVELOPING HUMAN FOREBRAIN. C. R. Neal, Jr., MD, PhD* and S. J. Watson, Jr., MD, PhD Mental Health Research Institute. University of Michigan Medical School. Ann Arbor, Michigan 48109.

In the extremely premature neonate, a large proportion of CNS development is undertaken ex utero, while exposed to several environmental and pharmacological stressors. Although there is a wealth of clinical information concerning long-term outcomes of premature survivors, there is little knowledge regarding the effects of environmental influences on their developing brain. Of particular interest to us is the developing opioid receptor system. Although some data has been acquired pertaining to the ontogeny of opioid receptors in the developing rat brain, such information is virtually non-existent regarding the human brain. Premature neonates are exposed to exogenous opiates (e.g., morphine, fentanyl) on a regular basis when critically ill and this may permanently alter their opioid receptor profile as adults. In order to better study this, it is important to first learn the expression of opioid receptors during normal human CNS development. Fresh post-mortem brain specimens were obtained at various gestational ages (16 to 36 wk). Tissue was blocked, immersed in Zamboni's fixative for 90-120 days, soaked in 10% sucrose for 96 hours, fast frozen at -40°C in isopentane, then cryostat cut into 15μm sections. Tissue was incubated in primary rabbit antiserum directed against the C-terminal sequence of the rat mu receptor protein then, using a tyramine-enhanced avidin-biotin-peroxidase complex, visualized with nickel chloride and diaminobenzidine. Pre-absorption controls were performed using 2μM protein absorption of primary antiserum prior to tissue incubation. Immunostaining in regions similar to those described previously in rat was observed in corpus striatum, entorhinal and cerebral cortex, and several additional forebrain and brainstem nuclei. Comparisons to distribution of mu receptor in rat brain and between gestational ages is discussed. Supported by NIDA Grant T32DA07268.